Risk analysis of Bovine viral diarrhea and Bovine herpesvirus-1 introduction based on biosecurity measures implemented in dairy cattle farms

Bibiana Benavides Benavides

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Tesi doctoral presentada per ***Bibiana Benavides Benavides*** per accedir al grau de Doctora en Veterinària dins del programa de Doctorat en Medicina i Sanitat Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona, sota la dirección del Dr. **Alberto Allepuz Palau** i el Dr. **Jordi Casal Fàbrega**

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Que la tesi doctoral titulada **"Risk analysis of Bovine viral diarrhea and Bovine herpesvirus-1 introduction based on biosecurity measures implemented in dairy cattle farms"** presentada per **Bibiana Benavides Benavides** per l'obtenció del grau de Doctora en Veterinària, s'ha realitzat sota la seva direcció a la Universitat Autònoma de Barcelona.

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LIST OF ABBREVIATIONS AND ACRONYMS

AI	Acute infection
AGID	Agarose gel immunodiffusion
BoHV-1	Bovine herpesvirus-1
BVDV	Bovine Viral Diarrhea Virus
BVD	Bovine Viral Diarrhea
DAFF	Department of Agriculture, Fisheries and Forestry
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
HACCP	Hazard Analysis and Critical Control Point
IFAT	Indirect immunofluorescent test
IBR	Infectious Bovine Rhinotracheitis
LI	Latent infection
MLV	Modified live virus vaccines
MD	Mucosal disease
OIE	World Organisation for Animal Health / Office International des Epizooties
PI	Persistently infected
PCR	Polymerase Chain Reaction
Se	Sensitivity
Sp	Specificity
TI	Transiently infected
TR	Trojan cows
VNT	Virus neutralization test

ABSTRACT

The general aim of this PhD thesis was to develop a risk assessment tool to support the decision making in relation to biosecurity measures prioritization in dairy farms.

In the first study, a stochastic risk analysis model was developed to quantify Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus type 1 (BoHV-1) introduction through animal movements. Purchasing cattle, rearing replacement heifers offsite and showing cattle at competitions, were considered in the model. Besides a review of the scientific literature, parameters were estimated using animal movement database, biosecurity surveys and the opinion of field veterinarians. In this model, 46 farms from Galicia and Catalonia that moved animals during 2017 were included. Results showed that the annual probability of BVDV and BoHV-1 introduction was very heterogeneous, being close to 0 in some farms and in others close to 1. The median of the probability of introduction of BVDV was 12% and for BoHV-1 9%, with an inter-quartile range from 1.2% to 28% for BVDV and 3% to 23% for BoHV-1. The highest probabilities were associated with local movements of cattle (i.e., inside the same autonomous community) and the fact of sharing the transport vehicle between farms. By evaluating the effect of biosecurity measures on a selected farm, implementation of a correct quarantine or not sharing transport with other farms greatly decreased this probability.

In the second study, the probability of BVDV and BoHV-1 introduction through indirect contacts was quantified also with a stochastic risk analysis model. Vehicles transporting calves, cattle to slaughterhouse, dead animals, and food mix, as well as visits by veterinarians and hoof trimmers, farm workers and contacts with neighbors were considered in the model. For this study were included the 127 farms that participated in the project. Data to estimate model parameters was obtained from the sources indicated before as well as from interviews with hoof trimmers, animal transporters and a rendering company. Results evidenced that the median annual probability of introduction for BVDV was 2.1% and for BoHV-1 3.9%, with an inter-quartile range from 1.3% to 3.5% and de 2.6% a 6.8% for BVDV and BoHV-1, respectively. The calf transport vehicle and veterinarians’ visits were the routes with the highest risk. The biosecurity measures with the greatest impact in reducing the probability of introduction of both viruses were the use of boots and clothing belonging to the farm and avoiding the driver that transport cattle coming into contact with the animals on the farm.

The two models allowed establishing the riskiest pathways for each studied farm and thus could be useful tool to prioritize biosecurity measures that must be implemented or improved to reduce the probability of BVDV and BoHV-1 introduction into a farm.

RESUMEN

El objetivo general de esta tesis doctoral fue desarrollar una herramienta de evaluación de riesgos para apoyar la toma de decisiones con relación a la priorización de medidas de bioseguridad en granjas lecheras.

En el primer estudio, se desarrolló un modelo de análisis de riesgo estocástico para cuantificar la probabilidad de introducción del Virus de la Diarrea Vírica Bovina (BVDV) y del Herpesvirus Bovino tipo 1 (BoHV-1) a través del movimiento de animales, es decir, compra o introducción de animales, recría criada fuera de la granja y movimientos a ferias de ganado. Los parámetros se obtuvieron a partir de literatura científica, así como de la base de datos de movimientos animales de la Administración, encuestas de bioseguridad y opinión de los veterinarios de campo. En el análisis se incluyeron 46 granjas de Galicia y Catalunya que movieron animales durante el 2017. La probabilidad anual de introducción de BVDV y BoHV-1 fue muy heterogénea, siendo cercana a 0 en algunas granjas y en otras cercano a 1. La mediana de la probabilidad anual de introducción de BDVD fue 12% y para BoHV-1 9%, con un rango intercuartílico entre 1.2% y 28% para BVDV y 3% a 23% para BoHV-1. Las probabilidades más altas estaban asociadas al movimiento local de animales (i.e., dentro de la misma comunidad autónoma) y al hecho de compartir el vehículo de transporte entre granjas. Al evaluar el efecto de las medidas de bioseguridad en una granja, la implementación de una correcta cuarentena y el no compartir el transporte con otras granjas disminuyo considerablemente esta probabilidad.

En el segundo estudio, se cuantificó la probabilidad de introducción de BVDV y BoHV-1 a través de contactos indirectos, para lo cual también se desarrolló un modelo de análisis de riesgo estocástico. Se consideraron los vehículos que transportan terneros, animales a matadero, cadáveres y mezcla de alimentación, así como los visitantes, entre los que se incluyeron veterinarios, podólogos, trabajadores de las granjas y vecinos. Para este estudio se evaluaron las 127 granjas que participaron en el estudio y la información para la estimación de los parámetros del modelo, además de las fuentes citadas anteriormente, se obtuvo de entrevistas a podólogos, transportadores de animales y una empresa de recolección de cadáveres. Los resultados mostraron que la mediana de la probabilidad anual de introducción para BVDV fue de 2.1% y para BoHV-1 del 3.9%, en un rango intercuartílico de 1.3% a 3.5% y de 2.6% a 6.8% para BVDV y BoHV-1, respectivamente. La entrada del vehículo de

transporte de terneros y las visitas de los veterinarios fueron las vías de entrada con mayor riesgo. Las medidas de bioseguridad con un mayor impacto en la disminución de la probabilidad de introducción de los dos virus fueron el uso de botas y ropa propios de la granja y evitar que el conductor que transporta animales entre en contacto con los animales de la granja.

Los dos modelos permitieron establecer las vías de entrada más riesgosas para cada granja estudiada y de esta forma pueden ser una herramienta útil para priorizar las medidas de bioseguridad que deben implementarse o mejorarse para reducir la probabilidad del ingreso de BVDV y BoHV-1en una granja.

PUBLICATIONS

The first study presented in this thesis has been published in international scientific peer-reviewed journal:

Study I: Development of a quantitative risk assessment of bovine viral diarrhea virus and bovine herpesvirus-1 introduction in dairy cattle herds to improve biosecurity

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Introduction

Chapter I

1.1. Routes of infectious diseases introduction to dairy farms

International trade, globalization, and global warming increases the probability of disease spread among countries and therefore enhance the importance of preventive measures against infectious diseases (Barkema et al., 2009). The prevention and control of infectious diseases relies on monitoring the health status of the herd, increasing host resistance to infection, removing reservoirs of infection and preventing contacts that result in transmission (i.e., biosecurity measures) (Smith and Grotelueschen, 2004).

On dairy farms, infectious diseases can cause important economic losses to producers. These can be due to direct costs such as treatment and production losses or indirect costs such as potential loss of market access for hazards with animal or public health risks (Wells, 2000; Van Schaik et al., 2002; Maunsell and Donovan, 2008).

Infectious diseases can enter a dairy herd through different routes. The most important route is through introduction of new animals, especially replacement heifers, or other type of contact between live animals (e.g., at pastures, markets, etc.) (Brennan et al., 2008; Nöremark et al., 2013). Transmission through people and vehicles visiting the herd, other animals beside cattle (e.g., cats, dogs, rodents), fomites (e.g., feeding utensils, farm equipment, veterinary material), vectors (either biological or mechanical) and contaminated water or feed, could be also important (Wells, 2000; Villarroel et al., 2007).

1.1.1. Cattle movements

Dairy farms around the world are undergoing a significant increase in their size and consequently important changes in their structure. In Europe, social, land space, and legislative and economic pressures constrain the growth of dairy herds in many parts of Western Europe (LeBlanc et al., 2006). However, the elimination of the European Union (EU) production quotas opened a new era of unrestricted milk production (Sayers et al., 2013). A collateral effect of the herd expansion in dairy farms is an increase in the cattle movements. On the one hand, young animals might be sent to external facilities to be raised and then they return before (or eventually after) the first calving. On the other hand, some farms might buy all the replacement heifers from other farms.

The introduction of cattle from external sources rather than internal herd growth implies the risk of introduction of pathogens (Maunsell and Donovan, 2008; Barkema et al., 2015). The most important source is through purchase of infected animals because not many of these animals are screened for infectious diseases before or shortly after arrival at the dairy farm (Faust et al., 2001; Hoe and Ruegg, 2006). In addition, in case of multiple-source purchase, herds-of-origin health histories are usually unknown (Maunsell and Donovan, 2008). This trend of herd expansion leads to an increased risk of disease transmission, due to commingling of animals from various sources or factors such as transport increasing stress levels potentially exacerbating latent disease conditions (Brennan et al., 2008).

Cattle movements due to reintroduction of cattle returning from out-farms (e.g. replacement heifers, common pastures) or that participated in cattle shows, or shared transportation with cattle from other farms, can play a significant role in the introduction of diseases (Van Schaik et al., 2002; Robinson and Christley, 2007; Mee et al., 2012; Gates et al., 2013). Furthermore, most farmers take insufficient measures to prevent spread of infections from animals reintroduced or returning from a calf-rearing facility or after a cattle fair/competition or market (Van Schaik et al., 2002; Villarroel et al., 2007).

Managing health risks when introducing animals to dairy herds should include control strategies before purchase and post-purchase management of animals (Maunsell and Donovan, 2008). Purchasing stock from certified disease-free source herds or high health status markets can greatly reduce the risk of introducing diseases (Wells, 2000; Sibley, 2010). Quarantine and testing before introduction are the most important biosecurity measures post-purchase. However, quarantine is very difficult on most dairy farms or ineffective implemented (e.g., the quarantine pens are adjacent to the existing herd or only is quarantined a portion of incoming cattle) (Faust et al., 2001; Villarroel et al., 2007).

Measures to reduce the probability of infection during transportation for animal movements include i) cleaning and disinfection of transport vehicles (Schnyder et al., 2019); ii) loading and unloading animals at the perimeter of the farms; iii) not mixing animals from different sources in the transport vehicle) (Maunsell and Donovan, 2008; Mee et al., 2012), and iv) minimizing transport distances and other stressors such as overcrowding (Greger, 2007). Legislation to control movement may also reduce the spread of infectious agents and is a frequent state-mediated reaction to exotic disease outbreaks (Valle et al., 1999; Van Schaik et al., 2002; Mee et al., 2012).

The best alternative, from a biosecurity point of view, would be maintaining a closed herd (i.e., without the purchase of new animals) because it eliminates infection risk from purchased cattle (Wells et al., 2002; Nöremark et al., 2013; Mee et al., 2012). However, even specific pathogen-free and closed herds experience disease breakdowns, reflecting the important role of other transmission routes (Van Schaik et al., 2002).

1.1.2. Indirect contacts

The role of indirect transmission of livestock diseases is still largely unknown. First, due to the highly diverse and complex nature of indirect contacts, it is difficult to assess the relative importance of these transmission pathways. On the other hand, because of privacy reasons, it is much easier to track cattle movements than that of farm operators and personnel for example (Rossi et al., 2017b). The probability of transmission given an indirect contact is considered to be much lower that direct animal–animal contacts. However, the likelihood of transmission is a combination of the probability of transmission given a contact and the frequency of such contacts and in the case of indirect contacts, they can occur at a relative high frequency (Ribbens et al., 2009).

Visitors to farms can introduce an infectious agent via their hands, but also their clothing, boots, equipment, and vehicles (Morley, 2002; Kirk et al., 2003). High-risk visitors include those people that usually have contact with dairy cattle such as veterinary practitioners, hoof trimmers, artificial insemination technicians, farm workers, other farmers and drivers of vehicles such as rendering or livestock vehicles (Sibley, 2010; Mee et al., 2012). Neighbors have a particular importance in densely populated areas and during the pasture season (Qi et al., 2019). In addition, farms that are contiguous are probably more likely to establish social relationships, facilitating sharing of equipment and potentially transmission of infectious agents via vehicles and personnel (Brennan et al., 2008). However, although visitors are known to be potential disease spreaders, there are very limited published studies that address this issue (Van Schaik et al., 2001; Rossi et al., 2017a).

Contaminated farm machinery, vehicles, veterinary equipment, (e.g. needles and syringes, nose tongs, halters, obstetrical equipment, dosing equipment, dehorning equipment, hoof paring equipment), and general farm equipment can all act as fomites for introduction of infectious agents into herds (Gunn, 1993; Lang-Ree et al., 1994; Niskanen and Lindberg, 2003; Mee et al., 2012). Various cattle pathogens, such as Bovine Viral Diarrhea Virus (BVDV) can survive in these fomites and therefore be implicated in the transmission chain (Stevens et al., 2011).

There are highly significant infectious diseases of cattle (e.g., respiratory diseases or foot and mouth disease) that can be carried and transmitted over long distances or between compartments of the farm by other animals either domestic ones (e.g., dogs and cats) or wildlife species (Sibley, 2010; Dewulf and Van Immerseel, 2018). Pathogens can be also transmitted by vectors (mosquitos, flies, ticks, etc.) either biologically (the pathogen replicates within the vector) or mechanically (without replication within the vector) where the transmission is influenced by several factor such as density of vectors (dependent on environmental conditions), and density of susceptible hosts (Dewulf and Van Immerseel, 2018).

Finally, water sources shared between farms could be an efficient way of spreading several pathogens amongst animals that drink the contaminated water (Sibley, 2010; Dewulf and Van Immerseel, 2018). Also, feed can be contaminated by spreading manure on fields where crops are grown or irrigating fields with contaminated water or crops that have been fertilized with manure from other dairies (Villarroel et al., 2007).

1.2. Biosecurity

Biosecurity consists of a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections, or infestations to, from and within an animal population (OIE, 2019). The goal of biosecurity measures is to prevent different transmission routes to break the spread chain (Wells, 2000; Villarroel et al., 2007). Biosecurity can be divided in two main components: external biosecurity or bioexclusion that prevent pathogens from entering a herd, and internal biosecurity or biocontainment which reduce the spread of pathogens within a herd (Villarroel et al., 2007; Laanen et al., 2013).

1.2.1. External biosecurity (Bioexclusion)

External biosecurity includes all practices devoted to preventing (risk reduction strategies) the farm's contact with the outside world avoiding the introduction of pathogens (hazards) (Mee et al., 2012). The main components of external biosecurity include measures related to: purchase of animals and animal products, transport of animals, other vehicles and equipment, removal of manure and cadavers, supply of fodder, water and equipment, access of personal and visitors, rodents and bird control, and location and environment (Dewulf and Van Immerseel, 2018). The principal actions are isolation, and diagnostic testing of new animals or animals returning to the farm, quarantine procedures, vehicle access control, avoid contacts with other herds and grazing areas, and control of visitor access (Kristensen and Jakobsen, 2011; Moore et al., 2008; Villarroel et al., 2007).

1.2.1.1. Measures related with purchase of animals and animal products

The general advice is to avoid purchasing cattle as much as possible (Sarrazin et al., 2018). However, if cattle are purchased, they should come from farms with a sanitary status that is equal to or higher that the own farm (Wells, 2000). If the previous options are not possible, another option is purchasing animals from herds of known disease status so that any risks can be managed and mitigated appropriately (Sibley, 2010). Other practices include reducing the number of animals and the number of herds from which the animals are purchased (Mee et al., 2012), avoiding purchases from markets or dealers (Kristensen and Jakobsen, 2011). To prevent BVDV, not buying pregnant animals reduces the risk of transmission of infection from an infected fetus (Lindberg and Houe, 2005). For animal products like sperm, embryos, or colostrum the health status of farms/institutions of origin must be considered (Sarrazin et al., 2018).

1.2.1.2. Quarantine procedures

Quarantine is the isolation of cattle in an area that prevents direct or indirect contact with other livestock. All incoming cattle can be grouped together in one or more receiving pens (even when originating from different sources) and be separated from the general population (Villarroel et al., 2007; Raaperi et al., 2014). The quarantine period should be long enough to allow detection of clinical signs. It has generally been recommended to be three or four weeks but will depend on the diseases of concern (Wells et al., 2002; Maunsell and Donovan, 2008). If animals have been tested, the quarantine should last until confirmation of the negative status of the animals is available.

Cattle in lactation should preferably be milked inside the quarantine area, and for pregnant cattle, calving should occur in the quarantine area and the new born should be tested immediately after birth (before the intake colostrum) and should be quarantined until test result are available. Finally, the all-in/all-out principle should be applied in the quarantine area (Sarrazin et al., 2018).

An appropriate quarantine area should be separate from the resident herd as much as possible. Housing should be clean and comfortable with good ventilation, and water sources. The facility should be designed so that routine health monitoring is easily done, a safe transition ration can be fed, and group health management procedures can be performed easily (Maunsell and Donovan, 2008). If the farm do not have such facilities, an alternative would be to house cattle in a separate group in the most remote location on the farm (i.e., pasture or an old building) (Villarroel et al., 2007; Dewulf and Van Immerseel, 2018).

Indirect disease transmission from cattle in quarantine is possible through people that enter the quarantine area, material that is used and through water and feed that are provided to the cattle (Sarrazin et al., 2018). Therefore, quarantine should be attended by specifically designated personnel. An alternative would be to assign trained personnel with strict hygiene and disinfection protocols and avoid these personnel to have immediate access to neonatal calves or maternity areas after working in the quarantine. In addition, is recommended to enter the quarantine area at the end of daily work routine (Villarroel et al., 2007; Dewulf and Van Immerseel, 2018). Specific clothing and boots should be available at the entrance of this area and everyone who entered the quarantine should make use of it. Specific material also should be available in the quarantine area and not be used in the other areas of farm (Edwards, 2010).

1.2.1.3. Testing animals

Testing animals on arrival is commonly recommended for many infectious diseases and can greatly enhance the sensitivity of detecting an infectious animal and therefore reduce risk (Wells, 2000; Maunsell and Donovan, 2008; Moore et al., 2008). The consequences of a false negative far outweigh those of a false positive; sensitivity should be favored over specificity for screening tests (Barkema et al., 2009).

Testing could be in a pool of samples of all cattle, a representative subset of cattle, or from all individual cows. Regardless the sampling strategy, the most important is do not allow any animal to be released into the general population until all submitted biological samples are found to have negative results (Wells, 2000; Villarroel et al., 2007). In addition, testing specific group of animals such as the progeny of females purchased while pregnant can enable the detection of infectious agents transmitted transplacentally, e.g., BVDV or Neospora (Sibley, 2010).

1.2.1.4 Vehicles and equipment

Transport of animals: Vehicles entering the farm also should be considered, especially those ones that pick up culled animals, cattle to slaughter or calves as they usually visit different farms to complete the capacity of the truck (Dewulf and Van Immerseel, 2018). Contact with animals with unknown diseases status from other farms added to the stressful situation increase the risk of becoming infected during transport (Mee et al., 2012). Smith et al. (2013) observed that when animals from different farms are transported separately but in successive shipments, the risk of transmission of infectious agents potentially increases. Therefore, ideally, only cattle designated for the farm should be present in the vehicle, otherwise animal transport vehicles should arrive empty, clean and disinfected before entering. When the farm uses their own transport vehicle, it is easier to control the cleaning and disinfection status of the vehicle (Sarrazin et al., 2018).

Appropriate cleaning and the application of a disinfection protocol for vehicles, as well as biosecurity measures at the farm, may be crucial for limiting the spread of disease via shared vehicles (Dee et al., 2004). Other measures at the farm level, such as move cattle leaving the farm to a separate building or loading area outside should prevent contact with the herd. The transporter should not be allowed to enter the stables in order to avoid contact with the animals of the farm (Sarrazin et al., 2018).

Other vehicles entering the farms include milking trucks, feed trucks, rendering trucks and visitor vehicles. The probability of transmission of pathogens will depend on the type of vehicle. The rendering company trucks are considered of higher risk than other type of vehicles (e.g., feed trucks) (Ribbens et al., 2009). Although feed and milk collection trucks are rarely exposed to the animals on farms (Nöremark et al., 2013), these vehicles could suppose a risk as they visit several farms in the same day (Ribbens et al., 2009). Feed should be delivered, and tank milk collected without the driver having to enter the stables (Sarrazin et al., 2018).

Biosecurity measures to avoid introduction of pathogens on vehicles include restricting access to areas of the farm where animals are housed. The receiving area at the farm should be located as far away from animals as possible and clearly identified at the entrance of the farm (Villarroel et al., 2007). On the other hand, all inbound and outbound traffic that serves multiple companies should be split up into a clean and a dirty section. The clean road for intra-farm movements and dirty road to manure transport (Dewulf and Van Immerseel, 2018). Cadavers should always be handled with specific attention (e.g., use gloves when manipulated) and stored in a separate place from the animals. After pick-up, the storage place should be cleaned and disinfected. This storage space should be located out of the farm to avoid vehicles of the rendering company having to enter the farm (Sarrazin et al., 2018).

Shared equipment between farms include tractors, trailers, and wagons, followed by machinery for harvesting and ploughing, and manure vehicles. Therefore, ensuring that the equipment is cleaned and disinfected when returned to the farm may be important in limiting this route of transmission (Brennan et al., 2008).

1.2.1.5. Avoid contacts with other herds

It should be avoided the contact between animals from different farms. These practices include pastures shared in the same season, or through an adjacent pasture, and farms with access to the same surface water in pastures. In those instances, double fences can reduce the risk of diseases transmission (Van Schaik et al., 2001; Dewulf and Van Immerseel, 2018). For animals that participate in fairs, markets or competitions, the unsold cattle and rearing replacement heifers offsite, should be quarantined when they return to the farm according to the same principles mentioned above (Van Schaik et al., 2002; Sarrazin et al., 2018).

1.2.1.6. Control of visitors

Visitors include professionals (i.e., veterinarians, artificial insemination technicians, cattle salesmen, feed suppliers, milk collectors, rendering companies, hoof trimmers) and non-professional (i.e., friends, family and neighbors). However, the veterinarian is the professional visitor that enters animal compartments most frequently and usually they can enter freely at the farm and stables where cattle are housed (Nöremark et al., 2013; Sarrazin et al., 2014; Sarrazin et al., 2018). Also, farm workers who have contact with animals on other farms or have animals in their house can also introduce diseases by acting as visitors (Villarroel et al., 2007). In addition, social visits that have contact with areas where animals are located, might also represent a risk for disease spread (Nielen et al., 1996).

Cattle farms receive several visitors that have close contact with animals. Therefore, variation in frequencies contact may affect the farm-level risk of pathogen transmission (Brennan et al., 2008). The main recommendation is fencing the farm and closing the entrance with a gate, making the phone number of the farmer visible so visitors can only enter the farm after notifying the farmer (Sarrazin et al., 2018).

General biosecurity measures for visitors, include maintaining an area separated from livestock and farm activities restricted for external visitors (Maunsell and Donovan, 2008; Sibley, 2010; Mee et al., 2012). The farm should provide personal protective equipment, e.g., gloves, footwear or clean boots and overalls that cannot be taken from the farm, and handwashing and boot washing facilities for maintaining proper hygiene (Morley, 2002; Van Schaik et al., 2002; Sibley, 2010). Therefore, a sanitary transition zone where visitors can change clothes and wash their hands should be located (Sarrazin et al., 2018). In addition, education and guidance for workers over standard operating procedures should be written and visible at the entrance of the facilities (i.e., basic hygiene procedures and in the areas of disease awareness, prevention, and control) (Villarroel et al., 2007).

1.2.1.7. Rodents, birds, dogs and cats control

An efficient rodent control should not allow them to breed in the barn surroundings, which includes removing hiding places near to the barns, also traps and poison are recommended for controlling rodents (Dewulf and Van Immerseel, 2018, Sarrazin et al., 2018). To avoid diseases transmission through domestic animals, access to the barns should not be possible and manure storage facility, and feed should be stored in well closed rooms (Sibley, 2010). On the other hand, bird droppings, urine and feces of wildlife could contaminate surface water, for this reason is necessary filtration and chemical sterilization of drinking water. In addition, periodic monitoring of water quality with analysis for chemicals, minerals, and bacteria should be carried out (Villarroel et al., 2007).

1.2.2. Internal biosecurity (Biocontainment)

Internal biosecurity can be defined as the measures taken to limit within-farm transmission of diseases between groups of animals, it is related to farm management and daily practice (Mee et al., 2012; Dewulf and Van Immerseel, 2018). Biocontainment also serves as an important backup system for biosecurity plans. When biosecurity is breached, biocontainment measures can prevent the spread of disease agents on a farm (Villarroel et al., 2007). The main components of internal biosecurity include: disease management, stocking density, work procedures, staff education, compartmentalization and working lines, cleaning and disinfection of barns, equipment and tools (Moore et al., 2008; Kristensen and Jakobsen, 2011; Dewulf and Van Immerseel, 2018).

1.2.2.1. Diseases management

It is recommendable to have a register with animal health data that includes different data such as animals sick, animals under treatment and which treatment they are receiving, or vaccination protocols, among others (Edwards, 2010, Sarrazin et al., 2018).

The strategy consists correct handling and treatment of diseased animals, including proper diagnostics, isolation, and diseases registration. Treatments of animals should be performed carefully to avoid iatrogenic transmission or contamination of material by environmental pathogens (Dewulf and Van Immerseel, 2018). Disease management will require resources and enthusiasm that can only be achieved by agreement between all workers involved in farm management (Sibley, 2010).

The farm should have hospital pens where sick animals are housed to prevent direct and indirect transmission of diseases to other animals. Its use should be exclusive to sick animals and should be cleaned and disinfected each time after its use (Maunsell and Donovan, 2009). When a lactating cow is diseased it should be milked in the hospital pen, otherwise as the last one in the regular milking in order to avoid contact with healthy animals (Hage et al., 2003).

1.2.2.2. Stocking density and work procedures

The number of animals should be according to the capacity of the pen to avoid stress caused by overpopulation that increases susceptibility to infections and rises the excretion of pathogens (Dewulf and Van Immerseel, 2018).

It is important to make work routines as easy as possible for employees, providing stations for hand and boot washing. These stations should be convenient located next to the work site. Brushes need to be in good working order and hoses there must be enough water pressure to wash effectively. Proper use of footbaths is necessary if they are to be effective. Boots should be rinsed off before using the footbath because inorganic material inactivates disinfectants and footbaths need to be inspected and changed at least daily and as often as necessary, to keep them clean (Dargatz et al., 2002).

1.2.2.3. Compartmentalization and working lines

The facilities design of a cattle farm can help prevent spread of pathogens, separating animals according to age groups in different areas. Workers should follow a work routine starting in the youngest animals' facilities, continuing with the adults and finally the quarantine and sick pens. (Dewulf and Van Immerseel, 2018). Susceptible animals in farm (i.e., periparturient cows, and newborns) should be located as far away from any hospital pens, and the flow of cattle movement within farm should be unidirectional (Villarroel et al., 2007). It is recommendable to use age-specific material for each age group and feeding utensils should only be used for feed. Equipment must be routinely cleaned and disinfected, and hygiene practices related with changing clothing, footwear and washing hands should be applied systematically (Villarroel et al., 2007, Dewulf and Van Immerseel, 2018).

1.2.3. Biosecurity programs in dairy farms

Biosecurity programs can be implemented at different levels such as country, region, or herd. At national level, minimizing the probability of introduction of exotic diseases and maintaining contingency plans are essential policies (Barkema et al., 2009). Animal identification, livestock tracing schemes, and advanced disease recording systems are important components for these policies as they allow tracing the spread of infections on a regional or national level, which is of paramount importance in the case of an outbreak of an exotic disease (LeBlanc et al., 2006). At the farm level, reducing the likelihood of disease introduction through adequate biosecurity practices would improve the animal health and welfare status of the herd, which in turn would improve pro-fits and job satisfaction (Barkema et al., 2009; Brennan and Christley, 2012). The implementation of biosecurity practices is an effective way to prevent and control many infectious diseases on farms (Ritter et al., 2017).

General biosecurity programs and protocols should be defined and applied on all dairy farms to prevent the introduction of diseases, they should include measures directed at reducing risk due to the most important transmission routes, at the same time, it can be applied other measures against specific pathogens. The general principles of biosecurity strategies include: i) separation of high and low risk animals and environments; ii) reduction of the general infection pressure and, iii) prioritize biosecurity measures against higher risk transmission routes (Wells, 2000; Dewulf and Van Immerseel, 2018). In this sense, standard framework, similar to the Hazard Analysis and Critical Control Point (HACCP) programs that are widely applied in food safety, could be adopted when designing a biosecurity program in dairy farms (Villarroel et al., 2007). These programs should be developed in collaboration with the herd veterinarian who knows the specific herd structure and the farmer (Villarroel et al., 2007; Brennan and Christley, 2012). Biosecurity programs should be monitored and regularly reevaluated and updated. Producers should be able to assess the effect of the implementation of the different biosecurity measures on the reduction of the risk of infection, health and the productive indices of farm (Raizman et al., 2006; Oidtmann et al., 2011; Van Steenwinkel et al., 2011).

The implementation of biosecurity plans on dairy farms is voluntary in almost all countries (Kristensen and Jakobsen, 2011), and its success is dependent upon participation of dairy farmers in these programs (Barkema et al., 2015). Some difficulties arise in practical implementation which are associated with attitudes and behaviors of farmers and veterinarians (Frössling and Nöremark, 2016; Moya et al., 2019), the previous experiences they have lived, and the importance they can attribute to the biosecurity programs (Renault et al., 2018). Although many farmers are aware that biosecurity measures are an effective way to prevent diseases, several studies demonstrated that most cattle farmers do not implement or implement incorrect biosecurity measures (Moore et al., 2008; Sayers et al., 2013). Therefore, farmers need to be motivated to change existing behaviors. Furthermore, farm workers participation is also necessary to implement effective biosecurity programs to decrease risk of pathogen transmission (Mee et al., 2012; Ritter et al., 2017).

Some studies have described biosecurity measures applied in dairy farms. Most of them have been conducted in the United States (Wells et al., 2000; Faust et al., 2001; Hoe et al., 2006; Villarroel et al., 2007); United Kingdom (Brennan y Christley, 2012; Gates et al., 2013); Finland (Sahlström et al., 2014); Sweden (Nöremark et al., 2010); Belgium (Sarrazin et al., 2014); Ireland (Sayers et al., 2013) and some European countries (Renault, et al., 2017). In United States reported that 45% of farms introduce cattle each year, 75.5% did not require any testing of new cattle, only 20.6% of dairy herds quarantine any cattle and 15% indicated use of quarantines for at least 7 days. Furthermore, 38% shared heavy equipment with other farms; 58.8% had at some point used the same equipment to handle manure and animal feed, and of these, 15.2% did not use any cleaning or disinfecting procedures for the equipment after manure was handled (Villarroel et al., 2007). In Europe, Sayers et al., (2013) described that in Ireland 12% of herds are closed, only 7.6% tested animals following purchase for diseases other than those under statutory control and just 14.5% farmers implemented correct quarantine procedures. Sarrazin et al., (2014) in Belgium, reported that only 12% of the herds purchasing cattle implemented quarantine measures; farm-specific boots and clothing for visitors were present in 70% and 66% of the farms, respectively. Despite their presence, only in 20% and 13% the studied herds, respectively, these measures were used. Luzzago et al., (2008) in Northern Italy concluded that dairy farms quarantine never was used. Renault et al., (2017) in Belgium, France and Spain reported that less than 10% applied quarantine to incoming animals. In relation to veterinarians, 11% wash their boots, 58% change clothes when they look dirty, and reusable material was sterilized after each animal by 67% of the veterinarians.

1.2.4. Measuring biosecurity on dairy farm

Measuring biosecurity is relevant to evaluate biosecurity level and identifying those measures that need to be improved (Dewulf et al., 2018). Several systems have been designed to evaluate the biosecurity status of livestock systems. They have been developed as check lists or standardized scoring systems based on expert opinion (e.g., farmers, veterinarians, and researchers) obtained through structured questionnaires. Evaluation tools allow quantifying biosecurity level and classify herds according to their level of implementation as well as benchmarking (Luzzago et al., 2008, Laanen et al., 2013). Different online tools for the evaluation of biosecurity have been developed. For example, the Biocheck from UGent, a risk-based scoring system to evaluate the quality of on-farm biosecurity (http://www.biocheck.ugent.be). This system focuses in aspects that are common to the transmission of many different types of infectious diseases (Dewulf et al., 2018). Other authors have developed scoring systems for specific cattle diseases such as Paratuberculosis (Raizman et al., 2006) or BVDV (Luzzago et al., 2008; Sarrazin et al., 2014) that would aid the implementation of a voluntary control program. This kind of tools usually shows where efforts can be made to improve biosecurity aspects on the farm. However, they do not quantify the risk of pathogens entry and, consequently, the impact of biosecurity measures on reducing the probability of introducing diseases can not be evaluated

A tool for decision making to reduce the risk to which a farm is exposed should reflect the probability of introduction (Moore et al., 2010). For this purpose, it may be desirable to undertake a quantitative analysis, for example, to identify critical steps or to compare sanitary measures. A quantitative risk assessment use mathematical models wich can represent reality and the impact of risk management options where the inputs and outputs are expressed numerically (Murray et al., 2004). In this context, the development of quantitative risk assessment can be a useful method to identify and justify enhanced biosecurity measures according to the probability of disease introduction based on objective parameters.

1.3. Bovine viral diarrhea virus (BVDV)

1.3.1. Etiology

Bovine viral diarrhea virus (BVDV) belong to the pestivirus genus within the Flavivirus family (Heinz et al., 2000). Three pestivirus species are described: bovine viral diarrhea virus 1 (BVDV-1), bovine viral diarrhea virus 2 (BVDV-2) and HoBi-like virus (often referred to as BVDV-3 or bovine atypical pestivirus (Bauermann et al., 2013). Phylogenetic analysis of the three bovine pestiviruses has further classified them into sub-genotypes and identified at least 21 BVDV-1, three BVDV-2 and four HoBi-like sub-genotypes (Yesilbag et al., 2017).

BVDV 1 and BVDV 2 are antigenically distinct, each genotype may exist as one of two biotypes, cytopathic (cp) and noncytopathic (ncp). Cytopathic is the biotype of choice for modified live vaccines because ncp viruses from either genotype can cross the placenta and establish persistent infections in the fetus (Houe, 1999; Evermann and Ridpath, 2002). However, the ncp biotype has been shown to predominate in the field. In persistently infected (PI) animals, both biotypes will result in the development of mucosal disease (MD) (Fulton et al., 2002). In Europe, at least 11 subgroups (BVDV types 1a–1k) of BVDV 1 have been described but their biological significance has yet to be examined (Vilcek et al., 2001).

1.3.2. Pathogenesis

BVDV replicates in draining lymph nodes and from there spreads via circulating lymphoid cells to the blood. The incubation period of bovine Pestiviruses, upon infection, is between 6- and 12-days post-exposure but can fluctuate depending on the strain of the virus, its virulence and the virus dose transmitted (Evermann and Barrington, 2005). Viremia can be detected 24 – 48 hours post infection. The course of the viremia is dependent upon the presence or absence of colostral antibodies that are believed to persist for up to 6 months of age (Baker, 1995).

1.3.3. Clinical manifestations

The clinical outcome of infection depends on the infecting viral strain as well as the age, immunological status, pregnancy status, gestational age of the fetus, the immune status (passive or active immunity from previous infection or vaccination), the level of environmental stress, and viral factors which include genomic and antigenic diversity among BVDV isolates (Baker, 1995; Grooms, 2004).

Infection of the dam in <40 days of pregnancy can result in embryonic or fetal mortality, whereas infection with a ncp strain between 30 and 120 days of gestation (prior to fetal immunocompetence) can result in fetal death, abortion, mummification and most importantly, the birth of calves which are immunotolerant to BVDV (i.e., persistently infected animals). These animals appear clinically normal, but excrete large quantities of virus into the environment throughout its life being the most important reservoir of virus, as a result, they are critical for the maintenance and circulation of BVDV in the field (Kahrs, 2001; Smith and Grotelueschen, 2004; Thurmond, 2005). In addition, PI animals are predisposed to secondary infections due to their reduced immune function and tend to have significantly reduced growth and production performance compared to their peers, thus, lifespan of PI animals is significantly shorter (Peterhans et al., 2010). However, some PI animals might remain clinically unaffected, seronegative and may breed satisfactorily, and they will then transmit the infection to the fetus, which will always be PI. In most infected bovine herds, the prevalence of PIs is estimated to be lower than 1%, although variation occurs (Houe, 1995).

Fetal infections during 120–180 days (mid to late gestation) result the birth of weak calves or calves with congenital or physical malformations, such as ocular (i.e., retinal atrophy, cataracts) or cerebellar lesions (i.e., cerebellar hypoplasia) and growth retardation with arrested bone development, and pulmonary hypoplasia (Grooms, 2004; Evermann and Barrington, 2005). Calves congenitally infected with BVDV are more likely to develop severe diseases in the first 4–5 months of life (Muñoz-Zanzi et al., 2003).

Cattle older than 6 months of age (without maternal immunity) could develop an acute infection after an incubation period between 5–7 days and a viremia that last up to 15 days

(Grooms et al., 2002). Transiently infected (TI) animals might present only mild clinical signs, but might suffer immunosuppression, increasing susceptibility to secondary infections (Grooms, 2004). TI cattle shed low levels of virus in body secretions and excretions from days 3 to 15 post infection, although shedding has been shown to last for up to 3 weeks (Thurmond, 2005).

Mucosal disease is a sporadic condition where <5% of herds are affected, but in outbreaks up to 25% of a herd may be infected. The incubation period elapsed 7–14 days and clinical signs include fever and profuse watery diarrhea. Erosions and ulcers may be present on the oral cavity, interdigital regions, teats, vulva, and prepuce (Grooms et al., 2002).

In camels and llamas, it has been described diarrhea, abortion, and ill thrift in pregnant llamas, and congenital defects in camel calves (Goyal et al., 2002; Yousif et al.,2004). In wildlife, such as axis deer, roe deer, and moose, include fever, corneal opacity, and depression. The severity of the disease appears to depend upon the viral strain, immune competence of the host animal, concurrent viral infection, and/or nutritional deficiencies, such as copper (Van Campen et al., 2001).

1.3.4. Epidemiology

1.3.4.1. Susceptible Hosts and reservoirs

The host range for BVDV includes all ungulates belonging to the order Artiodactyla (Van Campen, et al., 2001). Cattle and other domestic animals that can be infected with BVDV include sheep, goats, and members of the Camelidae family (camels, llamas, and alpacas) (Van Campen, et al., 2001; Goyal et al., 2002). Understanding the epidemiological importance of non-bovine hosts in the spread of BVDV is essential, particularly in countries where co-grazing of cattle and other susceptible species is common or those that have large populations of wild ruminants (Evans et al., 2019).

1.3.4.2. Virus transmission

Transplacental infection occurs when susceptible cows are exposed to BVDV during pregnancy, or when a PI cow becomes pregnant (Houe, 1995). Pregnant dams carrying PI calves are often referred to as “Trojan Cows” (Lindberg et al., 2001). Congenital infection of the fetus prior 125 days of gestation, it can result in the birth of a PI calf (Thurmond, 2005).

Horizontal transmission depends on the prevalence of PI animals, the rate of animal-to-animal contacts, the virulence of the virus strain(s), and the susceptibility of the cattle to the strains in the herd (Thurmond, 2005). Direct contact between an infected animal, particularly PIs and a susceptible animal is the most effective route for BVDV transmission, and usually result in transient infection (Niskanen and Lindberg, 2003). TI animals may remain infected for only a few days or weeks depending on the virulence of strains of BVDV (Niskanen et al., 2000). Thus, BVDV transmission and persistence of the infection within the herd will be limited (Lindberg and Houe, 2005). However, a few reports suggest that BVDV may persist in a herd in absence of PI animals (Moen et al., 2005; Collins et al., 2009) although other experimental studies do not support this (Nickell et al., 2011; Sarrazin et al., 2014). The full extent of viral shedding and persistence of virus in TIs and their ability to maintain infection within a population needs further investigation (Evans et al., 2019).

The main spread route of BVDV between herds is the contact with infected animals, through cattle movement (i.e., animal trade, common pastures, participation in cattle competitions), and indirect contact with secretions of infected animals contaminating bans, fomites, equipment, machinery and personal (Gunn, 1993; Lindberg and Alenius, 1999; Niskanen and Lindberg, 2003). The survival of BVDV in the environment and on equipment/clothing represent an important risk of indirect sources to the spread of BVDV within and between herds (Niskanen and Lindberg, 2003; Evans et al., 2019). The contribution of Trojan cows on the spread of infection between herds is limited, however they account for approximately 10% of PI births in the absence of effective control measures (Reardon et al., 2018).

Environmental stability of each of the viruses under different conditions (e.g., temperature, humidity, matrix) and their survival outside of the host is essential in understanding the role of fomites on the spread BVDV to susceptible hosts (Evans et al., 2019). In addition, conditions that favor crowding and aerosol transmission would increase the likelihood of BVDV transmission from calves with a respiratory form of BVDV infection (Baule et al., 2001).

Although the major routes of BVDV transmission are well known, cases of new infections appear despite strict biosecurity measures in areas with a systematic control. In 40-50% of these cases where new infections are detected in previously free herds, the route of transmission remains unidentified (Stahl and Alenius, 2012).

1.3.4.3. Geographical distribution

BVDV infection has a worldwide distribution, with large regional differences, being endemic in countries with higher bovine production, high herd density, and in all countries where no systematic control has been initiated (Houe, 2003; Lindberg et al., 2006).

In many European countries, the infection seems to have occurred endemically with about half the herds having PI animals and most herds having antibody carriers. The overall prevalence of PI animals is often in the range of 1–2% and the seropositive cattle in the range of 40–70% (Houe, 1995; Houe, 1999; Rüfenacht et al., 2000; Sarrazin et al., 2013). In European countries without BVD control programs there is a large regional variation, with a range of herd seroprevalence of 3.8-65.5% and within herd seroprevalence between 0.1-3.8% (Tab. 1). The prevalence of herds with PI animals varies between 1% and 50%, while the prevalence of PI animals in the herds has been reported to be between 0.06% and 1.3-2%. In the case of Spain, the studies carried out determined a seroprevalence at the herd level between 70.9-94.2% (Fig. 1) and 21-65.6% in the animals (Fig.2) (Sanibos-Anembe, 2018).

Country	Herd prevalence	Within herd prevalence
Belgium	3.8%-5%	0.1%-0.6%
France	21.5% (20.9%-22%)	2.90%
Germany	45.3%	0.9%-1.5%
Ireland	49.6%	0.75%
Italy	12.70%	
Portugal	9.7% (5.1%-16.3%)	
Spain	26%	0.7%
Sweden	0.8% (0-2.7%)	
Switzerland	2%	0.24%
The Netherlands	10-15%	2.22-3.86%
United Kingdom	65.5%	0.4%-1.8%

Table 1: Seroprevalence of Bovine viral diarrhea virus in Europe (Modified of Foddai et al., 2014; Santman-Berends et al., 2017)

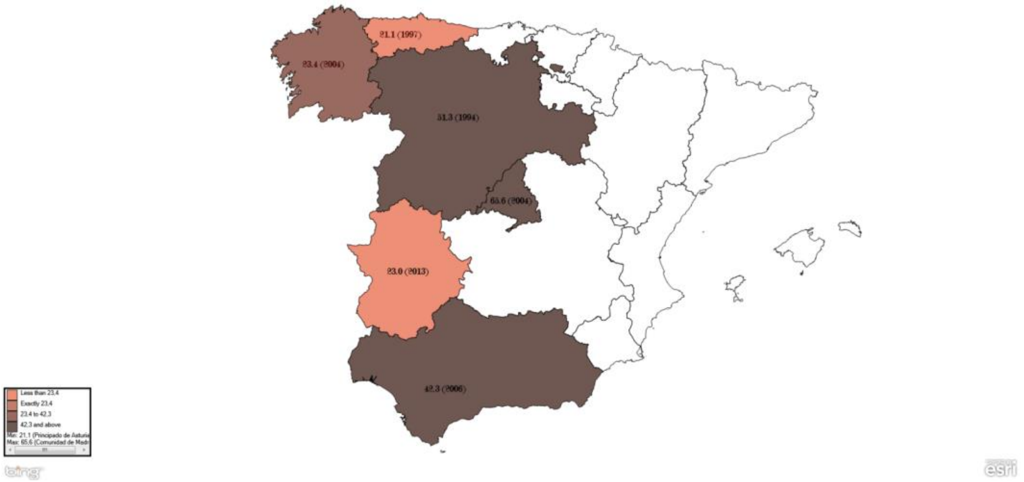

Figure 1: Herd BVDV prevalence in Spain. Available online: https://www.anembe.com/grupos-de-trabajo/sanibos

Figure 2: Within herd BVDV prevalence in Spain. Available online: https://www.anembe.com/grupos- de-trabajo/sanibos

1.3.4.4. Risk Factors

The most important risk factors identified for BVDV infection were acquisition of new animals (i.e., purchasing transiently or persistently animals, or pregnant cattle that give birth to PI calves) without control of viral status (Brock, 2004; Luzzago et al., 2008; Amelung et al., 2018 Lindberg and Houe, 2005) or being in contact with animals from other farms (Lindberg and Alenius, 1999). On the other hand, the relationship between herd size and the risk of infection suggests that large herds are at a higher risk of BVDV infection than small herds (Bishop et al., 2010; Amelung et al., 2018). The number of animals in a herd is a complex risk factor because it is difficult to isolate from other factors, such as cattle density, herd density and contact between different herds (Lindberg and Houe, 2005). However, the low density of herds and cattle are associated with the lower herd prevalence (Almeida et al., 2013), while higher prevalence's were found in regions with a high density of cattle (Ståhl et al., 2008). Finally, positive BVDV status in dairy farms also was associated with the number of contacts on dairy farms (Presi et al., 2011; Amelung et al., 2018).

1.3.5. Diagnostic

Antibody testing is commonly used as a screening tool for herds to identify animals which have been exposed to the virus and those which are susceptible to infection. Antibodies diagnostic tests include enzyme linked immunosorbent assay (ELISA), virus neutralization test (VNT) or, less often, agarose gel immunodiffusion (AGID) and indirect immunofluorescent test (IFAT) (Saliki and Dubovi, 2004). Other methods commonly used include antigen capture ELISAs (ACE) which can be used to test blood, milk and tissue samples; real-time polymerase chain reaction (RT-PCR) used to test blood, milk, semen and tissue samples and immunohistochemistry used to test fixed tissue samples such as ear notches. Those tests allow to identify PI animals and TI animals during period of viral excretion (4–15 days after infection) (Fulton et al., 2006; Hilbe et al., 2007; Dubovi, 2013).

To date there is no diagnostic test capable of detecting Trojan cows with high diagnostic sensitivity and specificity for preventing spread, persistence, and re-introduction BVDV infection (Lanyon and Reichel, 2014). Identifying Trojan cows prior to the birth of PI calves will provide significant improvements to control efforts (Evans et al., 2019). A preferable alternate strategy to representative random sampling of imported live animal populations might be to control testing all the calves born from imported pregnant animals (Alpay et al., 2019).

1.3.6. Control and Eradication

Bovine viral diarrhea has been placed on the OIE's list of notifiable diseases, mainly as a result of its potential for international spread, but is controlled mostly on a voluntary basis (Lindberg and Houe, 2005).While there are currently no formal reporting requirements for BVD, countries with national or regional control programs may have certain regulations for affiliated farmers that effectively restrict trade with animals of positive suspect or unknown BVD status (Marschik et al., 2018).

Control programs aim to reduce disease prevalence to a relatively low and manageable level, the fundamental principle of any BVD control and eradication program aims to provide a continued absence of the disease in the population based on reduce the prevalence of PI animals in a population and prevent the apparition of new PI animals (Houe et al., 2006). Several European countries with compulsory and systematic control programs differ in the way how PI animals are detected and in the role of vaccines (Bachofen et al., 2013). The strategies in Scandinavian countries are considered a general model of BVD control based on three central elements 1) biosecurity, to prevent introduction of infection into free herds; 2) elimination of PI animals in infected herds to reduce virus circulation; and 3) continuous monitoring of free herds for early detection of reinfection (Lindberg and Alenius, 1999).

At this moment, the Scandinavian countries are free of BVDV (Houe, 2005) and control or eradication programs are in place in Austria, Switzerland, the Netherlands, Germany, Ireland, and Scotland (Houe et al., 2006; Presi et al., 2011;B; Voas, 2012). Regional approaches to BVD control have also been implemented in Brittany in France, in some regions of Italy (Lecco, Como and Roma) as well as in Greece and in Spain such is the case of Galicia (Lindberg et al., 2006).

At farm level, biosecurity constitutes the most central element of BVDV control, the main measures are summarized in refuse the purchase of pregnant and seropositive cattle or to isolate the resultant calf should be isolated until testing can confirm it is not PI (Bitsch et al., 2000). Also, testing of individual calves as soon as possible after birth allows early removal of PI animals (Brock, 2004; Smith et al., 2014). Recommended measures include double fencing on boundary fences, testing of newly purchased animals, quarantining newly purchased animals and the cleaning of equipment and vehicles shared across properties (Evans et al., 2019). In general, the education of farmers is an important tool to avoid that new infected animals are introduced (Lindberg and Alenius, 1999).

- **Vaccination**

The original purpose of vaccination against BVD was prevention of clinical disease and transient immunosuppression caused by BVDV. Thus, vaccination did not necessarily prevent the emergence of new PI cattle, the virus reservoir was maintained, and infection of susceptible animals continued (Moennig and Becher, 2018). Currently, vaccination programs are designed to prevent clinical disease, protect against viremia and to prevent fetal infection. Although, the efficacy of these vaccines in field observations have been questioned, because birth of PI calves is not completely prevented after vaccination (Rodning et al., 2010; Stahl and Alenius, 2012). However, it has recently been shown that vaccinating calves against BVD can result in markedly different immune responses and improved growth rates compared to unvaccinated calves which are exposed to PI cattle (Grooms et al., 2014).

1.3.7. Impact over livestock industry

The overall disease impact depends of its effect in production/economics, animal welfare or human safety. However, the welfare implications have never been quantified in a systematic way (Lindberg et al., 2006). Several studies include main economic estimates in outbreaks and the major variation is believed to be due to biological variation rather than currency fluctuations. The most losses are associated with reproductive disorders and PI animals, in other cases losses from outbreaks due to BVD occurring simultaneously with other infections (Houe, 2003). In addition, BVDV infections have a significant impact on the competitiveness of European cattle industries (Lindberg, 2006).

The production losses include reduced milk production, reduced conception rate, respiratory disorders, other diseases, and death among animals acquiring acute infection. Fetal infection causes abortions, congenital defects, and growth retardation. On the other hand, fetal infection also leads to PI calves, which are often small and unthrifty, increased susceptibility to other diseases, and may eventually die from mucosal disease (Houe, 2003). Several studies reported the direct costs of a BVDV infection in dairy herds varied between €21 and €135 per cow but could be as high as €340 per cow when simultaneously other infections occurred or in case of highly virulent strains (Houe, 2003; Valle et al., 2005; Lindberg et al., 2006). The average annual net costs were estimated at €27.8 million for the dairy industry (Santman-Berends et al., 2015).

1.4. Bovine herpesvirus-1 (BoHV-1)

1.4.1. Etiology

Bovine herpesvirus-1 (BoHV-1) belongs to the genus Varicellovirus in the subfamily Alphaherpesvirinae under the family Herpesviridae. It is closely related to pseudorabies virus (Suid herpesvirus type 1) and varicella-zoster (chickenpox) virus (human herpesvirus type 3) (Biswas et al., 2013). According genomic analysis, BoHV-1 virus can be divided into several subtypes antigenically similar like BoHV-1.1 related to the respiratory syndrome, BoHV-1.2 related to genital infections, and BoHV-1.3/BoHV-5 associated with neurological disorders of the cattle (Keuser et al., 2004; Muylkens et al., 2007). BoHV-1 is resistant to environmental influences, at 4°C, the virus is stable for 1 month and may survive for more than 30 days in feed. The virus is sensitive to many disinfectants and is readily inactivated (Nandi et al. 2009).

1.4.2. Pathogenesis

BoHV-1 replicates in the periphery, i.e. the mucous membranes of either the respiratory or the genital tract. In the respiratory tract cause inflammatory changes such as rhinitis, laryngitis and tracheitis leading to destruction of the tracheal microvilli (Leite et al., 2002). Acute infection leads to high levels of virus production and secretion in ocular, oral, nasal, or genital cavities for 7–10 days after infection (Jones, 2019). After recovery, BoHV-1 establish a state of latency probably for the entire life of the host through enter the axons of local nerve cells, its reach the neuron bodies through trigeminal ganglion or pharyngeal tonsils following primary infection of the conjunctiva, oral and/or nasal cavities; in the sacral ganglia following genital infection (Winkler et al, 2000). The reactivation of the latent infection can be due to stressful conditions such as transport, parturition, animal movement and mixing, inclement weather concomitant infection, poor husbandry or diet, overcrowding or following treatment with corticosteroids and coinfection with other pathogens (Winkler et al., 2000; Muylkens et al. 2007). Latent infection should always be considered a potential source of infection, it can reactivate the virus, resulting in re-excretion of virus and a rise in neutralizing antibodies (Jones, 2019).

1.4.3. Clinical manifestations

After an incubation period of 2–4 days, the clinical manifestation of BoHV-1 include abortion, suboptimal fertility, respiratory disease, reduced milk production, and increased mortality (Muylkens et al., 2007), the clinical signs depend on subtype affecting:

- BoHV-1 subtypes 1 and 2a (respiratory infection), characterized by high fever, conjunctivitis, mucopurulent nasal discharge, and abortion at the end of the fifth month of pregnancy, with expulsion of dead fetus (Muylkens et al., 2007). Secondary bacterial (e.g.

M. haemolytica, P. multocida and H. somnis) or viral agents may result in severe respiratory diseases in young animals that can lead to the death (Holliman et al., 2005).

- BoHV-1. subtype 2b (genital infection) causes acute infectious pustular vulvovaginitis (IPV) in cows and infectious pustular balanoposthitis (IPB) in bulls. The incubation period for the genital forms of BoHV-1 is 2–6 day, usually develops 1–3 days after mating. Initial clinical signs are frequent urination and a mild vaginal infection, animals infected development pustules, ulcers in the mucosa of vulva and vagina, in the case of male lesions similar on the mucosa of the penis and prepuce (Muylkens et al., 2007).
- BoHV-1. subtype 3 (neurological disease) similar to encephalitis, but the importance is the development of latency (Woodbine et al., 2009).

1.4.4. Epidemiology

1.4.4.1. Susceptible hosts and reservoirs

BoHV-1 infected cattle and other Artiodactyla and cause disease in cattle, sheep, and goats (Biswas et al., 2013). However, sheep is unlikely to play any role in BoHV-1 transmission (Hage et al., 1997). Viruses antigenically related to BoHV-1 and BoHV-5 have also been isolated from several ruminant species including red deer, reindeer, mule deer, water buffalo, goats, elk, pronghorn antelope and wildebeest (Thiry et al., 2006). For this reason, buffalo and wildlife may play an important role in the maintenance of the infection (Boelaert et al., 2000).

1.4.4.2. Virus transmission

BoHV-1 is shed in nasal discharge for 10–14 days during acute respiratory infection and transmission occurs by contact with mucosal droplets from infected cattle (Kahrs, 2001). Infected cattle excrete the virus and can infect susceptible animals by nose to nose contact, and by coughing and sneezing aerosolized droplets (Raaperi et al., 2010). Survival of virus in secretions could contaminate clothing and fomites and presents a risk of indirect transmission between farms (Nettleton and Russel, 2017). Airborne transmission of BoHV-1 over short distances within a building has been also demonstrated (Mars et al., 2000). In addition, virus can be transmitted by contaminated semen during natural mating or artificial insemination (Van Oirschot, 1995).

1.4.4.3. Geographical distribution

BoHV-1 is widespread all over the world. There are significant differences in herd level prevalence and disease incidence between and within regions, depending on geography and cattle management practices (Woodbine et al., 2009). Herd prevalence in Europe has been described between 15-80% and within herd prevalence between 12-64% (Table 2). In Spain it is known there is a variation between 45.8-81.8% in herd prevalence (Fig. 3) and within prevalence between 25-45.8% (Fig. 4) (Sanibos-Anembe, 2018). In 2018, the latest report of the national control program describes herd prevalence of 33.71% (n=89) in dairy farms (https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/sanidad-animal/enfermedades/IBR.aspx).

Country	Herd prevalence	Within herd prevalence
Belgium	67%	35.9%
England and Gales	69%	42.5%
France	36%	1.40%
Ireland	77.3%	
Italy	84.3%-100%	35%-38.7%
Spain	50.4%-70.4%	38.4%-45.7%
The Netherlands	84%	30%

Table 2: Seroprevalence of Bovine herpesvirus 1 in Europe. (Modified to Raaperi et al., 2014)

Figure 3: Herd BoHV-1 prevalence in Spain. Available online: https://www.anembe.com/grupos-de-trabajo/sanibos

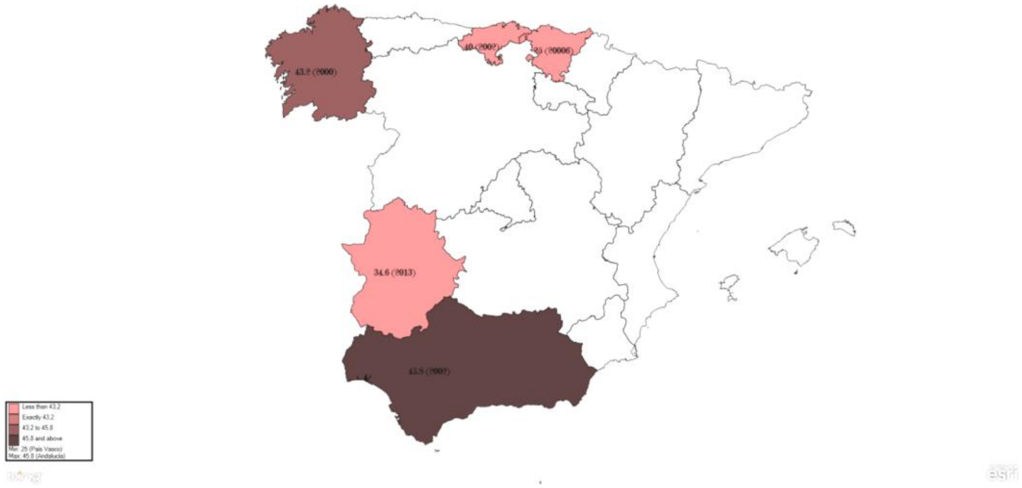

Figure 4: Within herd BoHV-1 prevalence in Spain. Available online: https://www.anembe.com/grupos-de-trabajo/sanibos

1.4.4.4. Risk factors

Several studies have identified risk factors for BoHV-1 seropositivity. Age (i.e., calves have a lower prevalence of infection), sex (males are more frequently positive than females) and herd size (large herds higher seroprevalence than small) have been reported (Boeleart et al., 2005; Cowley et al., 2011). In addition, larger herds, due to their higher number of contacts both within the herd and with other herds through cattle purchases and a higher number of visitors has been identified as a risk factor (Woodbine et al., 2009, Sayers et al., 2015). Farm density or cattle density may also increase the risk of BoHV-1 introduction because a continuous influx of susceptible animals keeps virus circulation (Van Schaik et al., 2002; Raaperi et al., 2010).

Contact with other animals during purchase of cattle and participation in shows allowing cattle to return to a farm if not sold at market or grazing cattle on other farm were also found to be important risk factors for the introduction of BoHV-1 increasing the probability to exposure to other viral types BoHV- 1 (Nardelli et al., 2008; Woodbine et al., 2009). In addition, the movement of animals to and mixing within new herds is stressful for cattle and can result in recrudescence of latent infection, and subsequently spreading to susceptible animals (Woodbine et al., 2009).

Other factors such as professional visitors not using farm protective clothing and herds situated close to other BoHV-1 positive herds (Van Schaik et al., 1998), and farms with mixed dairy and beef cattle have a higher risk of being seropositive than dairy-only holdings

(Boelaert et al., 2005). The presence of a breeding bull might also increase the risk of infection (Nettleton and Russel, 2017).

1.4.5. Diagnostic

Several serological tests are available for the detection of antibody against BoHV-1 infection. A variety of ELISAs have been employed to screen serum samples or bulk tank milk in dairy farms (Nandi et al., 2009). The disadvantage of bulk milk analysis, however, is that reflects data related to the lactating herd only, the disease status of which may differ from that of younger livestock in the herd (Sayers, 2017).

The PCR assay is as sensitive as virus isolation and is a practical alternative for the rapid detection of virus in nasal swabs, bovine fetal serum, and semen samples (Nandi et al., 2009). It has been shown to be the most effective way of confirming BoHV-1 in bovine abortions (Crook et al., 2012).Virus isolation in cell culture and fluorescent antibody tests have been replaced by PCR tests are useful for detecting small amounts of virus in diagnostic samples (Wang et al., 2007). The virus neutralization test (VNT) has been widely used and is the gold standard by which other techniques have been evaluated, but it requires cell culture facilities and is time-consuming (Biswas et al., 2013).

1.4.6. Control and Eradication

The control and eventual eradication of BoHV-1 is based on the detection and removal of infected animals, with or without the use of marker vaccines (Nettleton and Russel, 2017). Other control measures include three fundamental bans: purchasing positive animals, using whole-virus BoHV-1 vaccines, and inseminating cows with semen from positive bulls (Nardelli et al., 2008). OIE guidelines recommend 2–3 weeks quarantine period for newly introduced cattle after which only BoHV-1 seronegative cattle are admitted to the herd (Boelaert et al., 2005).

Several European countries have either successful BoHV-1 eradication programs (Denmark, Finland, Sweden, Austria, Switzerland, Norway, Federal State of Bavaria in Germany and province of Bolzano, Italy) and now are officially free of Infectious bovine rhinotracheitis (IBR). Others implemented an EU-approved compulsory program (Germany) (Nardelli et al., 2008), and in Spain since 2019 a national voluntary program is established (RD 554/2019). In addition, voluntary regional BoHV-1 control programs in specified herds are ongoing (Ackermann and Engels, 2006).

Figure 5: IBR situation in Europe 2018. (Mapa.gob.es, 2018)

- **Vaccination**

The vaccines reduce the severity of disease, virus replication and transmission, prevent the excretion of wild virus by naive animals and prevent re-excretion by latently infected ones, but they are not able to prevent BoHV-1 infection (Ackerman and Engels, 2006; van Drunen Littel-van den Hurk, 2006). There are four kinds of vaccines: modified live virus vaccines (MLV), inactivated vaccines, subunit vaccines and gE-deleted marker vaccines that are available to be used in cattle against BoHV-1 infections. MLV vaccines are potentially abortigenic and cannot be used in non-immune pregnant cattle and may develop the latent state that may lead to shedding of the vaccine virus (Nandi et al., 2009). Inactivated vaccines do not prevent the development of latency following exposure to field virus, but they are safe in pregnant animals, stable in storage and do not cause the shedding of virus (Castrucci et al., 2002). The use of marker vaccines is recommended as it offers the differentiation of vaccinated and naturally infected animals (Nandi et al., 2009).

Marker vaccines based on gE deletion mutants are widely used in Europe, either in live or inactivated forms. Live gE-negative vaccine induced better protection than inactivated gE-negative. In contrast, inactivated gE vaccines were more efficacious than attenuated gE vaccine at reducing virus excretion after reactivation. However, vaccination cannot prevent latency, either of the vaccine itself or of the wild virus. Therefore, to eradicate BoHV-1, repeated vaccination according to a strict schedule to reduce the possibility of wild virus excretion and strict management practices are required (van Drunen Littel-van den Hurk, 2006).

1.4.7. Impact over livestock industry

The introduction of BoHV-1 into a cattle farm can cause negative economic impact due to production losses and restrictions in the international trade of livestock. The main economic losses due to weight loss, decrease in milk production, abortions, a high insemination index in breeding animals, and increase in median calving date associated with inferior fertility (Nandi et al., 2009; Raaperi et al., 2012). Longer calving intervals and increased young stock and cow culling rates also are described (Raaperi et al., 2014).

Statham et al. (2015) reported a milk yield loss of 2.6 kg/d in BoHV-1 seropositive compared with seronegative dairy cows. A Dutch modeling exercise quantified losses of 0.92 kg of milk per cow per day during a BoHV- 1 herd outbreak (Van Schaik et al., 1999), and Sayers et al., (2007) found a 250 liter reduction in milk yield per multiparous cow per year in BoHV-1 positive herds.

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Objectives

Chapter II

The general aim of this PhD thesis was to develop a risk assessment tool to support the decision making in relation to biosecurity measures prioritization in dairy farms.

The specific objectives that have guided this PhD research are:

- To develop a quantitative risk assessment to estimate the probability of Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus type 1 (BoHV-1) introduction through animal movements based on the biosecurity measures applied in dairy farms in Catalonia and Galicia;

- To quantify the probability of BVDV and BoHV-1 introduction through indirect contacts using a quantitative risk assessment tool applied in dairy farms.

Study I

Chapter III

Development of a quantitative risk assessment of bovine viral diarrhea virus and bovine herpesvirus-1 introduction in dairy cattle herds to improve biosecurity

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3.1. Abstract

A quantitative risk assessment model was developed to estimate the annual probability of introduction of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) at the farm level through animal movments. Data from 2017 official animal movements, biosecurity questionnaires, scientific literature and expert opinion from field veterinarians were taken into consideration for model input parameters. Purchased cattle or cattle introductions, rearing replacement-heifers offsite, showing cattle at competitions, sharing transport vehicles with other herds, and transporting cattle in vehicles that have not been cleaned and disinfected were considered in the model. Results showed that the annual probability of introducing BVDV or BoHV-1 through infected animals was very heterogeneous between farms. The median likelihood of BVDV and BoHV-1introduction was 12% and 9%, respectively. Farms that purchased cattle from their same region (i.e., local movements) and shared transport with other farms had the higher probability for BVDV and BoHV-1 introduction. This model can be a useful tool to support decision making on biosecurity measures that should be prioritized to reduce the probability of introduction of these two viruses in dairy herds.

3.2. Introduction

Bovine viral diarrhea (BVD) caused by BVD virus (BVDV), and infectious bovine rhinotracheitis (IBR) caused by bovine herpesvirus-1 (BoHV-1) are two diseases of importance in dairy herds because of their impacts on reproductive performance, increased susceptibility to other diseases, early culling, and subsequent economic losses (Hage et al., 1998; Muylkens et al., 2007; Newcomer and Givens, 2016). Animals infected by BVDV can develop three different infection status: (i) persistently infected (PI) cattle, which are animals vertically infected during early pregnancy (30 to 120 days) that shed large amounts of virus all their life (Houe, 1999); (ii) transiently infected (TI) cattle, which are animals horizontally infected after birth that shed small amounts of virus for up to 15 days (Houe, 1999; Niskanen et al., 2000); and (iii) Trojan cows (TR), which are pregnant cows that carry a PI calf (Reardon et al., 2018). Cattle with BoHV-1 can develop acute infections (AI) shed the virus during a short period of time, followed by a latent infection (LI) for the rest or their lives, in when they can excrete the virus under stress conditions (Lazic et al., 2003). Cattle movements are considering the main routes of

BVDV and BoHV-1 spread between herds (Van Schaik et al., 1998; Lindberg and Alenius, 1999; Van Schaik et al., 2002).

Several European countries have implemented compulsory and voluntary BVD and IBR control and eradication programs (Lindberg et al., 2006; Nardelli et al., 2008). The implementation of biosecurity is considered an essential pillar in these programs. Different studies have described biosecurity measures in cattle farms (e.g., Sahlström et al., 2014; Sarrazin et al., 2014a), and several methods have been developed score different levels of biosecurity practices implemented on farms. Existing methods to evaluate biosecurity practices use checklists to support the development of on-farm biosecurity plans (https://www.farmbiosecurity.com.au), or to score the level of biosecurity measures implemented on farms based on those measures that are common to the transmission of different types of infectious agents, such as Biocheck.UGent™ (Laanen et al., 2014). In addition, several studies have identified biosecurity gaps as risk factors associated with disease outbreaks and, therefore, have provided relevant information for disease prevention (e.g., Almeida et al., 2013; Machado et al., 2016). However, the development of risk assessment models to evaluate the effect of biosecurity measures in the probability of disease introduction to support decision making on which practices should be prioritized according to farm-specific risks has received less attention. Biosecurity programs need to be flexible and adaptable to the particular situations on each farm (Wells, 2000; Brennan and Christley, 2012). In this context, quantitative risk assessment (Murray et al., 2004) can be a useful method to identify and justify the enhancement of biosecurity measures based on the probability of disease introduction.

The aim of the present study was to develop a quantitative risk assessment model to identify farm-specific biosecurity measures that should be implemented to reduce the probability of BVDV and BoHV-1 introduction into dairy cattle herds through animal movements.

3.3. Materials and Methods

3.3.1. Data

A structured questionnaire was used to obtain data on biosecurity measures on 34 and 93 farms from Catalonia and Galicia, respectively, which voluntarily participated in the project. The biosecurity questionnaire (available under request in Spanish) was structured in four parts: i) general data of the farm; ii) animal movements (e.g., origin of the animals, frequency of introductions, test, quarantine facilities, external rearing farms, cattle fairs, pasture, etc.); iii) vehicles (e.g., vehicles enter inside farm perimeter, vehicles can come with other animals, etc.); and iv) visitors and staff (e.g., external workers, frequency of visitors, use of protective clothing, etc.). The Autonomous Governments of Catalonia and Galicia provided records of cattle movements (national and international) of year 2017. Movements were registered at the animal level, and included the following information: calving date, movement date, country of origin, and a unique code of destination. Based on the calving date and movement date, the age of each purchased animal was calculated. For BVDV, we classified introduced cows and heifers into one of three groups: i) less than 12 months old; ii) from 12 to 24 months old; and iii) greater than 24 months old. Based on published research reports, PI animals were classified into one of the first two groups, while TI animals were into any group. Trojan cows could only be classified into one of the last two groups. For BoHV-1, introduced cattle were classified into one of two groups (i.e. < 24 months, ≥ 24 months), as disease prevalence can vary by age (Mars et al., 2001; Lassen et al., 2012; Sayers et al., 2015). The pregnancy status of introduced animals and an estimate of their days of gestation were provided for each study farm.

3.3.2. Risk release pathways

Field veterinarians responsible for the health management of farms involved in the project were invited to participate in the development of the risk assessment model. One discussion group was organized to debate about risk release pathways and risk mitigating measures that should be included in the model. Five veterinarians attended a group discussion meeting (Appendix Table A1). After the group discussion, variables for introduction of new animals through purchase of cattle, movements of reared replacement heifers offsite, movements to cattle competitions, share transport vehicles with others farms, and transport of cattle in vehicles that have not been properly cleaned and disinfected (i.e., contaminated transport vehicle) were considered for inclusion in the model. Movements to pasture were included in the biosecurity questionnaire, but they were not considered in the model because it was not a common practice in dairy cattle in the area under study. In Figure 1, parameters considered in the pathway for the ‘purchase of animals’ are shown. Parameters considered for the movement of animals to cattle competitions and reared replacement heifers offsite are presented in Appendix Figure A1 and Figure A2.

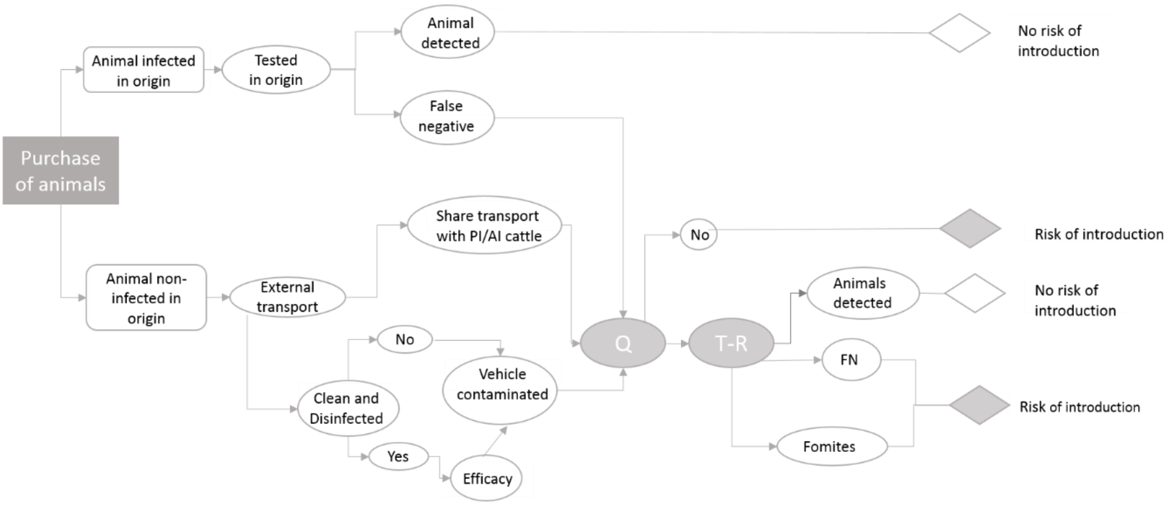

Figure 1: Pathway for bovine viral diarrhea virus and bovine herpesvirus-1 introduction in dairy cattle herds through the purchase of animals; Q: Quarantine; T: Test on quarantine; R: Quarantine routines. PI: Persistently infected with Bovine Viral Diarrhea Virus; AI: Acutely infected with Bovine Herpesvirus-1; FN: False negative

3.3.3. Model development

To estimate the probability of introduction of BVDV and BoHV-1 into a dairy cattle farm in a 12-month period, a stochastic risk assessment model was developed by using the mc2d package (Pouillot and Delignette-Muller, 2010) implemented in R (R Development Core Team 2008). Monte Carlo simulations (10,000 iterations) were performed, and all non-fixed input parameters were included as uncertain parameters.

Probability of purchasing an infected animal

In the analysis, using the cattle movement database, the origin of cattle purchased from other farms was France, The Netherlands, Catalonia, Galicia, or the rest of Spain. Accordingly, cattle movements were classified into one of these five origins. We assumed that the number of cattle purchased in each batch throughout the year had the same number of animals. Therefore, the number of animals purchased each time (i.e., the size of the batch) from each country/area, group age and farm (data obtained from the biosecurity questionnaires) was calculated using the equation [1] (Appendix Table A2).

The probability that at least one animal from a batch coming from a single farm of a certain country/area was already infected with BVDV or BoHV-1 was estimated using the equation [2] (Appendix Table A2). Herd and within-herd prevalence values were obtained through a literature review and personal contacts with experts from the countries. Values from Catalonia and Galicia were obtained from previous work conducted in these areas (Benavides et al., 2018). In the case of BVDV, herds with presence of young cattle that tested seropositive or detection of the virus within cattle herds were considered an indication the virus was circulating in those herds.

In Table 1, the different input distributions used in the model are described. Probability distributions were used to account for uncertainty in parameter estimates. Uniform distributions were reported as minimum and maximum values, and they were used as inputs obtained from the literature (i.e., BVDV and BoHV-1 prevalence, diagnostic test performance, BVDV transmission probability). PERT distributions were used for BVDV survival in different materials where the minimum, maximum, and the most likely value was the time in hours that the virus could survive.

Parameter	**Distribution/Values**	**References**
Bovine Viral Diarrhea Virus		
Herd prevalence in France	Uniform (0.21-0.22)	Santman Berends et al., 2017
Herd prevalence in the Netherlands	Uniform (0.16-0.18)	Van Duijn et al., 2019 ; Santman-Berends et al., 2015
Herd prevalence in Spain	Uniform (0.26-0.71)	Foddai et al., 2014 ; Gómez-Pacheco et al., 2009
Herd prevalence in Catalonia	Uniform (0.54-0.57)	Benavides et al., 2018
Herd prevalence in Galicia	Uniform (0.03-0.16)	Benavides et al., 2018
Viremic animal	Uniform (0.022-0.029)	Foddai et al., 2014 ; Meyling et al. 1990
PI[1] animal prevalence (<12 months)	Uniform (0.0002- 0.02)	Bachofen et al., 2013 ; Joly et al.2005
PI animal prevalence (12 to 24 months) *	Uniform (0.0001-0.01)	Ezanno et al., 2007
Sensitivity of ELISA (Abs)[2]	Uniform (0.93-0.98)	Hanon et al., 2018
Specificity of ELISA (Abs)	Uniform (0.94-0.99)	Hanon et al., 2018
Sensitivity of ELISA (Ag)[3]	Uniform (0.97-1)	Mars and Van Maanen et al., 2005
Specificity of ELISA (Ag)	Uniform (0.99-0.995)	Mars and Van Maanen et al., 2005
Sensitivity of PCR	Fixed Value (0.99)	Hilbe et al., 2007
Specificity of PCR	Fixed Value (1)	Hilbe et al., 2007
Efficacy of the cleaning and disinfection	Pert (0.80-0.90-1)	Foddai et al., 2014
Probability surviving in rubber (boots)	Pert (0.886-0.75-0.536)	Stevens et al., 2011
Probability surviving in galvanized metal	Pert (0.002-0.004-0.039)	Stevens et al., 2011
Probability surviving in soil	Pert (0.007-0.014-0.12)	Stevens et al., 2011
Probability of transmission from TI[4] animal	Uniform (0.001-0.05)	Very low-DAFF, 2004
Probability of transmission from PI[1] animal	Fixed value (0.66)	Niskanen and Lindberg, 2003
Bovine Herpesvirus-1		
Herd prevalence in France	Uniform (0.098-0.11)	Gache 2014, GDS 2016

Herd prevalence in the Netherlands	Uniform (0.15-0.156)	Scopaff 2018, www.gdanimalhealth.com/monitoringsurveillance
Herd prevalence in Spain	Uniform (0.50-0.70)	Raaperi et al., 2014
Herd prevalence in Catalonia	Uniform (0.27-0.57)	Benavides et al., 2018
Herd prevalence in Galicia	Uniform (0.06-0.11)	Benavides et al., 2018
Infected animal (<24 months)	Uniform (0.15-0.20)	Santman Berends et al., 2018
Infected animal (>24 months)	Uniform (0.53-0.58)	Santman Berends et al., 2018
Sensitivity of ELISA (Abs)	Uniform (0.72-0.927)	Raaperi et al., 2014
Specificity of ELISA (Abs)	Uniform (0.92-1)	Raaperi et al., 2014
Efficacy of the cleaning and disinfection	Uniform (0.95-1)	Nandi et al., 2009
Probability of transmission from AI[5] animal	Pert (0.1-0.15-0.2)	Mars et al., 2000
Probability of reactivation of LI[6] animal	Fixed value (0.07)	Noordegraaf et al.,1998
Animal transport		
Visited farms in each country/area	Uniform (2-4)	Expert opinion
Loaded animals in each farm	Uniform (9-18)	Expert opinion
Loaded animals in own-farm share vehicles	Fixed value (4)	Expert opinion
Probability of cleaning/disinfection of the transport vehicle		
France	Uniform (0.4-0.6)	Expert opinion
The Netherlands	Uniform (0.9-0.95)	Expert opinion
Catalonia, Galicia, and other parts of Spain	Uniform (0.05-0.1)	Expert opinion

Table 1: Input parameters, distribution, and values to estimate the probability of introduction to Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) in study farms. *****On average half the PI[1] animals die before 1 year of age. [1]PI=persistently infected; [2]Abs=antibodies; [3]Ag=antigen; [4]TI= transiently infected; [5]AI= acutely infected; [6]LI=latently infected.

The expected numbers of BVDV and BoHV-1 animals already infected in the farm of origin were calculated based on the probability that a single animal was infected using equations [3.1] and [3.2] (Appendix Table A2). The expected number of non-infected animals for each age group was therefore obtained by subtracting the expected number of animals infected in each age group from the total number of purchased animals.

Probability of false negatives

Based on group discussion with attending veterinarians, animals were routinely tested for the detection of BoHV1 and BVDV antibodies by using an ELISA prior to cattle purchase. Using ELISA, positive antibody was considered useful for detection of TI, TR or BoHV-1 seropositive cattle, but not PI. For the sensitivity (Se) and specificity (Sp) of the ELISAs, values reported by Hanon et al., (2018) and Raaperi et al., (2014) were used for the detection of BVDV and BoHV-1 antibodies, respectively. For the detection of PI antigen, the values for the ELISA antigen as reported by Mars and Van Maanen, (2005) was used (Table 1). The probability that one infected animal yielded a false negative result was calculated based on equation [4] (Appendix Table A2). Therefore, the probability that at least one infected animal was present in the batch of animals, and the probability that at least one infected animal was purchased from at least one of the farms from which animals could be introduced was calculated using equations [5.1] and [5.2] (Appendix Table A2).

Probability of infection during transport from an infected animal

Non-infected cattle could be infected during transport by sharing the transport vehicle either with a PI (for BVDV) or AI (for BoHV-1) animal, or because of being transported in a contaminated vehicle. TI animals were not included because the probability of transmission from a TI is very low (Table 1). The likelihood of contact with other cattle during the transport was obtained from the biosecurity questionnaires. Unfortunately, data about the number of farms attended by the same vehicle per day in the country/area of origin, nor the average number of animals transported in each movement were not available. Thus, based on group discussion with attending veterinarians, the maximum capacity of the vehicle was 36 animals, and a number of farms visited in each country/area of origin ranging between two and four was considered. Therefore, the number of animals loaded by farm would be between 18 (in the case of two origins), or nine (in the case of four origins). To calculate the probability of sharing a transport vehicle with a PI or AI animal when purchasing animals from a country/area equations [6.1] and [6.2] were used (Appendix Table A2).

We assumed that if purchased animals shared the same transport vehicle with PI or AI animals, the probability of infection was 100%, similar to a model reported by Santman-Berends et al., (2017). In the case of BVDV, the infection during transport would yield a TI animal, except for pregnant animals between 30 and 120 days of gestation. In this case, the infection during transport could yield a TR cow.

The probability that at least one non-infected (healthy) purchased animal of some age group yielded an infection status due to sharing transport vehicle with a PI or AI animal from each country/area was estimated with equation [7] (Appendix Table A2). In the case of BoHV-1, we considered animals greater or less than 24 months would have the same probability of being infected; thus, the age group calculation was not included. An active infection of 1% of the BoHV-1 seropositive animals was assumed based on Santman-Berends et al. (2018). Finally, the probability of being infected by considering the number of farms from which animals could be introduced was calculated.

Probability of infection due to being transported in a contaminated vehicle

The probability that at least one non-infected animal was infected due to being transported in a vehicle coming from some country and develop an infectious status was calculated using equation [8] (Appendix Table A2). In this equation the following parameters were included: i) the probability of cleaning and disinfecting the vehicle between transports, obtained in the group discussion with the field veterinarians, by the opinion of personnel working in the official veterinary services, and from the biosecurity questionnaires; ii) the efficacy of the cleaning and disinfection for BVDV as reported by Foddai et al. (2014) and for BoHV-1 based on descriptions reported by Nandi et al. (2009); iii) BVDV survival on different surfaces reported by Stevens et al. (2011) (Appendix Table A3). It was assumed that the time interval between transports had a minimum time of 4 hours, a most probable value of 12 hours, and a maximum of 24 hours. These values were justified based on input from the veterinarians involved in the study. The BoHV-1 survival was not included in the calculation because it was considered that it can survive during whole duration of the transport. Finally, the probability of infection of a susceptible animal in a contaminated surface was based on results from Niskanen and Lindberg (2003), who conducted an experiment in which three animals entered a pen where a PI had been previously, and two were infected. For BoHV-1, a value of daily aerogenic transmission of 0.15 between an infectious animal and another susceptible at a distance of 4 meters, as reported by Mars et al. (2000) was used as a proxy of indirect transmission.

The probability of purchasing at least one PI, TI, TR, or BoHV-1 seropositive animal was calculated by summing the probability of purchasing infected animals at origin and the probability of infection during transport.

Risk mitigation based on biosecurity measures implemented in the farm

Prior to estimating the probability reduction arising from the implementation of biosecurity measures in the farm, the probability that one animal from the purchased batch was a PI, TI, TR or BoHV-1 seropositive animal was estimated, taking into account the total number of animals of each age group introduced using a similar approach to that in the equation [3.1] (Appendix Table A2). The following biosecurity measures were considered in the model:

Quarantine period. TI animals would be infectious for up to 18 days (Santman-Berends et al., 2017), and would take between two and four days to become infectious (Muylkens et al., 2007). Thus, a duration of more than 24 days would reduce the probability of introduction to a negligible value. For PI, TR, and BoHV-1 seropositive cattle we did not considered the quarantine period. In addition in case the quarantine had a duration of less than 24 days, the probability of indirect transmission was estimated proportional to the duration (i.e., shorter durations would have a higher risk than longer ones). If tests were used in the quarantine, the probability that an animal yielded a false negative result was calculated using a similar equation to [4] (Appendix Table A2).

Quarantine routines. If farmers or farm workers were not taking care of quarantined animals at the end of the working day, the possibility of indirect transmission by fomites (i.e., boots) due to movement of farm workers was also considered. The impact of this measure was estimated by multiplying the probability that the TI or PI animal yielded a false negative result with the survival probability of BVDV in rubber (i.e., boots) and the probability of indirect transmission (Table 1). The time interval between visiting the quarantine and the rest of the farm was about two hours (with a minimum of 1 hour and a maximum of 4 hours) according to field veterinarian opinion.

As a proxy for the probability of indirect transmission, the value reported in the experiment conducted by Niskanen and Lindberg (2003) for BVDV, and the one conducted by Mars et al. (2000) for BoHV-1 was used. In the case of TI animals, considering that they intermittently eliminate a low amount of virus (Lindberg and Houe, 2005; Sarrazin et al., 2014b), the probability of transmission should be very low (Table 1). A quantitative estimate for this probability was obtained following the semi-quantitative methodology for import risk analysis described in the Department of Agriculture, Fisheries and Forestry (DAFF, 2004). In the case of BoHV-1, a proportion of the seropositive animals that could excrete the virus during quarantine due to the stress of transportation was considered. According to Noordegraaf et al. (1998), 7% of the seropositive cows could be reactivated and excrete the virus after the transport.

Number of purchased cattle

Finally, the annual probability of infection with BVDV or BoHV-1 due to purchasing animals was calculated by taken into account the number of animals introduced in each batch and the number of times animals were introduced in a 12-month period from each country/area. Therefore, the annual probability of BVDV or BoHV-1 infection in each study farm due to purchasing animals was calculated following equation [9] (Appendix Table A2).

Movements to cattle competitions

Some study farms moved cattle to national or regional competitions. To participate in these competitions, it is compulsory to certify that animals have tested negative for BoHV-1 antibodies and BVDV antigen. Therefore, for BVDV, the probability that false negative PI or TI animals could attend the competition would be negligible (as the sensitivity antigen detection tests is close to 100%). Thus, it was decided to only include this scenario in the pathway for BoHV-1.

From the movement database, the number of movements to cattle competitions and the age of moved animals was extracted. Herd prevalence was assumed that of regions where the study farm was located (i.e., Galicia or Catalonia). Movement to competitions was assumed to be carried out by using external vehicles (from a company) based on discussion with the field veterinarians. For BVDV and BoHV-1, a similar approach to equation [8] (Appendix Table A2) was used to calculate the probability that animals transported to a competition were infected in a contaminated transport vehicle. In this case, animals in a transport vehicle could have one or two origins, and the maximum number of individuals loaded per farm was considered to be four.

For BoHV-1, the probability that the animals shared the same vehicle with false negative AI animals or had direct or indirect contact with false negative AI animals at competition was also calculated using a similar approach to equation [7] (Appendix Table A2). Animals returning from the competition could enter directly into the farm or be quarantined. These data were obtained from the biosecurity questionnaires to calculate the probability of BVDV and BoHV-1 infection after implementation of biosecurity measures as previously described.

Reared replacement heifers offsite

Some study farms bred their own replacements offsite from the milking/lactating cattle farm. The number of these movements and age of moved animals were obtained from the movements' database; calculations were performed using a similar approach to equation [8] (Appendix Table A2). When the movement was carried out using an own vehicle, it was considered that transport could be shared with only one other farm, loading a maximum of four heifers. Risk mitigation derived from the quarantine was calculated as previously described. If heifers were reared in a multi-origin farm, these movements were considered as if they were from a different origin. Therefore, calculations described in the pathway for the introduction of reared replacement heifers' offsite were performed using similar approach to equation [9] (Appendix Table A2).

3.3.4. Sensitivity analysis

A sensitivity analysis was performed to evaluate the influence of uncertainty of input parameters in the model output for all study farms. The Spearman correlation coefficient was selected for these calculations. In addition, in all farms three uncertain input parameters were tested. The first parameter evaluated was herd prevalence and prevalence of infected animals, and the second parameter was the number of visited farms in each country/area and loaded animals in each farm. Both parameters were reduced to half of those used as default. The third parameter was the probability of cleaning and disinfection of the transport vehicle with a range of 90% to 95%, being higher than default value.

3.3.5. Risk mitigating strategies

The impact of some biosecurity measures on the probability of BVDV and BoHV-1 introduction was evaluated in selected farm as follows:

Quarantine

The quarantine fulfills following conditions: testing cattle on arrival, duration period is at least 24 days and, the quarantine is visited at the end of the workday. For BoHV-1 it was also considered a higher value for sensitivity and specificity of ELISA antibodies equal to 98.41% and 99.76 %, respectively (Bertolloti et al., 2015).

Test in origin

All purchased animals are tested prior to entering the farm. As previously described, higher sensitivity values were assumed for BoHV-1 ELISA.

Transport

Transport vehicle is not shared with animals from another farm.

3.4. Results

3.4.1. Animal movements and biosecurity measures

Among 127 study farms, 46 farms that moved cattle during 2017 were included in the risk assessment model. A description of cattle movements (number of entrances, number of purchased cattle, origin, age, other factors) by study farms is shown in Table 2.

Origin of purchased animals	**Number of Farms***	**Min**	**Median**	**Max**	**Total**
Number of cattle introduced/purchased per farm					
France	11	1	1	14	29
The Netherlands	6	1	1	2	6
Catalonia	12	1	3	16	65
Galicia	15	1	2	6	44
Rest of Spain	4	1	3	15	22
Number of origins per group of cattle purchased					
France	11	1	3	10	42
The Netherlands	6	1	1	1	5
Catalonia	12	1	2	24	44
Galicia	15	1	1	3	24
Rest of Spain	4	1	1	3	6
Total number of animals purchased					
France	11	1	8	541	687
The Netherlands	6	1	9	42	90
Catalonia	12	1	14	169	418
Galicia	15	2	4	40	108
Rest of Spain	4	2	8	96	113
Age of the animals (months)					
<12	15	2	6	7	113
12 to 24	22	1	2	81	325
>24	39	1	6	537	978
Movements of reared replacement heifers offsite					
Number	15	1	11	28	176
Heifers moved		14	134	357	1962
Movements to competitions					
Number	8	1	2	2	13
Cattle moved		1	2	6	27

Table 2: Description of animal movements in study farms. *Some farms purchased cattle more than one location. Thus, the sum of farms is higher than the number of study farms (n=46).

Cattle transport was carried out mainly with vehicles by an external company, and several farms shared transport vehicles with cattle from other farms (Table 3).

Type of movement	**Number of farms that use own transport vehicle**	**Number of farms that use vehicles from external company**
Purchase of cattle	2 (0) *	34 (20) *
Reared replacement heifers offsite	9 (1) *	6 (5) *
Cattle competitions	0	8 (8) *

Table 3: Characteristics of transport vehicles used for animal movements in study farms. * Number of farms that shared transport vehicle with cattle from other farms.

Of the 36 farms that purchased animals, 50% and 39% tested the animals for BVDV and BoHV-1 at the origin farm, respectively, prior to transportation. Eight out of 36 farms had quarantine facilities, and five of them tested animals in the quarantine for both diseases. Among the eight farms with quarantine facilities, six visit the quarantine at the end of the workday, and in seven farms, the quarantine duration period was more than 24 days. Among farms with cattle returning from competitions, only one farm placed animals in quarantine facilities. Of movements made with farm-owned transports, five (55%) cleaned and disinfected vehicles after being used.

3.4.2. Probability of BVDV and BoHV-1 introduction through animal movements at farm level

The model calculated the probability of virus introduction for each farm according to their characteristics. As an example, model results from one selected farm, together with the number of movements and biosecurity measures of that farm, are shown in Figure 2 and Table 4.

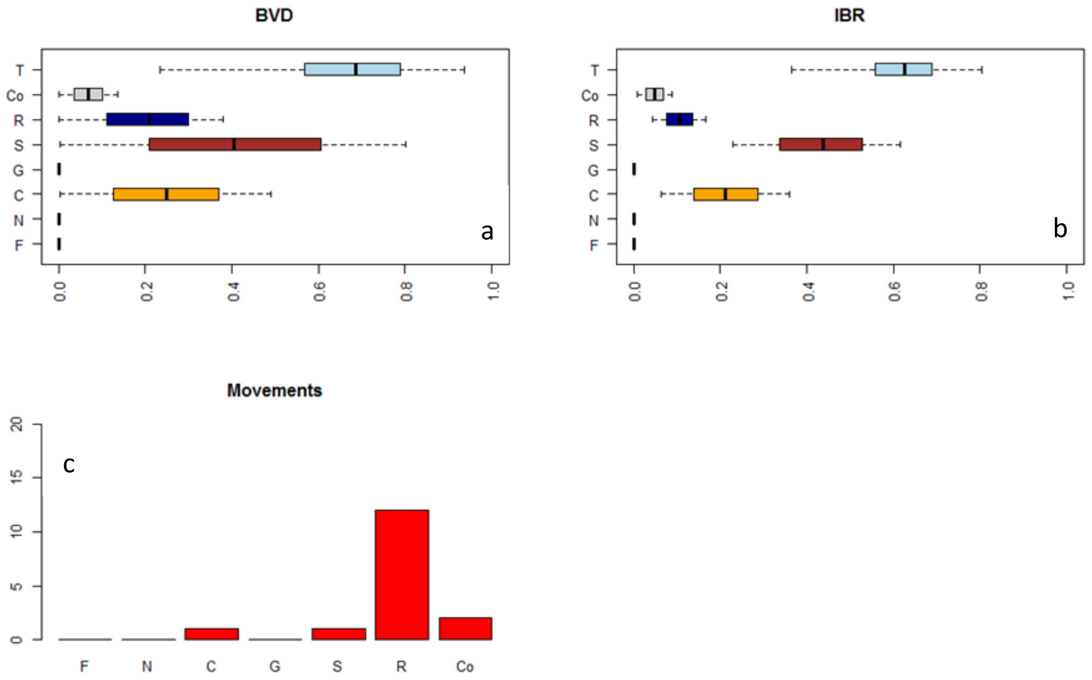

Figure 2: Probability distribution of Bovine Viral Diarrhea (BVD) and Infectious Bovine Rhinotracheitis (IBR) introduction for each movement performed by selected farm. a) Model results for BVD; b) Model results for IBR; c) Number and type of movements. T: Total probability. Probability of introduction through Co: cattle competitions; R: reared replacement heifers offsite; S: purchase of cattle from Spain; G: purchase of cattle from Galicia; C: purchase of cattle from Catalonia; N: purchase of cattle from The Netherlands; F: purchase of cattle from France. Movements: number of cattle movements from F: France; N: The Netherlands; C: Catalonia; G: Galicia; S: movements from Spain; R: replacement movements, and Co: movements to competitions.

The selected farm (table 4) purchased cattle from Catalonia and other regions of Spain, attended cattle competitions, and reared replacement heifers offsite. All movements corresponded to non-pregnant cattle.

	Age (months) of animals*			**Origins**	**Movements**	**Test in origin**	**Vehicle**	**ST[1]**	**Q[2]**
	<12	**12-24**	**>24**						
Catalonia	0	1	0	1	1	No	External company	Yes	No
Other regions of Spain	0	0	2	1	1	No	External company	Yes	No
Competitions	0	0	7	Na[3]	2	Yes	External company	Yes	No
External replacement	71	0	0	1	12	No	Own-farmed	No†	No

Table 4: Analyzed variables for one randomly selected farm. * All introduced/purchased cattle were non-pregnant animals †The vehicle was not cleaned and disinfected after animals were unloaded; [1]ST=Shared transport; [2]Q=Quarantine; [3]Na=Not apply.

The risk assessment model estimated that the farm had a high annual probability of introduction of BVDV and BoHV-1, with median values of 75% and 62%, respectively. The purchase of cattle from other regions of Spain was the route with higher probability values for both diseases, while movements to cattle competitions, reared heifers offsite, and purchase of cattle from other farms of Catalonia were higher for BVDV only. Based on model results, the farm had a negligible probability of purchasing PI or TR animals (Figure 3).

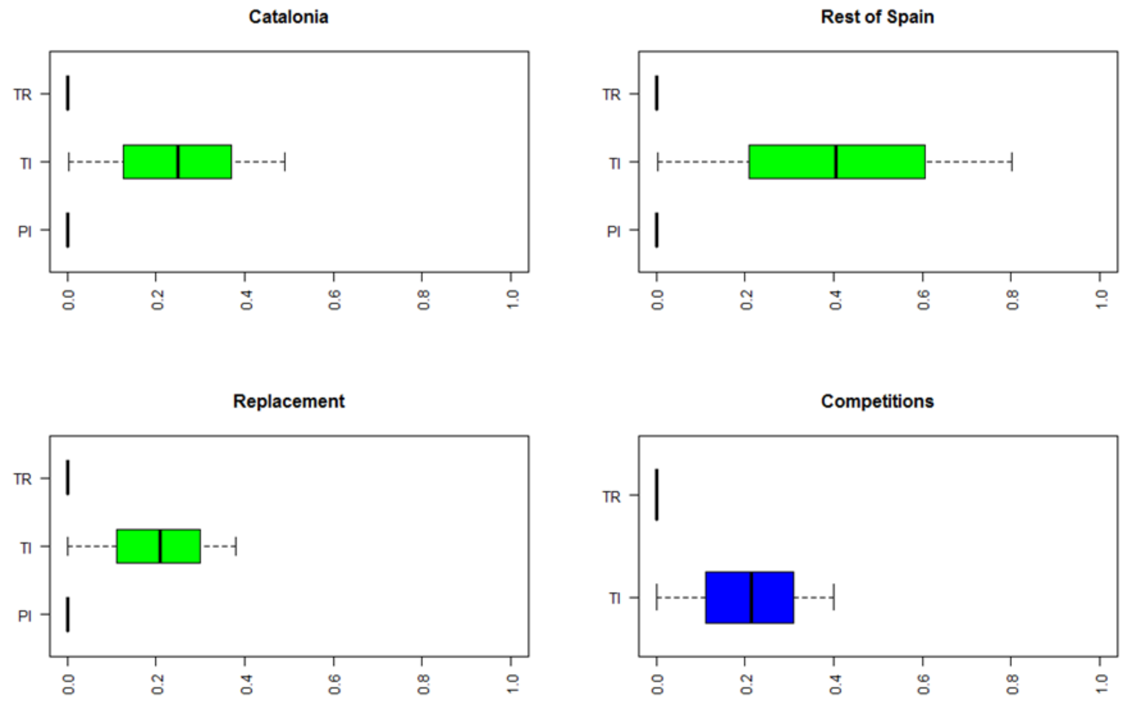

Figure 3: Probability of Bovine Viral Diarrhea introduction in a selected farm due to movements of animals within Catalonia, rest of Spain, replacement heifers and competitions. TR: Probability of Trojan cow introduction; TI: Probability of transiently infected cattle introduction; PI: Probability of persistently infected cattle introduction.

As shown in Table 4, different biosecurity measures could be implemented to reduce the probability of introducing BVDV or BoHV-1 infected animals into the selected farm. The purchase of new animals was conducted without any testing prior to movement and, more importantly, they were transported in a shared transport vehicle with cattle from other farms and loaded into the farm without being quarantined. Reared replacement heifers offsite had no contact with other animals. However, transportation took place with an own-farm vehicle that was used for other farms' movements, without cleaning and disinfection after each transportation.

3.4.3. Probability of BVDV and BoHV-1 introduction in the 46 dairy farms analyzed

Figure 4 shows the distribution of median probability values for the 46 study farms. The annual probability of introducing BVDV or BoHV-1 infected animals was very heterogeneous, being close to zero in some farms, while close to one in others (Figures 4a and 4d). In twenty-three farms, the median probability of BVDV introduction was ≤ 12%, (first quartile = 1.2%; third quartile = 28%). The median probability of BoHV-1 introduction was lower than 9% (first quartile = 3%; third quartile = 23%). Farms that purchased cattle from their same region (i.e. local movements) had a higher probability for introduction of BVDV and BoHV-1, followed by farms that introduced animals from other regions of Europe, reared replacement heifers offsite, or showed cattle in competitions (Figures 4a and 4d).

Sharing transport vehicles with other farms was the cause of a higher probability of infection for most study farms, followed by transport of animals in contaminated vehicles. The contribution of purchasing animals infected at origin was low for most study farms in the case of BVD, but not of IBR (Figures 4c and 4e). As regards BVDV infection status, in most of the farms the highest probability was the introduction of TI animals (Figure 4b).

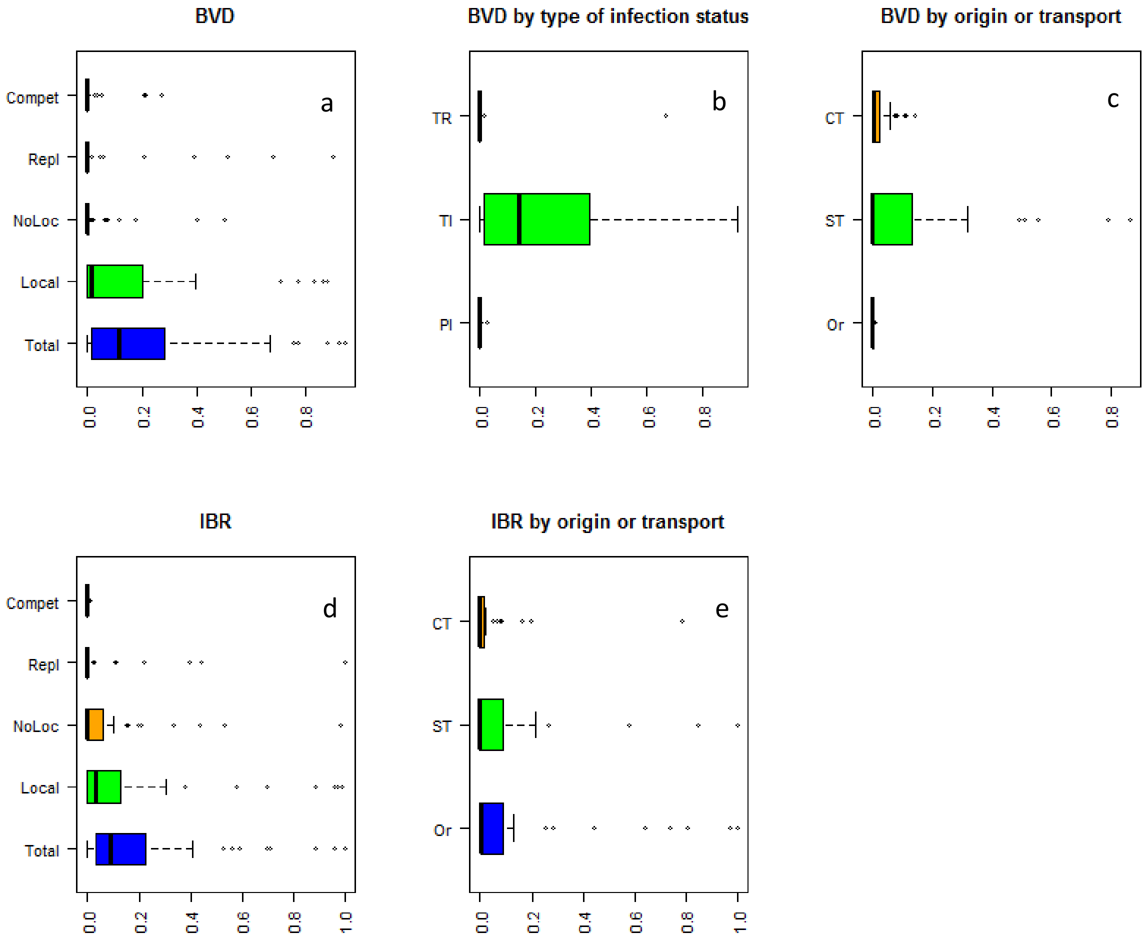

Figure 4: Distribution of median probability values of Bovine Viral Diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) introduction of all study farms. Figures 4a, 4b and 4c Probability of BVD introduction by Compet: cattle competitions; Repl: reared replacement heifers offsite; NoLoc: France, Spain and the Netherlands movements; Local: Catalonia and Galicia movements; Total: total probability by animal movements; TR: Trojan cows; TI: transiently infected cattle; PI: persistently infected cattle; CT: transport of cattle in contaminated vehicles; ST: share of transport vehicles with other farms, and Or: animal infected in origin. Figures 4d and 4e Probability of IBR introduction by Compet: cattle competitions; Repl: reared replacement heifers offsite; NoLoc: France, Spain and the Netherlands movements; Local: Catalonia and Galicia movements; Total: overall probability by animal movements; CT: transport of cattle in contaminated vehicles; ST: share of transport vehicles with other farms, and Or: animal infected in origin.

3.4.4. Sensitivity analysis

The Spearman correlation coefficient was close to zero in all farms for different input parameters (i.e., range -0.04 to 0.03). Therefore, the analysis showed that uncertainty in input parameters did not have any influence in model results. However, model results were sensitive to alterations in number of visited farms, number of loaded animals, herd prevalence, prevalence of infected animals and probability of cleaning/ disinfection of animal transport vehicle. Figure 5, shows the distribution of median probability values for the 46 study farms to alternative values to those parameters. Prevalence of infected herds and infected animals were the parameters that had a higher influence in model results.

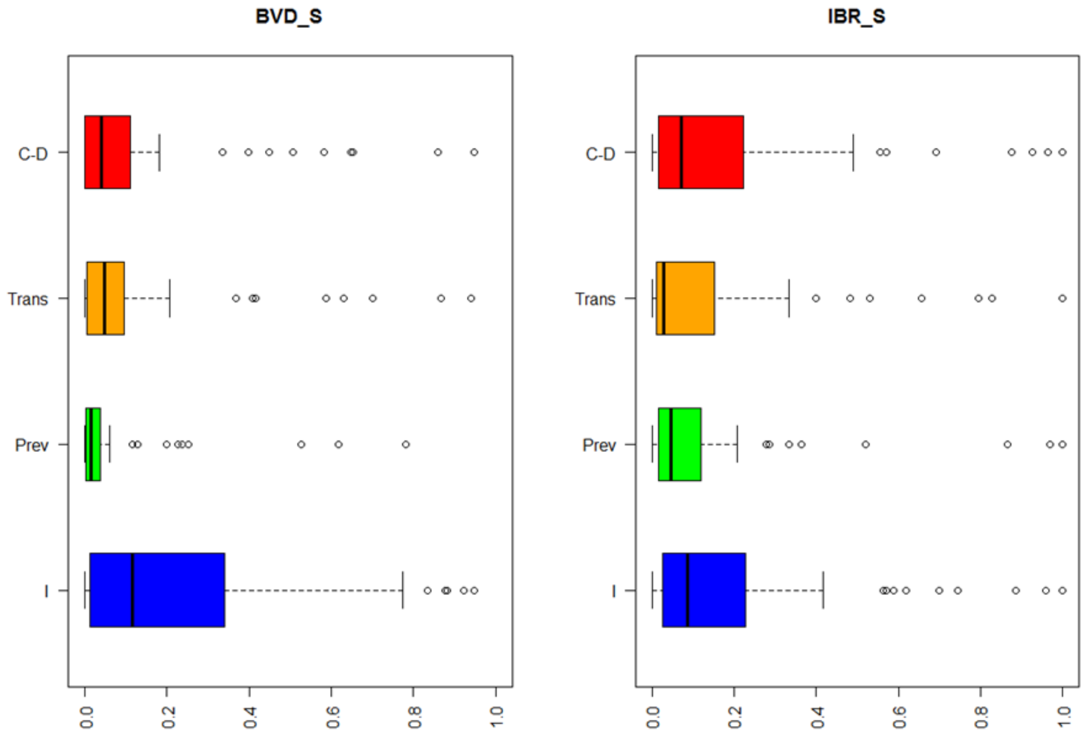

Figure 5: Distribution of median probability values of all study farms of Bovine Viral Diarrhea Virus (BVD_S) and Infectious Bovine Rhinotracheitis (IBR_S) introduction with alternative values. I: Initial probability of introduction with default values; Prev: Lower herd prevalence and infected animals prevalence (i.e., half of default values);Trans: Lower number of farms visited in each country/area and lower number of animals loaded in each farm (i.e., half of default values); C-D: Higher probability of cleaning/disinfection of transport vehicle (i.e., range of 90 – 95%).

3.4.5. Risk mitigating strategies

When selected farm met expectations described for quarantine, there was a notable reduction in the probability of introducing BVDV and BoHV-1 (<15%). Not sharing transport with cattle from other farms had a great influence in the probability of BVDV and BoHV-1 introduction, as decreased to 30% and 45%, respectively. On the other hand, the impact of testing all purchased animals at origin had a low impact in the probability of disease introduction for BVDV. Figure 6 shows the effect of these risk mitigating strategies.

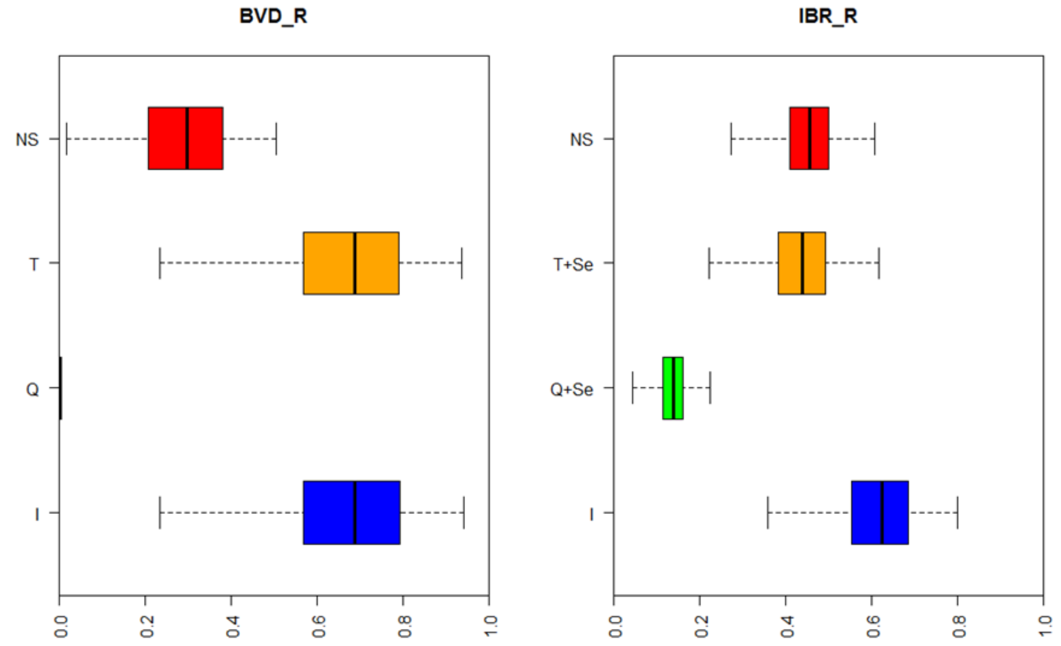

Figure 6: Reduction in probability of Bovine Viral Diarrhea Virus (BVD_R) and Infectious Bovine Rhinotracheitis (IBR_R) introduction by implementation of biosecurity measures in selected farm. I: Initial probability of introduction with default values; Q: Quarantine; Q + Se: Quarantine and higher sensitivity of antibodies ELISA; T: Test in origin; T + Se: Test in origin using antibodies ELISA with higher sensitivity; NS: Animal transport vehicle not shared with other farms.

3.5. Discussion

The risk assessment model showed that several farms had a high annual probability of introduction of BVDV and BoHV-1. Farms that purchased cattle from their same region (i.e. local movements) and shared transport with other farms had the higher probability for BVDV and BoHV-1 introduction. Results evidenced that biosecurity practices should be improved in dairy farms from Spain.

Quantitative risk assessment models can provide an accurate estimate of the probability of virus introduction, as they take into account those factors that influence the likelihood of disease transmission, such as the survival of the virus in the environment, amount of pathogen excreted, frequency of contacts and, biosecurity measures and other factors that can modify the probability of transmission for a given contact. Consequently, these kinds of models have a higher degree of complexity and need quantitative data that are not always available. Therefore, most of developed models that measure level of biosecurity at farm level in cattle and other species, have been based only on opinions and perceptions, and do not provide an estimate of probability of disease introduction (Pinto and Urcelay, 2003; Holtkamp et al., 2013; Laanen et al., 2013; Allepuz et al., 2018). Such models can be very useful as educational tools, for developing skills in risk-based prioritization, and increase awareness (Sternberg-Lewerin et al., 2015), as well as to benchmark farms in relation to their biosecurity level (Dewulf and van Immerseel, 2018). However, they cannot be used to assess probability of virus introduction, or to identify measures that should be prioritized based on their impact on that probability.

The use of quantitative risk assessment models can be useful to promote the improvement of biosecurity in dairy cattle farms and support disease control programs. However, complexity and lack of understanding of logic behind development of quantitative risk analysis models by end-users (e.g., field veterinarians) hampers use of these models in practice, and therefore limits impact in improving biosecurity and/or supporting disease control programs. We tried to overcome this issue by using a participative approach with the objective of developing a risk assessment tool that could be adapted to end-user needs. With that purpose, we invited field veterinarians to identify input parameters of the model, obtain some data (e.g., data related with animal transport practices), and evaluate the reliability of the obtained results. A successful implementation of biosecurity programs requires the participation of farmers, industry, and veterinarians (Barkema et al., 2015), so further efforts to discuss model results and, if needed, to incorporate suggested modifications with these stakeholders would be of paramount importance to improve biosecurity.

A parameter with a high degree of uncertainty was BVDV and BoHV-1 herd prevalence in Spain. We combined reported data in several epidemiological studies in order to consider differences between places with or without voluntary control programs for both diseases. Currently, in Spain, BVDV and BoHV-1 control programs are voluntary, and only implemented in some regions. As a matter of fact, in Galicia (north-western Spain) there is a voluntary program for both diseases (Lindberg et al., 2006; Eiras et al., 2009). However, since September 2019 a voluntary control program for BoHV-1 has been created in Spain (Royal Decree 554/2019). Due to the lack of recent studies about herd prevalence in the different regions of Spain, this parameter ranged between 26% and 71%. Consequently, the probability of virus introduction through this route also had a very wide distribution. Reducing uncertainty on the herd prevalence of BVDV and BoHV-1 infected herds would also be beneficial for increasing the accuracy of the model results.

In relation to cattle transport, we only had information on moved animals, but no specific information about routines of the cattle transport vehicle (e.g., number of farms visited per day, number of animals by transport vehicle, etc.). Lack of availability of this data is also common in other countries in Europe, as reported previously in other studies (Bronsvoort et al., 2008; Santman-Berends et al., 2018). Consequently, in this study the information was obtained through the discussions held with attending veterinarians. The sensitivity analysis evidenced that variation on these parameters influenced model results. Therefore, incorporation of data from animal transport companies in the risk assessment model would increase the accuracy of the estimations.

Interestingly, model results highlighted the important role of the animal transport vehicles in the spread of these viruses between dairy cattle farms, especially in the case of local movements when purchasing replacement heifers. In addition, testing animals in origin did not have a great influence in reducing the probability of disease introduction, as shown in the analysis conducted in one selected far. This lack of efficiency was also related to the role of animal transport, as negative animals in origin could get infected during transport. On the one hand, as a voluntary control program for both diseases, there are no legal requirements to transport cattle in relation to their health status. On the other hand, the cleaning and disinfection of transport vehicles is compulsory, but the efficacy is probably low, and sometimes these are not applied. Efforts on the development of more adequate disinfection points within Spain and with more rigorous monitoring, would be beneficial to reduce the probability of disease transmission through contaminated vehicles, as demonstrated previous studies that include international transport (Bronsvoort et al., 2008; Fountain et al., 2018). Furthermore, animal transports in which cattle from different farms were mixed, was quite common and increased the probability of disease introduction in several farms. The low number of animals moved by some farms hampers the possibility of not mixing due to economic reasons. A discussion should be set up in order to evaluate how this practice could be reduced, without compromising the viability of transport companies.

In the model, we assumed that all animals that shared transport vehicle together with a PI animal or AI animal, got infected. This represents a worst-case situation, because the limited duration of transport does not necessarily result in all cattle being infected by the PI or the AI animal. On the one hand, efficient transmission of BVDV from acutely infected animals may require exposure to higher viral loads or for a longer period of time

(Falkenberg et al., 2018). On the other hand, in BoHV-1 infections, latently infected animals can reactivate due to transportation stress, posing a similar risk compared to AI animals. In addition, periods of time less than eight hours are rather short to become both infected and then progress to the subsequent infectious status (Santman-Berends et al., 2018). In the model, we assumed the duration of animal transport was not enough for reactivation and we only considered it during the quarantine.

Participation in competitions did not represent a significant probability of disease introduction for most of the farms attending them, similar to that found in Denmark for the case of participation in international competitions (Foddai et al., 2014). The exception could be in relation to BVDV in the case of sending pregnant cattle of between 30 and 120 days of gestation to these competitions. Therefore, the probability of introducing TR cattle due to movements to cattle competitions could be avoided by just not sending pregnant cattle to participate. On the other hand, some researchers have found that allowing cattle to return to farm after competitions is a risk factor for the introduction of BoHV-1 and BVDV (Van Wuijckhuise et al., 1998; Houe, 1999). For BoHV-1, gathering of large numbers of cattle is a stress factor that could result in virus reactivation, and the overcrowded barn would facilitate spread of infectious diseases. Moreover, cattle returning to farm of origin could infect other cattle on farm (Van Schaik et al., 1999). Nevertheless, compulsory testing of all animals attending cattle competitions reduced the probability of disease introduction by this route, again the vehicle for animal transport being the most critical point to be infected.

This study had some limitations. First, the participation of farms was voluntary, so our sample was not representative of the dairy farms in Spain. However, in this study we intended to develop a risk assessment model to improve biosecurity rather than providing an estimate of the probability of disease introduction in dairy farms from these areas. Second, only one experimental study (Niskanen and Lindberg 2003) was available for the probability of indirect transmission of BVDV (i.e., infection due to contact with contaminated surfaces). In addition, to our knowledge, there are no studies for BoHV-1 about the probability of indirect transmission and survival of virus in different materials. BoHV-1 is stable for 1 month at 4°C, can be inactivated at 37°C within 10 days, and at 22°C within 50 days, and may survive for more than 30 days in foodstuffs (Nandi et al., 2009). Thus, based on reported resistance in the environment, we assumed that the virus would survive time between movements. In the same way, we did not find data on efficacy of cleaning and disinfection for BoHV-1 and based on the virus characteristics described by Straub (1990) we assumed that efficacy of conventional disinfectants was high. Further studies analyzing how these viruses are transmitted would be beneficial in order to develop more accurate models.

In the studied farms, the probability of introducing BVDV and BoHV-1 could be reduced by the implementation of biosecurity measures. We believe that, despite the inherent limitations of the developed model, we have provided a useful tool that supports the decision making on which biosecurity measures should be prioritized in dairy cattle herds in order to reduce the probability of introduction of these viruses. In addition, further efforts should be made to estimate the probability of virus introduction through other routes, such as movement of people, non-animal transport, etc., in order to obtain a complete scenario for each farm.

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Study II

Chapter IV

Quantitative risk assessment of BVDV and BoHV-1 introduction through indirect contacts based on implemented biosecurity measures in dairy farms of Spain

Under review in Preventive Veterinary Medicine

4.1. Abstract

A stochastic quantitative risk assessment model was developed to estimate the annual probability of the introduction of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) on 127 dairy farms through indirect contacts. Vehicles transporting calves, cattle to slaughterhouse, dead animals, and mixture of feed, as well as visits by veterinarians and hoof trimmers, farm workers and contacts with neighbors were considered in the model. Data from biosecurity questionnaires of each farm, scientific literature and expert opinion from field veterinarians, animal vehicle drivers, hoof trimmers and personnel from rendering transport companies were used to estimate values for input parameters. Results showed that the annual probability of introducing BVDV or BoHV-1 through indirect contacts was very heterogeneous, with median values for each farm ranging from 0.5 to 14.6% and from 1.0% to 24.9% for BVDV and BoHV-1, respectively. The calf vehicles and visits by veterinarians were the routes with higher probability of infection. The model also identified that providing protective clothing and boots exclusively for the farm, not allowing the animal vehicle driver to come into contact with animals present on the farm and ensuring that the calf vehicles arrived empty, were the measures with the highest impact on the probability of infection for most farms. This model might be a useful tool to show the effect of biosecurity measures to the farmers and veterinarians, and to support decision making on the measures that should be prioritized in dairy cattle herds to reduce the probability of introduction of diseases.

4.2. Introduction

Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus-1 (BoHV-1) infections are endemic, and cause disease in cattle populations worldwide. Both BVDV and BoHV-1 are transmitted horizontally by direct contact and contaminated fomites (e.g., pathogens carried on clothes, equipment or vehicles visiting the farm) (Muylkens et al., 2007; Nöremark et al., 2013). Cattle movements are considered as the major cause of BVDV and BoHV-1 spread between-farms (Van Schaik et al., 1998; Lanyon et al., 2014). However, they can also be spread due to sharing of contaminated equipment, movement of vehicles, farm workers, and visitors (Van Schaik et al., 1998; Gunn, 1993; Niskanen and Lindberg, 2003).

Although indirect contacts are less efficient in transmitting infectious diseases compared to direct contacts (Bates et al., 2001), indirect contacts can occur more frequently (Rossi et al., 2017a). The role of indirect transmission is still largely unknown due to limited availability of data, the highly diverse and complex nature of indirect contacts, and for privacy reasons. It is much easier to track livestock movements than that of farm operators, visitors, or vehicles (Rossi et al., 2017a). Existing studies highlight the importance of visitors who come in close contact with livestock in disease transmission (i.e., veterinarians, artificial insemination technicians, milk trucks, transporters of livestock, and rendering trucks) (Bates et al., 2001; Rossi et al., 2017b). Employees who work on other farms and/or kept cattle of their own represent a risk for BoHV-1 transmission (Van Schaik et al., 2001; Bates et al., 2001), as well as those making social visits who have contact with areas where cattle can have access, also represent a risk for disease spread (Nielen et al., 1996). Exchanging or sharing equipment between farms (e.g., tractors) is another type of indirect contact that has been described in dairy farms (Brennan et al., 2008). Bates et al., (2001) in United States quantified with a range from 234 to 743, the number of indirect contacts/month between farms (i.e., number of contacts in farm by individuals and vehicles that visited multiple livestock facilities). While in the Netherlands the mean indirect contact rate was 91 (Nielen et al., 1996), in New Zealand was 50 (Sanson et al., 1993). These different studies showed that the number of contacts between farms varies greatly when considering factors such as type of enterprise and facilities, or number of animals on the farm. Finally, epidemiological analysis such as network analysis and Susceptible-Infectious-Susceptible (SIS) spread models have supported disease spread at local scale (Rossi et al., 2017a, 2017b).

Biosecurity measures might be effective in reducing disease transmission through indirect contacts between farms. Recommendations described in the literature include avoiding equipment sharing, or promoting equipment disinfection in case it is shared, positioning of vehicle bath systems, providing personal protective equipment and boots, and restricting visitor contact with animals (Morley, 2002; Van Schaik et al., 2002; Mee et al., 2012). Animal transport vehicles should arrive empty, clean, and disinfected before entering the farm. If cattle from other farms are present on the vehicle, it should not enter and the driver should not be allowed to come into contact with animals on the farm (Sarrazin et al., 2018).

Quantitative risk assessment at farm level is a useful tool that enables biosecurity measures to be prioritized in order to reduce the likelihood of disease transmission between farms. The aim of this study was to quantify the probability of BVDV and BoHV-1 virus introduction into dairy farms through indirect contacts, and to identify and prioritize the measures that should be implemented or improved.

4.3. Materials and Methods

4.3.1. Risk release pathways

Indirect transmission pathways and their parameters included in the model were justified in consultation with two discussion groups of ten field veterinarians (one group in Galicia and the other one in Catalonia, Northwestern and Northeastern Spain, respectively) (Annex B. Table A1). The model considered the following pathways: i) animal transport vehicles (i.e., slaughterhouse and calf transport vehicles); ii) other vehicles (i.e., rendering and feed); and iii) visitors (i.e., veterinarians, hoof trimmers, farm workers shared between farms and visits or shared material between neighboring farms).

4.3.2. Data

Biosecurity data, together with BVDV and BoHV-1 prevalence data from 127 dairy farms (34 in Catalonia and 93 in Galicia) were obtained from a previous study conducted in the area (Benavides et al., 2018). Data for other parameters were obtained from peer-review papers, the above-mentioned discussion groups with field veterinarians and by telephone interviews with animal vehicle drivers, hoof trimmers and personnel from rendering transport companies. Parameters included in the model and their distributions are summarized in Table 1.

Parameter	**Distribution**	**References**
BVDV		
Herd prevalence in Catalonia	Uniform (0.54, 0.57)	Benavides et al., 2018
Herd prevalence in Galicia	Uniform (0.03, 0.16)	Benavides et al., 2018
PI[1] prevalence (<12 months)	Pert (0.0022, 0.0024, 0.0026)	Galician diagnostic laboratory, (data from 2018)
PI prevalence (12 to 24 months) *	Pert (0.0011, 0.0012, 0.0013)	Galician diagnostic laboratory, (data from 2018)
Efficacy of cleaning and disinfection	Pert (0.80, 0.90, 1)	Foddai et al., 2014

Probability surviving on galvanized metal (1-2 hours)	Uniform (0.096, 0.22)	Stevens et al., 2011
Probability surviving on soil (8-12-24 hours)	Pert (0.056, 0.104, 0.13)	Stevens et al., 2011
Probability of BVDV indirect transmission	Fixed Value (0.1)	Viet et al., 2004
Proportion of farms from Catalonia without PI control	Pert (0.37, 0.53, 0.68)	Benavides et al., 2018
Proportion of farms from Galicia without PI control	Pert (0.19, 0.27, 0.37)	Benavides et al., 2018
Proportion of tested animals in farms with a PI control program	Fixed value (0.5)	Expert opinion
BoHV-1		
Herd prevalence in Catalonia	Uniform (0.27, 0.57)	Benavides et al., 2018
Herd prevalence in Galicia	Uniform (0.06, 0.11)	Benavides et al., 2018
Infected animals (<24 months)	Uniform (0.15, 0.20)	Santman Berends et al., 2018
Infected animals (>24 months)	Uniform (0.53, 0.58)	Santman Berends et al., 2018
Probability of acute infection in infected animals	Fixed value (0.01)	Santman Berends et al., 2018
Efficacy of cleaning and disinfection	Uniform (0.95, 1)	Nandi et al., 2009
Probability of BoHV-1 indirect transmission	Pert (0.09, 0.10, 0.13)	Vonk Noordegraaf et al., 2002
Slaughterhouse transport vehicle		
Number of farms visited in Catalonia and Galicia**	Pert (2, 3, 5)	Expert opinion
Animals loaded in each farm from Catalonia	Pert (7, 8, 10)	Expert opinion
Animals loaded in each farm from Galicia	Pert (3, 7, 10)	Expert opinion
Calf transport vehicle		
Probability of cleaning/disinfection	Pert (0.21, 0.29, 0.38)	Benavides et al., 2018
Number of farms visited in Catalonia**	Pert (7, 10, 15)	Expert opinion
Number of farms visited in Galicia**	Pert (5, 10, 20)	Expert opinion
Number of animals loaded in Catalonia	Pert (25, 30, 35)	Expert opinion
Number of animals loaded in Galicia	Pert (10, 20, 50)	Expert opinion
Rendering vehicle		
Number of bovine farms visited**	Pert (6, 10, 12)	Expert opinion
Number of dead cattle loaded per farm	Pert (2, 4, 7)	Expert opinion
Mortality rate of PI calves (First year)	Fixed value (0.5)	Ezanno et al., 2007
Mortality rate of calves due to BoHV-1	Fixed value (0.02)	Moeller et al., 2013
Mortality rate of heifers due to BoHV-1	Fixed value (0.084)	Walker et al., 2012
Probability of transmission given the vehicle do not enter inside the farm perimeter	Uniform (0, 0.000001)	Negligible-DAFF, 2004
Feed vehicle		
Number of visited farms**	Pert (4, 6, 8)	Expert opinion
Visitors		
Probability of cleaning and disinfection of veterinary material	Pert (0.78, 0.86, 0.91)	Renault et al., 2017
Probability of sharing material between farms	Fixed value (0.2)	Expert opinion

Probability of BVDV surviving on enameled metal and rubber (20 minutes-1-2 hours)	Pert (0.75, 0.88, 1)	Stevens et al., 2011
Proportion of farms providing protective clothing and boots	Pert (0.043, 0.078, 0.138)	Benavides et al., 2018
Number of farms visited by any veterinarian in Catalonia**	Uniform (1, 5)	Expert opinion
Number of farms visited by clinical veterinarians in Galicia**	Uniform (4, 10)	Expert opinion
Number of farms visited by rest of veterinarians in Galicia**	Uniform (4, 6)	Expert opinion
Probability of cleaning and disinfection of hoof trimmers material	Uniform (0.9, 0.95)	Expert opinion
Number of farms visited by hoof trimmers in Catalonia**	Pert (3, 4, 5)	Expert opinion
Number of farms visited by hoof trimmers in Galicia**	Pert (4, 5, 10)	Expert opinion
Number of annual visits by hoof trimmers in farms with ≤250 animals	Fixed value (4)	Expert opinion
Number of annual visits by hoof trimmers in farms with ≤600 animals	Fixed value (12)	Expert opinion
Number of annual visits by hoof trimmers in farms with >600 animals	Fixed value (24)	Expert opinion
Probability of farm worker was contaminated given did not share boots between farms	Uniform (10^{-6}, 0.001)	Extremely low-DAFF, 2004
Number of days worked by farms workers	Fixed value (250)	https://www.dias-laborables.es
Neighbors		
Number of annual visits by neighbors	Fixed value (50)	Expert opinion
Number of annual times that farms>100 cattle share material	Fixed value (12)	Expert opinion
Number of neighboring farms from which visits can be received	Maximum fixed value (3)	Expert opinion

Table1: Input parameters to estimate the probability of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) introduction by indirect contacts in the study farms

4.3.3. Model development

To estimate the probability of introduction of BVDV and BoHV-1 into the 127 study farms in a 12-month period, we developed a stochastic risk assessment model by using the mc2d package (Pouillot and Delignette-Muller, 2010) implemented in R (https://www.R-project.org/). Monte Carlo simulations (10,000 iterations) were performed, and all non-fixed input parameters were included as uncertain parameters.

Probability of infection due animal transport vehicles

Animal transport vehicle pathway is shown in Figure 1. Based on interviews with vehicle drivers, calves for fattening and culled cows for slaughterhouse were not mixed in the same transport. Therefore, although a vehicle could be the same, we estimated the probability of infection in vehicles picking-up dairy cattle for slaughterhouse separately from those used for calves. Further, some farmers reported in the biosecurity questionnaire that calves were transported in owned farm vehicle. Thus, for such vehicles both options (i.e., external company and own farm) were considered, whereas for slaughterhouse vehicles, it was assumed an external company provided transportation.

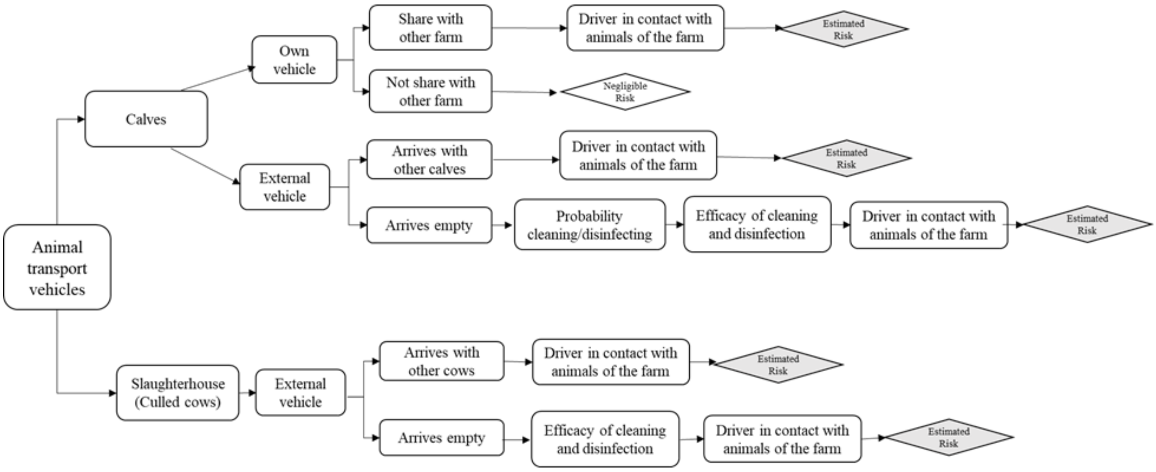

Figure 1: Pathway of entrance through animal transport vehicles

For BVDV, we only considered the likelihood of transporting persistently infected animals (PI), since transiently infected animals (TI) are not highly contagious (Lindberg and Houe, 2005). In Spain, according to field veterinarians, most farms with a PI control program only test females (i.e., replacement animals that will stay in the farm) and positive animals are slaughtered on the farm. Therefore, PI animals could be loaded on animal transport vehicles if they are males, or if they are females from an infected farm without a PI control program. In slaughterhouse vehicles, we assumed that PI animals were >12 months, as younger animals are more likely sent to fattening using calf vehicles. The estimated proportion of PI animals was that in PI calves detected in all calf births during 2018 in on farms from Northwestern Spain, involved in BVDV control program. For BoHV-1, we used the prevalence of infected animals <24 months old for calf vehicle and >24 months old for the slaughterhouse vehicle, and seropositive animals with an acute infection (AI) was assumed at 1% according to reported by Santman-Berends et al. (2018). Finally, the probability of loading at least one BVDV or BoHV-1 infected animal on calf vehicles (PC1) or slaughterhouse vehicles (PS1) was calculated using equation [1] in Table 2.

In farms where the animal transport vehicle always arrived empty (i.e., without animals), the probability that calf vehicles (PC2) or slaughterhouse vehicles (PS2) were contaminated on arrival was estimated using equation [2] in Table 2. We assumed that all the slaughterhouse vehicles were clean and disinfected when departing from a slaughterhouse, as it is compulsory by law, and facilities for cleaning and disinfection are in place in all of them. For calf vehicles, the proportion of farms in which a vehicle arrived clean and disinfected was obtained from biosecurity data. In addition, based on interviews with vehicle drivers, a time interval between 1 and 2 hours from departure to arrival at the farm was considered. BVDV survival on a metal surface was included for this time interval based on Stevens et al., (2011), and for BoHV-1 it was assumed that the virus would also survive 1 or 2 hours (Nandi et al., 2009). Finally, if animal transport vehicles belonged to an external company, the probability that at least one infected animal was loaded on the farm was estimated taking into account the number of farms each type of vehicle can visit in one day (Equation [3] in Table 2). And for the own-farm calf vehicles it was consider that, if shared, it was shared with a single farm.

We assumed that BVDV or BoHV-1 could enter the farm through the vehicle driver and thus, the probability that the driver was contaminated was equal to values estimated for vehicles in equation [2] in Table 2. The probability that they were contaminated during each day was estimated taking into account the number of farms visited every day using equation [3] in Table 2. According to biosecurity data, animal transport vehicles entered within the perimeter in all the studied farms. Therefore, the only biosecurity measure that could reduce the probability of infection was for the vehicle driver to avoid contact with cattle present at the farm (i.e., those that were not loaded on the vehicle transport).

The probabilities of indirect transmission (PT) for BVDV reported by Viet et al., (2004) and BoHV-1 Vonk Noordegraaf et al. (2002) were considered to estimate the probability of transmission given a contact between contaminated driver and cattle from the farm. If the driver did not have any contact with animals present at the farm, we assumed that organic matter of the driver's boots could be a source of contamination on the farm through farm workers. In this case, we considered the probability of indirect transmission in two steps. The annual probability that the driver a calf vehicle (PC4) and the slaughterhouse vehicle (PS4) could infect the farm was calculated using equation [4] in Table 2.

Probability of infection due other vehicles

Other vehicles included rendering transport vehicle and feed vehicle (i.e., vehicle that provides the mixture of food) (Figure 2). For the rendering transport vehicle, we considered the likelihood that the driver would become contaminated by contact with a dead animal infected with BVDV (i.e., only PI animal) or BoHV-1 from a random farm while loading it inside the vehicle. For BVDV, the probability that the vehicle driver had contact with a dead PI animal was influenced by the existence of PI control programs. For farms without a PI control program, we considered that half of the PI animals would die during the first year of life (Ezanno et al., 2007). If the farm did have a PI control program, we considered that half of the PI calves could be found in the dead animals collecting point as these animals were slaughtered at the farm. For BoHV-1, we used mortality rates described in calves and heifers by Moeller et al. (2013) and Walker et al. (2012), respectively. To estimate the likelihood that the vehicle driver from the rendering vehicle was contaminated due to a contact with a PI or a BoHV-1 dead animal, PT was used. Feed vehicles providing the mixture of food to several farms was used in some of them (according biosecurity data), and their drivers did not have direct contact with the cattle of the farm. Therefore, to estimate the probability that drivers from these vehicles became infected when visiting a random infected farm, we assumed that two consecutive steps should occur: i) a farm worker becomes contaminated by stepping on a contaminated area at the farm and ii) the driver was contaminated when passing through a farm worker stepping area. Thus, we used the PT squared.

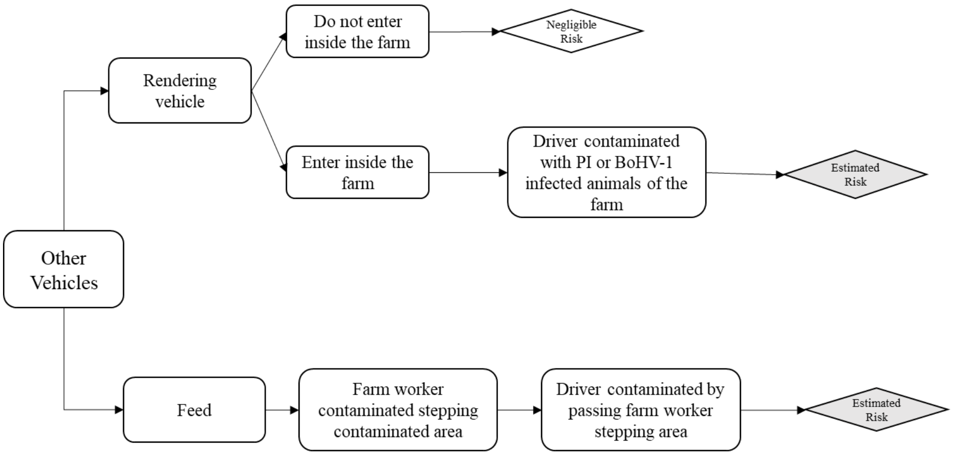

Figure 2: Pathway of entrance through rendering vehicles and feed vehicles

If the time interval between farms was less than 1 hour for both vehicles the virus would survive. Therefore, the probability that a driver from a rendering vehicle (PR1) or a driver from a feed vehicle (PF1) was contaminated either by BVDV or BoHV-1 was estimated using equation [5] in Table 2. The probability that the driver of a rendering vehicle or a feed vehicle was contaminated each day was estimated taking into account the number of farms visited every day using equation [6] in Table 2. If a rendering vehicle did not enter inside the perimeter of the farm to pick-up dead animals, the probability of infection was considered negligible. And finally, the probability that the farm became infected due to the rendering vehicle (PR3) or the feed vehicle (PF3) was estimated using the equation [7] in Table 2.

Probability of infection due to visitors

Veterinarians, hoof trimmers, farm workers that work in other cattle farms or have cattle at home and neighbors (i.e., cattle farms within a radius of 1 kilometer of each farm) were considered in the visitors pathway (Figure 3). Sharing pastures among neighboring farms was excluded because in the Spanish context it is an uncommon practice on dairy farms. In addition, according to field veterinarians, visitors from neighboring farms are frequent in small farms (i.e., <100 animals) for handling animals or help in birth, but very rare in larger farms. Therefore, for farms with <100 cattle we considered the likelihood of transmission from neighbor's visits while in larger farms we considered the probability of transmission due to sharing material with neighbors (data obtained from biosecurity data). Neighbors (for small farms) or shared material (for larger farms) were both calculated in the same way (PN1). Veterinarians (PV1), farm workers (PW1) and neighbors (PN1) could have had contact with cattle of any age while for hoof trimmers (PH1) it was assumed that they only would have had contact with animals >24 months old. Then, the probability that visitors had contact with at least with one infected animal when visiting a random farm was calculated using equation [8] in Table 2.

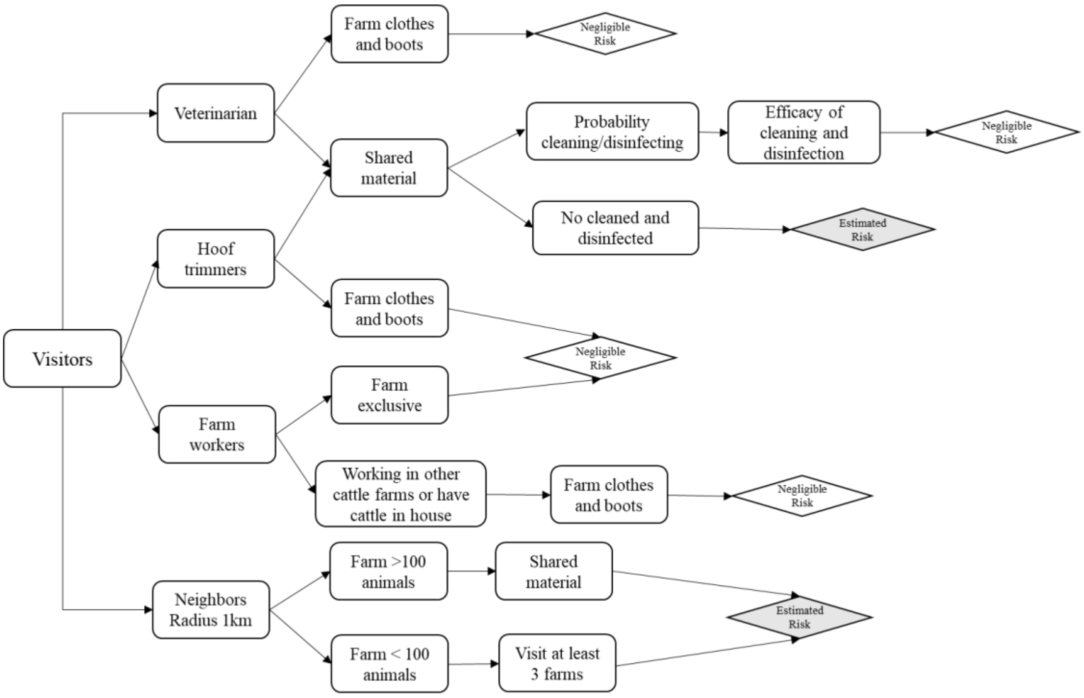

Figure 3: Pathway of entrance through visitors

In the next step, we considered the likelihood that their boots or reusable material (e.g., material for C-sections) were contaminated when leaving the farm. For farm workers, we only considered the likelihood of contamination of the boots, as it was assumed that they would not share reusable material between farms. For hoof trimmers, we assumed that they always shared material between farm visits. The proportion of farms providing boots to their visitors was obtained from biosecurity data, and the proportion of times a veterinarian used reusable material when visiting a farm was estimated as 20% by interviewed field veterinarians. The probability that veterinarians cleaned and disinfected the reusable material before going to the next farm was obtained from Renault et al. (2017); in hoof trimmers this probability was obtained through interviews with them. Thus, the probability that veterinarians and hoof trimmers reusable material (i.e., PV2 and PH2, respectively) was contaminated when leaving from a random farm was estimated using equation [9] in Table 2. Similarly, the probability of contaminated boots from veterinarians, farm workers and hoof trimmers (i.e., PV3, PW2 and PH3, respectively) was estimated using equation [10] in Table 2.

Considering the time interval between visited farms by veterinarians and hoof trimmers (i.e., between 20 minutes and 2 hours), reported by field veterinarians, BVDV survival in rubber and enameled metal (Stevens et al., 2011) was included to estimate the probability that veterinarians (PV4) and hoof trimmers (PH4) arrived contaminated at the next farm using equation [11] in Table 2. In Northwestern Spain, based on group discussions with field veterinarians, the number of farms visited by clinical veterinarians every day was higher (80%) than for the other veterinarians (20%). We assumed farm workers could only work on a second farm and, therefore, they could just visit one additional farm the same day. Then, the probability that an attending veterinarian or hoof trimmer became contaminated each day was estimated taking into account the number of farms visited by them every day, using equation [12] in Table 2. For neighbors, regardless the number of neighbors, it was assumed that visitors (or material) would come from a maximum of three farms and we estimated the probability (PN2) using equation [13] in Table 2.

The only biosecurity measure included in this pathway was the provision of protective clothing and boots to visitors. When this measure was applied, the probability that an attending veterinarian or hoof trimmer was a source of contamination on arrival would be attributable to reusable material. In farm workers that did not use same boots in multiple farms, the probability of contamination was considered to be extremely low. For neighbors visits this measure was not considered, because based on group discussion with field veterinarians, it was assumed that they would not use boots from the visited farm even if the farm had this biosecurity measure implemented for general visitors.

For the annual frequency of visitors that could have had direct contact with animals (reported in biosecurity data), we assumed that half of the visits corresponded to veterinarians. For farm workers, it was assumed they worked on the farm 250 days per year (annual business days). For neighbors, the frequency of visitors was set at 50 times per year while for shared material it was set at 12 times per year (PN3). In addition, for hoof trimers, the number of visits depended on the number of animals on the farm (Table 1) according to the interviews with hoof trimmers. The annual probability of infection due to veterinarians (PV6), farms workers (PW3), hoof trimmers (PH6) and neighbors (PN3) was estimated using equation [14] in Table 2.

Finally, the probability that the farm could become infected in one year by indirect contacts (PIND) was estimated using equation [15] in table 2.

Equation	Description
Animal transport vehicles	
1.Probability of loading at least one BVDV or BoHV-1 infected animal in the calf (PC1) or slaughterhouse vehicle (PS1) $PC1\ (j,k) = 1 - (1 - HP(j,k) *\ [(PPI * CPI + PPI * (1 - CPI) * B)\ \ or\ PIBR(j) * AcI])\text{\textasciicircum}n(j)$ $PS1\ (j,k) = 1 - (1 - HP(j,k) * ([PPI2 * CPI\ or\ PIBR2(j) * AcI])\text{\textasciicircum}n(j)$	• *j:* subscript for the region (Catalonia or Galicia) • *k:* subscript for the virus (BVDV or BoHV-1) • *HP:* herd prevalence • *PPI:* prevalence of PI animals <12 months • *CPI:* proportion of farms without a PI control program • *B*: proportion of cattle tested in farms with a PI control program (i.e., 50%) • *PIBR:* proportion of seropositive BoHV-1 animals <24 months • *AcI:* probability of having an acute infection • *n:* number of animals loaded on each farm • *PPI2*: prevalence of PI animals >12 months • *PIBR2:* proportion of seropositive BoHV-1 animals >24 months
2. Probability that calf vehicle (PC2) or slaughterhouse vehicle (PS2) was contaminated on arrival $PC2\ \ (j,k) = PC1\ (j,k) *\ (1 - PCD) * sm +\ PC1(j,k) * PCD *\ (1 - ECD) * sm$ $PS2\ \ (j,k) = PS1\ (j,k) *\ (1 - ECD) * sm$	• *PCD:* probability of cleaning and disinfection • *ECD:* efficacy of cleaning and disinfection • *sm:* BVDV survival in galvanized metal (1-2 hours)

3. Probability that at least one infected animal was loaded in the calf vehicle (PC3) or the slaughterhouse vehicle (PS3) according to the number of farms that each vehicle of external company can visit in one day $PC3(j,k) = 1 - \left(1 - PC2\ (j,k)\right)^{n(i)}$ $PS3(j,k) = 1 - \left(1 - PS2\ (j,k)\right)^{n(i)}$	- *n:* number of farms visited per day - *i:* subscript for each farm
4.Annual probability that the driver of the calf vehicle (PC4) or slaughterhouse vehicle (PS4) could infect the farm $PC4(i,k) = 1 - (1 - [PC3(j,k)\ or\ PC3(j,k) * PTIC\ or\ PTIC{}^\wedge 2\ ]){}^\wedge(AF(i))$ $PS4(i,k) = 1 - (1 - [PS3(j,k)\ or\ PS3(j,k) * PTIC\ or\ PTIC{}^\wedge 2\ ]){}^\wedge(AF(i))$	- *PTIC:* probability of transmission given an indirect contact - *AF:* number of times the vehicle arrived at each farm in one year
Other vehicles	
5.Probability that the driver from a rendering vehicle (PR1) or a feed vehicle (PF1) was contaminated either by BVDV or BoHV-1 $PR1(j,k) = 1 - \big(1 - HP(j,k) * ([PPI * CPI(j)\ or\ PIBR(j) * AcI] * MC(k) * PTIC(k) + \left[PPI * \left(1 - CPI(j)\right) * B\ or\ PIBR2(j) * AcI * MH\right] * PTIC(k))\ \big)^{nd(i)}$ $PF1\ (j,k) = 1 - (1 - HP\ (j,k) * [PPI\ or\ PIBR(j)] * PTIC{}^\wedge 2)$	- *MC:* mortality rate in calves - *MH*: BoHV-1 mortality rate in heifers - *nd:* number of dead cattle loaded per farm
6. Probability that the driver of rendering vehicle or the feed vehicle was contaminated each day according the number of farms visited every day $PR2(j,k) = 1 - \left(1 - PR1\ (j,k)\right)^{n(i)}$	

$PF2(j,k) = 1 - \left(1 - PF1\ (j,k)\right)^{n(i)}$	
7.Probability that the farm become infected due to the rendering vehicle (PR3) or the feed vehicle (PF3) $PR3(i,k) = 1 - (1 - PCA2\ (j,k) * [PTIC\ or\ Negl])\hat{}(AF(i))$ $PF3(i,k) = 1 - (1 - PF2\ (j,k) * PTIC)\hat{}(AF(i))$	- *Negl:* probability of transmission if the rendering vehicle did not enter inside the perimeter of the farm
Visitors	
8. Probability that visitors contacted at least with one infected animal when visiting a random farm, veterinarians (PV1), farm workers (PW1), hoof trimmers (PH1), and neighbors (PN1) $PV1\ (j,k) = 1 - (1 - HP\ (j,k) * [PPI\ or\ PIBR(j) * AcI]) * (1 - HP\ (j,k) * [PPI2\ or\ PIBR2(j) * AcI])\ )$ $PW1\ (j,k) = 1 - (1 - HP\ (j,k) * [PPI\ or\ PIBR(j) * AcI]) * (1 - HP\ (j,k) * [PPI2\ or\ PIBR2(j) * AcI])\ \ )$ $PH1\ (j,k) = 1 - (1 - HP\ (j,k) * [PPI2\ or\ PIBR2(k) * AcI])$ $PN1\ (j,k) = 1 - (1 - HP\ (j,k) * [PPI\ or\ PIBR(j) * AcI]) * (1 - HP\ (j,k) * [PPI2\ or\ PIBR2(j) * AcI])\ \ )$	
9. Probability that reusable material of veterinarians and hoof trimmers were contaminated when leaving from a random farm $PV2\ (j,k) = \ 0.2 * \left(\ PV1\ (j,k) * PLD * (1 - ECD\ ) +\ PV1(j,k) * (1 - PLD)\right)$ $PH2\ (j,k) = PH1\ (j,k) * PLD * (1 - ECD\ ) +\ PH1(j,k) * (1 - PLD)$	- *PLD:* probability of cleaning and disinfecting reusable material either by veterinarian or hoof trimmer
10. Probability that boots of veterinarians, hoof trimmers and farm workers were contaminated when leaving from a random farm $PV3\ (j,k) = PV1(j,k) * (1 - PB)$	- *PB:* proportion of farms providing boots and protective clothing to their visitors

$$PW2\ (j,k) = PW1(j,k) * (1 - PB)$$

$$PH3\ (j,k) = PH1(j,k) * (1 - PB)$$

11. Probability that the veterinarian and the hoof trimmer arrive contaminated at the next farm

$$PV4\ (j,k) = 1 - (1 - PV2(j,k) * srm) * (1 - PV3\ (j,k) * srm)$$

$$PH4\ (j,k) = 1 - (1 - PH2(j,k) * srm) * (1 - PH3\ (j,k) * srm)$$

- *srm:* BVDV survival in rubber and enameled metal (20 min -2 hours)

12. Probability that the veterinarian (PV5), hoof trimmer (PH5) became infected each day according the number of farms visited by them every day

$$PV5(j,k) = 1 - (0.8 * \left(1 - PV4\ (j,k)\right)^{n(j)\ or\ nclin(j)} + 0.2 * \left(1 - PV4\ (j,k)\right)^{n(j)*or\ nrest(i)})$$

$$PH5(j,k) = 1 - \left(1 - PH4\ (j,k)\right)^{n(j)}$$

- *nclin*: number of farms visited by clinical veterinarians
- *nrest*: number of farms visited by the rest of veterinarians

13. Probability that neighbors became infected each day according the number of farms visited by them every day

$$PN2(i,k) = 1 - \left(1 - PN1\ (j,k)\right)^{nn(j)}$$

- *nn:* number of neighboring farms

14. Annual probability of infection due to veterinarians (PV6), farms workers (PW3), hoof trimmers (PH5) and neighbors (PN3)

$$PV6(i,k) = 1 - (1 - PV5\ (j,k)\ * PTIC)^{AFV(i)}$$

$$PW3(i,k) = 1 - (1 - PW2\ (j,k)\ * PTIC)^{AFV(i)}$$

$$PH6(i,k) = 1 - (1 - PH5\ (j,k) * PTIC)^{AFV(i)}$$

$$PN3(i,k) = 1 - (1 - PN2\ (j,k) * PTIC)^{AFV/AFM(i)}$$

- *AFV:* number of times that the veterinarians, farm workers, hoof trimmers or neighbors visited each farm in one year
- *AFM:* number of times that material was shared between neighboring farms in one year

15. Probability that the farm could become infected in one year by indirect contacts (PIND) $PIND\ (i,k) = 1 - \left(1 - PC4(i,k)\right) * \left(1 - PS4(i,k)\right) * \left(1 - PR3(i,k)\right) * \left(1 - PF3(i,k)\right) * \left(1 - PV6(i,k)\right) * (1 - PW3\ (i,k) * (1 - PH6(i,k))* (1- PN3(i,k))$	• *PC4:* probability of infection due the calf vehicle • *PS4:* probability of infection due the slaughterhouse vehicle • *PR3:* probability of infection due the rendering vehicle • *PF3:* probability of infection due the feed vehicle • *PV6:* probability of infection due veterinarians • *PW3:* probability of infection due farms workers • *PH6:* probability of infection due hoof trimmers • *PN3:* probability of infection due neighbors

Table 2: Equations used to estimate probabilities inside the model to evaluate the probability of BVDV and BoHV-1 introduction in a dairy farm

4.3.4. Impact of biosecurity measures in the probability of BVDV and BoHV-1 introduction

The impact of biosecurity measures (IBM) to reduce the probability of BVDV and BoHV-1 introduction through indirect contacts was evaluated by calculating the probability of infection if each measure (rm) was applied in all study farms (i) compared with the median of original results (or). In percentage terms we used the follow estimation:

$IBM(i) = 1 - (median(rm(i)) / median(or(i))) * 100$

And the follows measures were included in this estimation:

Animal transport vehicles. Calf and slaughterhouse vehicles did not share transport with other cattle during the same trip (i.e., arrived empty at the farm), and vehicles drivers did not have contact with cattle present on the farm.

Rendering vehicle. The vehicle did not enter inside the farm perimeter to collect dead animals.

Protective clothing and boots. Each farm provided boots and protective clothing to all visitors who were expected have direct contact with animals.

4.3.5. Sensitivity Analysis

A sensitivity analysis was performed to evaluate the influence of uncertainty of input parameters in the model output for all study farms. Six uncertain input parameters were tested: i) the probability of BVDV and BoHV-1 indirect transmission; ii) the probability of clean and disinfected of material used by veterinarians and hoof trimmers; iii) number of visited farms by animal transport vehicles; iv) number of farms visited by veterinarians and hoof trimmers; v) the probability of sharing material during veterinarian visits; and vi) number of farms visited by feed vehicles and rendering vehicles. For the latter, the number of cadavers collected was also included. The six parameters listed above were reduced to half of the default value, and their effect in the model was evaluated independently.

4.4. Results

4.4.1. Characteristics and application of biosecurity measures in study farms

The herd size of the 127 study farms ranged from 15 to 954 (median=110). The annual frequency of indirect contacts by each farm considered in the model ranged from 66 to 712 (median=124). Rendering vehicles came once per month in most farms, while animal transport vehicles came mostly between one and two times per month. Veterinarians visited farms two to five times per month, and hoof trimmers made four visits per year. Most farms (n=124) used vehicles from an external company either to transport culled cattle to a slaughterhouse or calves for fattening and could arrive with cattle from other farms in 94.4% of slaughterhouse vehicles and 92.7% of calf vehicles. In addition, the driver could have had contact with animals present on the farm while helping to load cattle on the truck in 88.7% of slaughterhouse vehicles and 86.3% of calf vehicles. A rendering transport vehicle entered inside the farm perimeter in 80.4% of the study farms. Shared feed vehicles providing the mixture of food to the farm were used by 37.1% of the farms. Farm staff could also work in another bovine farm or had cows at home in 10.2% of the studied farms. Finally, only 7.9% of the farms provided boots and protective clothing exclusively for the farm.

4.4.2. Probability of BVDV and BoHV-1 introduction through indirect contacts in the 127 dairy farms analyzed

Figure 4 shows the distribution of the probability of BVDV and BoHV-1 introduction through indirect contacts to each study farms, and Table 3 presents summary statistics of median probability values (in percent) of BVDV and BoHV-1 introduction through indirect contacts to each farm. The distribution of median values was very heterogeneous. Median values varied from 0.5 to 14.6% and from 1.0 to 24.9% for BVDV and BoHV-1, respectively. In most farms, the median value was higher for BoHV-1 (3.9%) than for BVDV (2.1%). In 75% of farms, the median value was below 3.5% and 6.8% for BVDV and BoHV-1, respectively. Calf vehicles and veterinarians visits were routes with a higher probability of infection.

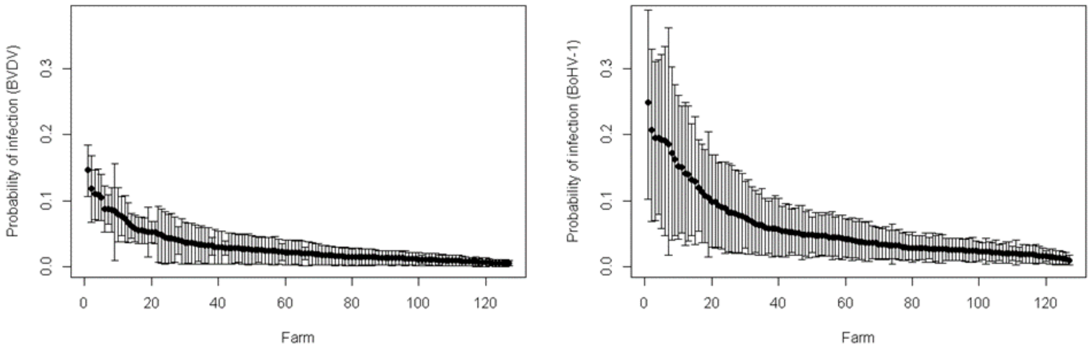

Figure 4. Distribution of the probability of Bovine viral diarrhea virus (BVDV) and Bovine Herpesvirus I (BoHV-1) introduction through indirect contacts to each of the studied farms (n=127).

		Bovine Viral Diarrhea Virus					**Bovine Herpesvirus I**				
Pathway	Route	Min[1]	Q1[2]	Median	Q3[3]	Max[4]	Min	Q1	Median	Q3	Max
Animal transport	Slaughterhouse vehicle	6.0^{-6}	2.6^{-4}	2.8^{-4}	8.3^{-4}	1.01	9.4^{-6}	0.35	0.37	1.05	7.72
	Calf vehicle	5.9^{-5}	0.46	0.49	1.23	8.42	7.4^{-5}	0.63	0.68	1.57	11.3
Other vehicles	Rendering vehicle	5.0^{-6}	$7.0^{-0.5}$	$8.0^{-0.5}$	$9.0^{-0.5}$	0.51	1.7^{-6}	2.8^{-3}	3.0^{-3}	3.2^{-3}	2.70
	Feed vehicle	0.0	0.0	0.0	0.1	0.37	0.0	0.0	0.0	0.18	1.01
Visitors	Veterinarians	7.0^{-4}	0.4	0.69	1.5	6.90	0.11	0.69	1.17	2.58	14.0
	Hoof trimmers	1.0^{-4}	3.2^{-4}	3.3^{-4}	7.9^{-3}	0.48	5.2^{-3}	7.3^{-3}	7.5^{-3}	0.22	2.20
	Farm workers	0.0	0.0	0.0	0.0	4.50	0.0	0.0	0.0	0.0	7.78
	Neighbors	4.9^{-5}	1.4^{-5}	9.9^{-5}	0.53	0.99	4.9^{-5}	3.3^{-4}	2.0^{-3}	1.03	1.71

Table 3: Summary statistics of the median probability values (in percent) of Bovine viral diarrhea virus and Bovine Herpesvirus I introduction through indirect contacts in the studied farms (n=127).

4.4.3. Impact of biosecurity measures on the probability of BVDV and BoHV-1 introduction through indirect contacts in the 127 dairy farms analyzed

Figure 5 shows the percentage of reduction in the median probability of BVDV and BoHV-1 introduction for studied farms. In general, wearing boots and protective clothing exclusively for the farm (BO), and not allowing the driver of the animal vehicle to come into contact with animals present at the farm (DR), were the measures with a higher impact on the probability of infection in most of the farms. And for BVDV, ensuring that the calf vehicles arrived empty to the farm (CE) also could reduce the probability of infection. While not allowing the rendering vehicle to enter inside the farm perimeter (REN) and ensuring that a slaughterhouse vehicle arrived empty to the farm (SLE) were the measures with a lower impact on both diseases.

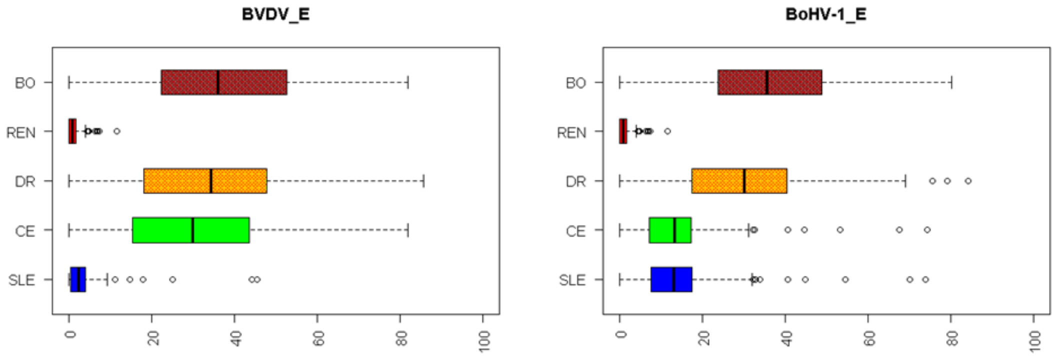

Figure 5: Percentage of reduction in the median probability of Bovine Viral Diarrhea Virus (BVDV_E) and Bovine herpesvirus-1 (BoHV-1_E) introduction after implementation of biosecurity measures in the studied farms (n=127). BO: farm provide boots and protective clothing to visitors; REN: rendering vehicle do not enter inside the farm perimeter; DR: drivers of animal transport vehicles do not come into contact with the animals present at the farm; CE: calf vehicle arrives empty; SLE: slaughterhouse vehicle arrives empty.

4.4.4. Model results and impact of biosecurity measures on probability of BVDV and BoHV-1 introduction a selected farm

The selected farm had a high probability of BVDV and BoHV1 introduction. The farm had about 150 cows in lactation, and replacement animals were reared at an external location. The animal transport vehicle was from an external company (for both the calf vehicle and the slaughterhouse vehicle) and could arrive with other cattle; the driver helped to loading animals on the truck and could have come into contact with cattle present at the farm. Calf and slaughterhouse vehicles arrived four and two times per month, respectively. The rendering vehicle visited the farm once per month but did not entered inside the perimeter. Veterinarians and hoof trimmers visited the farm four times per month and four times per year, respectively. The farm provided boots to visitors but not protective clothing. Farm staff included nine workers; one of them also worked at another farm, and this worker used the same boots on both farms. There five additional cattle farms in the area (i.e., 1 km radius) which did not share equipment or supplies with the selected farm.

The risk assessment model estimated that selected farm had an annual probability of BVDV and BoHV-1 introduction through indirect contacts of 14.6% (95%CI: 11.9%-17.9%) and 24.9% (95CI: 16.7%-32.9%), respectively. Routes with highest probability of diseases introduction were i) the calf transport vehicle: 8.3% (95%CI: 6.6%-17.9%) and 11.2% (95%CI: 4.7%-17.8%), respectively; ii) farm workers: 4.5% (95%CI: 3.9%-5.1%) and 7.7% (95%CI: 4.7%-11.1%), respectively; and iii) visits by veterinarians: 1.8% (95%CI: 0.1%-3.6%) and 4.2% (95%CI: 0.3%-8.1%), respectively. In this particular farm, the model evidenced that efforts should be devoted to avoid contact between the animal transport vehicle driver and cattle from the farm followed by the use of exclusive farm boots and protective clothing, as these measures had the highest impact on the probability of introduction (Figure 6).

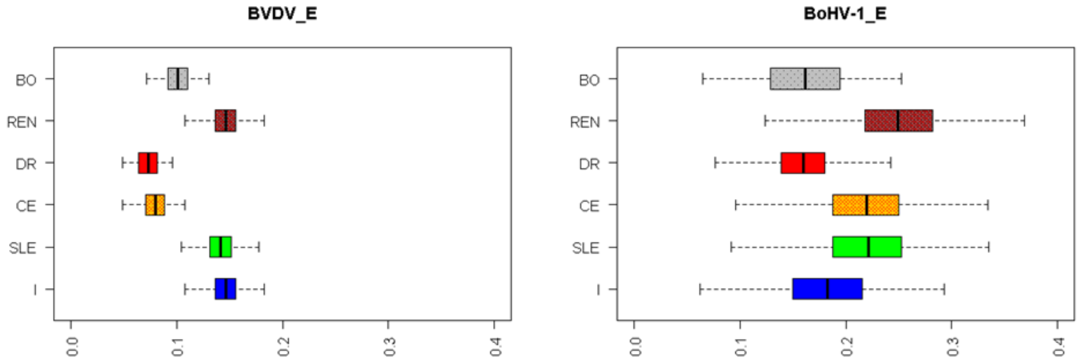

Figure 6: Probability of Bovine viral diarrhea virus (BVDV_E) and Bovine herpesvirus-1 (BoHV-1_E) introduction after implementation of biosecurity measures in selected farm. BO: farm provide protective clothing and boots to visitors; REN: rendering vehicle do not enter inside the farm perimeter; DR: drivers of animal transport vehicles do not come into contact with the animals present at the farm; CE: calf vehicle arrives empty; SLE: slaughterhouse vehicle arrives empty; I: initial median probability of BVDV and BoHV-1 introduction.

4.4.5. Sensitivity analysis

Figure 7 shows the proportion of change in median probability values of introduction to each of the studied farms, after changing values in selected parameters in the sensitivity analysis. The probability of transmission given an indirect contact (PT) was the most influential parameter in both diseases. When reducing PT value by half the probability of introduction decreased from of 21% to 71% for BVDV and 47% to 77% for BoHV-1. Changes in the number of visited farms by animal transport vehicles (VA) and the number of farms visited by veterinarian and hoof trimmers (VIS) also had a great influence on model results. The influence of VA was higher for BVDV. Changes in the proportion of farms where veterinarians shared material (MAT), in the number of farms visited by feed or rendering vehicles (OV), or in the probability of cleaning and disinfection of reusable material between farms by veterinarians and hoof trimmers (LDVIS) did not have a great influence on model results.

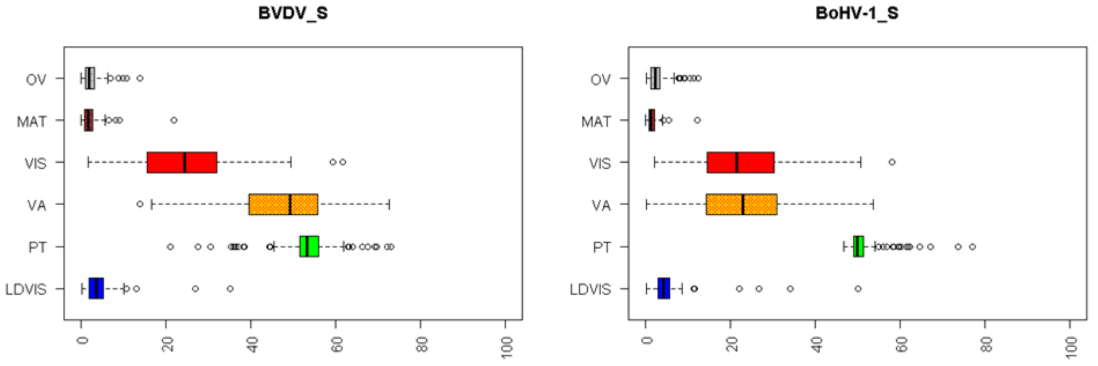

Figure 7: Proportion of change in the median probability values of Bovine Viral Diarrhea Virus (BVDV_S) and Bovine herpesvirus-1 (BoHV-1_S) introduction with alternative values (sensitivity analysis) in the studied farms (n=127). OV: number of farms visited by feed vehicle and rendering vehicle; MAT: proportion of farms with veterinarian material shared; VIS: number of farms visited by veterinarians and hoof trimmers; VA: number of visited farms by animal transport vehicles; PT: probability of BVDV and BoHV-1 indirect transmission; LDVIS: probability of clean and disinfection of material used by veterinarians and hoof trimmers.

4.5. Discussion

The results of this study showed that indirect contacts such as on-farm visitors and shared animal transport vehicles between farms can increase the probability of BVDV and BoHV-1 introduction on dairy farms. Among visitors, veterinarians were identified as having a higher probability of introducing BVDV and BoHV-1 into dairy farms studied. On the one hand, veterinarians were the most frequent visitors, and in the case of clinicians they made consecutive visits to multiple farms on a given day. Therefore, their potential of transmission of disease from one farm to another could be higher than other visitors. On the other hand, the time between one visit and another according to the discussion groups was short (e.g., 10-20 minutes), which probably made it to apply the recommended biosecurity protocols for their vehicle, equipment, and for themselves (i.e., cleaning and disinfection of their boots and material). Several studies also identified the veterinarian as the visitor with the highest risk of disease transmission between farms, because they visit more frequently different farms on the same day and have direct contact with the animals (Sibley, 2010; Nöremark et al., 2013; Rossi et al., 2017b) despite that they usually make the greatest biosecurity efforts among people visiting farms (Nöremark et al., 2010; Nöremark et al., 2013). For minimizing the probability of disease transmission by visitors, the use of boots and protective clothing is the best-known biosecurity measure, but unfortunately in the farms studied only ten provided them to visitors that were going to be in contact with animals. This result is in accordance with observations made in other countries. As an example, in Belgium, Sarrazin et al. (2014) reported that despite protective clothing and boots being present on several farms, they were infrequently used by visitors. Even farmers and farm workers carried out these hygienic measures with much lower frequency themselves. The risk assessment model developed in this study evidenced that implementing this biosecurity measure would have an important impact in reducing the probability of both viruses introduction through indirect contacts in most of the farms. Therefore, efforts should be made to raise awareness in visitors of the importance of using farm-specific protective clothing and boots when visiting it.

This study also identified the risk of the animal transport vehicles to introduce BVDV and BoHV-1 when these vehicles were shared between farms or when the driver had contact with the animals present on the farm. Previous studies reported that farmers sharing animal transports with other farmers is a common practice (Nöremark, 2010), as well as the risk that assumed by animals from different farms transported separately but in successive shipments (Bigras-Poulin et al., 2007; Smith et al., 2013). Although, to ensure the adequate cleaning and disinfection of the transporters between pickups is recommended (Alexandersen et al., 2003; Dee 2004; Smith et al., 2013), one study in Switzerland found that only 44% of calf transports are cleaned after each use (Schnyder, 2019), this proportion is similar to that found in this study. When evaluating biosecurity measures, the probability of introduction of both viruses is greatly reduced when the vehicle was not shared between farms (i.e., the vehicle arrived empty), because it reduces the risk of infected animals entering in the truck, and could contaminate the farm facilities or boots and clothing of drivers, especially for BVDV as PI animals shed large amounts of virus resulting in consistent and reliable transmission, even through contaminated objects (Niskanen and Lindberg, 2003).

As regards the vehicle drivers, the main measure recommended in the literature is requiring them to remain on the vehicles and outside the collection point during the loading process. Lowe et al. (2014) found this biosecurity measure may have a dramatic effect on limiting the transmission of infectious agents, and we obtained similar results in this study. The driver avoiding contact with cattle present in the farm had a high impact in reducing the probability of infection in several farms. Therefore, if cleaning/disinfection cannot be assured or cattle from other farms are present in the transport vehicle when visiting the farm, requiring the driver to remain in the vehicle would significantly reduce the probability of disease transmission.

Despite that indirect contacts had a lower probability of BVDV and BoHV-1 transmission compared to direct ones (i.e., movements of animals), 12% for BVDV and 9% for BoHV-1 (Benavides et al., 2020), 63% (81/127) of farms had no movements of animals in the study period, there was viral circulation, which shows the importance of indirect transmission routes for the viruses studied. Therefore, including direct and indirect transmission pathways allowed to have a complete picture about the different ways by which both viruses can be introduced into each farm, and quantifying the probability of disease transmission through direct and indirect contacts, allows prioritizing the implementation of biosecurity measures according to the current situation in each farm, since most farms just focus on those measures associated with the most apparent risks. For farms that moved animals, the most important measure was the correct application of quarantine, but in the farms that did not enter animals, it was a secondary measure, while the use of boots and personal protective clothing, together with animal transport drivers avoiding contact with cattle from the farm, were the most relevant measures. It is worth a mention that animal transport greatly influenced the probability of disease transmission in both models (i.e., direct and indirect contacts).

There were limitations to this study. First, few studies have evaluated for how long BVDV and BoHV-1 can survive in fomites and farm environments to understand livestock infection dynamics (Evans et al., 2019), which mean that accurate estimates could not be made of the probability that they survived between visits. In addition, there is little information as regards contamination rates of personnel, vehicles, or equipment after visiting infected farms and there are very few estimates on the probability of transmission given an indirect contact. Second, the frequency of visits was based on farm registers and the characteristics of the last farm visited by the people arriving to the farm was not available (just the frequency of visits) so, it was assumed that visits came from a random farm of the population. Third, farms in the study and people interviewed (e.g., transporters) were selected according to their willingness to participate. There might, therefore, be a classification bias in the estimations, as they might tend to answer what should be done and not what is done. Further studies to better characterize biosecurity practices among visitors (such as drivers) might be desirable to improve model accuracy. Despite the above-mentioned limitations, the model has the advantage in that it is flexible and biosecurity measures can be easily incorporated to study their effect in each particular case. Therefore, it might contribute to evaluate the effectiveness of the measures in each farm and to provide advice to farmers about the importance of biosecurity measures. It might also update veterinarians in biosecurity topics and infectious diseases, which could in turn improve acceptance and enforcement of biosecurity practices at farm level.

4.6. Conclusion

The developed risk assessment tool enables prioritization based on the impact of farm-specific biosecurity practices in the probability of BVD and BoHV-1 introduction on dairy cattle farms. This tool could help to develop structural, feasible and reasonable biosecurity protocols between farmers and veterinarians focusing on prevention under daily field practice conditions. Results in the studied farms from Spain provide evidence that efforts should be made to review biosecurity practices in relation to animal transport and visitors in most of the farms. Providing farm specific protective clothing and boots and avoiding animal transport drivers having contact with animals present in the farm would highly reduce the probability of infection through indirect contacts.

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General discussion

Chapter V

The importance of biosecurity is increasingly recognized due to the benefits related to animal health, increasing the profitability, and reducing the costs of diseases. A biosecurity program should be specific to each animal species or production system and it should differ for all farm operations because even within a group, risks vary by the type of management. The swine and poultry industries have implemented to a greater extent biosecurity programs with specific and detailed biosecurity guidelines. However, other production industries, such as sheep and cattle, have less explicit recommendations with fewer guides regarding biosecurity practices (Moore et al., 2008). The results shown in this doctoral thesis provide useful information for the development of biosecurity programs at the farm level according on-farm specific characteristics, that might contribute to maintaining a low risk of introduction of infectious diseases and that allows prioritizing the measures that should be applied in the daily routine.

Livestock biosecurity and its implementation are complex. The application of biosecurity measures depends largely on the attitude and perception of risk (Casal et al., 2007; Gunn et al., 2008; Kristensen and Jakobsen, 2011). Despite awareness of biosecurity may exist, its implementation at farm level is often poor (Faust et al., 2001; Hoe and Ruegg, 2006; Brandt et al., 2008; Gunn et al., 2008; Mee et al., 2012), because farmers ignore the potential risks to their farms and/or they have a lack of understanding of the value of implementing recommendations (Moore et al., 2008). Some of the obstacles to the implementation or improvement of biosecurity at farm level are associated with knowledge about the mechanisms of disease transmission (Sayers et al., 2014; Pritchard et al., 2015) and efficacy of biosecurity measures (Gunn et al., 2008).

Risk analysis (Murray et al., 2014), which has been applied mainly in the context of international trade to assess the risk of entry of pathogens into a country (for example, Santman-Berends et al., 2017) allows to estimate the risk of introduction and to evaluate the impact of control interventions. Therefore, it might be also a useful methodology to evaluate the efficacy of biosecurity measures on the probability of disease introduction in a farm, based on the mechanisms of disease transmission and farm specific characteristics. The quantification of the probability of infection for each farm based on its characteristics is the best starting point for decision-making, as allows prioritizing biosecurity measures according to risk which together with the availability of resources (financial, labor, and farm infrastructure) might motivate farmers and field veterinarians to implement an efficient biosecurity program (Wells et al., 2002; Mee et al., 2012).

Because of the strengths of risk analysis, we decided to follow this methodology in this doctoral thesis.

Results obtained through this thesis showed that the probability of entry of a pathogen can be differentiated based on the characteristics of the farms and the transmission routes. In general, direct contacts through animal movements had a higher probability of infection than indirect contacts, which is in accordance to the importance of animal movements in disease spread (Van Schaik et al., 2002; Niskanen and Lindberg, 2003; Mee et al., 2012). However, most of the dairy herds included in the study, did not have introduced cattle in their farms, and were exposed only to indirect contacts. In several of these farms, BVDV and / or BoHV-1 circulation was found, suggesting the importance of indirect transmission pathways. On the other hand, it was found that there is an increased probability of infection during transport by direct contact between animals, by contamination of the vehicle, or by the presence of other animals in the vehicle, showing the importance to control all routes of transmission to prevent disease introduction to the farm.

Biosecurity measures related to visitors in dairy farms, are not applied usually, such as the presence of perimeter fences, the registration of visitors or the use of protective clothing and boots, in comparison with intensive production system (Moore, et al., 2008; Nöremark et al., 2010). Despite that, most of the biosecurity measures which farmers perceive as important are related to the reduction of risks attributable to the entrance of people and vehicles that could act as carriers of infectious agents (Sarrazin et al., 2014). Casal et al., (2007), reported in pig farms that farmers considering important to restrict visits did not apply any basic measures to reduce them, this fact could indicate that some farmers may be aware that, as a concept, visits may imply a risk, but they do not see a visitor as a real risk. In dairy farms this could also happen, as there were very few farms that provided protective personal equipment to visitors, having this measure a high impact in reducing the probability of disease introduction through indirect contacts. The developed models might contribute to increase awareness to implement such biosecurity measures.

According literature, vehicles such as rendering company vehicles should be considered as a biosecurity risk because disease transmission through excreta of cadavers, and indirectly via fomites contaminated by them (Falkenberg et al., 2018); or the feed vehicle because they visit several herds on the same day (Ribbens et al., 2009; Nöremark et al., 2013). However, in this thesis, the estimated risk attributed to these vehicles was low, mainly due to the low probability of transmission of BVDV and BoHV-1 through cadavers. On the other hand, the feed vehicles that entered to the corridors where animals have their feed was described in few farms (49/127). Furthermore, the potential of transmission by these vehicles could be affected for many external factors such as the virus survival on different surfaces of the vehicle (e.g., tires), persistence of the virus in the ground and the ability to infect an animal from this type of substrate.

The impact of the application of the most common biosecurity measures for the studied viruses was also evaluated. For the model of animal movements, the impact of main biosecurity measures was evaluated in a single farm, therefore it cannot be generalized. However, the results were in accordance with what is widely recommended for the entry of new animals into the herd, ranking in the first place the correct use of quarantine, in terms of facilities, duration and management within the daily work routine (Villarroel et al., 2007; Maunsell and Donovan, 2008). Taking into account the heterogeneity of movements in the analyzed farms, it would be interesting to evaluate those biosecurity measures in all farms studied as was done in the indirect contacts model. In addition, it should be included in the analysis other common practices such as reducing the number of animals purchased and the number of herds from which the animals can be purchased (Kristensen and Jakobsen, 2011; Mee et al., 2012), or evaluate the effect of combining different measures. For example, for visitors the use of boots and clothing exclusive of the farm greatly reduced the probability of BVDV and BoHV-1 introduction, which could also be considered in fulfill quarantine for animals entering. On the other hand, the measure with the greatest impact was not identified, because in the current study we did not establish whether its impact was greater than or equal to the other evaluated measures. Therefore, further analysis by using these models should be performed in order to evaluate the measure that have the greatest impact in each farm, and the measures that would be more worthwhile based on their cost.

In the development of the models we had the support of field veterinarians to identify the main pathways to BVDV and BoHV-1 introduction in dairy farms and to clarify some field practices about which we had no information, such as the transportation of animals in the study areas or the number of daily visits made by a veterinarian. This highlights the absence of systematized records of routine dairy farm activities, such as the entry of vehicles, people, etc., and for transport companies, veterinarians, hoof trimmers or visitors in general, recording daily routes. Previous studies identified the value of recording and sharing information to improve the risk classification of animals that enter in cattle farms (Hobbs, 2004; Damiaans, et al., 2019). Moreover, this information would be especially useful for estimating input parameters with greater precision, facilitating the development of more robust epidemiological models on transmission, spread and prediction of infectious diseases outbreaks.

On the other hand, digitalization of this information could provide accurate data on disease risks for all stakeholders in animal health. For example, for improving cattle trading decisions based on the disease risk of animals that will be purchased by farmers or risk-based trading markets for cattle, in which disease-free livestock bring an economical compensation to the farmer. Building confidence for consumers generate greater social responsibility in livestock traders, livestock keepers and decision makers (Enticott, 2016).

Currently, livestock systems have access to technologies to generate more precise information, such as system detecting automatic estrus in dairy cows (Rutten et al., 2014) or the automatic body weight scoring system to estimate body weight in dairy cattle (Bewley et al., 2010) or detect earlier stages of diseases. These technologies in primary production should be understand as components that influence food supply chain through traceability and generate evidence of safety that could increase the trust of consumers (Rojo-Gimeno et al., 2019). In these types of cooperative systems, social or community trust is one component in building a successful system for disease prevention like biosecurity programs based on effective communication between stakeholders (Frössling and Nöremark, 2016). For these reasons, we believe that the usefulness of tools such as those generated in this thesis promote the use of accurate information that can be captured, analyzed, and used for decision making in the manner proposed for the use of the Big Data in farming operations (Wolfert et al., 2017).

In addition, farmer education and updating veterinarians in biosecurity topics and infectious diseases could improve acceptance and enforcement of biosecurity practices at the farm level. A lack of understanding of how infectious diseases can spread and in what way the biosecurity measures are supposed to break the different routes of transmission are necessary to raise awareness of the implementation of biosecurity measures. Veterinarians should help famers to better understand the routes of transmission for diseases that are most threatening to their operations. To develop optimal plans aimed at preventing disease transmission, they should ensure that farmers understand reasons behind their advice and giving farmers the opportunity to express their viewpoints, contributing to build teamwork between the farmer and the veterinarian to structure a reasonable protocol focusing on prevention under daily field practice conditions (Brennan and Christley 2012; Laanen et al. 2014; Ritter et al., 2018). Furthermore, education, training, and the involvement of all stakeholders are essential for the success of biosecurity at the enterprise, regional, and national levels, so, hence particular improved knowledge transfer so that all stakeholders can identify their own role and improve farm-level biosecurity (Robertson, 2020).

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General conclusions

Chapter VI

1. The annual median probability of BVDV and BoHV-1 introduction through animal movements (in farms that entered animals) was very heterogeneous for both diseases. For some farms, the probability was close to 0% whereas for others was 99%. Nevertheless, in 75% of the studied farms such probability was below 28% and 23% for BVDV and BoHV-1, respectively.

2. Shared transport with cattle from other farms had the highest probability of BVDV and BoHV-1 introduction. Therefore, animal transport practices should be improved to reduce such risk.

3. The annual probability of introducing BVDV or BoHV-1 through indirect contacts ranged between 0.5% and 15% and from 1% to 25% respectively, in the 127 farms that were evaluated.

4. Among indirect contacts, allowing drivers from calf vehicles to contact with cattle present at the farm and visits of veterinarians had the highest probability of BVDV and BoHV-1 introduction. Therefore, biosecurity measures against these routes should be improved.

5. A correct quarantine (i.e., testing cattle on arrival, duration period of at least 24 days and quarantine visited at the end of the workday) and providing boots and protective clothing to visitors greatly reduced the probability of infection by direct and indirect contacts, respectively. Therefore, their implementation in dairy farms should be encouraged.

Appendix

Chapter VII

Annex A – Supplemetary Information Study I

DEVELOPMENT OF A QUANTITATIVE RISK ASSESSMENT OF BOVINE VIRAL DIARRHEA VIRUS AND BOVINE HERPESVIRUS-1 INTRODUCTION IN DAIRY CATTLE HERDS TO IMPROVE BIOSECURITY

B. Benavides, J. Casal, J.F. Diéguez, E. Yus, S.J. Moya, R. Armengol, and A. Allepuz

Background	Years of expertise	Number of dairy farms advised
Animal health, reproduction, and bovine clinic	40	25
Animal health, reproduction, and technical management	15	22
Animal health and reproduction	4	15
Animal health and reproduction	28	26
Animal health, reproduction, and milk quality	20	20

Table A1. Characterization of field veterinarians which participated in the discussion groups

Equation	Description
Probability of purchasing an infected animal	
1. Number of animals purchased each time from country/area “i” and age group “k” $n\ (i,k) = \frac{\text{animals (i, k)/ times (i)}}{\text{farms (i)}}$	• *animals (i, k):* number of animals purchased throughout the year from country/area “i” of group age “k” per farm. • *times (i):* number of times animals have been purchased from country/area “i” in one year.

	- *farms (i):* number of farms from which animals can be purchased in each country/area “i”.
2. Probability that at least one animal from a batch coming from a single farm of a certain country/area “i” was already infected with BVDV or BoHV-1“j” (being “j” a subscript indicating if the animal was a PI, TI, TR or BoHV-1 seropositive animal). $Pinf\ (i,j) = 1 - (1 - HerdPrev\ (i) * \llbracket WithiPrev) \rrbracket\ \hat{}(n(i,k) * gest(i)\ )$	- *HerdPrev (i):* herd prevalence in country/area “i”. - *WithinPrev:* the proportion of seropositive (BoHV-1) and viremic (BVDV) animals - *gest (i):* proportion of pregnant animals imported from country/area “i” (this parameter was only used for BVDV calculations, and in the case of animals greater than 12 months).
3.1 Probability that a single animal was infected $Pinf\_Animal\ (i,j) = 1 - (1 - Pinf\ (i,j)\ )^{\frac{1}{n}(i,k) * gest(i)}$ 3.2 Expected number of BVDV and BoHV-1 animals already infected in the farm of origin $Animals\_Infected\ (i,j) = Pinf\_Animal\ (i,j) * n(i,k) * gest(i)$	
Probability of false negatives	
4. Probability that one infected animal yielded a false negative result $P\_fal\_neg(i,j) = \frac{Pinf\_Animal\ (i,j) * (1 - Se)}{((1 - Se) * Pinf\_Animal\ (i,j) + (Sp * (1 - Pinf\_Animal\ (i,j))}$	- *Pinf_Animal:* Probability that a single animal was infected - *Se*: sensitivity - *Sp*: specificity

5.1 Probability that at least one infected animal was present in the batch of animals $P\_fal\_neg\_batch\ (i,j) = 1-(1-\ \llbracket P\_fal\_neg\ (i,j)\ )\rrbracket\ \hat{}(Animals\_Infected\ (i,j)))$	- *P_fal_neg:* Probability that one infected animal yielded a false negative result - *Animals_Infected:* Expected number of BVDV and BoHV-1 animals already infected in the farm of origin
5.2 Probability that at least one infected animal was purchased from at least one of the farms from which animals could be introduced $Pinf\_batch\ (i,j) = 1-(1-\ P\_fal\_neg\_batch\ \ (i,j)\ )^{farms\ (i))}$	- *P_fal_neg_batch:* Probability that at least one infected animal was present in the batch of animals
Probability of infection during transport	
6.1 Probability of sharing a transport vehicle with at least one PI or AI animal in country/area “i” $P\_share\ \ (i)\ \ =\ \ \llbracket HerdPrev\ (i) * (1-(1-\ WithinPrev(i) * P\_AI\ or\ P\_PI)\rrbracket\ \hat{}loaded$	- *P_AI:* probability of an active infection in a group of BoHV-1 seropositive animals - *P_PI:* probability of PI animal - *Loaded*: number of animals loaded by farm
6.2 Probability of sharing a transport vehicle with a PI or AI animal when purchasing animals from country/area “i” $P\_I\_V\ (i) = 1-(1-P\_share(i))\hat{}Visited$	- *P_share:* Probability of sharing a transport vehicle with at least one PI or AI animal in country/area “i” - *Visited*: number of farms visited by country/area
7. Probability that at least one non-infected purchased animal of the age group “k” yielded an infection status “j” due to sharing transport vehicle with a PI or AI animal from each country/area “i” $P_{BVD_{Shared}}(i,j) = 1-(1-\ \llbracket P\_I\_V\ (i))\ \rrbracket\ \hat{}(healthy(i,k) * gest(i)\ )$	- *P_I_V:* Probability of sharing a transport vehicle with a PI or AI animal when purchasing animals from country/area “i” - *Healthy*: non infected purchased animal - *gest (i):* proportion of pregnant animals imported

$$P\_IBR\_Shared\ (i) = 1-(1-\ P\_I\_V(i))^{\ healthy(i)}$$

Probability of infection due to being transported in a contaminated vehicle	
8. Probability that at least one non-infected animal was infected due to being transported in a vehicle coming from country ‘i’ and develop an infectious status ‘j’ $P\_I\_C\ (i,j) = 1-(P\_I\_V(i)*C(i)\ *(1-E)*S*PT\ +\ P\_I\_V(i)*(1-C(i))*S*PT)^{\ healthy(i,k)}$	• *P_I_V:* Probability of sharing a transport vehicle with a PI or AI animal when purchasing animals from country/area “i” • *C*: probability of cleaning and disinfecting the vehicle between transports in country/area “i”. • *E*: efficacy of the cleaning and disinfection • *S*: survival of BVDV in metal • *PT*: probability of infection of a susceptible animal in a contaminated surface.
Annual probability of infection by BVDV or BoHV-1 due to purchasing animals	
9. $P\_BVD\_Purchase = 1-\prod_{i=1}^{5}\ (1-PI\_Y(i))*\prod_{i=1}^{5}\ (1-TI\_Y(i))*\prod_{i=1}^{5}\ (1-TR\_Y\ (i))$ $P\_IBR\_Purchase = 1-\prod_{i=1}^{5}\ (1-IBR\_Pos\_Y(i))$	• *PI_Y:* annual probability of purchasing at least one PI • *TI_Y:* annual probability of purchasing at least one TI • *TR_Y:* annual probability of purchasing at least one TR • *IBR_Pos_Y:* annual probability of purchasing at least one TR

Table A2: Equations used to estimate probabilities inside the model to evaluate the probability of BVDV and BoHV-1 introduction in a dairy farm

Fomite	Treatment	Survival time (Hours)				
		1	2	4	12	24
Rubber	Mucus	88.6%	75%	53.6%	10.4%	5.6%
Galvanized metal	Mucus	21.5%	9.6%	3.9%	0.4%	0.2%
Soil	Mucus	47.9%	26.2%	12.0%	1.4%	0.7%

Table A3: Probability of Bovine Viral Diarrhea Virus survival after application to the potential fomite *Treatment: Prior to application to the potential fomite, the virus was diluted in PBS that included 20% synthetic mucus. Source: Stevens et al., 2011.

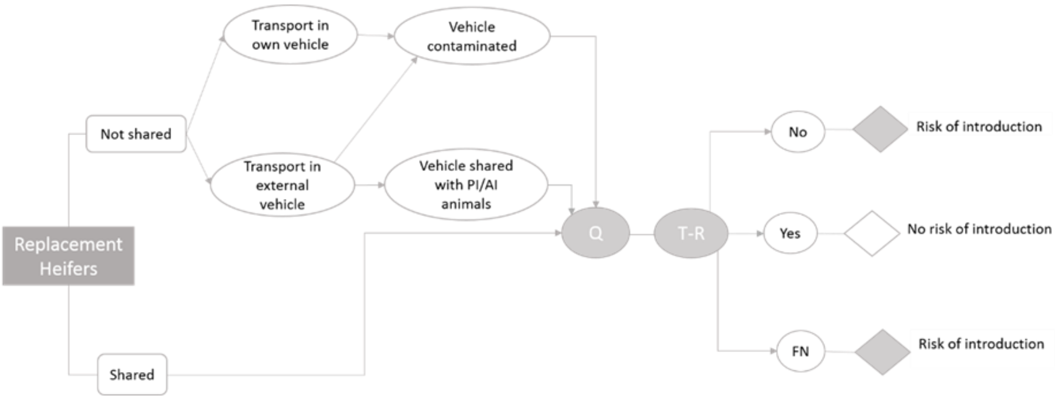

Figure A1: Pathway of entrance according types reared replacement heifers offsite. Shared/Not shared: when place to reared heifers is shared or not with animals of other farms; PI: Persistently infected with Bovine Viral Diarrhea Virus; AI: Acutely infected with Bovine Herpesvirus-1; Q: Quarantine; T: Test on quarantine; R: Quarantine routines; FN: False negative.

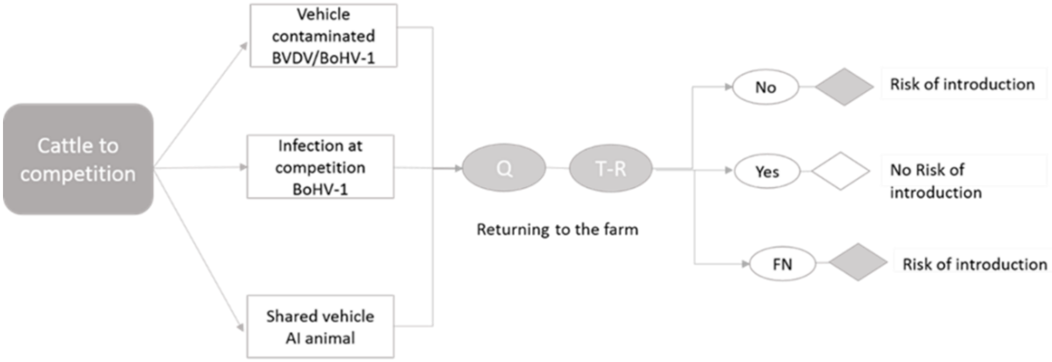

Figure A2: Pathway of entrance according to the scenarios considered for the movements to cattle competitions; AI: Acutely infected with Bovine Herpesvirus-1; Q: Quarantine; T: Test on quarantine; R: Quarantine routines; FN: False negative.

Annex B – Supplemetary Information Study II

QUANTITATIVE RISK ASSESSMENT OF BVDV AND BoHV-1 INTRODUCTION THROUGH INDIRECT CONTACTS BASED ON IMPLEMENTED BIOSECURITY MEASURES IN DAIRY FARMS OF SPAIN

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Veterinarian profile	Years of expertise	Number of dairy farms advised
Animal health, reproduction, and bovine clinic	40	25
Nutrition and economic advice	5	25
Animal health, reproduction, and milk quality	20	20
Animal health	35	125
Animal health	13	90
Farm health management	15	1
Bovine clinic	19	NC*
Nutrition, bovine clinic, and livestock association manager	33	1750**
Bovine clinic	9	50
Animal health and production laboratory	27	NA***

Table A1. Characterization of field veterinarians who participated in discussion groups. *NC: Non countable; ** Number of members of livestock association; ***NA: Not apply.