Bovine Coronavirus and Bovine Respiratory Syncytial Virus Infections in Dairy Herds

Prospects for Control

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Abstract

Bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) are significant causes of enteric and respiratory disease in cattle throughout the world. The aim of this thesis was to gain further knowledge of the epidemiology of these infections in Swedish dairy herds.

Two studies were conducted of the association between BCV/BRSV antibody status and health and performance, on herd (79 herds) and individual animal level (65 herds). The analyses were carried out using survival, linear regression, and logistic regression models. Herds that were antibody-positive to BCV and/or BRSV had higher bulk tank milk (BTM) somatic cell count than herds negative to both BCV and BRSV. Cows in herds with recent BRSV infection had a lower milk yield than cows in BRSV-free herds, and cows in herds with clinical signs of BRSV infection had a higher somatic cell count than cows in herds without clinical signs.

In the third study, the effect of herd-level risk factors on antibody status was quantified in 257 herds using logistic regression models and spatial analysis. Large herd size, being located in southern Sweden, and not providing boots for visitors were found to be associated with antibody positivity to BCV and BRSV, while short distance to nearest cattle herd was additionally associated with positivity to BCV. Providing boots for visitors likely reflects herds with a high level of biosecurity measures. Neither BCV-positive nor BRSV-positive herds were spatially clustered, indicating that local spread and airborne transmission are not of great importance.

The fourth study investigated the long-term dynamics of BCV and BRSV infections in 20 herds from each of two southern and two northern areas. There was a high prevalence of BCV and BRSV amongst the herds in the southern areas, and a lower prevalence in the northern areas; one of the latter was free from BRSV at the end of the study. There was also self-clearance of BCV and BRSV, manifesting as conversion from antibody positive to negative in pooled milk samples and BTM.

The results of this thesis suggest that it is possible to develop inexpensive strategies for controlling BCV and BSRV infections, based on biosecurity and antibody monitoring. Such a control strategy would improve cattle and calf health.

Keywords: BCV, BRSV, antibody detection, performance, biosecurity, cattle, control, herd health, risk factors, spatial correlation

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Dedication

To my family

”Det går aldrig – och dessutom är det omöjligt”
Öländsk devis

“Who's to say
What's impossible
Well they forgot
This world keeps spinning
And with each new day
I can feel a change in everything
…
I want to turn the whole thing upside down
I'll find the things they say just can't be found
I'll share this love I find with everyone”
Jack Johnson, Upside down (Curious George 2005)

Contents

List of Publications

This thesis is based on the work contained in the following papers, which are referred to in the text by Roman numerals:

I Ohlson, A. Emanuelson, U., Tråvén, M., Alenius, S. (2010). The relationship between antibody status to bovine corona virus and bovine respiratory syncytial virus and disease incidence, reproduction and herd characteristics in dairy herds. *Acta Veterinaria Scandinavica* 53:37.

II Beaudeau, F., Ohlson, A., Emanuelson, U. (2010). Associations between bovine coronavirus and bovine respiratory syncytial virus infections and animal performance in Swedish dairy herds. *Journal of Dairy Sciences* 93 (4), 1523-1533.

III Ohlson, A., Heuer, C., Lockhart, C., Tråvén, M., Emanuelson, U., Alenius, S. (2010). Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds. *Veterinary Record* Aug 7; 167(6): 201-6.

IV Ohlson, A. Tråvén, M., Emanuelson, U., Alenius, S. (2010). A longitudinal study of the dynamics of bovine coronavirus and bovine respiratory syncytial virus infections in dairy herds (submitted).

Abbreviations

AI	artificial insemination
BCV	bovine coronavirus
BRSV	bovine respiratory syncytial virus
BTM	bulk tank milk
ELISA	enzyme-linked immunosorbent assay
HRSV	human respiratory syncytial virus
Ig	immunoglobulin
NADRS	national animal disease recording system
SCC	somatic cell count
SOMRS	Swedish official milk recording system

1 Introduction

Bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) are two contagious pathogens affecting beef and dairy cattle worldwide (Valarcher & Taylor, 2007; Clark, 1993). Infection with BCV causes diarrhoea and various degrees of respiratory tract disease, while BRSV infection manifests as respiratory tract disease, fever, and in severe cases subcutaneous emphysema and death. These infections are widespread in Swedish cattle herds, with negative consequences both for animal welfare and for the economic situation of the farmer (Elvander, 1996; Tråvén *et al.*, 1993; Larsson *et al.*, 1991; Jacobsson *et al.*, 1989).

A common belief among Swedish farmers and veterinarians is that the inter-herd spread of BCV and BRSV cannot be controlled. Some even believe that regular infection is beneficial, in that it confers immunity on the animals. Observations show, however, that there are dairy herds that have stayed free from BCV and BRSV infections for many years. To develop effective control strategies for BCV and BRSV the epidemiology needs to be clarified, including risk factors for transmission and accurate estimates of the associated effects on herd health and performance.

The overall aim of this doctoral research was to gain further knowledge of the epidemiology of BCV and BRSV infections in Swedish dairy herds. Chapter 2 gives a general background to BCV and BRSV infections in dairy herds; chapters 3–6 are devoted to the studies conducted during this doctoral work; and chapter 7 summarises areas identified for further research.

2 Background

2.1 Beef and dairy cattle in Sweden

In 2009 there were roughly 1.5 million cattle in Sweden, 360 000 of which were dairy cows and 190 000 suckler cows (Swedish Board of Agriculture, 2010). These dairy cattle were organised into 6020 herds with a mean size of 59 cows and a mean milk yield of 9162 kg per cow-year. The development of dairy herds in recent decades has been towards fewer but larger herds; between 1980 and 2008, the number of herds decreased by 85% but the number of dairy cows by only 46%. As a comparison, the number of beef holdings was 11 900 in 2009, whit a mean size of 16 cows. There is a stable season and an outdoor season; farmers are bound to have all adult cattle on pasture during summer (at least 2–4 months, depending on location). There are great regional differences in herd density; it is higher in the southern parts of Sweden and low or sparse in the north (Figure 1).

Veterinarians are obliged to report individual treatments, vaccinations, and diagnosis for all production animals to the Swedish Board of Agriculture via the national animal disease recording system (NADRS; Emanuelson, 1988). More than 90% of Swedish dairy cows are additionally enrolled in the Swedish official milk recording scheme (SOMRS) which record individual data on production and reproduction (Mörk *et al.*, 2010; Mörk *et al.*, 2009; Olsson *et al.*, 2001).

Sweden has a long history of successful control programs against infectious diseases in cattle (National Veterinary Institute, 2009). Swedish cattle herds are declared free from bovine herpes virus 1 and bovine leukaemia virus, and currently 99.8% of the cattle herds are free from bovine viral diarrhoea virus (BVDV) according to the rules of the national eradication program (Lindberg & Alenius, 1999). Mycobacterium avium subspecies paratuberculosis has never been detected in Swedish dairy cattle,

and there have only been sporadic cases in beef herds, all associated with imported animals.

2.2 Bovine coronavirus

2.2.1 Aetiology

Coronaviruses are enveloped, single stranded and positive sense RNA virus of the genus *Coronavirus* in the Coronaviridae family (Clark, 1993). It has a pleomorphic to spherical shape with prominent surface projections, resembling the corona of the sun (Lai, 1990). The envelope consists of a lipid layer derived from the plasma membrane of the host cell; if this is disrupted, the virus completely loses its infectivity. Five major structural proteins are encoded, one being the spike (S) protein, which is important for virus-host interaction and antigenicity and is also used for comparative analysis in molecular epidemiology (Liu *et al.*, 2006; Gallagher & Buchmeier, 2001; Kubo *et al.*, 1994; Yoo *et al.*, 1991). There are reports of small genetic differences between isolates from winter dysentery (the clinical name for coronavirus-induced enteritis in adults), and calf diarrhoea (Kourtesis *et al.*, 2001; Dea *et al.*, 1995; Tsunemitsu & Saif, 1995), and between isolates from enteric and respiratory origin (Hasoksuz et al., 2002; Hasoksuz et al., 1999; Reynolds *et al.*, 1985), but no consistent antigenic or genetic markers have yet been identified (Saif, 2010).

Coronaviruses are divided into three groups based on phylogeny; BCV belongs to the second group together with porcine haemagglutinating encephalomyelitis virus, equine coronavirus, and human coronavirus OC43, among others (Woo *et al.*, 2009). Coronaviruses are adaptable, which has led to a diversity of strains and genotypes and more closely related coronavirus has been observed due to recent interspecies jumping. This may be the cause of zoonotic outbreaks with catastrophic consequences, for instance the severe acute respiratory syndrome (SARS) epidemic in 2003.

2.2.2 History

BCV was first associated with diarrhoea in calves (Stair *et al.*, 1972), and serological evidence later emerged for it also being the causative agent for winter dysentery (Alenius *et al.*, 1991; Emanuelson *et al.*, 1989). This evidence, which was presented in 1989, confirmed previous results indicating this correlation (Espinasse *et al.*, 1982; Takahashi, 1980).

The first report of winter dysentery came from the USA in 1915 (Jones & Little 1931), and was followed by disease reports from several countries. In 1946 there was an acute outbreak of winter dysentery among cattle in the

south of Sweden, and by March 1948 this had spread all over the country (Hedström & Isaksson, 1951).

The infection now appears to exist worldwide, with reports from Brazil, Turkey and South Korea among others (Brandao *et al.*, 2006; Hasoksuz *et al.*, 2005; Jeong *et al.*, 2005), as well as from of the Scandinavian countries (Gulliksen *et al.*, 2009a; Hägglund *et al.*, 2006; Liu *et al.*, 2006; Härtel *et al.*, 2004).

2.2.3 Pathogenesis

BCV virus enters via the oral and respiratory routes, and replicates in the surface epithelial cells, particularly in the lower small intestine but also in the colon and in the respiratory tract (Clark, 1993; Saif *et al.*, 1986; Reynolds, 1983). Infected cells die and are replaced by immature cells. The diarrhoea that follows is caused by malabsorption due to a loss of surface area, which decreases the digestive and absorptive capacities and leads to osmotic imbalance. The infection is usually self-limiting; the crypt epithelial cells are rarely attacked and can produce new cells resistant to virus.

2.2.4 Clinical picture

The clinical manifestation of BCV is watery diarrhoea, possibly mixed with blood, and possibly with a characteristic odour (Clark, 1993; Alenius *et al.*, 1991; Saif *et al.*, 1988). The incubation period is 2–6 days, and the diarrhoea commonly lasts for 3–6 days. A decrease in milk yield has been described in both experimental (Tråvén *et al.*, 2001) and field studies (Tråvén *et al.*, 1999; Jactel *et al.*, 1990; Saif *et al.*, 1988). In addition to diarrhoea, various degrees of respiratory symptoms can occur in both calves and adults (Saif, 2010). A concurrent BVDV infection has been shown to aggravate the clinical symptoms of BCV (Niskanen *et al.*, 2002; Alenius *et al.*, 1991). Treatment is usually not necessary, but in severe cases rehydration and rebalancing of electrolytes are needed (Clark, 1993).

BCV infections has a high morbidity but usually a low mortality — it is effectively spread to all susceptible animals within the herd (Bidokhti *et al.*, 2009; Hägglund *et al.*, 2006), and in an experimental study it was not possible to prevent the spread of BCV from the infected calves to the control group (Niskanen *et al.*, 2002).

2.2.5 Immune response

The first antibody isotope produced after an infection is IgM, which is detectable in serum and milk during the acute phase from day 2–7 after infection and remains detectable for 3–6 weeks, as measured after an

experimental infection of seronegative animals (Tråvén *et al.*, 2001). IgA is produced to protect mucosal surfaces from day 7–9, and by day 9-11 IgG antibodies can be detected in milk and serum. The IgG levels may remain high for at least a year after the infection even without reinfection (Alenius *et al.*, 1991). Antibodies are passed to the offspring via the colostrum; these are detectable in the sera until approximately 5–6 months of age, and can disturb the immune response to a natural infection (Alenius *et al.*, 1991; Heckert *et al.*, 1991). The efficiency and duration of a naturally-acquired immunity is unknown (Saif, 2010).

2.2.6 Diagnostic methods

BCV infection can be diagnosed either directly, by detection of virus or viral nucleic acid in faecal samples and nasal swabs, or indirectly, by antibody detection in milk and serum.

Particles of BCV can be demonstrated via direct or immune electron microscopy (Heckert *et al.*, 1989; Saif *et al.*, 1986). Isolation of BCV in tissue or cell culture is a technique used mainly for research purposes, since it is difficult and time-consuming. Reverse transcription polymerase chain reaction (RT-PCR) and nested PCR are sensitive methods to detect BCV RNA (Cho *et al.*, 2001a).

Detection of antibodies by indirect enzyme-linked immunosorbent assays (ELISA) is widely used, especially for epidemiological research such as prevalence studies and herd monitoring (Beaudeau *et al.*, 2010; Hägglund *et al.*, 2006; Tråvén *et al.*, 1999; Paton *et al.*, 1998). Isotype-capture ELISA to detect BCV-specific IgA and IgM is used to distinguish primary infection from reinfection (Näslund *et al.*, 2000).

2.2.7 Epidemiology

BCV is usually endemic in the cattle population; nationwide surveys of antibodies in bulk tank milk (BTM) show a prevalence of 100% in England and Wales (Paton *et al.*, 1998) and 70-100% in Sweden (Tråvén *et al.*, 1999). A study of 112 herds in Ithaca, New York revealed a 33% incidence over a 9-month period (White *et al.*, 1989). A high incidence rate of BCV among Swedish calves has also been shown (Hägglund *et al.*, 2007; Hägglund *et al.*, 2006).

Diarrhoea is a major health problem in calves worldwide. The association between diarrhoea in calves and reduced weight gain, increased mortality, and future reduced fertility has been quantified (Virtala *et al.*, 1996; Wittum *et al.*, 1993; Waltner-Toews *et al.*, 1986). However, these studies did not focus specifically on BCV, nor did they cover adult cattle. A study of feedlot

cattle have shown a decreased weight gain associated with BCV shedding (Cho *et al.*, 2001b).

BCV is shed via faeces and nasal discharge (Reynolds *et al.*, 1985). Its survival outside a host has not been evaluated, but the virus is considered to be labile and sensitive to inactivation (Clark, 1993). Most outbreaks occur during the winter season; viral survival is longer when temperature and UV radiation levels are lower and humidity is higher (Clark, 1993; Saif, 1990). Increased host contacts and stress during the winter season, associated with the animals being housed inside, is also likely to contribute to the infection pattern.

Studies on molecular epidemiology show that the virus differs between outbreaks both spatially and temporally, indicating that there is a new introduction of virus rather than latency or carrier animals (Liu *et al.*, 2006). Other studies have demonstrated viral shedding in the faeces of clinically healthy cows (Collins *et al.*, 1987; Crouch *et al.*, 1985; Crouch & Acres, 1984) which leads to the hypothesis that BCV may remain in the herd by means of carrier animals. Coronavirus isolated from wild ruminants in Ohio has been shown to be closely related to BCV and capable of infecting gnotobiotic calves (Tsunemitsu *et al.*, 1995). Bovine-like coronaviruses have also been isolated from elk-calves, water buffalo-calves and recently from a giraffe (Decaro *et al.*, 2008; Hasoksuz *et al.*, 2007; Majhdi *et al.*, 1997).

2.3 Bovine respiratory syncytial virus

2.3.1 Aetiology

BRSV is an enveloped non-segmented negative-stranded RNA virus incorporated in a helical nucleocapside (Valarcher & Taylor, 2007). It is classified in the *Pneumovirus* genus of the Paramyxoviridae family. The name refers to the characteristic syncytia formation in cell culture due to its cytopathic effect (Chanock *et al.*, 1957). The genome encodes at least ten proteins, of which the fusion protein and the glykoprotein play important roles in attachment and entry of host cells (Valarcher & Taylor, 2007).

BRSV is closely related to the human respiratory syncytial virus (HRSV), which is a major cause of respiratory disease in young children (Hall, 2000; Van der Poel *et al.*, 1994). Humans and cattle are considered to be the natural hosts of HRSV and BRSV, respectively, but antigenically-related RSV has been isolated in sheep and goats (Evermann *et al.*, 1985; Lehmkuhl *et al.*, 1980), and antibodies to BRSV have been detected in other species (Van der Poel *et al.*, 1995).

2.3.2 History

HRSV was first isolated in laboratory chimpanzees in 1955 (Morris *et al.*, 1956). By 1960 it was clear that HRSV was an important cause of respiratory disease in children (Chanock *et al.*, 1957), and ten years later BRSV was isolated from calves in Switzerland (Paccaud & Jacquier, 1970). In 1976 there was an epizootic outbreak of respiratory disease in Norwegian cattle herds, and BRSV was isolated (Odegaard & Krogsrud, 1977). This was followed by only sporadic cases until 1995, when there was another epizootic of BRSV in central Norway (Norström *et al.*, 2000). In 1988, BRSV was isolated in Sweden during an outbreak of respiratory disease in cattle (Elvander, 1996; Jacobsson, 1989).

Today, BRSV is recognised as one of the most important causes of respiratory disease in beef and dairy calves, both worldwide (Brodersen, 2010; Valarcher & Taylor 2007) and in Scandinavian countries (Gulliksen *et al.*, 2009b; Autio *et al.*, 2007; Hägglund *et al.*, 2006; Härtel *et al.*, 2004; Uttenthal, 2000).

2.3.3 Pathogenesis

BRSV replicates in the epithelial cells of the respiratory tract (Viuff *et al.*, 2002). Infected cells activate an inflammatory response involving chemokines and cytokines; this then attracts neutrophils, macrophages, and lymphocytes to the airways (Valarcher & Taylor, 2007). In vitro studies have demonstrated a cytopathic effect of BRSV in tissue culture. However, this effect was absent when using epithelial cells, indicating that immune-mediated mechanisms have a dominating impact on the pathogenesis (Valarcher & Taylor, 2007; Larsen *et al.*, 2000). The infection causes an interstitial pneumonia, and mucopurulent exudate and haemorrhage are often observed in the bronchi and bronchioles (Kimman *et al.*, 1989; Bryson *et al.*, 1983).

2.3.4 Clinical findings

The incubation period for BRSV infections is usually between 2 and 5 days (Valarcher & Taylor, 2007). The clinical manifestation can involve a fever of 40°C or higher, decreased appetite, and signs of upper and lower respiratory disease including coughing, nasal discharge, abdominal breathing, increased lung sounds, and high respiratory rate (Elvander, 1996; Harrison & Pursell, 1985; Verhoeff, 1984). Severely affected animals can develop subcutaneous emphysema and die in acute pneumonia. A BVDV co-infection can increase the severity of a BRSV infection (Elvander *et al.*, 1998). Respiratory viruses enhance bacterial colonisation of the respiratory tract, and it has been

estimated that 90% of bacterial pneumonias develop after viral infection (Babiuk *et al.*, 1988). Treatment includes supportive therapy, non-steroidal anti-inflammatory drugs, and antibiotic therapy against secondary bacterial pneumonia (Larsen, 2000).

The intra-herd spread of BRSV is effective, usually all naïve animals are affected (Bidokhti *et al.*, 2009; Valarcher & Taylor, 2007; Hägglund *et al.*, 2006); in one experimental study, the BRSV infection also spread to the control group (Elvander *et al.*, 1998).

2.3.5 Immune response

In experimentally infected colostrum-deprived calves, IgM and IgA antibodies were detected in serum, secretions, and faeces from day 8 post-infection. IgG1 was detectable in serum from day 13, and IgG2 from 1–3 months post-infection (Kimman *et al.*, 1987). A similar response was seen in experimentally infected colostrum-fed calves and IgG1 titers increased 6–10 days after infection (Uttenthal *et al.*, 2000). Maternally-derived IgG1 antibodies have been detected up to 6 months of age; these can largely suppress the humoral immune response (Baker, 1986; Kimman, 1987).

Little or no clinical signs are observed in re-infected animals (Kimman, 1987). Reinfection associated with increased antibody levels is commonly observed in younger cows, and it has therefore been hypothesised that immunity increases with re-exposure to BRSV (Van der Poel *et al.*, 1995; Verhoeff, 1984).

2.3.6 Diagnostic methods

BRSV can be isolated from nasal secretions in the early stages of an infection, and from lung lavage and transtracheal aspirate in later stages (Larsen *et al.*, 1999; Van der Poel *et al.*, 1997). Antigen can be detected by immune fluorescent antibody staining, which is a rapid, sensitive, and reliable test commonly used for clinical samples (Larsen *et al.*, 2000). Isolation of BRSV in tissue culture is seldom used, since the virus is difficult to culture. RNA detection and amplification can be carried out by conventional RT-PCR assays and real time fluorogenic RT-PCR assays (Hakhverdyan *et al.*, 2005; Achenbach *et al.*, 2004; Larsen *et al.*, 1999; Vilcek *et al.*, 1994).

The most commonly used methods for antibody detection are the virus neutralisation test and ELISA (Elvander *et al.*, 1995).

2.3.7 Epidemiology

Surveys have shown an endemic spread of BRSV, with a 100% prevalence of antibodies in BTM in England and Wales (Paton *et al.*, 1998) and a 41–89%

prevalence in Sweden (Elvander, 1996). Recent studies have shown a high incidence of seroconversion in Swedish beef and dairy calves (Hägglund *et al.*, 2007; Hägglund *et al.*, 2006). BRSV is one of the most important welfare and economic problems in the Danish beef industry, associated with a high mortality (15–30%) among calves and a high rate of antibiotic treatment (Larsen *et al.*, 2010).

BRSV is shed by nasal discharge and droplets, and has been observed up to 12 days after experimental infection (Castleman *et al.*, 1985; McNulty *et al.*, 1983; Elazhary *et al.*, 1980; Jacobs & Edington, 1975). Since BRSV is an enveloped virus, it is more sensitive to inactivation compared to naked viruses (Baker, 1997). A study on HRSV in nasal secretions from infants revealed that the virus remained infectious for 6 hours on countertops, for 1.5 hours on rubber gloves, for 30–40 minutes on clothes and tissues, and for 20 minutes on skin (Hall, 1982). Hand washing is known to be very effective at controlling HRSV (Hall, 2000). An in vitro study showed that generic liquid hand dishwashing detergents were 100 times more effective than antibacterial soaps (Contreras *et al.*, 1999). No corresponding studies have been carried out for BRSV. Airborne transmission was shown in an experimental study designed with two separate stables connected by a tube in the wall (Mars *et al.*, 1999).

Outbreaks occur mainly during autumn and winter (Hägglund *et al.*, 2006; Van der Poel *et al.*, 1993). It is not known how the virus survives during the summer season, but clearly there are limited options: (1) latency and re-activation, (2) viral circulation also during summer but at a low level, or (3) presence of some kind of viral reservoir. Viral sequencing showed that the virus was identical within herds during the same outbreak, but varied both temporally and spatially between outbreaks; the investigators concluded that there is a new introduction of virus rather than latency or carrier animals (Larsen *et al.*, 2000). Van der Poel (1997) used corticosteroid treatment in an attempt to activate a latent infection, if present, in seropositive animals; there was a rise in antibody titre, but sentinels were not infected and the virus was not isolated. Valarcher et al. (2001) demonstrated BRSV persistence in lymph nodes up to 71 days after an experimental infection of calves.

2.4 BCV and BRSV vaccines

Live vaccines against BRSV are used around the world, but their efficiency has been questioned (Larsen *et al.*, 2001; Valarcher *et al.*, 2000). There is also a risk that live vaccines may become contaminated by other pathogens such

as BVDV (Falcone et al., 2003; Barkema et al., 2001). A report from Belgium describes deaths of respiratory distress among calves being vaccinated with inactivated BRSV vaccine (Schreiber *et al.*, 2000). Formalin inactivated vaccine against HRSV and BRSV have shown to enhance disease in infants and cattle respectively (Brodersen, 2010).

There is one commercial vaccine available for BCV; a combination of inactivated vaccine against diarrea caused by BCV, rotavirus, and Escherichia coli that is mainly used in beef cattle herds. There are, so far, no vaccines to prevent BCV induced respiratory disease in cattle (Saif, 2010). The only vaccine against BRSV that is commercially available in Sweden is an inactivated combination vaccine for against BRSV, Mannheimia heamolytica, and bovine parainfluensa virus 3, which was found to be inadequate in a recent study (Larsen *et al.*, 2010). Experimental trials with immune-stimulating complex (ISCOM) vaccines against BRSV have shown promising results, but these vaccines are not yet commercially available (Hägglund *et al.*, 2004). New sophisticated vaccines are oncoming with a molecular approach i.e. use of DNA as a part of the composition (Brodersen, 2010).

3 Aims

The overall aim of this research was to gain further knowledge of the epidemiology of BCV and BRSV infections in Swedish dairy herds.

The specific aims were:

- to study the effects of BCV and BRSV infections on milk yield, reproduction, and herd health parameters;
- to evaluate possible risk factors for the inter-farm spread of BCV and BRSV;
- to investigate the possibility of controlling BCV and BRSV infections in dairy herds via biosecurity measures;
- to describe the long-term dynamics of BCV and BRSV infections in dairy herds; and
- to study the prevalence of antibodies and frequency of conversions in pooled milk samples of primiparous cows and BTM.

4 Materials and Methods

This section summarises the overall issues regarding study populations, data collection, and research methods. A more detailed description is given in each individual paper (I-IV).

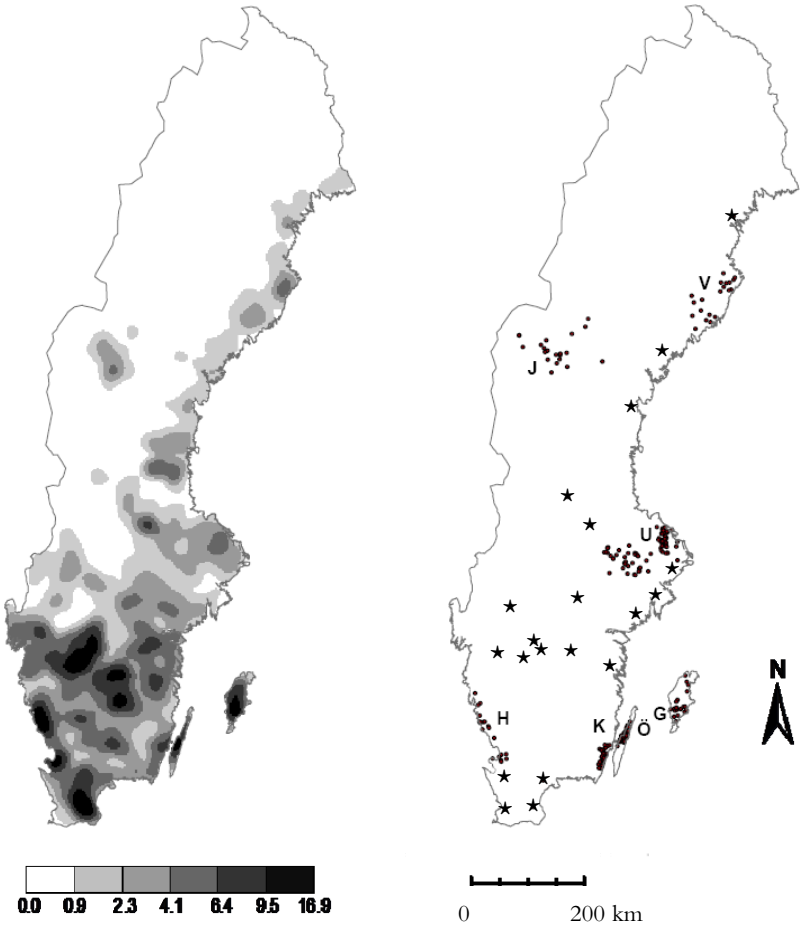
4.1 Study populations

We aimed to include 280 herds within seven areas of Sweden as study population. The areas were located within the counties of Halland, Kalmar, Öland, Gotland, Jämtland, and Västerbotten. These counties were selected for two reasons; (1) previously-estimated differences in the prevalence of BCV and BRSV antibodies in BTM; 90–100% and 84–89% respectively in Halland and Gotland, and 70–79% and 41–51% respectively in Jämtland and Västerbotten (Tråvén *et al.*, 1999; Elvander, 1996), and (2) structural differences.

Three different selection strategies were applied. In the counties of Halland, Gotland, Jämtland, and Västerbotten, we selected a convenience sample of ten herds with 30–80 cows and ten herds with more than 80 cows. From Kalmar, Öland, and two neighbouring veterinary districts in Uppland, we included approximately 50 herds, representing the majority of the existing herds within a limited geographical area. Finally, we included 20 herds distributed throughout Sweden, each with at least 180 cows, owned by farmers who were considered by the local veterinarian to be progressive farmers with high management skills. These herds are henceforth referred to as the "special group".

Herds were eligible for inclusion if they were members of the local livestock association and enrolled in NADRS and SOMRS (Olsson *et al.*, 2001; Emanuelson, 1988). All herds were free from BVDV according to the rules of the Swedish eradication program (Lindberg & Alenius, 1999).

For studies I and II we used the herds located in Uppland as study population, for study III all herds were included, and for study IV we used the herds located in Halland, Gotland, Jämtland, and Västerbotten.



(a) (b)

Figure 1. Maps of Sweden, showing (a) the density of dairy farms (expressed as the number of farms per 100 square kilometres) recorded by the Swedish Board of Agriculture in 2005, and (b) the locations of the study herds in the seven study areas; G: Gotland, H: Halland, J: Jämtland, K: Kalmar, Ö: Öland, U: Uppland, V: Västerbotten. The stars represent the herds referred to as the special group, defined in the text.

4.2 Sampling

The herds were sampled before (September/October) and after (April/May) the winter season over three years, September 2006 to May 2009. A pooled milk sample from five home-bred primiparous cows and a BTM sample were obtained from each herd at each of the six sampling occasions, except for the Uppland areas and the special group. For these, we used the samples from the monthly milk recording scheme collected by Steins laboratory in spring 2008 and spring 2009, with the samples originating from primiparous cows being pooled by herd. These herds were not sampled in autumn 2008. The individual samples from primiparous cows were additionally used to evaluate the correlation between individual and pooled milk samples (Ohlson *et al.*, 2009).

4.3 Antibody detection

The milk samples were analysed for the presence of IgG antibodies to BCV and BRSV, using commercially available indirect ELISAs (Svanova biotech) (Elvander *et al.*, 1995; Alenius *et al.*, 1991). The optical density (OD) at 450 nm was corrected by subtraction of the negative control antigen OD. To adjust for possible day-to-day variations we calculated the percent positivity (PP) using the following formula: (corrected OD/positive control corrected OD) × 100. For the BTM samples, a PP value of < 5 was considered as negative. This closely corresponds to the corrected OD of 0.05 which has previously been used as a cutoff for detection of BCV and BRSV antibodies in BTM (Tråvén *et al.*, 1999; Paton *et al.*, 1998; Elvander, 1996). For the pooled milk samples, a PP value of < 20 was regarded as negative, closely corresponding to the corrected OD of 0.20 which is the cutoff for negative individual milk and serum samples as recommended by the manufacturer.

The sensitivity is estimated to be 84.6% for BCV and 94.6% for BRSV and the specificity to be 100% for both, according to the manufacturer's manual. The estimates for BRSV are based on a parallel analysis with the ELISA used at the National Veterinary Institute, Sweden and the ELISA used at the Central Veterinary Laboratory, England (Elvander *et al.*, 1995). The BCV ELISA was developed by Alenius et al. (1991), with estimates of sensitivity and specificity based on a comparison between the ELISA and a virus neutralisation test. In Elvander et al (1995) there was a good agreement between milk and serum samples regarding IgG antibody titer to BRSV, and unpublished data confirms this for both BRSV and BCV.

We used 10 ml test tubes containing 1.5 mg of the preservative agent Bronopol (2-bromo-2-nitropropane-1.3-diol). The samples were not diluted or centrifuged, and were stored at -20°C until analysis.

4.4 Information provided to farmers

The farmers were informed by mail regarding the antibody status (positive/negative) of the herd based on the pooled milk sample, and those with antibody-negative herds were encouraged to let visitors know their status. All farmers received basic information regarding clinical symptoms, and advice on how to protect their herd via biosecurity routines regarding visitors and purchase of animals. The local veterinarian also received the results of each sampling occasion.

4.5 Questionnaires

In connection to spring sampling in 2007 and 2008, the farmers completed a questionnaire in which they were asked to declare the occurrence of any clinical signs which could be related to BCV or BRSV infections, defined as signs of diarrhoea or respiratory disease affecting at least 25% of the cows within 1 week. These questionnaires were used in study II, to evaluate the effects of BCV and BRSV infections on individual performance.

In spring 2007, the farmers also completed a questionnaire on herd routines; this was used in study III to quantify the effects of potential risk factors on seropositivity to BCV and BRSV.

4.6 Additional data

Herd data on milk production, SCC, disease records, reproductive performance, and disease incidence were acquired from SOMRS and NADRS. For study I we used herd-level data from September 1st 2005 to August 31st 2006, and for study II we used individual data from September 1st 2006 to May 31st 2007 and from September 1st 2007 to May 31st 2008.

To facilitate spatial analysis in study III, the geographical X and Y coordinates of the herds were obtained from the Swedish Board of Agriculture.

4.7 Statistical methods

In study I, we used linear and logistic regression models to assess differences between antibody-positive and antibody-negative herds regarding herd health and other herd characteristics. The outcome variable was the parameter of interest, and the explanatory variables were antibody status (defined as 0 if negative to both BCV and BRSV and 1 otherwise) and other variables of biological importance for the outcome.

In study II, we used multilevel linear and logistic regression models to evaluate possible differences in individual milk yield, SCC, and reproductive performance between the defined groups (based on antibody status before and after the winter season and the presence/absence of clinical signs). Each model had performance as outcome variable and the defined BCV/BRSV status groups together with factors known to influence the performance under study as explanatory variables. Cow and herd were included as random effects to account for repeated measurements and within-herd cluster respectively. Differences between the groups in young stock mortality were evaluated with the Cox proportional hazard model. The data were fitted by using the robust sandwich estimate in order to account for clustering within the herd.

To quantify the effects of potential risk factors for seropositivity in study III, we used logistic regression models with antibody status as outcome variable (defined as 0 negative and 1 positive) and herd level factors obtained in the questionnaires as explanatory variables. We also used two spatial analysis techniques: the K-function and Moran's I statistic. Each herd was considered as a point according to its X and Y coordinates. The K-function was used to test if there was aggregation of antibody-positive herds over negative, and Moran's I statistic was used to identify the possible presence of dependency (autocorrelation) in the residuals between neighbouring herds.

In study IV, we used multilevel logistic regression models to evaluate possible seroconversion differences between the areas; seroconversion was the outcome variable (with 0 defined as no seroconversion and 1 defined as seroconversion) and area, season, and herd size were the explanatory variables. Herd was included as a random effect to correct for repeated measurements.

Statistical analyses were performed using the statistical software Stata (Stata statistical software: Release 9.2 and 10.0; College Station, TX, USA: StataCorp LP.), SAS (SAS Institute Inc., Cary, NC), and R (TEAM, 2008). Maps were generated using R and the geographical information system software ArcView version 9.1 (ESRI Inc., Redlands, California, USA).

5 Results & discussion

This chapter provides a summary and general discussion of the results, together with methodological considerations. A more detailed discussion is given in each individual paper.

5.1 Health and performance

The main findings associated with herd health and performance were:

- Herds that were antibody-positive to BCV and/or BRSV had a significantly higher BTMSCC compared with herds that were negative to both BCV and BRSV (median cells/ml: 218 000 and 163 000 respectively). All other health variables analysed were in favour of the antibody-negative herds, though the differences were not statistically significant (I).
- Cows in herds recently infected with BRSV (seroconverting from negative to positive) and cows in herds where the farmer reported clinical signs suggestive of a BRSV outbreak had a significantly lower milk yield (0.57 and 0.91 l/day, respectively) compared with cows in antibody-negative herds (II).
- Cows in herds where the farmer reported clinical signs of a BRSV outbreak had a significantly higher SCC (12.000 cells/mL) compared with cows in herds without clinical signs of BRSV (II).

One concern relevant to the present thesis is whether the lower BTMSCC/SCC and higher milk yield among antibody-negative herds was a direct effect of infection, or whether the results were confounded by management factors. A disadvantage of cross-sectional studies is that they are not particularly useful in evaluating causal associations. Well-managed herds

are more likely to have a lower SCC, a higher milk yield, and a higher level of biosecurity measures (i.e. avoiding introduction of contagious infections). We believe, however, that these results are not solely a reflection of management differences. The absence of these infections for at least two years, as indicated by antibody-negative primiparous cows, is likely to be associated with a truly positive effect on herd health. The medians for all other analyzed health and reproductive parameters in study I were consistently in favour of the herds negative to both viruses although the differences were not statistically significant. Median herd size was numerically higher amongst the herds in the negative group (57) compared to the herds positive to BCV and/or BRSV (43).

The antibody-positive herds investigated in study I had been infected at least around 2 years prior to the sampling time, and the data used in the analysis was 1-year retrospective. In study II, the analysis covered data from a 7-month period; we know that the herd was infected during this time, but not when. In addition, the antibody status was measured on a herd level, and older cows might have immunity to these infections. In the light of this, the difference between the defined groups in studies I and II might be underestimated.

The disease and reproduction data collected from NADRS and SOMRS were reported by farmers, AI technicians, and veterinarians. The farmers were not necessarily homogeneous in either their willingness to report or the criteria used to decide whether to call out a veterinarian. It is possible that farmers of well managed herds are better at reporting and well managed herds might also have a higher biosecurity level and are therefore more likely to avoid infectious diseases. A recent evaluation of the validity of NADRS (Mörk *et al.*, 2010) revealed a moderate correspondence between manual herd registers and the NADRS database. However, it is unlikely that antibody status of a herd would systematically affect the veterinarians' and technicians' level of reporting. In additions, the major findings were related to BTMSCC, individual SCC, and milk yield, which are objective measurements.

It is likely that antibody negativity to BCV and/or BRSV as well as a recent infection would have similar effects on the analysed parameters also in dairy herds located in other parts of Sweden, and the target population for study I and II is therefore all Swedish dairy herds.

In addition to the data analysed in studies I and II there exist unpublished data from the questionnaires in which the farmers described outbreaks possibly related to BCV and BRSV infections. The results are summarised in Table 1. Among the herds experiencing enteric disease, 28% seroconverted

from antibody-negative to antibody-positive in the pooled milk samples, while 94% of those experiencing respiratory disease seroconverted to BRSV. This could be associated with fewer differential diagnoses for respiratory signs compared to diarrhoea, but could also reflect a difference in protective immunity between BCV and BRSV. Van der Poel et al. (1995) concluded in an observational study that there were neither clinical signs of disease nor a significant drop in milk yield associated with BRSV reinfection, as measured by increased antibody titer.

Table 1. *Summary of 2007 and 2008 questionnaire data in which farmers described possible outbreaks of bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) and their suspicion of transmission routes; number of herds for each parameter. Only estimates from herds that seroconverted from antibody negative to positive in a pooled milk sample of primiparous cows are shown.*

Description	BCV	BRSV	Transmission	BCV	BRSV
Clinical signs	94	16	Unknown	11	5
Seroconverted	26	15	Visitor	7	6
Drop in milk yield	25[a]	10[b]	Slaughter lorry	4	2
Veterinary visit	6	6	Milk lorry	2	1
Abortion	4	4	Airborne	1	1
Dead calves	3	1[c]	Animal introduction	1	0
Dead cows	3	1[c]			

[a] 5-50% decrease (median 20%) in bulk tank milk for 1-5 weeks (median 2 weeks).

[b] 5-20% decrease (median 15%) in bulk tank milk for 2-5 weeks (median 2 weeks).

[c] One herd had three dead calves, two dead cows, and two abortions.

5.2 Between-herd transmission

The main findings related to inter-farm transmission were:

- A significantly higher proportion of herds antibody-negative to BCV used external technicians for artificial insemination instead of farm personnel (29/33), compared to their antibody-positive counterparts (28/46; I).
- Antibody-positive farms were not spatially aggregated over antibody-negative farms, regarding either BCV or BRSV (III).
- Large herd size, being located in southern Sweden, and not providing boots for visitors were found to be associated with antibody positivity to BCV and BRSV. Short distance to nearest cattle herd was also associated with antibody positivity to BCV (III).

The positive association between external technicians and BCV antibody negative herds may seem contradictory, but also suggest that it is possible to avoid infection even in a herd which has regular visitors. In the Uppland area, a typical 50-cow herd has two technician visits per week, and a technician visits approximately six herds per day. The result is in agreement with a study by Bidokhti et al. (2009), where the use of external technicians for insemination was significantly associated with BCV and BRSV antibody-negative herds.

The lack of spatial autocorrelation indicates that local spread and airborne transmission are not the most important factors for the inter-farm spread of BCV and BRSV, at least not in areas with moderate herd density under Swedish conditions.

Not providing boots for visitors was the only management factor associated with antibody positivity in study III. We believe that providing boots for visitors and *always* using them strongly symbolises farms with high biosecurity measures, and this therefore lend support to the hypothesis that herds antibody-negative to BCV/BRSV are significantly different from antibody-positive herds regarding biosecurity measures.

Region was associated with antibody positivity to BCV and BRSV, with decreasing odds ratios moving from south to north. The results from Moran´s I showed, that the unexplained variance (the model residuals) was not due to spatially-correlated factors. In a recent nationwide study on Swedish beef herds, there were a positive association between herd density and prevalence of BCV and BRSV, however, a high density area with low prevalence was also identified and the investigators therefore concluded that other factors than herd density, such as regional differences in biosecurity and management routines, have impact on the prevalence of BCV and BRSV (Beaudeau et al., 2010). The additional significant factor for BCV, distance to nearest cattle herd, was not significant for BRSV. This difference could be explained by the fact that BCV is shed via faeces, which might be more easily spread between herds than nasal discharge.

The seven areas chosen as source population in study III were selected to reflect the target population; dairy herds in Sweden. The source population is reasonably valid for the target population. The main consideration is whether the study population reflects the source population (and hence the target population) since the herds included were randomly selected which may have influenced the internal validity. For Uppland, Kalmar and Öland this should not affect the results inasmuch as the majority of the existing herds within limited areas were included. In Halland, Gotland, Jämtland and Västerbotten, the selection could lead to a bias, but because the antibody

status of the herds was unknown before sampling and the selection was not based on management skills or lack thereof, we do not regard this as a major concern. The 16 herds selected to go into the special group, however, could be seriously biased by selection. The results of the analyses remained unchanged, however, when the models were run without these herds.

In the outbreak questionnaires, we asked the farmers about possible transmission routes (Table 1). There was only one report of animal introduction for BCV and no such reports for BRSV. This suggests a difference in epidemiology between these viruses and BVDV, which is also an enveloped virus. BVDV seems to spread primarily by direct contact, and the control program in Scandinavia has been successful by targeting animal movements (Lindberg & Houe, 2005).

5.3 Long-term dynamics of BCV and BRSV

The results of study IV show that the long-term dynamics of BCV and BRSV differed between the herds located in the two southern areas (Halland and Gotland) and the herds in the two northern areas (Jämtland and Västerbotten). In the southern areas the percentage of antibody positive herds (pooled milk sample) was continuously at a high level (85–100%), whereas in the northern it varied for BCV from 38% to 80% and for BRSV from 0% to 80%. At the last sampling occasion, there was evidence of self-clearance for BRSV in the Jämtland area. This might be due to natural fluctuation, but could also be the result of higher awareness and improved biosecurity measures in these herds. It is possible that the restriction on animal movement from southern Sweden, due to blue tongue virus, also contributed to this positive development.

During the study period, there was evidence of self-clearance in herds manifesting as a change in antibody status from positive to negative in the pooled milk sample; 28 times in 27 herds and 32 times in 32 herds for BCV and BRSV respectively. Two herds were antibody negative to BCV in BTM and four herds to BRSV throughout the study period. In addition, three herds for BCV and three herds for BRSV became antibody negative in BTM during the study period. These herds were all located in Jämtland and Västerbotten, except one herd negative to BCV that was located in Halland. One of these herds, located in Västerbotten, became positive to BCV whereas the other herds remained negative. One herd in Jämtland, comprised of 147 milking cows, was antibody negative to BCV and BRSV in BTM throughout the study period.

The results from the logistic regression models showed differences between BCV and BRSV regarding conversions from antibody negative to positive. For BCV, there were no differences between the areas or between the two herd-size groups. Conversely, for BRSV the new infection risk was greater in the two southern areas compared to the north, and also greater in larger herds (>80 cows) compared with herds of average size (30-80 cows). Herds negative in the pooled milk sample had been free from the infections for at least two years. This indicates that in herds seroconverting from negative to positive there was a new introduction of virus rather than a continuous within-herd circulation or activation of virus from carrier animals. The risk of converting from negative to positive was higher during the winter season for both infections. A few conversions were observed between April/May and September/October suggesting that there might also be a circulation of the virus, albeit at a low level, during the summer (outdoor) season.

The results of study IV support our hypothesis that it is possible to establish herds free from BCV and BRSV. The hypothesis that areas can have self-clearance of BRSV cannot be rejected, while the corresponding situation for BCV needs further investigation. The target population was herds located in areas with similar structure and similar prevalence of BCV and BRSV, i.e. southern and northern parts of Sweden.

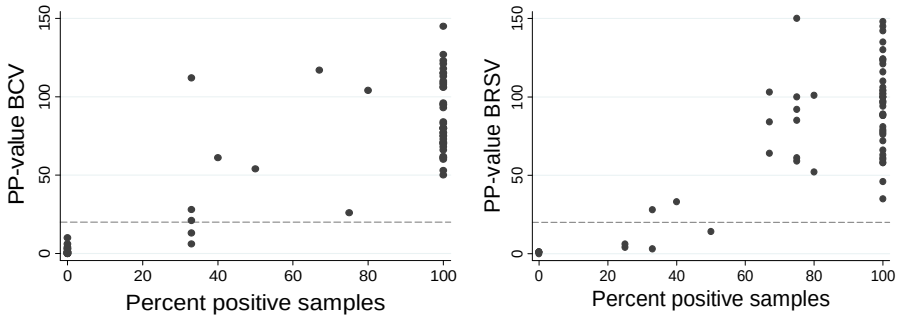
For Kalmar (40–50 herds) and Öland (50–52 herds) the percentage of antibody positive herds to BCV in the pooled sample was 80–90% and 88–98% respectively, for the six sampling occasions. Corresponding number were 57-75% for Uppland (36-90 herds) and 75-89% for the special group (11-20 herds); two herds were antibody negative to BCV in the pooled milk sample throughout the study period, consisting of 220 and 300 cows located in central and northern Sweden respectively.

For BRSV, the percentages of antibody positive herds in the pooled milk sample were; 78–92% for Kalmar, 94–100% for Öland, 75–81% for Uppland and 63–82% for the special group.

5.4 Antibody detection in pooled milk samples and BTM

The relationship between antibody status in individual and in pooled milk samples was evaluated using the individual samples collected from 64 herds in Uppland by Steins laboratory (Ohlson *et al.*, 2009). Ten milk samples were randomly selected from each herd. In the majority of the herds with mixed results there was a clear age difference; cows with lower lactation

number were antibody-negative and older cows antibody-positive. The primiparous cows were pooled by herd, with 3–5 cows in each pool. None of the pooled milk samples that consisted of 100% negative individual samples were positive, and none of the pooled samples that consisted of 100% positive individual samples were negative, regarding both BCV and BRSV (Figure 2). In the majority of the herds (83% for BCV and 74% for BRSV), all primiparous cows were either all positive or all negative.



(a) (b)

Figure 2. The y-axis shows the percent positivity (PP) value of IgG antibodies to (a) bovine coronavirus (BCV) and (b) bovine respiratory syncytial virus (BRSV) in pooled milk samples of 3-5 primiparous cows, and the x-axis shows the corresponding percentage of positive individual samples included in the pooled sample. The dashed line is the cutoff at a PP value of 20.

In study IV, we evaluated the association between the antibody titre in BTM and the status of the younger cows. We used 432 pooled samples from primiparous cows with corresponding BTM. All BTM samples that were antibody-negative (PP<5) had a corresponding negative pooled sample (PP<20). In addition, for both BCV and BRSV the median PP in BTM was lower in the samples that had a negative corresponding pooled sample (t-test, P<0.001); this difference remained significant when the BTM-negative samples were excluded. The majority of the negative pooled samples, however, had a corresponding positive BTM sample.

Our conclusion is that detection of antibodies to BCV and BRSV in BTM is a good method for estimation of herd exposure historically or in areas where the viral circulation has been very low or absent for some time. The use of pooled milk samples is a favourable approach for identifying susceptible herds in early control programs in endemic areas and for animal

trading. It is crucial that the cows included in the pool are home-bred, in order to get a true reflection of the herd status.

5.5 Prospects for control

5.5.1 Possibility of controlling the spread of BCV and BRSV

The findings reported in this thesis indicate that local factors such as daily visiting milk trucks, wild animals, and airborne transmission are unlikely to be major important transmission routes of BCV and BRSV infections. We believe that contacts through networks (visitors, equipments etc) are more important for the inter-farm spread of these viruses. Therefore, it seems clear that there is a possibility of controlling the spread of BCV and BRSV in a cost-effective and practical way via increased biosecurity.

Jämtland was likely to be free from circulating BRSV in the end of the study period. This demonstrates that it is possible to have self-clearance of BRSV, that is, clearance without strict intervening activities such as vaccination or regulation of transport and contacts, not only within a herd but also within an area. Although no clearance of BCV occurred in any of the studied areas, the prevalence of antibodies in the pooled milk samples was low among the study herds in Jämtland in 2007 and in Västerbotten in 2008. In addition, study IV revealed self-clearance (via conversion from antibody-positive to antibody-negative in the pooled sample) in herds, for both of BCV and BRSV.

Given that Sweden and the other Scandinavian countries have successfully eradicated BVDV, a possible and tempting challenge would be to establish areas with freedom from BCV and BRSV. To our knowledge, there have been no previous efforts to establish BCV or BRSV free herds on a regional or national level.

5.5.2 Proposals for controlling the spread of BCV and BRSV

The current situation in the northern parts of Sweden is appropriate for the initiation of a voluntary control program based on increased awareness and biosecurity measures targeting indirect contacts and purchase of animals. In this program, the antibody status of the herds would be monitored before each winter season. Pooled milk samples from primiparous cows would be preferable at the start, but could be replaced by BTM monitoring once freedom from infection is established. Detecting antibodies to BCV and BRSV in milk samples by an indirect ELISA is cheap, and the samples are easy to collect. The farmers would receive information on their BCV and BRSV antibody status, along with biosecurity advice. All veterinarians, technicians,

and others that frequently visit dairy herds in the area would be informed about the program, provided with basic information on BCV and BRSV infections, and advised on good biosecurity routines. These recommendations would be developed together with farmers, veterinarians and other professions in the field in order to achieve a high level of implementation.

Our hypothesis is that with increased biosecurity, the basic reproduction rate for the transmission of BCV and BRSV between herds in a susceptible population will be below one, and therefore the infection spread will be halted. An outbreak of BCV or BRSV in the study area would be easily detected, since all farmers and all professionals in the field would be involved in the project. Information about herd antibody status is likely to increase the interest in biosecurity and the acceptance of new routines.

5.5.3 Benefits of preventing BCV and BRSV infections

We believe that it is not consistent with a sustainable healthy cattle population to have BCV and BRSV outbreaks every year when there is a possibility of controlling the spread. Despite the fact that routines related to good biosecurity have been recommended for many years (Larsson *et al.*, 1995), and that due to the BVDV program Swedish farmers are probably more aware of and more likely to practise these measures than farmers in many other countries, a recent survey revealed that there is still a need for improvement (Nöremark *et al.*, 2010). Increased biosecurity would not only prevent the spread of BCV and BRSV, but also make Swedish cattle farms better prepared for future epizootics and prevent the spread of zoonotic infections as well as other contagious infections. Due to the contagious nature of BCV and BRSV, they are good indicators of biosecurity at the farm level. A reduced incidence of BCV and BRSV outbreaks would benefit the farmer economically, enhance animal welfare, and reduce the use of antibiotics.

6 Conclusions

- Herds that were antibody-negative to both BCV and BRSV had a lower BTMSCC compared to farms that were antibody-positive to BCV and/or BRSV. Cows in recently BRSV-infected herds had lower milk yield and higher SCC compared to cows in BRSV-free herds.

- BCV and BRSV antibody-positive herds were not spatially aggregated compared to antibody-negative herds, indicating that local spread and airborne transmission are not the most important factors for the inter-farm spread of BCV and BRSV, at least not in areas with moderate herd density under Swedish conditions.

- Providing boots for visitors and ensuring that these boots were always used was associated with a lower herd prevalence of both BCV and BRSV; this factor is likely to reflect a high level of biosecurity and indicates that the introduction of virus into a herd is not just by chance.

- There was evidence of self-clearance of BRSV within an area; that is, clearance without strict intervening activities such as vaccination or regulation of transport and contacts, but with increased awareness and biosecurity recommendations.

- The results of this thesis suggests that it is possible to develop inexpensive strategies for controlling BCV and BSRV infections, based on biosecurity and antibody monitoring.

7 Further research

- Studies regarding the attitudes of farmers and veterinarians towards new information in the veterinary field in general and new information on biosecurity in particular would be highly desirable. In order to achieve widespread adoption of recommendations gained from research, there must be a strategy for communicating with the farmers and veterinarians and ensuring their ongoing engagement in the research process. Farmer participation in crucial phases of the research, beginning with problem formulation, testing of options in the field, and the implementation of control or prevention measures, would ensure that the research findings are meaningful to the end users, that new knowledge is made available in forms appropriate to a wide cross-section of the farmers, and most importantly, that the outcomes are implemented in their herds.

- A study to investigate the possibility of establishing long-term freedom from BRSV and BCV within a geographical area, via increased biosecurity, would give evidence for the possibility of cattle production without these viruses, and also allow evaluation of the effect on the incidence of other contagious infections.

- Another valuable research route would be to investigate BCV and BRSV outbreaks using reliable dates for the onset and only including seroconverting animals, in order to accurately quantify the short-term and long-term loss of production and reproduction performance on an individual level.

- One way to obtain deeper knowledge regarding the epidemiology of BCV and BRSV infections would be to use molecular analysis for contact tracing. This technique was successfully used in the final phase of the Swedish BVDV programme.

- Development of efficient vaccines against BCV and BRSV infections would be desirable. A vaccine could be used alongside biosecurity measures to reduce the infection pressure, especially in larger farms that frequently introduce new animals.

- Routine collection of pooled milk samples of primiparous cows from the milk recording scheme is technically feasible and would provide excellent and unique possibilities for nationwide epidemiological studies of BCV and BRSV and for the control of these infections.

8 Populärvetenskaplig sammanfattning

8.1 Bakgrund

Varje år drabbas ett stort antal svenska mjölkkobesättningar och besättningar i den specialiserade köttproduktionen av infektioner med bovint coronavirus (BCV) och bovint respiratoriskt syncytialt virus (BRSV). BCV ger upphov till diarré hos kalvar och vuxna djur (vinterdysenteri) och kan även orsaka varierande grad av luftvägssjukdom. En infektion med BRSV ger hög feber och luftvägssjukdom och det händer att djuren dör i akut lunginflammation. Drabbade djur behandlas ofta med antibiotika pga. sekundära bakteriella lunginflammationer. Några bevisat säkra och effektiva vacciner mot dessa virusinfektioner finns för närvarande inte tillgängliga.

Rapporter visar att BCV och BRSV är spridda över hela världen. Förekomsten av antikroppar i tankmjölk var 100% för BCV och BRSV i England och Wales, i Sverige har motsvarande studier visat en förekomst på 70-100% för BCV och 41-89% för BRSV.

BCV och BRSV är smittsamma infektioner och oftast infekteras alla mottagliga djur i besättningen. Smittspridingen mellan besättningar sker både direkt från djur till djur och indirekt via t.ex. besökare och redskap. De är känsliga för avdödning med desinfektionsmedel och överlever inte någon längre tid utanför djuret.

Den nuvarande uppfattningen bland många djurägare och veterinärer är att det inte går att förhindra smittspridning mellan besättningar, och att det är bra med upprepade infektioner för att upprätthålla djurens immunitet. Observationer visar dock att det finns mjölkbesättningar som tack vare bra smittskyddsrutiner och en medveten hållning har hållit sig fria från BCV och BRSV infektioner i åratal. Ett utmärkande drag för dessa besättningar är att de uppvisat en hög produktion och en god kalvhälsa.

8.2 Sammanfattning av avhandlingsarbetet

Det övergripande syftet med avhandlingsarbetet var att få ytterligare kunskap om BCV och BRSV infektioners epidemiologi i Svenska mjölkbesättningar.

Vi inkluderade besättningar från Halland, Gotland, Jämtland och Västerbotten (ca 20 från varje område) samt Kalmar, Öland och två områden i Uppland (ca 50 från varje område, motsvarande majoriteten av de befintliga mjölkbesättningarna inom ett begränsat område) och slutligen ca 20 gårdar spridda över Sverige mer än 180 kor och som den lokala veterinären ansåg vara i frontlinjen vad det gäller skötsel och kunskap.

Vi samlade in samlingsprover på mjölk från fem förstakalvare och tankmjölksprover före och efter stallsäsongen under tre år (2006-2009). Efter en genomgången infektion är antikroppar mätbara under lång tid, tankmjölken speglar därför långtidshistoriken medan ett samlingsprov på förstakalvare visar om besättningen varit utsatt för smitta de senaste två åren. Proverna analyseras på förekomsten av antikroppar mot BCV och BRSV med ELISA. Efter varje provtagning fick djurägarna veta besättningens antikroppsstatus (negativ/positiv) tillsammans med information om infektionerna och smittskyddsråd. I informationen till djurägarna samt i studie I-IV baserades besättningarnas antikroppsstatus på samlingsprovet av förstakalvare.

Hälsoparametrar och mjölkavkastning

I den första delstudien utvärderades association mellan antikroppsstatus och hälso- och reproduktions parametrar på besättningsnivå. Vi använde oss av besättningarna från de två uppländska områdena. Tio besättningar som var antikroppsnegativa mot både BCV och BRSV jämfördes med 69 besättningar som var positiva mot BCV och/eller BRSV. Besättningsstorleken (median) var för de negativa besättningarna 57 kor och för de positiva 43 kor. Data analyserades ett år retrospektivt från provtagningstillfället. Vi fann att de fria gårdarna hade lägre celltal i tankmjölken, med statistisk signifikans (median 163 000 celler/mL och 218 000 celler/mL) mätt över en ettårs period. De fria besättningarna låg konsekvent bättre till på alla analyserade hälso- och reproduktionsparametrar, jämfört med de antikroppspositiva besättningarna, men skillnaden var inte tillräckligt stor för att vara statistiskt säker.

I den andra studien analyserades data på individnivå, gällande mjölkavkastning, mjölkens celltal, reproduktions parametrar och mortalitet hos ungdjur, inkluderande 65 uppländska mjölkgårdar. Kor i besättningar som nyligen haft en BRSV infektion hade signifikant lägre mjölkproduktion jämför med antikroppsnegativa gårdar (0,7 liter per dag och ko mätt över en 7-månaders period). I besättningar där djurägaren noterat kliniska symptom

på BRSV infektion hade korna en signifikant högre cellhalt i mjölken jämfört med kor i besättningar utan rapporterade symptom (18 000 celler/mL högre i snitt vid varje mätning).

Smittspridning mellan besättningar

I studie 1 hade 88% av de BCV negativa besättningarna externa seminörer istället för egen semin, detta var en signifikant högre andel jämfört med de BCV positiva besättningarna (61%). Detta indikerar att seminörerna i upplandsområdet hade ett fungerande smittskydd men kan också spegla att de besättningar som använder sig av professionella seminörer var mer noggranna med sina skötselrutiner. Resultatet visar att det är möjligt att hålla sig fri från BCV infektion trots regelbundna besök.

I den tredje studien identifierades riskfaktorer för BCV och BRSV introduktion, genom att jämföra rutiner mellan antikroppsnegativa och antikroppspositiva besättningar. I denna studie ingick 257 gårdar från alla områden. Att tillhandahålla stövlar för besökare som *alltid* används var en skyddande faktor för både BCV och BRSV. Detta var den enda skötselfaktor som var signifikant associerat med antikroppsstatus och vi anser att den speglar en hög nivå av smittskydd. Vi undersökte även den geografiska spridningen genom spatial analys och fann att smittade gårdar inte bildade kluster jämfört med negativa gårdar. Detta fynd indikerar att luftburen och lokal (t ex via gnagare) smittspridning inte är av den viktigaste vägen för överföring av BCV och BRSV mellan gårdar.

Infektionsdynamik

I den fjärde studien beskrivs infektionsdynamiken under en tre års period. Här ingick besättningarna från Jämtland och Västerbotten i norr samt Halland och Gotland i söder. I de två södra landskapen var det kontinuerligt hög andel gårdar som var antikroppspositiva (85-100%), medan det i norr varierade; för BCV 38-80% och BRSV 0-80%. Bland de jämtländska besättningarna sjönk förekomsten av antikroppar mot BRSV under studieperioden och Jämtland bedömdes vara fria från cirkulerande BRSV vid sista provtagningen våren 2009. Detta indikerar att det går att få ett område fritt från BRSV.

Av de besättningar som var antikroppsnegativa i tankmjölken vid första provtagningen hösten 2006, alternativt blev antikroppsnegativa under studieperioden (5 för BCV och 8 för BRSV), förblev alla negativa utom en besättning i Västerbotten som serokonverterade mot BCV. Dessa besättningar återfanns i de norra områdena utom en gård i Halland som var negativ mot BCV.

8.3 Möjlighet till kontroll

Baserat på resultaten i denna doktorsavhandling tror vi att det på ett kostnadseffektivt och praktiskt genomförbart sätt, genom ett ökat smittskydd, går organisera ett frivilligt kontrollprogram mot BCV och BRSV. Norra Sverige är lämpligt för en start av ett sådant program på grund av det fördelaktiga smittoläget. Övervakningen kan till en början ske genom analys av antikroppar i samlingsprover av mjölk från förstakalvare för att sedan (förutsatt att förekomsten minskar) gradvis övergå till tankmjölksprover. Vi tror att vetskapen om besättningens antikroppsstatus tillsammans med information om infektionerna kommer leda till ett ökat intresse för smittskydd och en ökad mottaglighet för nya rutiner hos alla aktörer. För att få ut nya rekommendationer baserat på forskningsstudier måste det finnas en strategi för kommunikation mellan forskare och djurägare/veterinärer m.fl. Därför bör djurägare och personer verksamma i fältet vara med och utforma de nya rutinerna.

För att få uthållig djurhållning måste smittskyddet skärpas i svenska besättningar. Trots att smittskyddsrutiner har rekommenderats i många år är de fortfarande otillräckliga i många besättningar enligt en nyligen publicerad studie (Nöremark et al., 2010). Ett stärkt smittskydd skulle inte bara förhindra spridningen av BCV och BRSV utan också minska spridningen av andra smittämnen. En minskad frekvens av BCV och BRSV infektioner skulle förbättra djurvälfärden, vara ekonomiskt viktigt för djurägaren och minska användningen av antibiotika.

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