Surveillance analysis Tool for Outcome-based Comparison of the confidence of FREEdom

European Food Safety Authority

STOC free: WP1, Deliverable 1

Guidelines for the design of conceptual models representing the infectious process at different levels, from animal to region, with an application to BVD

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1 Conceptual models aims and design strategy

1.1 Definition of the conceptual model

The aim of the STOC free project is to design and validate a framework that enables a transparent and standardized estimation of a probability of freedom from infection and its associated uncertainty from heterogeneous information. An essential step in this estimation will be to connect the available information to a probability of infection.

The conceptual model will map the different types of information that exist for a given infectious disease onto the true status regarding infection. It is conceptual in the sense that it connects:

- The biological system: the true status regarding infection which is of interest for different levels of analysis: animal, herd and territory.
- Information that is extremely diverse. Conceptually, 2 types of information that are different in nature can be distinguished:
    - Information generated and collected to specifically detect the infection or the pathogen of interest such as test results from control programmes
    - Information associated with an increased probability of pathogen presence such as risk factors for infection

The conceptual model will be made of diagrams and text explanations. It will eventually be used to design statistical models that will integrate different pieces of information (data) for the estimation of probabilities of being in each single state of interest (outcome) at different levels.

1.2 Motivations for the conceptual model

For some non-regulated endemic infectious diseases of cattle, control programmes have been developed and implemented in several territories. These programmes can differ in terms of objective and means. As an example, bovine viral diarrhoea (BVD) is a disease against which several control programmes exist worldwide. Some of these programmes, at least initially, aim at the control of the infection while others aim at its eradication. Some use bulk tank milk testing for disease screening, while others resort to individual screening methods such as serum or tissue tag testing. Some operate at a regional scale while others are conducted at a national level. Some are mandatory while others are voluntary. Some only test once every year while others test on multiple moments (bi-annually, quarterly or monthly). This heterogeneity makes estimations of probabilities of freedom from infection hard to compare between territories operating under different control programmes, because information used or available to determine these probabilities are different. This complicates animal trade between regions when buyers need to evaluate the risk taken when purchasing an animal or a group of animals from another region. Avoiding the introduction of an infectious disease can be important, especially in regions that have managed to successfully control or eradicate the disease.

In this context, the aim of the STOC free project is to design and validate a framework that enables a transparent and standardized estimation of a probability of freedom from infection and its associated uncertainty from heterogeneous information. This is a type of surveillance, known as outcome-based surveillance, in which the focus is on the surveillance outcome regardless of the

means used to arrive at this outcome. Therefore, we will use heterogeneous information to estimate a probability of freedom from infection which will be the outcome. Information that can be used to estimate a probability of freedom from infection depends on the type of data that are collected and available and varies between regions, types of production (notably beef vs. dairy) and control programmes.

The available information can relate to either consequences or potential causes of the infection. Usually, the status of animals, herds or territories regarding an infection is evaluated by performing biological tests. These tests measure consequences of infection. But risk factors relating to the probability of introducing the infection could also be included. These risk factors are conceptually different from biological test results because they are associated with the cause of the infection, which may have occurred or not, while test results are associated with a possible infection which is a past event.

Information can be obtained on different biological phenomena associated with the infection process. For instance, biological tests can seek to identify antibodies, antigens or nucleic acids. For the same biological phenomenon, the information obtained can be different either because the test used is different or because it is performed on a different matrix, such as blood, milk, faeces or skin tissue.

Regarding the level at which the information is available, biological tests can be performed at the individual level or on pooled samples such as bulk milk. Therefore, there is a methodological challenge in being able to estimate an outcome that is comparable, i.e. a probability of freedom from infection and associated uncertainty, regardless of the inputs, that may be extremely variable. In this work, this process will be completed for BVD. The first step will consist of representing the features of the infection that do not vary between countries such as course of infection in a bovine and then to connect to these features to the different types of information that can be used to estimate a probability of freedom from infection by BVDV.

The representation of the (true) states regarding infection and their connection to available observations **is what we call a conceptual model.** Depending on the level of interest (animal, herd or territory), there can be several conceptual models for the same disease. Each level is composed of two layers: the first layer is the representation of the different possible (true) states of the system and the second layer represents the different types of observations that can be used to determine the state of the system. This model will serve as the basis to construct the statistical models that will integrate all the available information on all three levels in order to estimate the probability of freedom from infection and associated uncertainty.

The first layer of the conceptual model that is independent from observations is the representation of the BVDV biological system. At animal level this is the infection process. The infection process first involves the course of infection within an animal: how an infectious agent is transmitted to an animal, how the animal responds to the infection and how it recovers from infection. This course of infection is agent dependent (in this case BVDV), it will be linked to infectious agent characteristics: the routes of transmission, the clinical disease associated to the infection and the ability of hosts to become immune (lifelong or not). If vaccination is available, the course of infection in animals can be modified by vaccinating the animal. At herd level, the infection process is impacted by herd

husbandry. Depending on agent characteristics, the structure and the management of the herd can enhance or reduce the probability of (re-) introduction and/or spread and/or the delayed detection of the virus within the herd. Finally, at the territory level, contact structure (both within and between territories), prevalence, control programmes and policies can impact the introduction and the spread of the agent through a territory.

The second layer of the model will represent the available information or observations and how it can be connected to the infection process. Direct observations of the evolving infection process are generally not available. So, we have to use available information or observations that can inform or enhance our understanding of the actual process, the state of the system. Those can be for example diagnostic test results, demographic/geographic parameters and surveillance or control programme information.

This conceptual model, mapping information onto the true status regarding infection, will enable us to have a better understanding of available information and how to interpret them. The next sections describe the step-by-step design of the conceptual model. In section 2, 3 and 4 the developed conceptual model for BVDV infection at animal, herd and territory level respectively. The last section will discuss how the conceptual models will be translated into the statistical model.

1.3 Step for the design of the conceptual model

Three levels are considered: animal, herd and territory. For each level, the first step of the conceptual model is the representation of the biological features of infection. The work is based on bibliographic research. The representation has to include disease biology, dynamics of infection and transmission, and characteristics of the pathogen of interest (survival rate in the environment for example). This work requires a good overview of the susceptible population, the infectious agent, the disease and associated risk factors (Victora *et al.*, 1997). Then, quantitative information about the infection (such as duration of infection, duration of shedding …) will be added.

The next step will be the description and connection of all the possible available observations to the different states of our systems. Observations can be either causes of infection, such as risk factors for introduction or transmission; or consequences like diagnostic test results. They can take the form of aggregate observations like prevalence for a territory, a herd, a group of animals.

In non-regulated livestock diseases, the susceptible population will be farmed animals. To focus on the infection dynamics, we can study it at different levels. The first level will be the animal level, which explains the transmission of infectious agent and the course of infection in an animal. This level will be based on good knowledge of the disease biology and its progression, after the animal is infected by the pathogen. It describes different types of infection (i.e. vertical or horizontal), of disease (i.e. clinical, subclinical or mucosal disease in persistently infected animals), possible ways of transmission (i.e. direct or indirect) and the development of immunity. In this part we also include the possibility of vaccination and its efficiency and possibly interference with testing results.

Then, we will focus on herd level. Herd structure, herd dynamics and specific farming practices can influence (re-)introduction, virus spread within the herd and delayed detection of infectious agent in the herd. For example, if the transmission of infection can occur through direct contact between animals, the density of animals in a herd will influence the transmission of virus within the herd. Finally, we can also consider the territory level. In fact, this is often the level where the programmes

are practically applied. This means that herds within a territory, even if they can be different, will have common surveillance practices and measurements. Therefore, the second level of the model will be to explain the dynamics of infection at each level, including connection with risk factors.

Finally, in parallel with the description of the infection dynamics at each chosen level, the conceptual model must list, describe and link all the possible available observations of our system to each level. Observations can be either diagnostic test results, geographic parameters, like density of animals or density of herds. They can also take the form of aggregate observations like prevalence for a territory, a herd or a group of animals.

2 Conceptual model for BVD at the individual animal level

In this section we first describe the epidemiological states of individual animals regarding infection with BVDV. We then describe how the animals can move between these states, i.e. the course of infection. The last part details how biological test results can be used to elucidate the epidemiological status of an animal.

2.1 Epidemiological states of the system

In order to quantify the probability that an animal is free from infection with BVDV, 4 mutually exclusive categories of animals are considered and described: persistently infected, (PI), transiently infected (TI), immune (R) and susceptible individuals (S).

2.1.1 Persistently infected (PI)

Persistently infected animals are the most important source of BVDV infection. PIs are infected in utero, between 30 and 120 days of gestation, while their immune system is immature. As a consequence, they are immunotolerant (they do not produce antibodies against homologous virus), become persistently infected and shed large amounts of virus throughout their lives. A calf born to a PI cow will always be PI but if a cow has a non-PI calf she cannot be a PI.

At birth, PI calves can appear either clinically healthy or small, weak and ill-thrifty (Baker, 1995) and may show stunted growth and chronic ill thrift (Voges *et al.*, 2006). Furthermore, PI animals are regularly reported to be particularly susceptible to secondary infections (Voges *et al.*, 2006), suggesting poor immune function. This results in the fact that PI animals have a poor survivability rate (Houe, 1993). Only PI calves can develop mucosal disease, which is inevitably fatal. This disease appears after the acquisition of a cytopathogenic strain of BVDV that can occur with a mutation of a non-cytopathogenic BVDV biotype circulating in the PI animal or through infection by a cytopathogenic strain (Brownlie, Clarke and Howard, 1984).

2.1.2 Transiently infected (TI)

Animals that are infected by BVDV after birth or during the last trimester, when the immune system is able to fight the infection, develop a transient infection. A transient viremia will start approximately 3 days after the infection (Pedrera *et al.*, 2012) until immunity develops around 2 weeks later (Meyling, Houe and Jensen, 1990). The transient infection is most of the time subclinical but usually comes with a transient immunosuppression, especially in calves. After a transient infection, the immunity developed against the BVDV is considered to be lifelong.

2.1.3 Immune post infection

After infection by BVDV, all animals apart from PIs remain immune for the rest of their lives. After obtaining immunity, cows cannot produce PI calves anymore. It is worth adding that non-PI female cows that give birth to PI calves are always immune (seroconversion occur during gestation) and that immune female from natural infection before insemination will not give birth to PI calves.

2.1.4 Susceptible

Susceptible animals are animals that haven't been infected with BVDV and that have not developed antibodies. Hence, they are naïve (not immune). These animals can get infected and pregnant females can give birth to PIs if they are infected during the gestation window of susceptibility for development of PIs.

2.2 Course of infection

BVDV transmission can occur from different sources and through different routes of infection. There are two types of BVDV infections: infection after birth (i.e. horizontal) and in utero infection (i.e. vertical). This part will describe all the aspects of the course of infection by BVDV.

2.2.1 Sources and routes of infection

The BVD virus is shed through a wide range of body fluids: nasal discharge, urine, milk, semen, saliva, tears and foetal fluids (Meyling, Houe and Jensen, 1990). Faeces appears to be a poor source of virus but can be infectious (Brownlie *et al.*, 1987).

The most common means of transmission is from nose to nose contact with a permanently infected (PI) individual as they shed large amounts of virus. Although, they shed lower amounts of virus, transiently infected animals can also be involved in the transmission (Niskanen and Lindberg, 2003).

2.2.2 Infection after birth

Susceptible animals that are infected after birth become transiently infected. After immunity has developed, after around two weeks, they become immune.

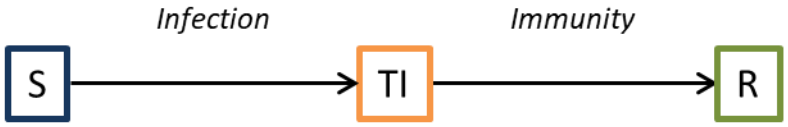

Figure 1: Representation of the course of infection thought horizontal transmission: susceptible animal (S) can be infected by BVDV thought different means of infection and become a transiently infected (TI) bovine. Around 2 weeks post infection, as a result of immunity development this bovine will become immune to the virus and become recovered (R).

2.2.3 In utero infection

When susceptible pregnant cows are infected they become transiently infected: the virus multiplies in the cow and can infect the foetus. The impact of the infection on the foetus depends on the stage of gestation. Usually, during the first 30 days post conception, embryonic infection leads to embryonic death (Moennig and Liess, 1995). Between 30 and 120 days of gestation (susceptible window for PI creation), before the development of the immune system in the foetus, infection can lead to the birth of persistently infected (PI) calves (Brownlie *et al.*, 1998). Later in pregnancy the effect of foetal infection is variable from no effects to teratogenic effects, foetal death and abortion. Foetuses that are immunologically competent at the time of infection can be born either transiently infected or immune. Recent work show a long term impact of pre-natal infection with many possible congenital defects in the central nervous system (Givens and Marley, 2013).

During their entire life, PI animals will shed large amounts of virus in all excretions and secretions: milk, semen, saliva, nasal secretion, urine, faeces, blood and aerosol (Brownlie *et al.*, 1987; Nettleton and Entrican, 1995). Only a small proportion of female PI calves reaches adulthood and gets pregnant. However, calves born of PI cows are also PI.

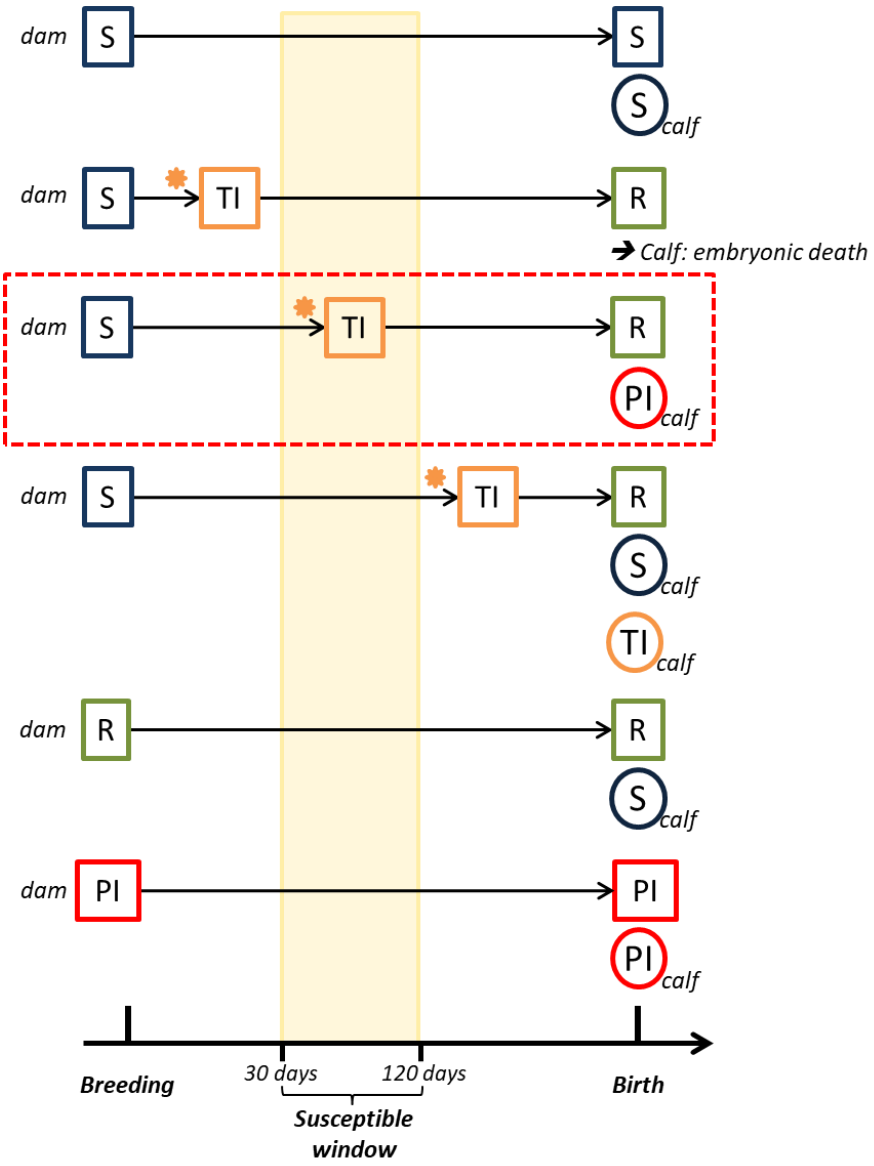

Figure 2: Impact of in utero infection on the status of the calf at birth. Squares represent dams' statuses and circles calves' statuses at birth. The calf's status at birth depends on whether its dam got infected during the gestation and on the stage of gestation at which the infection occurred. No seroconversion during gestation leads to the birth of a susceptible calf, either while the dam is either S or R. Transient Infection during gestation can lead to different calf states at birth depending on the stage of gestation when infection occurs. Only transient infection of the dam that occurs during the windows of susceptibility (30 to 120 days of gestation) leads to PI calf. S: susceptible, TI: transiently infected, R: recovered, PI: persistently infected.

2.2.4 Maternally derived immunity

New-born calves can acquire passively derived immunity against BVDV through serum antibodies present in colostrum (Moerman *et al.*, 1994; Chamorro *et al.*, 2015). The duration of this immunity can vary depending especially on the amount of antibodies ingested and absorbed (Fulton *et al.*, 2004) and can last for 3 to more than 6 months (Fux and Wolf, 2012; Fulton, 2013). The decline in maternally derived immunity over this period will increase the susceptibility of calves to acute infections. It is worth noting that passive maternally-derived immunity can modify diagnostic test results of PI calves as it can create false negative results particularly when testing blood samples for presence of BVDV by ELISA (Fux and Wolf, 2012).

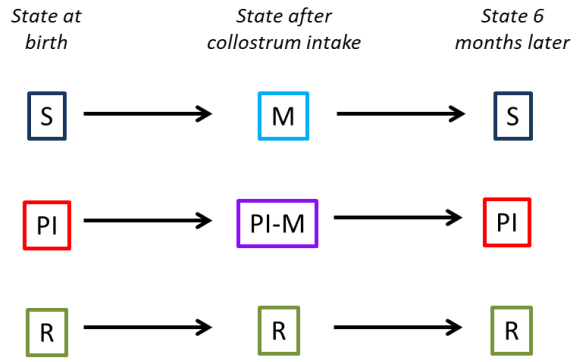

Figure 3: Evolution of the calf's status at birth, after colostrum intake and 6 months later. After colostrum intake, susceptible (S) calves will be protected by maternal antibodies (M) for around 6 months and then will be S again. Persistently infected (PI) calves will stay PI after colostrum intake but with maternal antibodies (PI-M) for around 6 months. Finally in recovered (R) calves, colostrum intake will not change the status of the calf.

2.2.5 Vaccination

In individual animals, the course of infection can be modified by vaccination. Vaccination against BVDV is mainly used to prevent transplacental infection of the foetus and thus to reduce the formation of more PIs (Frey et al., 2002; Patel et al., 2002; Meyer et al., 2012). Vaccines that contain both BVDV1 and BVDV2 strains are available. Two types of vaccines have been developed: inactivated and modified-live viral (MLV) vaccines. MLV vaccines lead to higher and quicker onset of immunity with a more consistent antibodies response and usually need only one dose for immunization, but some have the potential to create PIs if used in pregnant cattle.

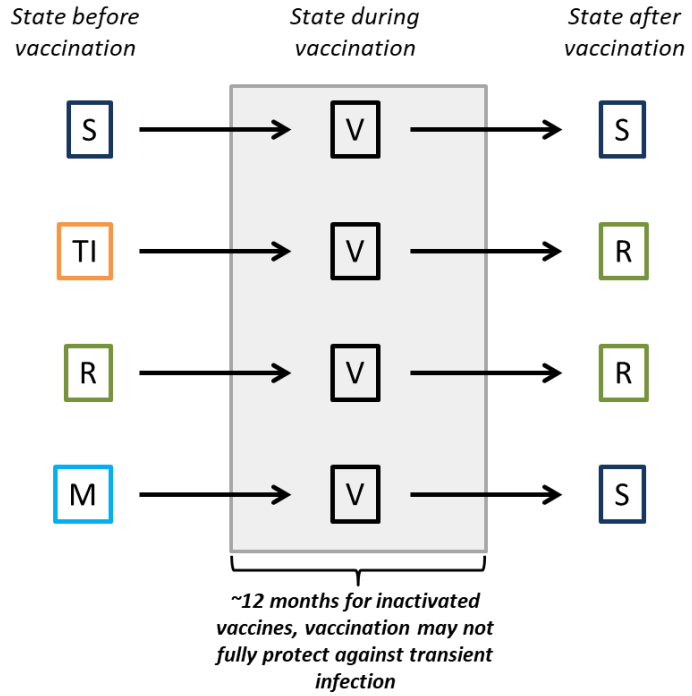

Figure 4: Vaccination of cattle and results of vaccination in bovines from different statuses. After vaccination susceptible (S), transiently infected (TI) and immune (R) and protect with maternal antibodies (M) bovine become vaccinated (V). Vaccinated transiently infected and immune bovine will become immune when susceptible and protect with maternal antibodies bovine will become susceptible once duration of protection thanks to vaccination is over. Vaccination of dams with a specified vaccine should prevent transplacental infection and production of PI cattle by vaccinated dams should be limited under the period of protection.

2.3 Available information at individual animal level to observe the system

Available tests can be divided into two groups: tests that detect an on-going infection through the detection of the virus (viral antigens: Ag ELISA or viral RNA: PCR) and tests that detect an immune response against the virus through the detection of circulating antibodies (Ab ELISA). Both can be used for the diagnosis of BVDV depending on the purpose and context.

Sensitivity and specificity of Ag ELISA and PCR are relative to virus isolation as the gold standard. Sensitivity and specificity of Ab ELISA are relative to serum neutralization test as the gold standard.

2.3.1 Antigen detection tests: ACE

Antigen-capture ELISA tests (ACE) need to target a highly conserved Ag across BVD strains. Two tests have been developed against two BVDV proteins: NS3 (formerly p80) and E^{RNS} (formerly E^{0}). The approved samples that can be tested with ACE are: serum (plasma), tissue (skin biopsy, ear notch) and individual milk samples.

As an ACE detects viral antigens, this test is able to detect infected animals that shed the virus: TIs and PIs. TIs can be challenging to detect as they shed lower amounts of virus during a short time period. Using RT-qPCR as a reference test, an Ag ELISA test was able to detect only 10 out of 57 TIs but correctly detected 17 out of 17 PIs (Hanon *et al.*, 2014). However, once an Ag ELISA returns a positive result, interpretation of the state of animal, without any other information will be TI or PI as this single test is not able distinguish between them (Hanon *et al.*, 2014) but the test value can be predictive of the state of infection. Repeating the test three weeks/ 1 month later will clarify whether the animal is TI (negative Ag-ELISA) or PI (again positive Ag-ELISA).

2.3.2 Nucleic acid detection: RT-qPCR

Reverse transcriptase polymerase reaction (RT-PCR) (Hertig *et al.*, 1991) is widely used for BVDV diagnosis. A wide range of samples can be used in these test: blood, milk, saliva and tissue (Bhudevi and Weinstock, 2003; Kim and Dubovi, 2003; Kliučinskas *et al.*, 2008). Moreover, some RT-PCR tests can distinguish BVDV type I and type II (Letellier *et al.*, 1999). Quantitative RT-PCR (qRT-PCR) has been developed for BVDV diagnosis, as there exists a relationship between threshold cycle (CT), cycle number at which the fluorescence generated is higher than the threshold, and the quantity of viral RNA present (Bhudevi and Weinstock, 2001). qRT-PCR can be used to make a distinction between TI and PI in term of CT, knowing that PI will shed a larger quantity of virus.

2.3.3 Antibody ELISA (enzyme-linked immunosorbent assay) (Ab ELISA)

Ab ELISA is an immune-enzymatic technique that allows the detection of antibodies in a sample. Sample antibodies will bind specifically to an antigen present on a surface and the binding will be visualised following an enzymatic coloured reaction. Relative to the SNT, specificity and sensitivity of Ab ELISA for BVDV detection is high: up to 99% and 98% respectively (Cho *et al.*, 1991; Kramps *et al.*, 1999; F. Beaudeau *et al.*, 2001) . Both serum and milk can be used as matrices. This test is also able to detect, but not differentiate colostrum-derived antibodies in suckling calves (Fux and Wolf, 2012).

A positive Ab-ELISA can be associated with either an immune state resulting from a natural infection, the presence of maternal antibodies in calves under 6 months or with vaccination. A single test result may not be able to distinguish those three categories. However, repeated testing can clarify the true BVD status in that maternal and vaccination derived antibodies will decrease with time.

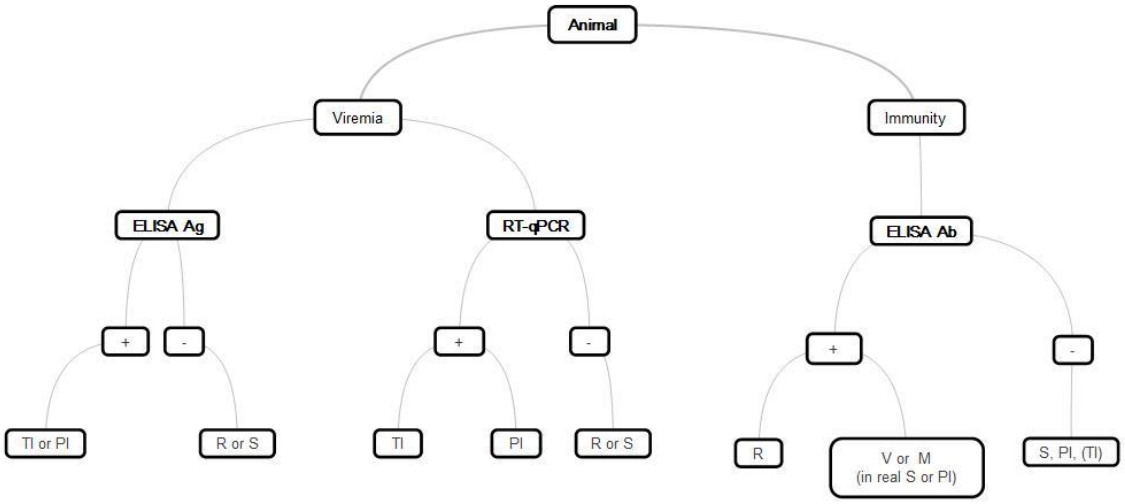

Figure 4: Representation of test result interpretation at the animal level with a single test.

2.3.4 Test combinations

Test combinations can be defined as: different diagnostic tests at the same time or several identical tests at different times on the same animal, or both. Different diagnostic tests can inform both the immunological and viremia state of the animal. Several identical tests can inform about the evolution of the animal's state. For example, two ELISA Ag test results with an interval of 3 weeks can discriminate between TI and PI. Two positive Ag ELISA results identify/indicate a PI animal while a positive and a negative result identify/indicate a TI.

3 Conceptual model for BVD at the herd level

The probability of a herd selling an infectious (PI or TI) animal depends on both the probability of this herd having introduced the infection as well as on the within herd dynamics of the infection once it has been introduced and on the ability to detect the change. BVDV introduction can occur through different routes (i.e. purchase, contact at boundaries fences ...). Once the infection has been introduced, the within herd dynamics depends on herd demographic and contact structures as well as on herd management. Important differences exist between beef and dairy herds which need to be taken into account. These differences can be represented in terms of herd structure and herd management.

3.1 Epidemiological state at the herd level

There are, at least four different states at herd level depending on the situation of the herd regarding BVDV infection.

3.1.1 Virus free and seronegative herds

Naïve free herds are herds that are not currently infected and that have not been recently (in the past +/- 10 years) infected by BVDV. They are composed of susceptible cattle that are not immune against BVDV (S=100%).

3.1.2 Herd infected with at least one transiently infected animal (absence of persistently infected animal, either alive or in the foetal stage)

Herds in this category are infected by at least one transiently infected animal. They are composed of S and TI animal and as the herd infection progresses the proportion of S and TI declines and R cattle will rise. In general, immune animals (R>0) will be found in these farms.

3.1.3 Herd infected with at least one persistently infected animals (either alive or in a foetal stage)

Persistently infected herds contain at least one persistently infected animal alive or to be born (Trojan cow). They are composed of susceptible, transiently infected and at least one persistently infected animal and as the herd infection progresses by an increasing number of immune cattle. In general, immune animals (R>0) and transiently infected animals (TI>0) will be found in these farms.

3.1.4 Virus free and partly seropositive herd (at least one animal is seropositive)

This state occurs:

- When all infectious animals (PI, TI) are removed (by death, sale, converted to recovered animals) and there are still animals with antibodies (R) present. (R>0; TI=0; PI=0);
- after vaccination of parts of the herd (V>0; TI=0; PI=0)
- by a combination of both (R>0 & V>0; TI=0; PI=0).

Herds in this state can become “virus free and seronegative herd” once all the immune and vaccinated animals have left the herds.

3.2 Course of infection at herd level

3.2.1 Risk factors for introduction of BVDV in a herd

BVDV introduction into a herd can occur through different routes. Those possible routes are described and quantified in terms of probability of transmission between herds in table in Annex I. We can separate them into 3 categories: introduction of infectious animals, contact with infected animals direct from another herd and indirect transmission through contaminated material (biological or equipment).

3.2.1.1 Purchase and introduction of infectious animals

3.2.1.1.1 Persistently infected

Introduction of a PI to a herd (directly or through a Trojan cow, see below) is the main source of introduction of BVDV in a herd in endemic situations in the absence of control measures. As a PI will shed a high level of virus (Brownlie *et al.*, 1987) throughout its entire life, transmission/infection of the herd can occur quickly, continuing whilst the PI animal remains in the herd.

3.2.1.1.2 Trojan cow

Trojan cows are non PI, immune cows that have been transiently infected by BVDV during their first semester (day 40 to 120) of pregnancy. As a TI individual, the dam will clear the infection in approximately two weeks but will carry a PI foetus. These cows can be sources of new infections in a herd at the birth of the PI calf. As the dam is healthy and immune, Trojan cows are a high risk for introduction or reintroduction of BVDV.

3.2.1.1.3 Transiently infected

Transiently infected animals can be a source of introduction of BVDV into a herd. Nevertheless their relative role as a source of infection is much lower than the role of the PI animal. In fact, they shed lower amounts of virus and the period of shedding is short (around 2 weeks). The relative importance of TI in (re-) introduction of BVDV in a herd is under discussion: some argue that TIs are unlikely to be a source of infection (Niskanen, Lindberg and Tråvén, 2002; Sarrazin *et al.*, 2014) while others suggest that BVDV can be maintained in a herd without presence (or at least identification) of PIs (Moen, Sol and Sampimon, 2005).

3.2.1.2 Contact with animals from neighbouring/other herds

Direct contact with infected cattle from another herd is also an important means of introduction of BVDV. These contacts can occur through shared grazing or adjacent herd pasturing areas, animal shows and markets. Annex I Tables I and II list animal contact on pasture or across boundaries as a risk factors for introduction of BVDV, especially when the susceptible cattle comes in contact in early pregnancy (at risk of producing a PI). A survey in Danish dairy herds showed that contact with cattle from another herd and pasturing within 5m were positively associated with seroconversion to BVDV (Houe, 1999).

3.2.1.3 Person contacts

Introduction of BVDV in a herd can also occur through indirect transmission by contaminated persons, when they have contact with animals (e.g. veterinarian, farmers, claw cutters, inseminators). It is essential that persons that have contact with the animals follow strict hygiene rules.

3.2.1.4 Contaminated materials and products

Introduction of BVDV in a herd can also occur through indirect transmission through contaminated products or materials. Compared to direct contact with infected cattle, indirect routes may play a minor role in transmission. However, towards or at the end of an eradication programme, when introduction of BVDV through purchases and contacts is limited/rare, indirect transmission can become relatively more important (Hult and Lindberg, 2005). BVD virus can be preserved in cryopreserved semen of infected bulls, so artificial insemination with contaminated semen of PI and TI can lead to dam infection (Meyling and Mikél Jensen, 1988; Rikula *et al.*, 2008). Other products like contaminated vaccine or contaminated veterinary materials like needles and tongs can also lead to new infections (Gunn, 1993; Niskanen and Lindberg, 2003). Finally, sharing equipment e.g. trailers during transport can also be a source of infection.

3.2.2 Within herd dynamics of BVDV

Within-herd spread can be influenced by herd management once BVDV has been introduced. First, the course of infection within a herd after introduction of BVDV will be presented. Then, herd factors that can influence spread will be described.

3.2.2.1 Representation of the course of infection within a herd

The course of infection within a herd will start with the introduction of BVDV through different routes (cf. part below: Introduction risk factor for BVDV). Depending on the route of introduction, the proportion of bovines newly infected in the herd can vary. Once an infectious animal is introduced it will shed virus and infect other animals. Then, those newly infected animals will also shed virus and in turn will infect other susceptible animals. If some infected cows are pregnant between 30-120 days of gestation, the infected foetus will become a PI calf. If nothing is done to limit the infection, the virus will continue to spread within the herd with a negative impact on reproduction. After a while, a large proportion of cows within the herd will become immune against BVDV and will no longer be susceptible to BVDV.

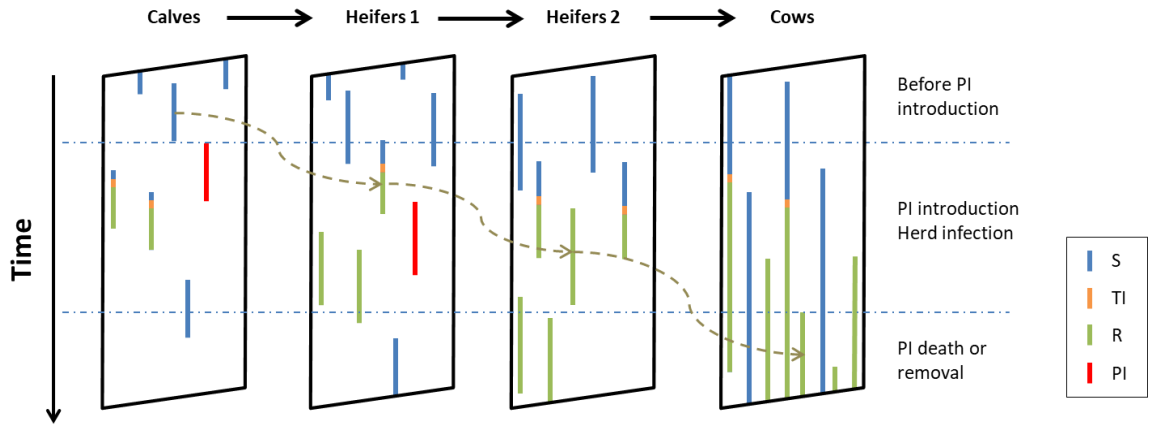

Figure 5: Representation of the course of infection after the introduction of a PI in a virus free and seronegative herd. This example herd is composed of 4 groups of animals: calves, heifers 1, heifers 2 and cows. Verticals lines represent individual bovine and the length of the line the time spend in each category. As time passes, animals will move to other groups. Grey dotted arrows represent movements between groups through time. Once a PI animal is introduced to the herd the infection spreads in all groups. The transmission thought different groups within a herd is linked to the herd structure and how animals from different groups are separated.

3.2.2.2 Herd structure and management that influence the course of infection

Herd husbandry can affect infection transmission between groups of animals. Herd characteristics will be explained here: type, structure, contact structure and management.

3.2.2.2.1 Type of herd

Only breeding beef and dairy herds through generation of PI calves will be considered in this work because they will be the main source of infection for other farms. Farms that specialise in fattening cattle will not be studied because the animals they sell are sent directly to slaughter and not to other farms. However, fattening units within beef and dairy farms can act as sources of infection for breeding units on those farms, and will therefore be included as risk factor.

3.2.2.2.2 Herd contact structure

In cattle herds, animals live in separate groups. For example, in dairy herds, there are usually groups of calves, heifers and lactating cows. Animals in the same group have higher probabilities of contact than animals in separate groups. Furthermore, within a herd, the different groups can have more or less contact. For example, in beef herds, calves stay with their dam until weaning which can happen at up to 9 months, whereas in dairy herds, calves and dams are quickly separated. This results in PI calves being in close contact with the breeding herd for much longer in beef than in dairy herds. Within a herd, the different groups can be kept apart in different barns or on different pastures. The separation between groups can be quite different and herd specific.

Herd level structure associated with a risk of introduction, within-herd transmission or persistence of infection of BVDV:

- Size of the herd (or number of cows as a proxy) (Graham *et al.*, 2013)
- The age at which calves are separated from their dam. In breeding herds, calf stay with dams until weaning, meaning that if the calf is a PI it can transmit infection to other dams during the risk period of early pregnancy. In dairy herds, calves are separate from dams at birth meaning that transmission can only occurs between calves.
- The age at first calving: in dairy herds, the age at first calving is usually 24 months while it may be up to 36 months in beef herds. This implies that there are at least 2 groups of heifers in dairy herds and 3 in beef herds.
- The replacement rate determines the proportion of female calves born on the farm that are kept to replace breeding cows. The lower the replacement rate is, the higher the probability that a present PI calf is sold rather than kept as replacement.
- The proportion of time spent at pasture for the different groups determines the probability of being in contact with animals from neighbouring herds. Inversely proportional to the time spent indoors.
- Number of neighbouring herds (Graham *et al.*, 2016)
- Within herd biosecurity : how many barns for the different groups

3.2.2.2.3 Herd Management

Some farm management practices are of major importance in the dynamics of BVD in these herds.

- Calving distribution can be seasonal, that means that all calving will occur in a short period (3 months). This is associated with most pregnant cows being in the window of susceptibility

for the formation of a PI calf at the same time. If an infectious animal is in contact with these cows during the window of susceptibility, this can result in a high number of PI calves. However, seasonal calving allows the identification and removed of pre-breeding cattle. Conversely, extending calving and breeding means that a PI born at any time of the year may have the opportunity to contact a pregnant animal in early gestation.

- The number of cattle purchased by the herd can lead to multiple re(-introduction) of BVDV in the herd
- Within herd biosecurity and hygiene measures can limit the spread of infection.
- Location: separation of calves from pregnant animals

3.2.2.2.4 Vaccination

Vaccination can modify the course of infection within a herd as it can reduce or prevent in utero infections and limit the production of PIs. Cattle vaccination will also impact monitoring options for the presence of BVD infection in herds. With vaccination, animals may produce antibodies and all screening which is based, for example, on surveillance of antibodies in cows through bulk milk tank testing, may not be possible for a certain time period depending on the vaccine used and original immune status of the herd. Moreover, farm-level information about vaccination against BVDV is not readily available.

3.2.2.2.5 Differences between dairy and beef herds

Table I shows differences between dairy and beef herds linked to risk factors for spread of BVDV that have been described above.

Table I: Example of the main differences between dairy and beef herds in France

Risk factor	Dairy herds	Beef herds
Age at which calves are separated from their dam	At birth	After weaning
Age at first calving	24 months (at least 2 groups of heifers)	36 months (at least 3 groups of heifers)
Replacement rate	~25-40%	~20-30%
Proportion of time spent at pasture	Depend on : Herd size Region	Often Can be Seasonal
Vaccination	Variable	Often
Seasonal calving	Often none	Often

3.3 Available observations at herd level

At the herd level there are three types of available observations. First, risk factor information that can explain possible causes of infection wihtin the herd. Then, results from biological tests will inform about the consequences of this infection. Finally, factor that can lead to delayed detection

3.3.1 Risk factors associated with herd characteristics

Herds can be described by several characteristics that can be involved in BVDV dynamics. These characteristics can inform about the contact structure within the herd and with neighbouring herds and can be linked to the risk of introduction or the risk of transmission within the herd.

3.3.1.1 Risk factors for BVDV introduction

- Number of neighbouring cattle herds with common boundaries, size of the common boundaries
- Number of cattle purchased and number of purchased animals that are pregnant?
- Time spent at pasture
- Biosecurity measures for professional and visitors (i.e. farmers, veterinary, AI technicians, traders)
- Herd size

3.3.1.2 Risk factors for BVDV transmission within a herd

- Surface area (km²):
    - Building
    - Pasturing area : a large surface of pasturing area can increase the number of potential neighbours
- Number of animals
- Density of animals (km²)
- Calving distribution
- Contact structure within the herd (individual within herd biosecurity)
- Age at which calves are separated from their dam

3.3.2 Results from biological tests

Herd diagnosis can be conducted at the level of the individual or a group of animals. It can involve testing of samples individually (refer to part 2.3.Available information at individual animal level to observe the system) or in pools. Depending on context and territory, programmes can have different aims and lead to different screening strategies. Furthermore herd type can impact the strategy used. The main difference between beef and dairy herds is the use of bulk tank milk (BTM) to monitor BVDV infection. Diagnostic strategies at the herd level involve antibody detection and virus detection.

3.3.2.1 Detection on Bulk milk test

3.3.2.1.1 Ab ELISA

Monitoring BTM can detect seroconversion of a herd with Ab-ELISA. The level of BVDV antibodies in milk can even be correlated semi-quantitatively to the prevalence of seropositive animals in the dairy cows (F Beaudeau *et al.*, 2001; Eiras *et al.*, 2012b). Depending on the test and the assessment objective, the interpretation and the threshold value of tests can be quite different. Ab tests are widely used for herd diagnosis. Aims are either: (i) provide on-going evidence of freedom thought repeat negative tests or (ii) detect introduction of infection.

Ab levels in BTM can give an indication on the prevalence of sero-positive cows in the dairy herd (F Beaudeau *et al.*, 2001; Eiras *et al.*, 2012a) and variation in Ab levels can indicate a new infection of the herd (F Beaudeau *et al.*, 2001). One rare limitation to this test is if a PI contributes to the BTM : antigens can neutralize antibodies and can cause a negative BTM Ab ELISA (Sandvik, Larsen and

Nyberg, 2001) but this is not a major risk as PI often die or are removed before adulthood and requires a specific ratio of PI/seropositive cows. Serial testing allows the observation of the evolution of the herd status over time. Figure 9 show an example of infection of a herd and evolution of Ab-ELISA on BTM results.

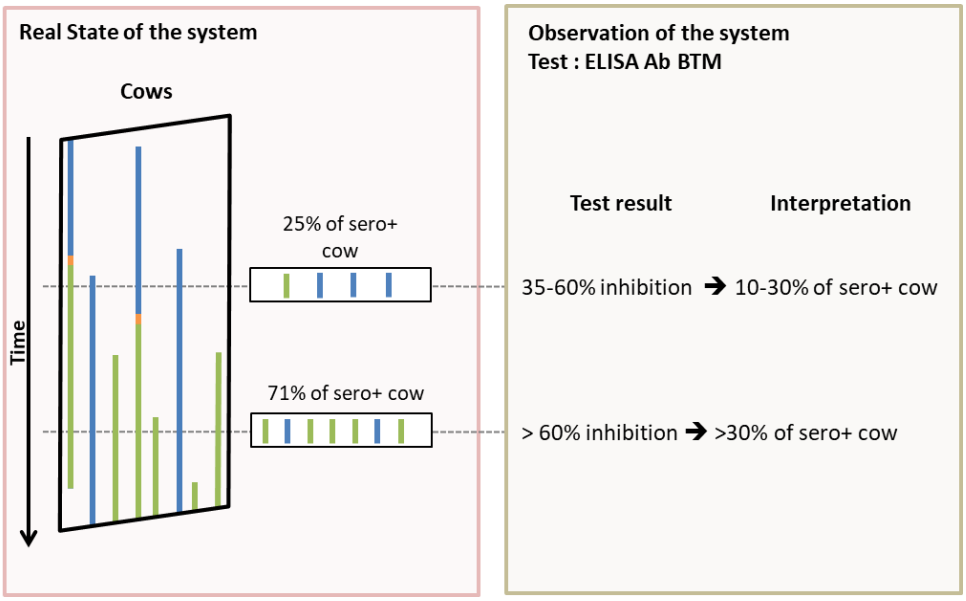

Figure 6: Example of Ab-ELISA test on BTM in cows. This diagram on the left is from the figure 5 and represent the cows compartment in a herd each vertical line represent one animal which can be susceptible (in blue), transiently infected (in orange) and immune (in green) bovines. This diagram represents the link between the real state of the system (meaning the proportion of immune animals) and available observation at herd level based on an ELISA BVDp80 kit (example of Brittany,(F Beaudeau et al., 2001)).

However, as this test relies on detection of antibodies to BVDV, its value is reduced in herds which apply vaccination against BVDV.

3.3.2.1.2 PCR

PCR can also be used on BTM (Muñoz-Zanzi *et al.*, 2000). In practice RT-PCR on BTM is very sensitive as it has been proven that this test can detect 1 PI in a herd of 132 cows (2 in a herd of 800) (Drew, Yapp and Paton, 1999; Renshaw, Ray and Dubovi, 2000; Hill, Reichel and Tisdall, 2010).

3.3.2.1.3 Spot test detection

3.3.2.1.3.1 Pooled milk

Ab-ELISA on pooled milk can be applied to cows that provide milk but that do not contribute to BTM, or can occasionally be applied to beef herds. This test can also be used to screen a specific age group (i.e. early lactation) as a negative result provides evidence of freedom even if BTM is positive. This test has the same limitations as the BTM testing.

3.3.2.1.3.2 Pooled serum sample

Ab-ELISA on pooled serum samples is usually applied to young stock and non-breeding beef cattle. This test is useful to predict presence or absences of PI in a dairy herd where BTM or first lactation tests are positive. Young stock will become Ab negative after the decrease of maternal antibodies,

after/at around 6 months of age. Testing those animals will give crucial information on the current situation within the herd. This variation highlights the importance of selection of animals for testing. In fact, the selection of animals tested is fundamental. Recently purchased animals have to be excluded from the test group and each separate group must be tested.

A PCR can also be applied on a pooled serum sample (Muñoz-Zanzi *et al.*, 2000). In such samples, PCR may be able to detect any individual infected up to a pooled sample of 50 individuals (Smith *et al.*, 2008; Yan *et al.*, 2011).

3.3.2.1.4 Vaccinated herd

Vaccination can limit detection of current infections when using tests based on Ab detection. A solution is to use unvaccinated sentinels and test them for Ab detection. Pillars and Grooms (Pillars and Grooms, 2002) have shown that serological testing of unvaccinated heifers within a vaccinated herd can be used to detect the presence of PI in a herd with a sensitivity and specificity of respectively 66% and 100%. Ab titers can also be useful to distinguish vaccinated herds with and without the presence of a PI animal. Houe *et al.*, 1995, show that the screening of 5 young stocks can distinguish vaccinated herd with or without PI. The probability to find at least 3 of (in) 5 animals with higher titer in a herd where killed-virus vaccine was used and in the absence of PI, was P<0.01, while it was P>0.99 in a similar herd in the presence of a PI.

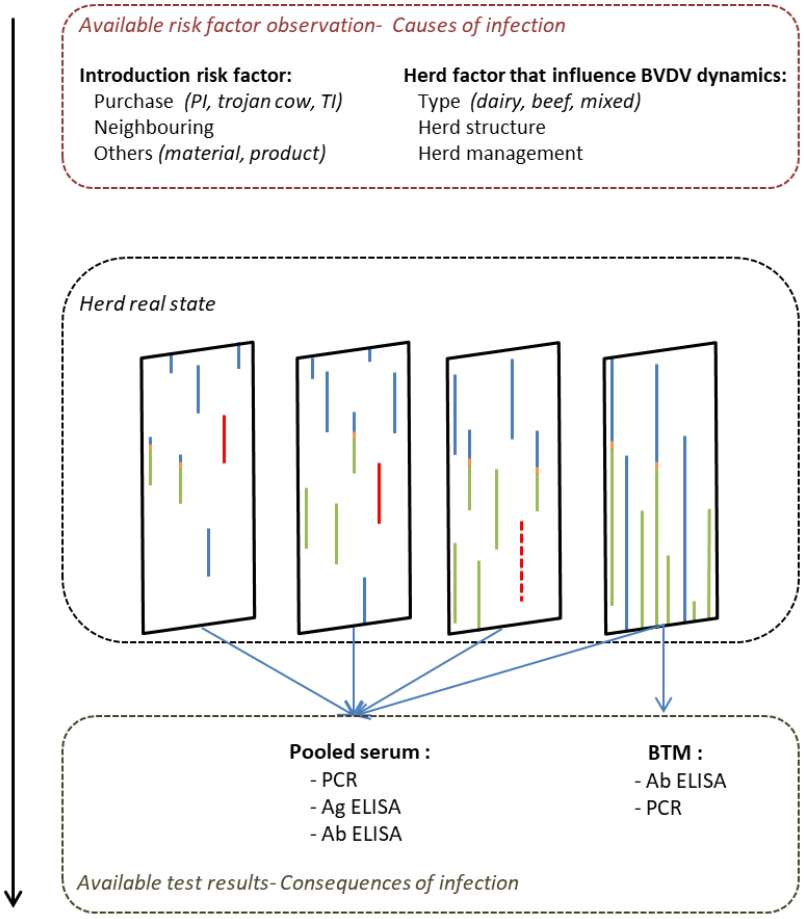

Figure 7: Available information at herd level: causes and consequences of infection by BVDV within a herd.

3.3.3 Delayed detection of BVDV in a herd

The time interval between the introduction of the infection and its detection can vary. As this interval increases the probability that infection spreads within the herd and between herds increases. The lengthening of this interval is what we call delayed detection.

Delayed detection can be associated with the design of the testing procedure such as test frequencies; animals tested and test performance (test characteristics).

3.3.3.1.1 Surveillance programme design

Surveillance programme design will determine how the presence of infection within the herd is detected. In fact, if the test is carried out just after introduction of a PI in a herd, depending on the test used, the result can be negative as the infection will take some time to spread in the entire herd over time and for individual animals to develop detectable levels of Ab. Serial testing allows herd monitoring and an increased frequency of testing can limit delayed detection. The length of time between two screening tests impacts the risk of delayed detection. Keeping the time low will reduce the risk of delayed detection.

3.3.3.1.2 Test performance

Test performance can contribute to delayed detection when sensitivity is not 100%. In general, test sensitivity is quite high for BVDV diagnostics, which should limit the impact of test performance to cause delayed detection. However, it worth noting that applications of these tests may result in a reduced sensitivity and specificity because of human errors during the diagnosis process (sampling, labelling, laboratory errors …).

4 Conceptual model for BVD at the territory level

Territory level can be either a region or a country depending on the way that BVDV eradication/control is managed. A territory is defined as an area where herds follow the same control measure (programme) and where information is gathered together. A territory has one BVD programme which can be based on different components (i.e. different component for dairy and beef herds).

Within the consortium, the BVDV control programmes for the Netherlands, Sweden, Ireland and Scotland, are applied on country level. For Germany and France, because of variability of types of herds, territory level will not correspond to country. For Germany it will be the Federal States and for France a region, or even a department.

4.1 Epidemiological state at territory level

There are at least three different BVD states at territory level.

4.1.1 Infection free and seronegative territory

A infection-free territory is defined as a territory composed of seronegative herds that are currently not infected by BVDV and where all cattle are susceptible.

4.1.2 Territory with infected herds

An infected territory is defined as a territory with at least one infected herd(s) meaning that the infection is present or spreading within the territory. In this defined territory, herds can be naïve and infection free, currently infected or seropositive (some or all animals). The proportion of herds in each state depends on the prevalence of BVDV infection and the control measure in place (endemic territory versus on-going eradication programme). Over time and depending on the contact between herds within the territory and the actions taken to trace and eradicate infected animals these proportions can change.

4.1.3 Post-eradication territory: Infection free and seropositive territory

A post-eradication territory does not have any infected herds within but can be composed of seropositive and seronegative herds.

4.2 Risk of introduction of BVDV into a territory

4.2.1 Cattle movement

As for herd level, cattle movement through purchase and market outside of a territory can be sources of (re-)introduction of BVDV into a territory. As PI animals are the main source of (re-) introduction of BVDV, purchasing young animals or pregnant dams (with a chance of being a Trojan cow) is particularly risky. More information about that risk can be found under herd level risk factors (section 3.2.1.1)

4.2.2 Infection prevalence in neighbouring territories

Infection prevalence in neighbouring territories can also be a risk factor for introduction of BVD within territories, when cattle are moved to/through or grazed in the neighbouring territory.

4.2.3 Wildlife (reservoir)

BVDV have been reported for over 40 different species, including domestic and wildlife species (Nelson *et al.*, 2016). As in domestic cattle, BVDV can induce persistent infections in 8 other species: white-tailed deer, mule deer, eland, mousedeer, mountain goats, alpacas, sheep, and domestic swine (Terpstra and Wensvoort, 1997; Scherer *et al.*, 2001; Duncan *et al.*, 2008; Nelson *et al.*, 2008; Bachofen *et al.*, 2013). Sources of infection for non-bovine species can be a spillover from cattle population by sharing environment or through direct contact (Nelson *et al.*, 2016). Despite this, infection through wildlife is not considered a major cause of introduction.

4.3 Within territory dynamics of BVDV

4.3.1 Territory representation

A territory is defined by an area where herds within the area follow the same programme against BVDV. Important territory characteristics that can vary from one territory to another and influence BVDV dynamics:

- Proportion of beef and dairy herds : as practises differ between beef and dairy herds
- Cattle density, herd density and degree of fragmentation of farms may influence the contact structure and potential contact between herds within the territory. Intensity of contact between herds can influence the transmission between herds once BVDV have been introduced.
- Purchase: the proportion of herds that purchase at least one animal and the total number of purchases can also influence transmission of BVDV once it has been introduced in the territory. If the proportion is high the transmission between herds is likely to be high.
- Infection prevalence within territory

4.4 Available observations at territory level

4.4.1 Territory structure information

Available information linked with territory structure can be listed as:

- Number of herd within the territory (number of herds)
- Density of herds within the territory (number of herds/km²)
- Surface area of the territory (km²)
- Proportion of dairy and beef herds (%)
- Infection prevalence of neighbouring territories
- Number of cattle purchased from outside of the territory and their source
- Participation in market/trade shows either inside or outside of the territory with participant from everywhere
- Information linked to wildlife: if available (qualitative/quantitative data) as the role of wildlife in BVDV dynamics is not considered significant.

4.4.2 Territory BVDV programme (surveillance/eradication)

Information at territory level will be derived from aggregation of observations at herd level. Seroprevalence of BVDV at territory level can be available. Programme information will also be available and will help to estimate the situation of the territory. First, the programme can be defined as compulsory or voluntary. In voluntary programmes not all herds within the territory are likely to

be involved in the programme. In this case information for herds that are in the territory but outside the programme can be missing. Vaccination campaign features like type and name of the vaccines used within territory may be also available.

Tests used in the programme will also be available. Type (sample and test) and performance (sensitivity and specificity) parameters will be provided. Other parameters involved in programme features such as time in between tests or group of cattle tested is referred at herds' level.

4.4.3 Delayed detection of BVDV in a territory

Delayed detection of BVDV within a territory can be linked to the efficiency of the surveillance/control programme. Depending on features of the programme, BVDV can spread within territory without being detected for a certain time period

4.4.3.1.1 Voluntary versus compulsory programme

Surveillance programme can be compulsory, meaning that all herds are participating, or voluntary meaning that only some herds of the territory are participating. If BVDV transmission occurs in non-participating herds in voluntary programmes, detection of BVD in participating herds can take more time than in a territory with a compulsory programme because it will take more time to find the herd at the source of infection. We can imagine that higher percentages of participation in the programme result in a lower risk of delayed detection.

4.4.3.1.2 Programme design

Characteristics of a programme may influence delayed detection. Performance of the chosen test (sensitivity and specificity), length of time between the test and group of animal tested can lead to misclassification. It case of false negative that can lead to delayed detection.

5 Conclusion

This work is a first step towards estimating probabilities of freedom from infection and their associated uncertainties from information that is diverse and heterogeneous. The conceptual models presented here map various pieces of information onto BVDV infection systems,which represent infection by the virus at animal, herd and territory level. The next step will be to translate these conceptual models into statistical models. From a statistical point of view, the challenge of translating heterogeneous inputs at different scales into uniform output has some specific characteristics that are important for the choice of the method to use.

The first feature of the challenge is its structure. This can be illustrated using the example of the probability for a calf of being born PI (Figure 9). The dam of such a calf, while susceptible to infection before her pregnancy, will have been infected by the BVDV during the window of susceptibility of her pregnancy (30-120 days of gestation). Therefore, she would have tested negative for an antibody test before the pregnancy and positive after. Omitting interference with the colostrum, the calf would multiply the virus and would not produce antibodies against it. This calf would therefore test positive to antigen ELISA or PCR tests and negative to antibody ELISA tests. With this example, we see that the information (test results), can be mapped onto the probability for a calf of being a PI using a conceptual representation of the infection epidemiology. Given the calf status, test results can differ depending on test characteristics as measured by sensitivity and specificity, but the underlying representation of how they are connected will not.

The second feature of our problem is the heterogeneity in the data. Under some control programmes, BTM antibody results are measured at regular intervals allowing the detection of herd seroconversion and thereby the probability of PI calf births. Other control programmes look for virus antigens or RNA in new-born calves. Going back to Figure 9, we would have information on either the dam statuses before and after pregnancy or on the calf status after birth. This heterogeneity in input can be a difficulty when our aim is to estimate a probability that is independent from the available data.

For statistical modelling, we will turn to Bayesian methods. A Bayesian representation of our problem will allow us to address these two features. In Bayesian statistics, models can be represented using directed acyclic graphs (DAG). Figure 9 is a DAG. Each box in the DAG (called a node) is connected to one or more other boxes with arrows (called edges). Each node can contain either observed data (test result) or unobserved/missing data (calf infection status, dam infection status, no test results (if no test has been performed)). The DAG describes the relationships between these nodes. Bayesian models go further by assigning statistical distributions to nodes and by providing mathematical descriptions of the relationships between nodes. For example, the node *PI calf* in Figure contains the calf's infection status. The calf is either PI or not PI. Being in one of two mutually exclusive categories is modelled using a Bernoulli distribution, which has a parameter p which is the probability for this calf of being PI. This probability p can be made dependent on the dam's status. In turn, the calf's status influences the probabilities of testing positive to specific tests, which will depend on test sensitivities and specificities. Bayesian models allow chaining these relationships within a single model.

The next step of our work will therefore consist in translating our conceptual models into Bayesian statistical models and then to parameterise these models so that they estimate a probability of freedom from infection regardless of the heterogeneity in input.

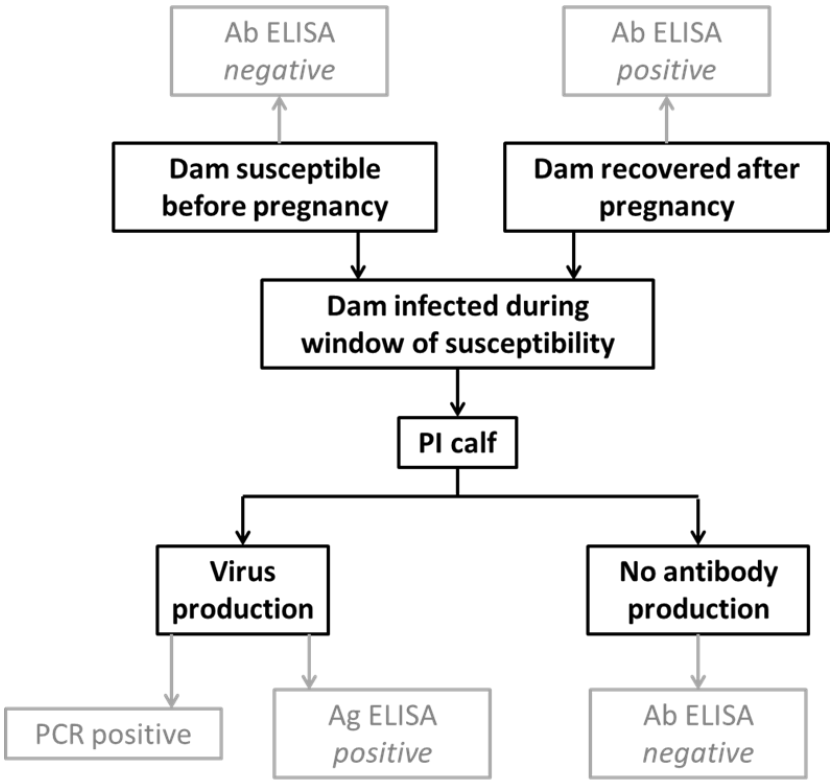

Figure 9: Representation in a DAG (Directed Acyclic graph) of the causes and consequences of a calf being born PI (black) and examples of associated test results (grey), omitting interference with maternally derived antibodies. Test results can differ depending on test characteristics.

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7 Annex

ANNEX I: Risk factors for introduction of BVDV at herd level

Table I: Risk factor for introduction of BVDV and their need for control. Reproduced from (Lindberg and Alenius, 1999).

Risk	Perceived need for control	Plausible ways through which BVDV is introduced into a non-infected herd	Comments
Livestock trade	Yes, imperative	Purchase of : 1) A PI animal. 2) A dam carrying a PI calf. 3) A seronegative animal in early pregnancy, infected during trade. 4) Other animal which has attained transient infection during trade and transmits virus to newly pregnant non immune animals in the destination herd.	(a) Effect on disease spread by PIs in the market will be multiplied if contacts with seronegative animals in early pregnancy can occur. (b) Prevalence of dams carrying PIs likely to be higher than prevalence of PI animals. The latter has been estimated to 1-2% in endemic situation (Houe, 1995). (c) Transiently infected animals are regarded as low impact transmitters (Niskanen *et al.*, 1996).
Exhibitions	Yes	1) Seronegative animal in early pregnancy becomes infected at the exhibition. 2) (An animal attains a transient infection and succeeds in infecting newly-pregnant non-immune animals after returning home.)	(a) PIs present at exhibitions will constitute a severe risk for farmers bringing seronegative animals in early pregnancy. (b) Transiently infected animals are regarded as low impact transmitters.
Animal contacts on pasture or over fences	Yes	1) Seronegative animals in early pregnancy become infected on pasture 2) (Some other animal attains a transient infection and subsequently transmits the infection to others, newly-pregnant non-immune animals in the herds.)	(a) Not controlling for release of PIs on common pastures will constitute a severe risk for farmers pasturing seronegative animals in early pregnancy. (b) PI carrying dams may spread disease if they abort or calve on pasture. (c) From a disease control point-of-view, and in terms of herd incidence, over-fence contacts will be less important than common pasturing.
Live vaccines	In the context of BVDV control, the use of live BVDV vaccines should be banned until proven safe.	At least one susceptible animal in early pregnancy becomes infected due to usage of live vaccine contaminated with non-cytopathogenic BVDV strains in the production process, or disease emerge as a result of recombination between vaccine and field strains (Ridpath and Bolin, 1995, Desport el al., 1997).	Risk of introducing strains new to the cattle population in question.

Risk	Perceived need for control	Plausible ways through which BVDV is introduced into a non-infected herd	Comments
Semen and embryos	Yes	At least one susceptible animal in early pregnancy becomes infected by other dams transiently infected due to AI with semen from PI bull or transiently infected bull, or persistent foetal infection develops in dam receiving AI with semen form PI bull or transiently infected bull.	Risk of introducing new strains to the cattle population in question. A case has been reported with a seropositive bull constantly shedding virus in semen, in the absence of general persistent infection (Voges *et al.*, 1998). Although this phenomenon is probably of low frequency occurrence, it should be noted that such bulls could only be detected by testing semen.
Visitors, including vets, AI technicians and herdsmen in the replacement system	Unlikely to be of major importance and impact, but preventive measures are appropriates in scheme rules.	At least one susceptible animal in early pregnancy becomes infected due to contact with inadequately cleaned and/or disinfected clothes, boots, and instruments and similar.	Risk for transmission will depend upon : - Interval time between visit in infected/non-infected herd (prevalence of infection in the area) - Types of vehicles (faeces, clothes instruments (Gunn, 1993), contaminated injectable) and amount of virus transmitted (Houe, 1999) - Pregnancy and immune statues of in-contact animal(s) in the herd
On farm collection of slaughter animals or brokered calves by professional transportation staff	Preventive measures are appropriate in scheme regulation.	At least one susceptible animal in early pregnancy becomes infected due to virus transfer by : - Transportation staff - Farmer entering transportation vehicle Risk for airborne transmission of virus from transportation vehicles parked close to stable entrances or air intakes has not been investigated	Risk of successful transmission will depend upon : - Number of infected animals in the vehicle, and type of infection (PI/transient) - Time interval between visit in infected/non-infected herd - Degree of handling at pick-up or delivery, i.e. degree of contact between transportation staff and cattle in the herd and/or between farmer and cattle in the vehicle - Pregnancy and immune status of in-contact animals in the herd.
Other species (sheep, goats, swine, deer, elks)	Preventive measures for sheep are appropriate in scheme regulation.	At least one susceptible animal in early pregnancy becomes infected due to contact with a persistently infected sheep/goat/pig/deer/elk.	No evidence exists that wild ungulates, swine or goats has transmitted the infection to cattle, even though interspecies transmission is possible (Nettleton, 1990). Strains proven to be involved in transmission from sheep to cattle have been of bovine origin(Paton *et al.*, 1995). BVD control was not compromised by sheep when implemented on the Shetland Islands (Synge *et al.*, 1999).
Vectors (ticks, mosquitos, flies)	No, at least not in the temperate climate zones.	At least one susceptible animal in early pregnancy becomes infected due to contact with virus-carrying vector.	Insects, such as biting flies have been shown to be capable of carry BVDV under experimental conditions (Tarry et al., 1991). Vector-borne transmission has never been described under natural

Table II: Types of contacts that can act as routes for transmission of BVDV infection between herds (from Lindberg and Houe 2005)

Characteristic of contact type			β_A	β_B	κ
Source of infectivity	Length of contact[a]	Type of recipient[b]	Probability of transmission A[c]	Probability of transmission $B\|A$[c]	Number of potential infectious contacts per time unit[d]
PI animals	Permanent			1	Low
	Transient	Not early pregnancy	Close to 1	Negligible	Low–very high
		Early pregnancy	Close to 1	Close to 1	
Dams pregnant with PI foetuses	Permanent			Close to 1	Low
Transiently infected animals	Permanent	Not early pregnancy	Very low	Negligible	Low–moderate
		Early pregnancy	Very low	Close to 1	
	Transient	Not early pregnancy	Negligible	Negligible	Low–very high
		Early pregnancy	Negligible	Close to 1	
Contaminated biologicals	n.a.	Not early pregnancy	Very low–very high, dependent on dose and way of administration!	Negligible	Low–extreme
		Early pregnancy		Close to 1	
Contaminated equipment, persons etc	n.a.	Not early pregnancy		Negligible	Low–moderate
		Early pregnancy		Close to 1	

[a] Permanent: more long-term introduction of the infectious animal into the herd; Transient: the infectious animal is in contact with other herds for a limited period of time, e.g. during an exhibition, an auction or a common pasture.

[b] Denotes whether the infectious animal is in contact with a susceptible animal in early pregnancy in the recipient herd for the duration of its infectious period, or not.

[c] A, the probability that the contact results in a transient infection in *the recipient(s)*; B, the probability that the contact results in a persistent infection in *the herd*. B is conditional on A for all scenarios except for permanent contacts with PI animals or PI carriers.

[d] Refers to the (potential) frequency of contacts between infected and a non-infected herds, by the contact type in question. Permanent contacts translates into intensity of trade, and transient contacts into intensity of contacts over fences, on common pastures, at auctions, exhibitions, etc.