From BVD Vaccination to Bleeding Calves

Analysis of the process of pharmacovigilance concerning the PregSure® Bovine Virus Diarrhoea Vaccine from Pfizer now known to cause Bovine Neonatal Pancytopenia

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Contents

Abstract

Bovine Neonatal Pancytopenia is a bleeding disorder occurring in the European Union and New Zealand. The disease affects neonatal or very young calves from PregSure® BVD vaccinated dams. Dams produce alloantibodies against impurities from the MDBK cell line on which PregSure® is produced, including MHC 1, present in PregSure® BVD vaccine. Only dams not recognizing the proteins as self produce those alloantibodies. If a calf produced by such a dam inherits paternal antigens resembling those present in PregSure® BVD and it receives colostrum from its own dam, the alloantibodies present in the sera and colostrum of the dam target the leukocytes, thrombocytes and haematopoietic bone marrow cells of the calf. After binding of the alloantibodies to the cells, the cells undergo phagocytosis through macrophages by complement activation. This results in bone marrow aplasia and haemorrhagic diathesis. Most of the affected calves die, but spontaneous recovery is possible and also subclinical Bovine Neonatal Pancytopenia occurs.

The disease Bovine Neonatal Pancytopenia is actually a severe adverse reaction to PregSure® BVD vaccine. This research internship report analyses the process of pharmacovigilance of PregSure® BVD vaccine and follows the developments around the vaccine and the disease through the years. Pharmacovigilance is applied to each veterinary medicine on the market and the process guards the balance between risk and benefits of medicines.
Aetiology and pathogenesis of Bovine Neonatal Pancytopenia is investigated thoroughly in the last decade, but still the exact cause of the disease is unknown. It is also not clear which protein or proteins is or are the exact target or targets of the colostrum derived alloantibodies of BNP-dams. Further investigation is necessary because Bovine Neonatal Pancytopenia is the first frequently occurring immunodeficiency caused by a prophylactic measurement like the PregSure® BVD vaccine. Development of the same pathogenesis in the future with other vaccines, human or animal, produced on the same production cell line as the target species can be prevented by extensive knowledge of the pathogenesis and aetiology of Bovine Neonatal Pancytopenia.

Keywords
Bovine Neonatal Pancytopenia, pharmacovigilance, bone marrow aplasia, haemorrhagic diathesis, alloantibodies.

Introduction

Bovine Virus Diarrhoea is a contagious bovine disease that can lead to high economic losses and difficulties in national and international trade of cattle. (Salt, 2004) Vaccination programmes against Bovine Virus Diarrhoea Virus to protect bovine livestock are therefore promoted by governments and several vaccines produced by different pharmaceutical companies are available on the European market.
One of those available vaccines against Bovine Virus Diarrhoea was PregSure® BVD produced by Pfizer Animal Health (since 2012 Zoetis™). This particular vaccine was eventually withdrawn from the European market because of its involvement in the pathogenesis of Bovine Neonatal Pancytopenia. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)
The process that led to the withdrawal of PregSure® BVD is subject of this research internship report. The aim of the research is to investigate and analyse the process of pharmacovigilance concerning the PregSure® BVD vaccine in relation to the onset of Bovine Neonatal Pancytopenia.

For each veterinary medicine on the market, including vaccines, pharmacovigilance is applied. Through pharmacovigilance the animal health is protected and the balance is kept between benefits and risks of veterinary medicines. (Dyer, 2010) The European Medicines Agency, represented by the Committee for Medicinal Products for Veterinary Use and the competent pharmacovigilance authorities in countries in the European Union, for example Paul-Ehrlich-Institute in Germany (Bastian, 2011) or the Veterinary Medicines Directorate in Great Britain (Dyer, 2010), guard the process of pharmacovigilance. (The European Parliament and the Council of the European Union, 2004)
The holder of the marketing authorisation of the veterinary medicine, in this case Pfizer Animal Health, and the competent pharmacovigilance authorities, work together to collect all information about suspected adverse reactions to a veterinary medicine. Animal owners and breeders can communicate adverse reactions to a healthcare professional (e.g. a veterinarian), who communicates the adverse reaction to the competent authority or the holder of the marketing authorisation, but they can also communicate to the competent authority or holder of the marketing authorisation directly. (The European Parliament and the Council of the European Union, 2004)
Sometimes a suspected adverse reaction can be a serious adverse reaction, this is the case when an adverse reaction is fatal, life-threatening, disabling, incapacitating or if the adverse reaction results in permanent or prolonged signs in the treated animal(s). (Dyer, 2010)
Those serious adverse reactions are recorded by the marketing authorisation holder and reported to the European Medicines Agency and the other member states of the European Union within fifteen days. The adverse reaction records kept by the marketing authorisation holder are submitted periodically to the European Medicines Agency, supported by a scientific evaluation with emphasis on the risk-benefit balance of the veterinary medicine. In case of communication to the general public concerning the pharmacovigilance of a veterinary medicine, the European Medicines Agency has to be notified first. (The European Parliament and the Council of the European Union, 2004)
A marketing authorisation for a veterinary medicine is first granted for a period of five years, in which the marketing authorisation holder collects pharmacovigilance data about the veterinary medicine. If the risk-benefit balance of the veterinary medicine is positive, the marketing authorisation is extended for an unlimited period. (The European Parliament and the Council of the European Union, 2004)

PregSure® BVD vaccine is now known to play an important role in the pathogenesis of Bovine Neonatal Pancytopenia. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)
The disease Bovine Neonatal Pancytopenia is a bleeding disorder. Bleeding disorders in general are relatively rare in cattle, with disorders in the primary haemostasis leading to petechiae, ecchymoses, bleeding after injections, melena, bleeding from the gastrointestinal tract, epistaxis and haematuria and disorders in the secondary haemostasis causing bleeding into body cavities, muscles, joints and the development of haematomas. Bovine

Neonatal Pancytopenia causes thrombocytopenia, which is a disorder in the primary haemostasis and the most common cause of bleeding disorders in cattle (Bell, 2011a)

In this research a chronological analysis of the process of pharmacovigilance of PregSure® BVD is written from May 2004 till June 2014. This does not mean that the process does not continue after this date. On the contrary, as can be read in the discussion, further research to this subject is wanted and needed to prevent a serious adverse reaction like Bovine Neonatal Pancytopenia to a vaccine in the future.

Material and methods

Information gathering started with a multi-field search in the search engine 'CAB Abstracts: Veterinary Science' (© 2000-2014 Ovid Technologies, Inc, Version: OvidSP_UI03.12.00.116, SourceID 60384). The terms entered in CAB Abstracts were "bovine [AND] neonatal [AND] pancytopenia" and the search resulted into 46 found publications.
Next the titles and abstracts of the search results were checked for relevance and the publication's references were searched for more relevant publications.

Nine publications of the 46 results of the initial search proved not to be relevant (2 out of 9), were written in a foreign language not being English, German or French (4 out of 9), or could not be obtained in the Utrecht University Library, through Google Search, or via personal relations (3 out of 9). The references search resulted into 82 publications, of which 14 publications proved not to be relevant (2 out of 14), could not be obtained (6 out of 14), or appeared to be double present into the list of publications (6 out of 14). The search resulted into a total of 103 publications, which were used in this research internship.

After completing the gathering of publications, analysis of the found publications started. This included a thorough reading of the publications and processing of the information of the articles into a chronologic timeline in Excel® 2010 (Microsoft Corporation, Redmond, USA). The timeline made in Excel® contained an extended summary of each publication, sorted by date.
A graphic visualisation of the timeline was made in Visio® Professional 2013 (Microsoft Corporation, Redmond, USA), in which the essence of most of the publications, summarized in one sentence, was labelled to a date on the timeline.

When a specific date was mentioned for an event concerning the pharmacovigilance of BNP this date was used in the timeline, otherwise the month of publication of the article was used.

Results

A graphic visualisation of the pharmacovigilance timeline of PregSure® BVD vaccine can be found in the attachments. The first attachment corresponds with the period 2004 to 2008, the second, third and fourth ones correspond respectively with the years 2009, 2010 and 2011. The last attachment shows 2012 to 2014. The attachments can be found at the end of this document starting at page 26.

2004 to 2008

The marketing authorisation for PregSure® BVD vaccine by Pfizer is granted first in Germany in May 2004 by the Paul-Ehrlich-Institute, the national competent pharmacovigilance authority of that country. (Bastian, 2011) The immune reaction triggered by PregSure® BVD protects cattle against Bovine Virus Diarrhoea Virus type 1 and 2 and prevents fertility problems in dams. (Salt, 2004) (Pfizer Ltd, 2004) Antibody levels triggered by vaccination with this vaccine are high, much higher than after vaccination with other available vaccines such as Bovilis® BVD, Bovidec® and Vacoviron® FS (Association for Veterinary Teaching and Research Work (AVTRW), 2005), and persist at least until twelve months after vaccination. Boosting the vaccination after twelve months leads to even more convincing level of antibodies. (Harmeyer, 2004) (Pfizer Ltd, 2004)
PregSure® BVD vaccine, which contains an inactivated Bovine Virus Diarrhoea type 1 virus strain 5960 and the adjuvant Procision-A^{tm}, becomes available in the other countries of the European Union in December 2004 (Pfizer Ltd, 2004) (European Medicines Agency, 2010b) and in New Zealand in May 2008. (Butler, 2011)
Animals can be vaccinated with the vaccine at all times, even during pregnancy and there is no withdrawal period for PregSure® BVD vaccine. Basic immunisation includes two 2 millilitre injections separated by a three week period. If the basic immunisation is completed at least fourteen days before insemination, the vaccine provides a one hundred percent protection of the foetus against in utero infection with Bovine Virus Diarrhoea Virus and thus prevents a calf from becoming a persistently infected calf. (Pfizer Ltd, 2004) (Harmeyer, 2004) (European Medicines Agency, 2010b)

Early in 2007 an unusual increase of young calves with bone marrow aplasia and haemorrhagic diathesis is noticed in Germany. (Friedrich, 2009) (Deutsche Buiatrische Gesellschaft, 2009) At the end of 2007 a serious accumulation of reports of the bleeding calves has been received in the German state Bavaria and by then the phenomenon goes by the name 'Blutschwitzen' (blood sweating disease) (Kappe, 2009) (Kappe, 2010) The French also start noticing cases of bleeding calves in 2007 (Schelcher, 2010). The Netherlands notices an increase in cases of blood sweating disease in 2008. (French Buiatrics Association, 2009b)
Retrospectively the first cases of calves with blood sweating disease have been noticed in Germany in 2005 (Bastian, 2011), in Belgium in 2006 and 2007 (Pardon, 2010) and in Poland in 2007. (Jaskowski, 2012)
Great Britain notices their first cases of calves with idiopathic thrombocytopenia with bone marrow aplasia in May 2008 in Scotland (SAC C VS Disease Surveillance Report, 2008) and during this year and the next year they keep on receiving sporadic reports of young calves dying of blood sweating disease. (Penny, 2009)
A common feature with all reported cases, in all countries, is that no cause can be found for the bone marrow aplasia and haemorrhagic diathesis, despite early research to investigate the cause in Germany and Belgium. (Penny, 2009) To help further investigation into the cause of blood sweating disease, the Bavarian 'Tiergesundheitsdienst' (Animal Health Service) urges farmers and veterinarians at the end of 2008 to report cases of bleeding calves to their service for further investigation. (Tiergesundheitsdienst Bayern, 2008)

2009

Early 2009 brings the first reports of blood sweating disease in England. (VLA Disease Surveillance Report, 2009a) (Association for Veterinary Teaching and Research Work (AVTRW), 2010) Bleeding calf syndrome, as the disease is called in Great Britain, is first

officially confirmed in England in May 2009, a month after the first officially confirmed case in Scotland. (Veterinary Laboratories Agency, 2011)
Investigation on eight Scottish herds shows an incidence of blood sweating disease of 3 to 5% amongst calves born this spring. A case is defined as a calf less than three weeks old with fever and spontaneous bleeding from body cavities, at injection sites and after ear tagging. Blood panel shows pancytopenia and post-mortem examinations show generalised bleeding. (SAC C VS Disease Surveillance Report, 2009c) Two months later the Bavarian Animal Health Service defines the case definition further to calves of two to three weeks old, which are normal at birth and during initial development, but start to bleed suddenly out of intact skin and body cavities and have heavy bleedings from injection sites and after ear tagging. The nasolabial planum and mucosa are increasingly pale and almost all calves die in the first hours or days after the onset of the first symptoms. At post-mortem examination heavy bleeding in the subcutis, intestines and other organs are seen, along with severe bone marrow aplasia causing thrombocytopenia. (Kappe, 2009)
At the same time in Scotland, the first careful ideas about the cause of the blood sweating disease start to emerge; they take an autoimmune thrombocytopenia caused by colostrum derived antibodies and a viral or toxic infection of the foetal bone marrow into account. (Penny, 2009) To investigate the syndrome, especially the appearance of the bone marrow typical for a blood sweating disease case (SAC C VS Disease Surveillance Report, 2009b), and its probable cause further, the Scottish Agricultural College and Veterinary Laboratories Agency urge farmers and veterinarians to report and offer suspected cases of blood sweating disease for further investigation, just like the Bavarian Animal Health Service did earlier in 2008. (Penny, 2009) (Emerging Diseases, 2009) (SAC C VS Disease Surveillance Report, 2009a) (Hollimar, 2010) (Association for Veterinary Teaching and Research Work (AVTRW), 2010) (Tiergesundheitsdienst Bayern, 2008)
Around June 2009, after investigating an increasing number of cases in Bavaria, it is noticed that the blood sweating disease occurs clustered in herds and regions. (Deutsche Buiatrische Gesellschaft, 2009) In this month the Paul-Ehrlich-Institut makes a pharmacovigilance questionnaire available for German farmers and veterinarians to report cases of blood sweating disease. (Reichmann, 2012) The syndrome is now being reported from Germany, France, Belgium, the Netherlands, Great-Britain (Deutsche Buiatrische Gesellschaft, 2009) and Italy. (French Buiatrics Association, 2009a)

As a, with a high dose of Dexamethasone and Enrofloxacine successfully treated and recovered case of blood sweating disease, is reported from Belgium (French Buiatrics Association, 2009a), investigators from the Ludwig-Maximilians-University of Munich report a further defined case definition of the syndrome. Calves are 12.7 to 17 days old at onset of the bleeding, they show bloody faeces, petechiae on mucosa, spontaneous bleeding without previous injury, bleeding after ear tagging and at injection sites, fever despite treatment with antibiotics and anti-inflammatories, depression in advanced stages of the disease, a blood panel with pancytopenia and most of the affected calves eventually die. Pathology shows generalised internal bleedings, which cause the anaemia in the calf. (Friedrich, 2009) (Kappe, 2010)
They also investigate the possible differential diagnoses of blood sweating disease. After ruling out genetic, toxic and physical, infectious and idiopathic causes they suppose vaccination of the dam against Bovine Virus Diarrhoea Virus could be a probable cause of the syndrome. The other differential diagnoses are ruled out respectively because the syndrome occurs in different breeds of cattle, toxins and physical agents could not be demonstrated and a dam can have more than one affected calf in consecutive years. A common infectious agent could also not be demonstrated and an idiopathic cause is not deemed a likely cause in so many widely distributed cases. (Friedrich, 2009) (Kappe, 2010)

In the meantime as the summer of 2009 has come and gone and autumn arrives more cases of blood sweating disease are being reported from Scotland and England (VLA Disease Surveillance Report, 2009c) (VLA Disease Surveillance Report, 2009d) (VLA Disease Surveillance Report, 2009b) (SAC C VS Disease Surveillance Report, 2010a) and the first Welsh case of blood sweating disease is reported in October 2009. (VLA/ GVS/ AGV Conference, 2010) (Veterinary Laboratories Agency, 2011) (VLA Disease Surveillance Report, 2010b)

In Germany in the meanwhile the development of the blood panel in the course of the syndrome is investigated. The blood panel is physiological at birth, but within three hours after first colostrum intake the amounts of thrombocytes and leukocytes drop dramatically. After a brief recovery of the blood panel in the first day, the thrombocytes and leukocytes drop further. This enables the early signs of the syndrome to be visual as early as the second day of life. Within 120 hours after the first colostrum intake the thrombocyte count drops below the critical value of $30x10^9$cells/litre and bleeding of increasing severity starts to occur. Treatment with blood transfusions and symptomatic treatment of secondary infections only temporarily restore the blood cell count. Bleeding resumes within a few days. (Deutsche Buiatrische Gesellschaft, 2009)
Post-mortem investigation reveals a severe bone marrow aplasia in calves that died of blood sweating disease, which contrast with a bone marrow hyperplasia in calves that recovered their blood cell counts and eventually survived the disease. The early onset of the drop of thrombocytes and leukocytes, after the first colostrum intake, supports the involvement of colostrum in the development of the bone marrow aplasia and haemorrhagic diathesis in blood sweating disease. (Deutsche Buiatrische Gesellschaft, 2009)

Blood sweating disease even becomes the sole subject of a Satellite Symposium at the European Buiatrics Forum in December 2009. It appears that the course of the syndrome is very similar in the different European countries and that vaccination of the dam with PregSure® BVD vaccine seems to be a returning factor. (Bell, 2010a) (Veterinary Laboratories Agency, 2011) (Pardon, 2010) (Friedrich, 2009) (Friedrich, 2011) (French Buiatrics Association, 2009b) (Brugere-Picoux, 2010) An increase in cases of blood sweating disease is noticed in France and Great Britain in 2009 (French Buiatrics Association, 2009b) and a mortality of 95% is reported from Great Britain. (Dyer, 2010) The Germans propose a new case definition; a calf with severe bleedings, tested negative for Bovine Virus Diarrhoea Virus, thrombocytopenia (< $200x10^9$cells/litre), leukopenia (< $4x10^9$cells/litre), aged less than four weeks and bone marrow aplasia at post-mortem investigation. (French Buiatrics Association, 2009b) At this gathering, investigators from the different countries agree on a new official name for the blood sweating disease: Bovine Neonatal Pancytopenia. (Friedrich, 2011)
With the first reported cases received from Northern Ireland at the end of 2009 (VLA/ GVS/ AGV Conference, 2010), over 400 of reports of Bovine Neonatal Pancytopenia, involving 2000 animals, have been received in the member states of the European Union were PregSure® BVD vaccine is registered. Most reports originate from Germany. (European Medicines Agency, 2010b) This number underestimates the actual amount of Bovine Neonatal Pancytopenia cases, due to underreporting. (French Buiatrics Association, 2009b)

2010

Although Bovine Neonatal Pancytopenia most often results in death for the calf, the disease is not obligatory deadly. Some calves survive and now even subclinical cases of Bovine Neonatal Pancytopenia are suspected to occur. (Research, 2010) The incidence of the disease differs somewhat between countries; it is for example in Belgium estimated to occur in 1.4 in 10,000 calves and in 1.5 in 10,000 calves in Great-Britain, but the incidence is estimated to be lower in France with 1.2 in 100,000 calves. (Theron, 2010)

February 2010 brings speculation in Germany about a probable involvement in the pathogenesis of Bovine Neonatal Pancytopenia of Porcine Circovirus genotype 2B. A virus resembling this Circovirus is found in case calves and Bovine Neonatal Pancytopenia resembles Chicken Infectious Anaemia, caused by another Circovirus. (Kappe, 2010) Researchers in Great Britain, also investigating the cause of the new disease, however suspect an immunological cause with colostrum involvement. (Association for Veterinary Teaching and Research Work (AVTRW), 2010) (Research, 2011) They cannot find any evidence for a Circovirus involvement in their cases and therefore reject Circovirus as a probable cause. (Willoughby, 2010) (Association for Veterinary Teaching and Research Work (AVTRW), 2010)
Researchers from Belgium redefine the case definition of Bovine Neonatal Pancytopenia to a calf aged less than one month, with bleeding from the skin, petechiae and/or melena, leukopenia of < $3x10^9$cells/litre, thrombocytopenia of < $100 x10^9$cells/litre, bone marrow

aplasia and absence of Bovine Virus Diarrhoea Virus. They observe a mortality of 91%, but also spontaneous recoveries in some calves and an immunological cause with colostrum involvement is suspected. (Pardon, 2010)

By April 2010 over 2300 cases of Bovine Neonatal Pancytopenia have been reported in the European Union, 1800 of them are German cases. (Schumann, 2011) In this month Pfizer Animal Health, the pharmaceutical company producing PregSure® BVD vaccine, voluntarily stops the sales of the vaccine in Germany, (European Medicines Agency, 2010b) after a report to the European Medicines Agency by the German Paul-Ehrlich-Institut. They doubt the safety of the use of PregSure® BVD vaccine in cattle because of a suspected connection between the vaccine and the development of Bovine Neonatal Pancytopenia in calves. (European Medicines Agency, 2010a) (European Medicines Agency, 2010b) A month later the first Bovine Neonatal Pancytopenia cases are reported from Ireland. (Sanchez-Miguel, 2010)
To assist in the research for the aetiology and epidemiology of Bovine Neonatal Pancytopenia, Pfizer Animal Health voluntarily stops all sales of PregSure® BVD vaccine in the European Union in June 2010. (Dyer, 2010) (European Medicines Agency, 2010b) The voluntary sales stop is followed by a suspension of the marketing authorisation of the vaccine in July 2010. The suspension is authorised by the Committee for Medicinal Products for Veterinary use of the European Medicines Agency because of the potential association of PregSure® BVD vaccination of the dam with the development of Bovine Neonatal Pancytopenia in calves. Estimated incidence of Bovine Neonatal Pancytopenia according to the European Medicines Agency based on the use of one dose PregSure® BVD vaccine in Europe is 0.016%. This incidence is only present in countries in which PregSure® BVD vaccine is available on the market, countries without a history of PregSure® BVD use, only have isolated incidents of idiopathic Bovine Neonatal Pancytopenia. This incidence of a potentially fatal disease as a possible adverse reaction to a prophylactic measure is unacceptable, hence the suspension. (European Medicines Agency, 2010b)
To remove the suspension, research has to prove that PregSure® BVD does not increase the risk of Bovine Neonatal Pancytopenia development, or until effective measures can be applied to use the vaccine safely. (European Medicines Agency, 2010a) (Dyer, 2010)

In the meantime a search for effective measures to prevent Bovine Neonatal Pancytopenia in calves is initiated. At a German farm preventive treatment of the calves with Dexamethasone and Enrofloxacine seems to be effective to significantly reduce the incidence of cases. (Klemt, 2010) Scottish measures including muzzling of the calf to prevent suckling of colostrum at the dam until 32 hours after birth and replacing the colostrum of the dam with colostrum from two other Bovine Neonatal Pancytopenia-free farms, are able to prevent Bovine Neonatal Pancytopenia developing in the new-born calves of dams, which have had an affected calf earlier. (Bell, 2010b)
Other research in Great Britain shows that bone marrow samples taken from the sternum of Bovine Neonatal Pancytopenia affected calves, serve best in proving the presence of bone marrow aplasia. Many of the British calves with a drop in leukocytes and thrombocytes two hours after colostrum intake develop the disease in their first four weeks of live. However analysis of blood samples taken in the first week of live from healthy calves that would not develop clinical signs of Bovine Neonatal Pancytopenia, shows that some of the healthy calves have a drop in their blood cell count too. This again suggests the existence of a subclinical form of the syndrome. (Chilean Buiatrics Society, 2010) (VLA/ GVS/ AGV Conference, 2010)
Risk factors determining if a calf gets affected by Bovine Neonatal Pancytopenia are, according to British research: vaccination of the dam against Bovine Viral Diarrhoea Virus and/or Blue Tongue Virus and the ingestion of colostrum from the own dam by the calf. (VLA/ GVS/ AGV Conference, 2010)
Scottish research at a farm with an incidence of clinical Bovine Neonatal Pancytopenia of 5% further confirms the presence of a subclinical form of the disease. They find that in this herd 58% of the one-day-old-calves had developed thrombocytopenia. The thrombocyte count of these calves proved to be recovered by the eleventh day of live. (Bell, 2010a)

The year 2010 concludes with the count of reported Bovine Neonatal Pancytopenia cases in the European Union rising to over 4000 cases, 3000 of them originating from Germany. (Bastian, 2011)

2011

A practical tip to facilitate diagnosing Bovine Neonatal Pancytopenia in the living calf by thrombocyte count, is taking a blood sample with needle and syringe, instead of using a vacutainer. The sample can best be transported in an EDTA tube after gently mixing. A fresh blood smear can also help to attain a reliable thrombocyte count. Furthermore calves suspected of the disease need to be carefully handled; each contact with the animal can cause haemorrhages or haematomas. (Bell, 2011b)
Several therapeutic measures have been used in Great Britain on affected calves, like blood transfusion, high doses of intravenous corticosteroids, clotting agents and antibiotics, with mixed results. (Bell, 2011b) A veterinarian in Great Britain sees a connection between Bovine Neonatal Pancytopenia and a syndrome in lambs and young goats. The lambs and young goats develop anaemia after ingesting bovine colostrum, though in this case mostly erythrocyte cell lines are affected, instead of the thrombocyte cell lines in Bovine Neonatal Pancytopenia. (Winter, 2011)
In February German research proves that Bovine Neonatal Pancytopenia can be caused in some susceptible neonatal calves by feeding them colostrum from dams that produced an affected calf in earlier gestations. The experiment shows a significant difference in blood cell counts between experimental and control calves. Al experimental calves showed a drop in thrombocytes and leukocytes in a few hours after the first colostrum ingestion. After a brief recovery of the blood cell counts, they dropped further in five of the six experimental calves, with three of the calves eventually developing clinical Bovine Neonatal Pancytopenia. The two calves with low blood cell counts, but no clinical signs were subclinical cases of Bovine Neonatal Pancytopenia. This was also supported by examining of bone marrow samples of these two calves. Further investigation into the aetiology of Bovine Neonatal Pancytopenia should focus on something present in the colostrum that is not infectious, not cellular, leads to a dramatic drop in thrombocytes and leukocytes in the circulation and causes a severe destruction of all bone marrow cell lines to complete aplasia. (Friedrich, 2011)

When comparing German herds with and without cases of Bovine Neonatal Pancytopenia, the use of PregSure® BVD vaccine has a high Odds Ratio in all analyses and thus is the factor most influencing the occurrence of the disease. (Carlin, 2011) (Sauter-Louis, 2012) This was also confirmed by British research that proves a significant association between the use of PregSure® BVD vaccination in the dam in the last five years and the development of Bovine Neonatal Pancytopenia in the calf. However since PregSure® BVD vaccination is no longer available, it is suspected that the number of Bovine Neonatal Pancytopenia cases will drop in the future. (Veterinary Laboratories Agency, 2011) (Emerging Diseases, 2011) At the moment, February 2011, the total count of case reports from the European Union reads 4623, with 3040 cases originating from Germany. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)

May arrives with research in Great Britain finding no evidence for an association between Major Histocompatibility Complex class2 diversity on the allelic DRB3 locus and the susceptibility of calves to Bovine Neonatal Pancytopenia. They tried to explain the clustering of the disease in herds and cattle families by the genetic diversity in this protein. (Ballingall, 2011b) (Ballingall, 2011a),
Later this month German research demonstrates a crucial role for colostrum derived alloantibodies in the pathogenesis of Bovine Neonatal Pancytopenia. They demonstrate the presence of alloantibodies in the sera of dams vaccinated with PregSure® BVD vaccine. The concentration of the alloantibodies is significantly higher in dams that in earlier pregnancies produced a calf affected with Bovine Neonatal Pancytopenia (BNP-dams) in comparison with dams that were vaccinated, but did not produce an affected calf. After feeding normal calves colostrum from BNP-dams, alloantibodies in the colostrum bind to the blood cells of the calf, with the level of bonded cells corresponding with the severity of the syndrome e.g. high levels equal clinical disease and median levels subclinical. The following pathogenesis is suggested for Bovine Neonatal Pancytopenia: colostrum derived alloantibodies bind after

colostrum ingestion by the calf to the calf's leukocytes and thrombocytes in the circulation, after which the cells perish. The same also happens to the leukocyte and thrombocyte cell lines in the bone marrow. The development, severity and possible recovery from Bovine Neonatal Pancytopenia depend on the concentration of alloantibodies present in the circulation of the calf and the calf's genetic based susceptibility. (Bridger, 2011) (Doll, 2011)

A second British investigation to investigate the risk factors of a calf developing Bovine Neonatal Pancytopenia finds that if the dam of a calf had been vaccinated with PregSure® BVD, it has 41 times higher possibility of developing Bovine Neonatal Pancytopenia. The herd of the calf originating from Scotland also increased its risk of developing the disease, but this is supposed to be caused by a higher awareness of Scottish farmers. (Lambton, 2012)

Like German research a month earlier, Belgian researchers also investigate sera of BNP-dams. They find that sera of BNP-dams bind significantly more to leukocytes of calves, in comparison with PregSure® BVD vaccinated dams not being BNP-dams and dams with no history of Bovine Neonatal Pancytopenia. Binding to different subpopulations of leukocytes was different, probably due to differences in expression of the target antigen. This suggests an immunological pathogenesis of the leukopenia in calves suffering from Bovine Neonatal Pancytopenia. It is proposed that the target antigen for the alloantibodies present in the sera of BNP-dams and their colostrum must be present on all leukocytes and thrombocytes. Furthermore feeding of pooled colostrum to calves and the paternal genetic background could play a role in the clustered presence of the disease in herds. (Pardon, 2011)

At the same time another research from Germany also finds that sera of BNP-dams and PregSure® BVD vaccinated dams not being BNP-dams, contain alloantibodies that can bind bovine leukocytes. Sera from BNP-dams induce the highest level of binding. After binding of the alloantibodies to the leukocytes *in vitro* induction of phagocytosis by macrophages is demonstrated in this research. Another find is the binding of alloantibodies of BNP-dams to Madin-Darby Bovine Kidney cells (MDBK cells). These cells belong to the production cell line of PregSure® BVD; the inactivated virus present in the vaccine is produced on this cell line. Sera of cows vaccinated with other vaccines against Bovine Virus Diarrhoea Virus or not vaccinated, do not bind to MDBK cells. It is thought that the highly potent adjuvant of PregSure® BVD, inducing high amounts of antibodies, in combination with residue of the production cell line present in the vaccine, might be the key in the development of Bovine Neonatal Pancytopenia in calves by also inducing high amounts of alloantibodies in vaccinated dams. PregSure® BVD vaccinated dams only produce alloantibodies if they recognise the MDBK cells as non-self. If a calf inherits a paternal genetic set of antigens similar to the MDBK cells, the alloantibodies present in the colostrum of the dam recognise the calf's blood cells and cell lines in the bone marrow as non-self after colostrum ingestion and cell destruction occurs after binding of the alloantibodies. This research therefore proves the involvement of PregSure® BVD vaccination in the induction of alloantibodies, associated with the development of Bovine Neonatal Pancytopenia. (Bastian, 2011)

Another German research continues onto the research described above and shows that colostrum derived alloantibodies bind significantly more to monocytes than granulocytes of calves. Furthermore it appears that the alloantibodies can bind differently to calves' leukocytes under the same circumstances. A target antigen for the alloantibodies could not be found, but the search for an antigen should focus on Pestiviruses (e.g Bovine Virus Diarrhoea Virus) of cell fragments of MDBK cells. (Schumann, 2011)

As the knowledge of the pathogenesis of Bovine Neonatal Pancytopenia increases, there also arise questions about the risk of human consumption of milk, colostrum or meat of BNP-dams or PregSure® BVD vaccinated dams or meat originating from calves that have recovered from the disease. There seems to be no microbiological risk for the food chain accompanying this disease (Advisory Committee on the Microbiological Safety of Food (ACMSF) Working Group on Newly Emerging Pathogens, 2010) because no infectious cause can be found. Bovine Neonatal Pancytopenia therefore cannot be classified as a zoonosis. (Veterinary Laboratories Agency, 2011) (Emerging Diseases, 2011) An immunological risk,

however very low, is present in consuming Bovine Neonatal Pancytopenia associated products. The risk is estimated to be very low because of the low incidence of the disease, the low level of still viable alloantibodies present in milk, colostrum or meat after dilution, processing and cooking of the raw products and hence the low exposure of humans to the alloantibodies. There is minimal absorption of bovine antibodies in the human gastrointestinal tract. Infants should in theory be more able to absorb the alloantibodies from their gastrointestinal tract than adults, but milk products for these young consumers are produced with extra care, to minimize the presence of bovine antibodies even further. Besides since the probable cause of Bovine Neonatal Pancytopenia seems to be the use of PregSure® BVD vaccine, that is no longer in use, the immunological risk will only drop further in the future. As of yet no increase in reports of humans with haemorrhagic diathesis due to bone marrow aplasia, that could be linked to Bovine Neonatal Pancytopenia, has occurred. (Advisory Committee on the Microbiological Safety of Food (ACMSF) Working Group on Newly Emerging Pathogens, 2010)

In the summer of 2011 a possible target antigen for the colostrum derived alloantibodies associated with Bovine Neonatal Pancytopenia is discovered: Major Histocompatibility Complex class 1 (MHC 1). MHC 1 is highly polymorph, bound to the membranes of cells and is especially numerously present on haematopoietic cells. With this new knowledge the pathogenesis is suspected to be as follows: a dam is vaccinated with PregSure® BVD vaccine and recognises the MHC 1 allotypes present in the vaccine as non-self which induces the production of alloantibodies in the cow. If this dam then produces a calf that has received paternal MHC 1 allotypes equal to those present in PregSure® BVD and the calf receives colostrum from this dam, the alloantibodies present in her colostrum and ingested by the calf will bind to the calf's blood cells and bone marrow cell lines, which results in destruction of the cells by phagocytosis by complement activation. En masse destruction of the cells results in bone marrow aplasia and haemorrhagic diathesis.
It is not yet known how many alleles of MHC I protein are involved in the susceptibility of calves to Bovine Neonatal Pancytopenia. Factors that do influence the development of the disease are the total ingested volume of colostrum by the calf, the level of alloantibodies present in the colostrum based on the number of PregSure® BVD vaccinations received by the dam, the time passed after the last vaccination with PregSure® BVD and the affinity of the alloantibodies for the MHC 1 allotypes of the calf. Again the extremely potent adjuvant is thought to play a crucial role in the alloantibody induction. (Deutskens, 2011) (Cooper, 2012) (Deutskens, 2012)
French research later this year confirms MHC 1 as target antigen for the alloantibodies from BNP-dams. They further explain that the feeding of pooled colostrum to calves increases the risk of developing Bovine Neonatal Pancytopenia, because of the increase of possible recognition and affinity of the alloantibodies for the MHC1 antigen allotype of the calf. The presence of MHC 1 in the PregSure® BVD vaccine alone could be enough to induce an alloimmune reaction in dams, but the reaction is in fact significantly higher because of the adjuvant present in the vaccine. This presents a potential risk for all vaccines produced on allogenetic production cell lines, including human vaccines.
A low incidence of Bovine Neonatal Pancytopenia is the result of the high level of variation in the MHC 1 genotype; therefore there is little chance of an antibody-antigen match. Incidental idiopathic cases of Bovine Neonatal Pancytopenia without a history of PregSure® BVD vaccination or feeding of pooled colostrum could be caused by leaking of antibodies through the placenta. (Foucras, 2011) (Cooper, 2012)

The feeding of pooled colostrum increasing the risk of Bovine Neonatal Pancytopenia development in calves is shown on a German farm. This farm has a policy of always feeding pooled colostrum to its new-born calves and consequently has a relatively high percentage of calves showing clinical signs of Bovine Neonatal Pancytopenia: 9.84% and another 10% of the calves are subclinically affected. Subclinical calves and calves that recovered from clinical disease have lesser growth than normal calves. (Witt, 2011)

In August 2011 Pfizer Animal Health stops sales of PregSure® BVD vaccine in New Zealand after the confirmation of the first cases of Bovine Neonatal Pancytopenia outside of the European Union in this country. (Zentis, 2011) (Butler, 2011) The company permanently

withdraws the marketing authorisation of PregSure® BVD vaccine in the same month. (Reichmann, 2012) The people of New Zealand are concerned about the public health risks of consuming Bovine Neonatal Pancytopenia and PregSure® BVD associated products, since infants in this country consume bovine colostrum and because of the use of colostrum in health foods. Also the consumption of superfluous dairy male calves within the first weeks of their lives is of the people's concern. (Zentis, 2011) (Butler, 2011) The public health risk is in reality very small as described earlier. (Advisory Committee on the Microbiological Safety of Food (ACMSF) Working Group on Newly Emerging Pathogens, 2010) This is confirmed by Pfizer and New Zealand's Ministry of Agriculture and Forestry. (Zentis, 2011) (Butler, 2011)

At the end of August, a month full of news concerning PregSure® BVD vaccine and Bovine Neonatal Pancytopenia, a veterinarian from the United Arab Emirates has been seeing a similar syndrome like Bovine Neonatal Pancytopenia in race dromedaries in the last fifteen years. It is not clear if there is truly a connection between the two syndromes. (Wernery, 2011)

Now the pathogenesis of Bovine Neonatal Pancytopenia becomes clearer, it is still not clear why the disease clusters in some regions or in some herds. German research finds that it only takes a little amount of very concentrated colostrum with Bovine Neonatal Pancytopenia associated alloantibodies to cause the disease. The ingested amount of colostrum and the individual absorption of the alloantibodies in the calf's gastrointestinal tract also influence the development of Bovine Neonatal Pancytopenia. Calves developing a fatal Bovine Neonatal Pancytopenia proved to absorb significantly more antibodies from the colostrum. Occurrence of the disease in a region or herd depends on the number of cows developing a bigger or smaller spectrum of alloantibodies after PregSure® BVD vaccination and on the degree of genotype differences between calves and cows. Feeding pooled colostrum is also a very influential factor. (Schröter, 2011)

Winter of 2011 concludes with German researchers not finding a connection between mutations in the genes of coagulation factor XI and the susceptibility of calves to Bovine Neonatal Pancytopenia and eventual development of the disease in calves. (Krappmann, 2011) (Ballingall, 2011a)
Further it is again noticed in Germany that farms that have used PregSure® BVD vaccine in the past, have significantly more cases of Bovine Neonatal Pancytopenia in their herds compared to farms that have used another vaccine against Bovine Virus Diarrhoea Virus or have not used vaccination in the past. New however is the discovery of a higher incidence of the disease in herds that have used PregSure® BVD vaccine longer and more often in the past, in comparison to low incidence farms with PregSure® use in the past. (Deutsche Buiatrische Gesellschaft, 2011) (Landesamt für Verbraucherschutz Sachsen-Anhalt Fachbereich 4 Veterinärmedizin, 2012)

2012 to 2014

As cases of Bovine Neonatal Pancytopenia since 2011 also are being reported from outside the European Union (New Zealand), vigilance for the disease is urged in Canada. Since PregSure® BVD has not been available in Canada, a surge in calves with Bovine Neonatal Pancytopenia is not expected but still farmers and veterinarians are urged to report young calves with haemorrhagic diathesis and bone marrow aplasia. (Cooper, 2012)

The knowledge of the pathogenesis of Bovine Neonatal Pancytopenia increases further in June 2012 through the discovery that colostrum derived alloantibodies produced by BNP-dams are IgG1 subclass antibodies. This is to be expected because this subclass of antibodies is actively transported from the bloodstream to the mammary glands. Hence 90% of antibodies present in colostrum are of the subclass IgG1.
The IgG1 alloantibodies bind all of the thrombocytes, granulocytes and monocytes, and many leukocytes show signs of alloantibody-binding in their cytoplasm as well as on their cell membranes. As the leukocytes of the myeloid cell lines are bonded more often than those of the lymphoid cell lines, this research doubts the MHC 1 to be the only target antigen. This doubt is supported by thrombocytes not presenting this protein on their membranes. MHC 1 is however, amongst other yet unknown antigens, able to be one of the

target antigens. Further it remains unclear why some PregSure® BVD vaccinated cows become BNP-dams and some do not. It is suggested that the type of immune reaction of the cow to the PregSure® BVD vaccine influences this development. Genetics, nature and dose of the vaccine antigen and the adjuvant present in the vaccine could also influence the dam becoming a BNP-dam, or not. (Assad, 2012)

Researchers in Poland find that the withdrawal of PregSure® BVD vaccine of the market, does not necessarily stop cases of Bovine Neonatal Pancytopenia occurring in the future. This is probably caused by the long persistency of the colostrum derived alloantibodies in the dam's circulation. (Associação Portuguesa de Buiatria, 2012a)
In Germany it has been noticed earlier that the development of a PregSure® BVD vaccine using farm becoming a high Bovine Neonatal Pancytopenia incidence farm, depends on the longer and more often use of the vaccine and the feeding of pooled colostrum to calves. Those high incidence farms also have an increase in the number and treatment of neonatal disease in general. This could be an effect of subclinical Bovine Neonatal Pancytopenia, because calves with subclinical disease are weaker than normal calves, have a tempered growth and have leukopenia. High incidence farms therefore appear to have much more financial damage by Bovine Neonatal Pancytopenia than is assumed until now. (Reichmann, 2012) (Associação Portuguesa de Buiatria, 2012a)
The high financial losses by subclinical Bovine Neonatal Pancytopenia can be prevented in high incidence farms. To achieve this prevention, farmers need to replace colostrum of BNP-dams, preferably with colostrum from dams in the same herd without a history of PregSure® BVD vaccination. (Schröter, 2012)

In august 2012 the number of Bovine Neonatal Pancytopenia cases has increased to 6913 in the European Union. There is however a decrease in new cases visible when comparing 2011 and 2012 to previous years. (Jones, 2013)
As mentioned earlier at the end of 2009 the number of actual cases is much higher, because only half of the Bovine Neonatal Pancytopenia cases are actually reported. The level of underreporting is therefore substantial. (Reichmann, 2012)

German research shows that the differences in incidence in their federal states can be explained by the differences in the use of PregSure® BVD vaccine. For example the Bavarian government stimulated the use of the vaccine according to the user manual (two doses of PregSure® BVD for basic immunisation and a booster vaccination with the same vaccine after twelve months), but the government of Lower Saxony only stimulated the use of a two-step combination vaccination of one vaccination with PregSure® BVD followed by two vaccinations with a live vaccine. The incidence of Bovine Neonatal Pancytopenia in Bavaria is high, the incidence in Lower Saxony low. The different government policies lead to dams receiving fewer vaccinations with PregSure® BVD than dams in Bavaria. This proves that repeated vaccination with PregSure® BVD is necessary to acquire significant levels of alloantibodies in dams and the development of BNP-dams, but still some dams only need one vaccination with PregSure® to become a BNP-dam. (Kasonta, 2012)

The development of the knowledge about the pathogenesis continues at the end of 2012 with the discovery of colostrum derived alloantibodies targeting not all haematopoietic cell lines in the bone marrow equally. Non-differentiated progenitor cells of the granulocyte and erythrocyte and macrophage and megakaryocyte (GEMM) cell line seem to be the primary target of the alloantibodies in the bone marrow. More differentiated erythrocyte and granulocyte/macrophage progenitor cells do not seem to be targeted in the initial phase of Bovine Neonatal Pancytopenia. Lymphocytes and thrombocytes seem to be the primary targets of alloantibodies in the circulation. This suggests a common target antigen on GEMM progenitor cells, lymphocytes and thrombocytes, which's targeting by alloantibodies starts immediately after colostrum ingestion. (Laming, 2012)
A suggestion for this common target antigen is made in January 2013; adenylaat kinase 2. This protein is present on thrombocytes, leukocytes and cells of the haematopoietic bone marrow cell lines. Mutations in this protein cause severe immunodeficiency in humans, in who haematopoiesis is disturbed by destruction of the haematopoietic progenitor cells in the bone marrow resulting in leukopenia and thrombocytopenia. The same disease occurring in

humans can be created in zebra fish by disabling of adenylaat kinase 2, which suggests a high conservation of the protein in the evolution and also pleads for adenylaat kinase 2 as target antigen.
This new target antigen suggested is discovered by researchers comparing and analysing the protein contents of PregSure® BVD and MDBK cells. PregSure® BVD's protein content appeared to be similar to MDBK cells, which suggests an insufficient purification of the vaccine antigens and thus a failure in the production process. The researchers deemed MHC 1 unlikely as target antigen because MHC 1 is the least common protein in PregSure® BVD. Besides MHC 1 is present on all leukocyte cells, which argues with the differences of alloantibody binding to different subsets of leukocytes. (Euler, 2013)

Differences in affection of haematopoietic cells by alloantibodies from BNP-dams are further defined by British research. Leukocytes and thrombocytes are most affected in the circulation and damage in the bone marrow is limited to all cells of the thrombocyte, lymphocyte and monocyte cell lines and just the primitive precursors of the cell lines of neutrophils, eosinophils and erythrocytes. After colostrum ingestion the lymphocytes, neutrophils and monocytes counts drop quickly due to phagocytosis by complement activation. Mature neutrophils and eosinophils from the bone marrow are then mobilised to restore the level of these cells. There is no haematopoietic response to the thrombocytopenia, but there is some mobilisation of thrombocytes out of the spleen. Erythrocyte levels remain within the normal range for a long time during the development of Bovine Neonatal Pancytopenia. (Bell, 2013)
Besides a role for the adjuvant used in PregSure® BVD in the development of high and persistent amounts of alloantibodies against PregSure® BVD components in BNP-dams, there is also the probability that the adjuvant multiplies maternal alloantibodies against paternal foetal antigens that would normally be induced by pregnancy. This type of maternal antibodies are boosted during each subsequent pregnancy and could contribute to the occurrence of Bovine Neonatal Pancytopenia despite the withdrawal of PregSure® BVD vaccine from the market in 2010. It is also thought that these maternal alloantibodies could be responsible for isolated idiopathic cases of Bovine Neonatal Pancytopenia without a history with PregSure® BVD vaccination (Bell, 2013), as reported by (Fukunaka, 2010), (Shimada, 2007), (Gosselin, 2011) and (Raczynski, 2011).

German research again warns against the use of pooled colostrum in February 2013, since this increases the risk of calves of the herd developing Bovine Neonatal Pancytopenia. It also appears that vaccination of the dam during the sixth month of gestation increases the risk of a calf developing the disease. There seems to be a genetic predisposition for clinical and subclinical Bovine Neonatal Pancytopenia. This is based on dams producing different alloantibodies due to quantitative and qualitative differences in the dam's individual immune responses to PregSure® BVD vaccination and the different susceptibility of the calf to the disease, influenced by paternal genes. (Doll, 2013c)

The specific nature and composition of PregSure® BVD vaccine is investigated in autumn 2013. PregSure® BVD induces a coordinated immune response to double stranded RNA (dsRNA) or a dsRNA analogue. This proves an impurity in the vaccine, because the immune response should be to a single stranded RNA (ssRNA) since Bovine Virus Diarrhoea Virus is an ssRNA virus. It could also be that a component of the adjuvant of PregSure® BVD vaccine acts as fake dsRNA. So as of jet the exact pathogenesis of Bovine Neonatal Pancytopenia remains unclear. (Demasius, 2013)

A multi-country case-control study at the end of 2013 identifies risk factors for the development of Bovine Neonatal Pancytopenia in Germany, the Netherlands, France and Belgium. As described above they find that vaccination of the dam with PregSure® BVD vaccine is strongly associated with the development of Bovine Neonatal Pancytopenia in the calf. Older dams have had more vaccinations with PregSure® BVD and consequently have a bigger chance of an affected calf because of an increase in risk of disease with the number of vaccinations received. Also the feeding of pooled colostrum to calves was found to be strongly associated with the development of Bovine Neonatal Pancytopenia as described earlier. (Jones, 2013)

Of calves succumbed by Bovine Neonatal Pancytopenia, 47% died of haemorrhagic anaemia and 50% died of infectious diseases (pneumonia, enteritis, septicaemia). Those infectious diseases are normal neonatal diseases substantially worsened by Bovine Neonatal Pancytopenia. The disease is not obligatory lethal, demonstrated by subclinical affected calves which have extreme low blood counts with even haemorrhagic diathesis, but eventually recover. It is still not clear why some calves die and other calves with the same blood cell counts and amounts of bleeding survive. (Henniger, 2014) (Henniger, 2013a) Clear is however that calves surviving Bovine Neonatal Pancytopenia do not suffer from permanent kidney damage. There is no difference between renal reference values of normal calves and formally Bovine Neonatal Pancytopenia affected calves. (Henniger, 2013b)

Discussion

In the last decade a lot has been discovered about the aetiology and pathogenesis of Bovine Neonatal Pancytopenia. Many institutes from a multitude of countries have made a contribution to the process of pharmacovigilance of PregSure® BVD, explaining its involvement in the pathogenesis of Bovine Neonatal Pancytopenia. An overview of the different institutes sorted by country can be found in attachment 6 at page 31.

Bovine Neonatal Pancytopenia is a disease that affects initially healthy calves. Within the first hours after the first colostrum intake, a drop in thrombocyte and leukocyte cell counts occurs, with the depth of the drop being different for each individual. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (French Buiatrics Association, 2009b) (Doll, 2011) (Buck, 2011) (Foucras, 2011) (Friedrich, 2011) (Schröter, 2011) (Witt, 2011) The first clinical signs of disease appear between seven and twenty-one days of age (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (French Buiatrics Association, 2009b) (Friedrich, 2009) (Kappe, 2010) (Pardon, 2010) (Veterinary Laboratories Agency, 2011) but in research settings initial blood traces in faeces and petechiae on mucosa can already be seen at two to nine days of age. Those early signs are most often missed in practice and the first clinical sign noticed is bleeding after ear tagging, followed by blood in the faeces, melena and signs of haemorrhagic diathesis. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Deutsche Buiatrische Gesellschaft, 2009) (Friedrich, 2009) (Klemt, 2010) Due to the leukopenia most of the calves also have secondary neonatal infections (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Friedrich, 2009) (Klemt, 2010) (Pardon, 2010) and mortality of Bovine Neonatal Pancytopenia varies between 50-60% (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Buck, 2011) and 89-90%. (Friedrich, 2009) (Pardon, 2010) (Veterinary Laboratories Agency, 2011)
During the first two to seven days of life the thrombocytes and leukocytes drop below the reference values in lethal cases of Bovine Neonatal Pancytopenia (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Deutsche Buiatrische Gesellschaft, 2009) (Friedrich, 2011) (Schröter, 2011), but sometimes the blood counts rise again for a short period to within the reference range because of mobilisation of cells from the spleen and bone marrow. In lethal cases the blood cell counts of thrombocytes and leukocytes drop again after this short rise till values as low as or lower as $15x10^9$cells/litre for thrombocytes and $2x10^9$cells/litre for leukocytes. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Deutsche Buiatrische Gesellschaft, 2009) (Bridger, 2011) (Friedrich, 2011) (Schröter, 2011) Erythrocyte counts descend parallel to the haemorrhages between the third and seventh day of life. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Deutsche Buiatrische Gesellschaft, 2009) (Doll, 2011) (Friedrich, 2009) (Friedrich, 2011)

Within 72 hours after the first colostrum ingestion mild damage to all cell lines in the bone marrow occurs. Macrophages are present (Deutsche Buiatrische Gesellschaft, 2009) (Bridger, 2011) and missing cells are replaced with a protein rich fluid and erythrocytes. The degree of bone marrow damage determines the development of lethal Bovine Neonatal Pancytopenia or recovery of the calf. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b)
Calves without signs of clinical disease can have a drop in their leukocyte and thrombocyte counts too, but obviously the signs of haemorrhagic diathesis do not occur. Those calves suffer from subclinical Bovine Neonatal Pancytopenia. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Klemt, 2010) (Doll, 2011) (Friedrich, 2011) (Schröter, 2011) The percentage of subclinical affected calves varies between 16.4% (Witt, 2011) and 58% (Bell, 2010a) and the blood cell counts of the calves recover between the tenth and twentieth day of life, after recovery of the bone marrow. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b)
Post-mortem investigation shows carcasses with anaemia, bleeding in the subcutis, petechiae and ecchymosis on the mucosa and massive internal bleeding in chest skin, breast skin, serosa, internal organs, intestinal walls and lumen, thorax, abdomen and limbs. There is a mediocre to severe depletion of lymphocytes from lymph nodes and spleen and neither damages in the vascular endothelium are visible nor does parenchymal damage

in organs occur. (Doll, 2013a) (Associação Portuguesa de Buiatria, 2012b) (Friedrich, 2009) (Bell, 2010a) (Kappe, 2010) (Pardon, 2010) (Buck, 2011)

Cases of Bovine Neonatal Pancytopenia appear clustered in regions and in veterinary practices, which is caused by differences in the use of PregSure® BVD vaccine in the protective vaccination programmes against Bovine Virus Diarrhoea Virus. Longer and more frequent use of PregSure® BVD vaccine leads to more cases of Bovine Neonatal Pancytopenia (Deutsche Buiatrische Gesellschaft, 2011), but genetics of calf and dam also play a role in the development of the disease. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)
Half of the herds with Bovine Neonatal Pancytopenia only ever observe one affected calf, the other half have incidences of the disease of about 10% (Klemt, 2010) (Foucras, 2011) (Witt, 2011) (Sauter-Louis, 2012), but incidental higher incidences also occur. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) Bovine Neonatal Pancytopenia can affect the calves of heifers (Pardon, 2010) but usually occurs in older dams. A BNP-dam has a higher risk of producing another affected calf in the next pregnancies. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) (Friedrich, 2009) (Sauter-Louis, 2012)
The occurrence of Bovine Neonatal Pancytopenia is strongly associated with the vaccination of the dam with PregSure® BVD vaccine. Odds Ratios for the association are 40.8 in Great Britain (Veterinary Laboratories Agency, 2011) (Lambton, 2012) and 426 to 1292 in Germany. (Sauter-Louis, 2012) Neither infectious (French Buiatrics Association, 2009b) (Friedrich, 2009) (Pardon, 2010) (Buck, 2011) (Schumann, 2011) nor toxicological causes (Friedrich, 2009) (Kappe, 2010) (Pardon, 2011) can be demonstrated in affected calves or their surroundings. An immunological cause of the disease is confirmed by the demonstration of colostrum derived alloantibodies present in the sera and colostrum of BNP-dams. (Bridger, 2011) (Bastian, 2011) (Foucras, 2011) (Schumann, 2011)

Significant amounts of alloantibodies, though different per individual, are only present in PregSure® BVD vaccinated dams. The alloantibodies produced by the dams bind to calf's leukocytes (Bastian, 2011) (Bridger, 2011) and thrombocytes (Foucras, 2011) (Assad, 2012), but the efficiency of the bondage is, just like the produced amount of alloantibodies, different for each individual due to a relative heterogeneity in the alloantibodies and the epitopes of antigens at which they are directed. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) Like binding to the leukocytes and thrombocytes of calves, the alloantibodies also bind to MDBK cells on which PregSure® BVD vaccine is produced. (Bastian, 2011) (Deutskens, 2011) (Foucras, 2011)
One of the probable target antigens of the colostrum derived alloantibodies is MHC 1. (Deutskens, 2011) (Foucras, 2011) (Deutskens, 2012) MHC 1 is a highly polymorph protein in cattle. PregSure® BVD vaccinated dams, not recognizing the MHC 1 variant present in PregSure® BVD as self, produce alloantibodies against this protein. If a calf of such a dam inherits paternal MHC 1 resembling the MHC 1 variant of PregSure® BVD and it receives colostrum of its own dam, its thrombocytes, leukocytes and hematopoietic bone marrow cells become targets of the alloantibodies and succumb. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) Because MHC 1 is highly polymorph and because of the role of paternal genes, the incidence of Bovine Neonatal Pancytopenia is relatively low. (Deutskens, 2011)

Dams develop alloantibodies after PregSure® BVD vaccination because of the presence of non-virus antigen in the vaccine (Deutskens, 2011) and the alloantibody titres are high and very persistent because of the efficient, immune complex stimulating adjuvant. (Salt, 2004) (Deutskens, 2011) (Bastian, 2011)
Since the end of 2011 the number of new Bovine Neonatal Pancytopenia cases reduces, because of the withdrawal of PregSure® BVD from the market, the replacement of PregSure® BVD vaccinated dams and the exclusion of colostrum produced by BNP-dams. The disease still occurs sporadically because of the persisting alloantibodies present in vaccinated dams. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) Prevention of a BNP-dam again producing an affected calf can be achieved by muzzling the calf (Bell, 2010b)or putting a net on the udder of the dam. The colostrum of the BNP-dam can at best be replaced with colostrum of the same herd from a dam with no history of PregSure® BVD

vaccination. Pooled colostrum needs to be avoided at all times because of the increased risk of Bovine Neonatal Pancytopenia development in the calf. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)
Symptomatic treatment of Bovine Neonatal Pancytopenia affected calves consists of blood transfusions, treatment of secondary infections (Friedrich, 2009) (Bell, 2010a) (Buck, 2011) and immune suppression by Dexamethasone (Klemt, 2010) (Pardon, 2011), but the results of treatment are inconclusive. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b)

Bovine Neonatal Pancytopenia is the first described, frequently occurring immunodeficiency that is actually a severe adverse reaction to a vaccine: PregSure® BVD. Unusual is the binding of colostrum derived alloantibodies to different cellular populations. Sporadic isolated idiopathic cases of Bovine Neonatal Pancytopenia without a history of PregSure® BVD vaccination or the use of pooled colostrum do occur. These cases are probably caused by normal maternal antigens induced by successive pregnancies or after minor damages in the placenta or at birth.
The aetiology and pathogenesis of Bovine Neonatal Pancytopenia is of importance for the production and use of vaccines in animals and humans. Further research needs to be done to further investigate the pathogenesis, find the exact cause of the disease and further identify the target antigen. This is necessary to prevent the same mechanism of pathogenesis to happen again in other vaccines produced on production cell lines of the same species as the target species for the vaccine. (Doll, 2013b) (Associação Portuguesa de Buiatria, 2012b) (Jones, 2013) (Henniger, 2013a) (Henniger, 2014)

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Marie-José Strik

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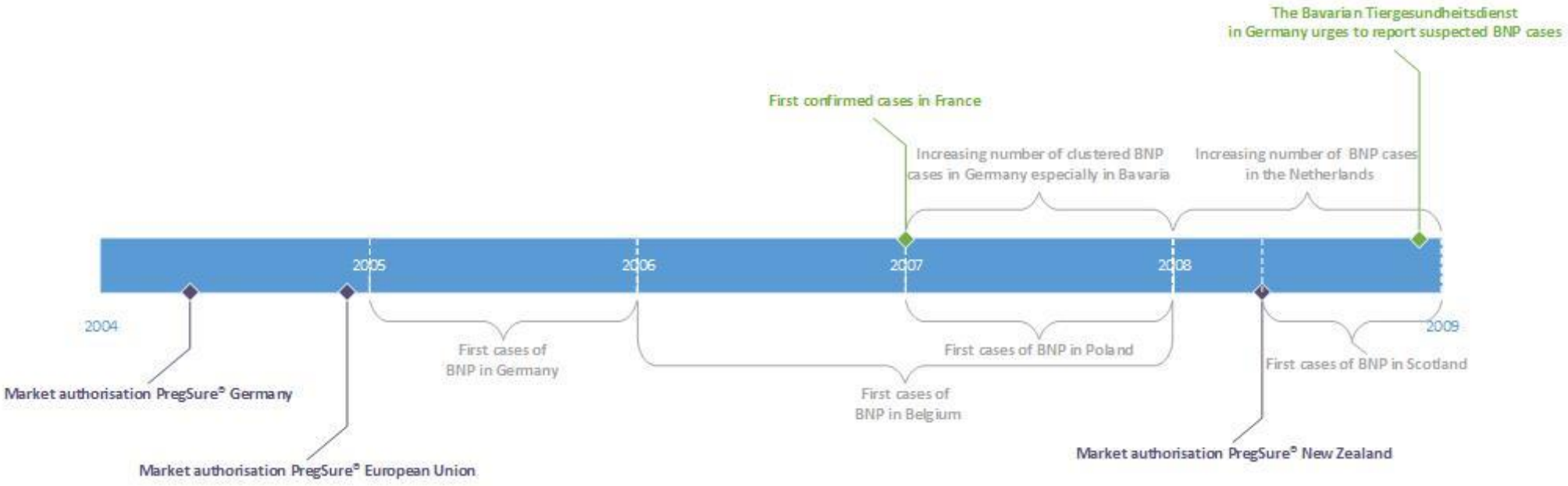
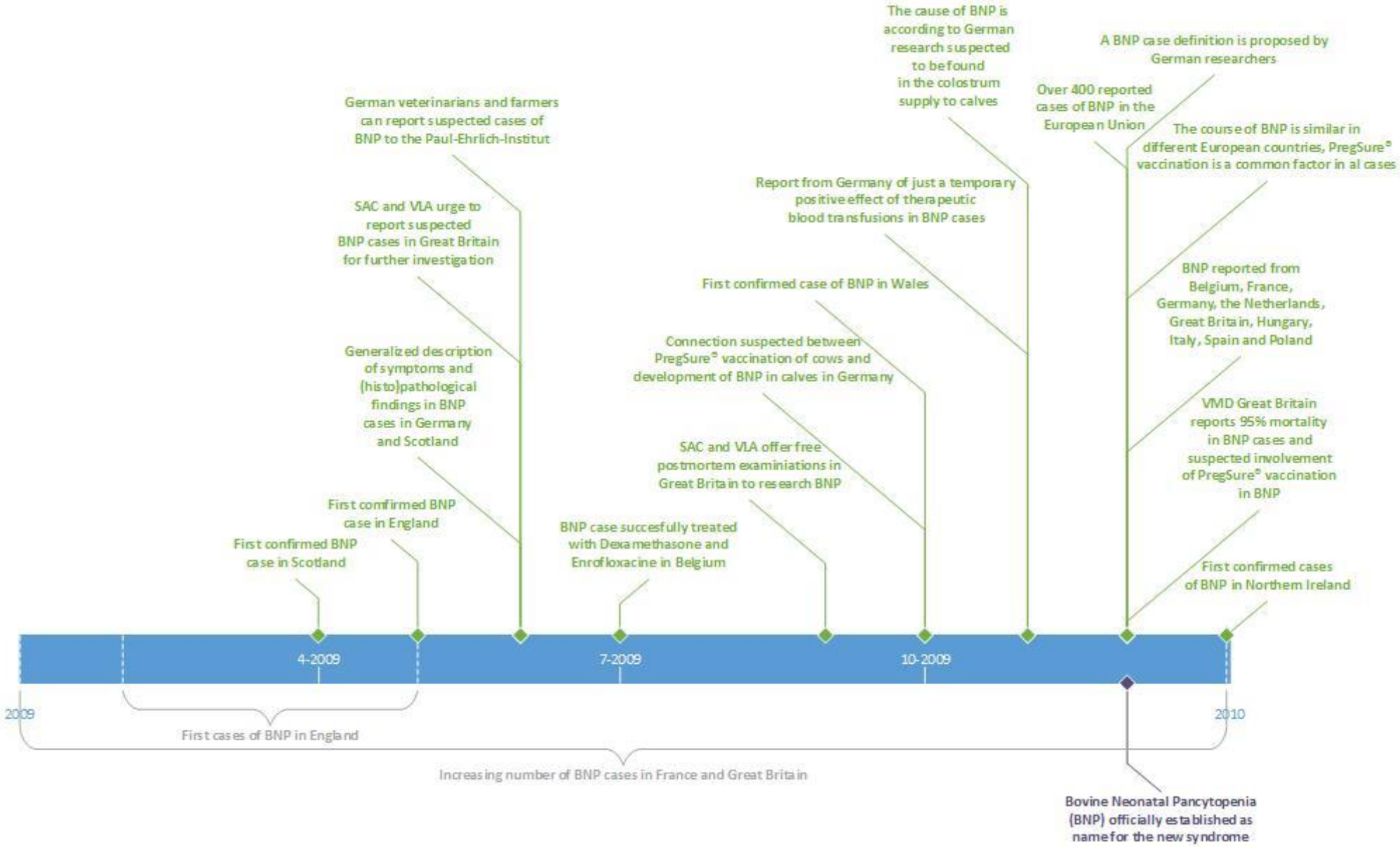
Attachments

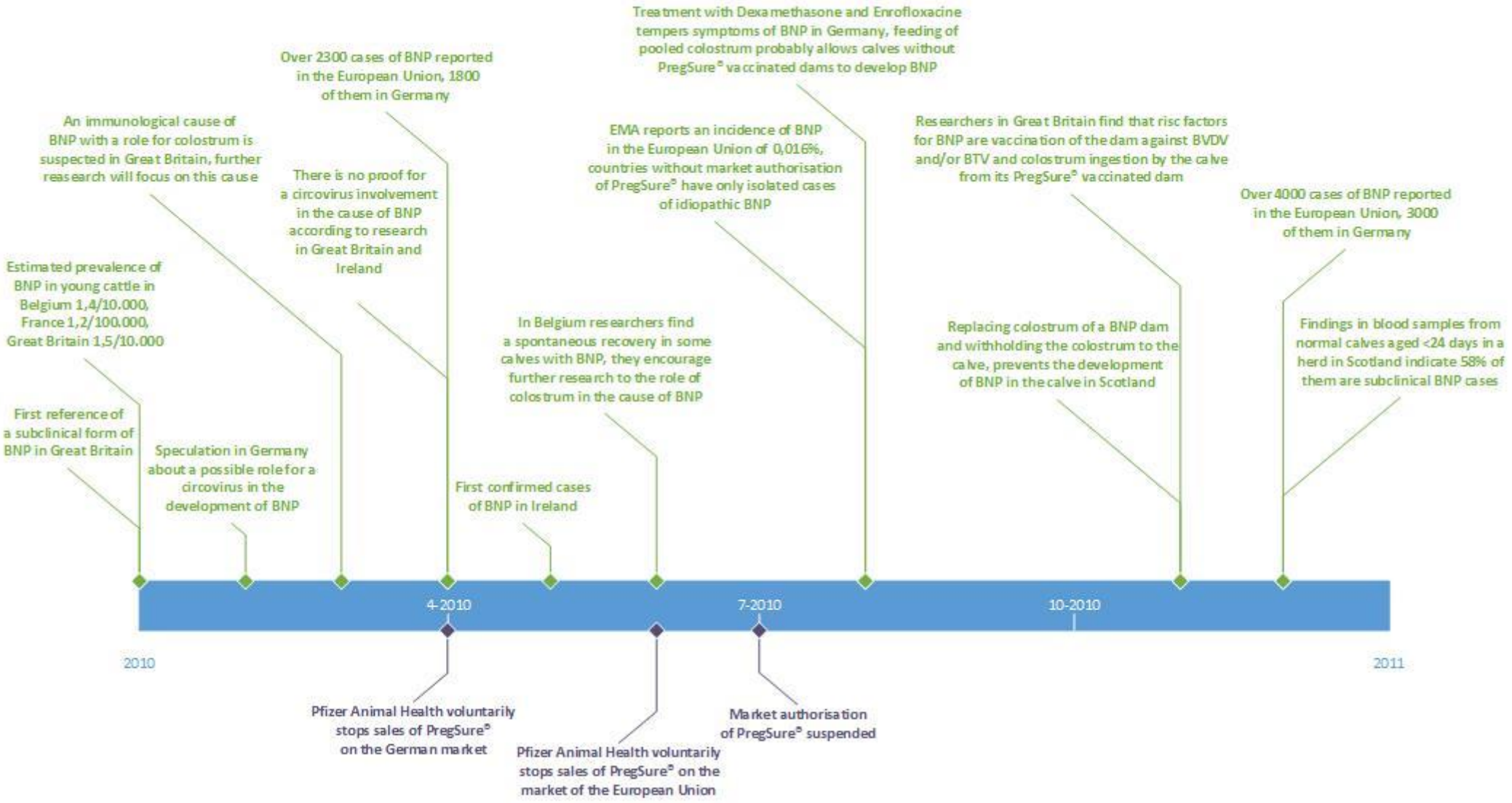
1 Pharmacovigilance timeline of PregSure® BVD 2004 to 2008



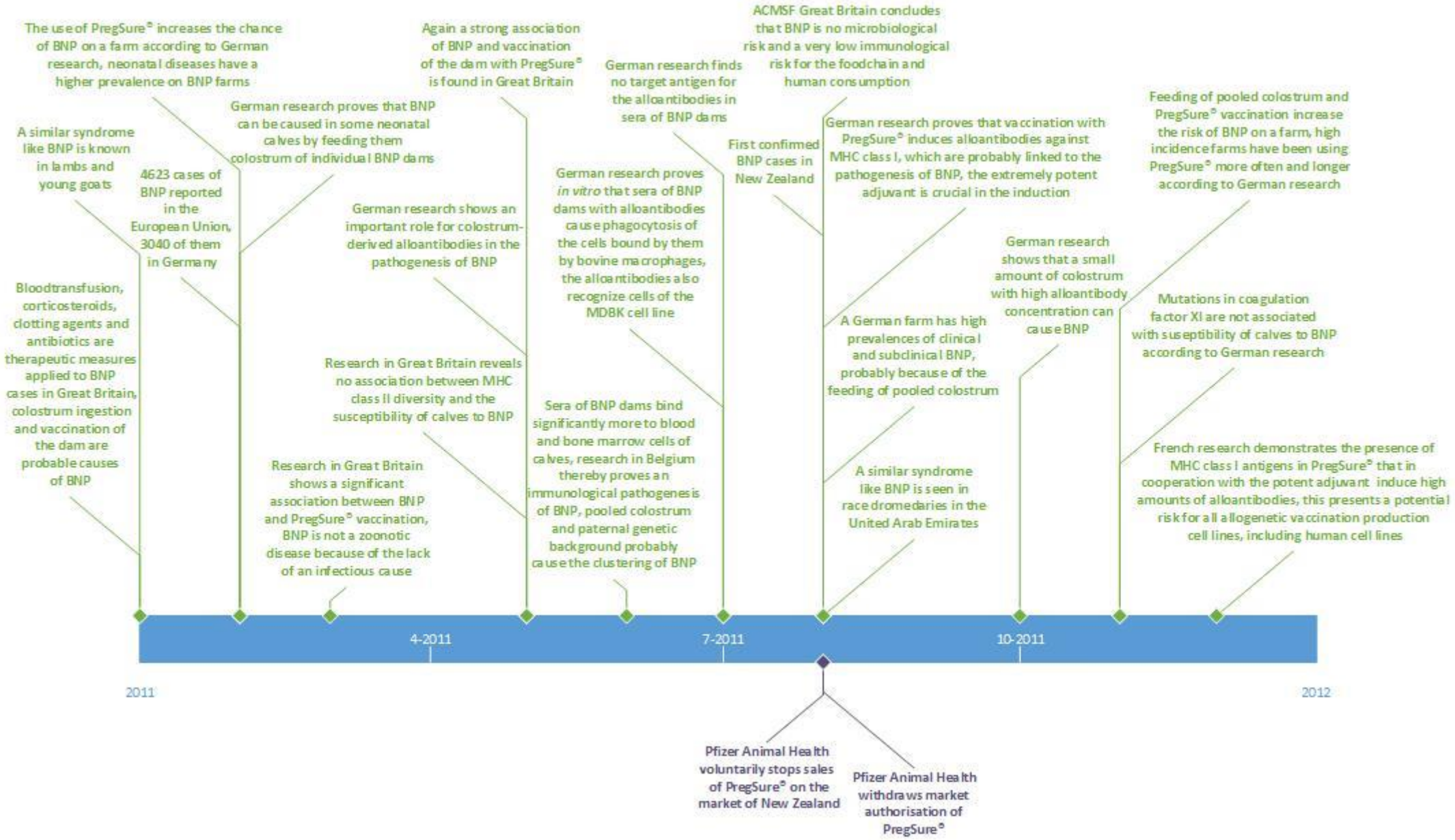
2 Pharmacovigilance timeline of PregSure® BVD 2009



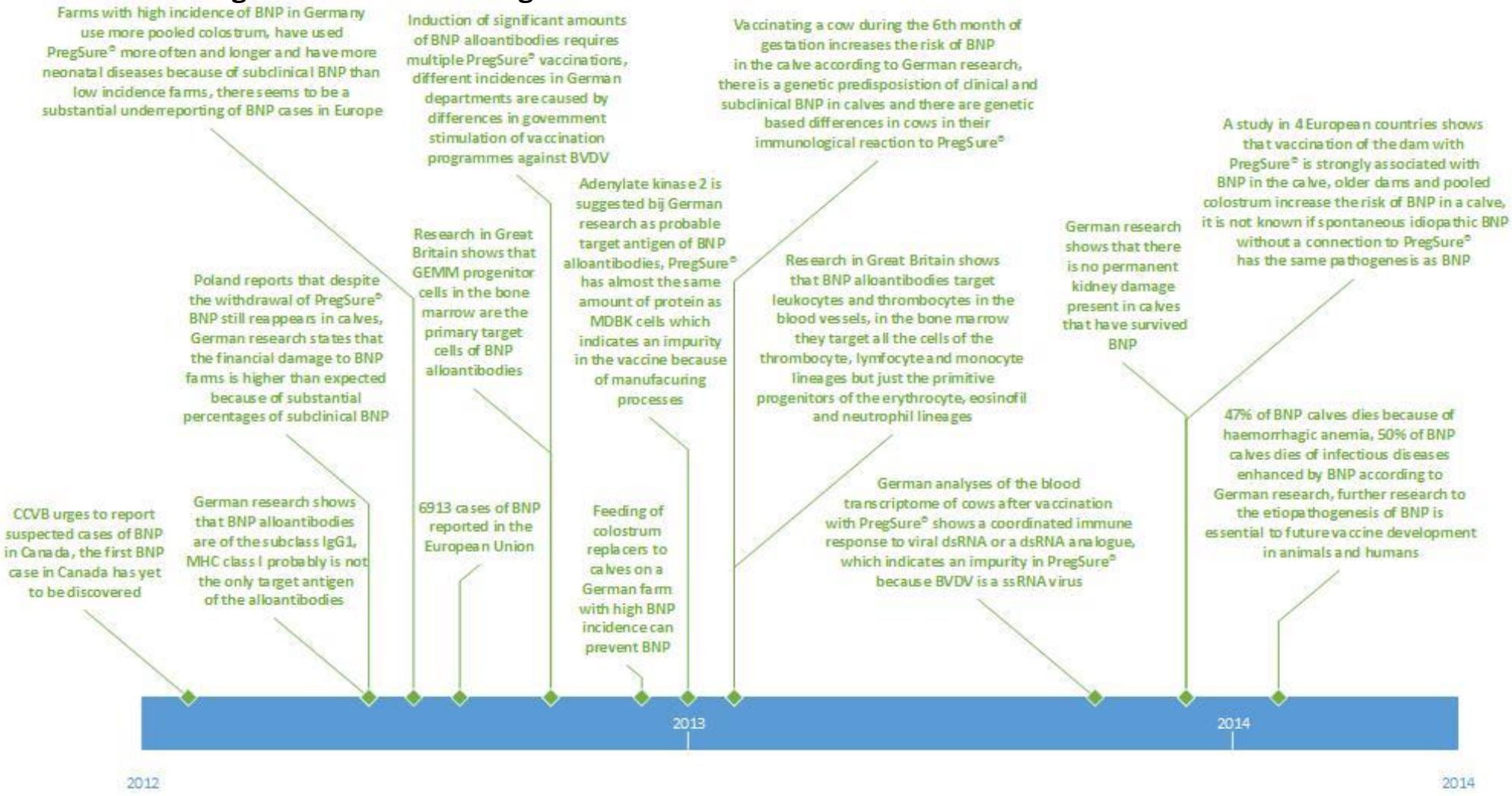
3 Pharmacovigilance timeline of PregSure® BVD 2010



4 Pharmacovigilance timeline of PregSure® BVD 2011



5 Pharmacovigilance timeline of PregSure® BVD 2012 to 2014



6 List of contributing institutes and their country of origin

Based on the first author of the found publications

Institute	Country
Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University	Belgium
Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University	Belgium
Service de thériogénologie et de médecine interne Département clinique des animaux de production, Faculté de médecine vétérinaire de l'université de Liège	Belgium
Canadian Centre for Veterinary Biologics, Canadian Food Inspection Agency	Canada
Centre Hospitalier Universitaire Vétérinaire, Université de Montréal	Canada
Chilean Buiatrics Society	Chile
European Medicines Agency	European Union
European Medicines Agency, Committee for Medicinal Products for Veterinary Use	European Union
The European Parliament and the Council of the European Union	European Union
Institut National de la Recherche Agronomique, Unité Mixte de Recherche 1225, Interactions Hôtes-Agents Pathogènes Toulouse	France
L'école nationale vétérinaire d'Alfort (Unité de pathologie médicale du bétail et des animaux de basse-cour)	France
Société Française de Buiatrie	France
Université de Toulouse, Institut National Polytechnique de Toulouse, Ecole Nationale Vétérinaire de Toulouse	France
Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Freie Universität Berlin	Germany
Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Justus-Liebig-University Gießen	Germany
Clinic for Ruminants, Centre of Clinical Veterinary Medicine, Ludwig-Maximilians University Munich	Germany
Deutsche Buiatrische Gesellschaft	Germany
Faculty of Veterinary Medicine, Justus-Liebig-University Gießen	Germany
Institut für Tierzucht und Vererbungsforschung, Stiftung Tierärztliche Hochschule Hannover	Germany
Institute for Genome Biology, Leibniz Institute for Farm Animal Biology	Germany
Institute for Hygiene and Infectious Diseases of Animals, Justus-Liebig University Gießen	Germany
Institute of Animal Physiology, Department of Veterinary Sciences, Ludwig-Maximilians University Munich	Germany
Institute of Virology, Faculty of Veterinary Medicine, Justus-Liebig-University Gießen	Germany
Klinik für kleine Klauentiere, forensische Medizin und ambulatorische Klinik, Stiftung Tierärztliche Hochschule Hannover	Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt, Fachbereich 4 Veterinärmedizin Stendal	Germany
Landwirtschaftskammer Nordrhein-Westfalen, Referat 34 Tiergesundheit	Germany
Paul-Ehrlich-Institut	Germany
Research Unit Molecular Biology, Leibniz Institute for Farm Animal Biology	Germany
Tierärztliche Praxis für Groß- und Kleintiere, Niesky	Germany
Tiergesundheitsdienst Bayern e.V.	Germany
Zentrum für klinische Tiermedizin, Department of Veterinary Sciences, Ludwig-Maximilians University Munich	Germany
Advisory Committee on the Microbiological Safety of Food	Great Britain
Animal Health and Veterinary Laboratories Agency	Great Britain

Association for Veterinary Teaching and Research Work	Great Britain
Association of Government Veterinarians	Great Britain
Department of Population & Production Health, Royal Veterinary College	Great Britain
Division of Epidemiology and Population Biology, Moredun Research Institute	Great Britain
Moredun Research Institute	Great Britain
Pfizer Animal Health	Great Britain
Pfizer Animal Health, Biologicals R & D	Great Britain
Royal (Dick) School of Veterinary Studies, Department of Veterinary Clinical Sciences, University of Edinburgh	Great Britain
Scottish Agricultural College Veterinary Services	Great Britain
Suspected Adverse Reaction Surveillance Scheme at the Veterinary Medicines Directorate	Great Britain
The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh	Great Britain
Veterinary Pathology Unit, Royal (Dick) School of Veterinary Studies, University of Edinburgh	Great Britain
Cork Regional Veterinary Laboratory, Department of Agriculture	Ireland
Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine	Japan
Tokachi Agricultural Mutual Aid Association	Japan
New Zealand Veterinary Pathology	New Zealand
The Ministry of Agriculture and Forestry	New Zealand
Department of Veterinary Medicine, Faculty of Animal Breeding and Biology, Poznań University of Life Sciences	Poland
Associação Portuguesa de Buiatria	Portugal
Central Veterinary Research Laboratory Dubai	United Arab Emirates
International Society for Infectious Diseases	United States of America