A Study of Bovine Coronavirus (BCV) and Bovine Respiratory Syncytial Virus (BRSV) Infections in Dairy Herds in Sweden

Mehdi Rajabimoghaddam Bidokhti

Faculty of Veterinary Medicine and Animal Science
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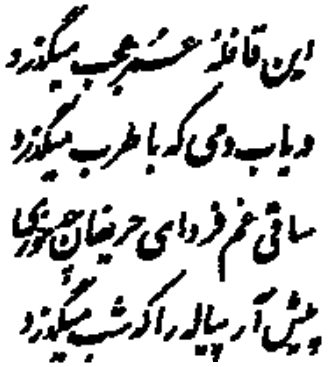
The present thesis is a partial fulfilment of the requirements for the Master of Science (MSc) Degree in Veterinary Medicine for International Students at the Swedish University of Agricultural Sciences (SLU), in the field of Ruminant Medicine and Veterinary Epidemiology.

Mehdi Rajabimoghaddam Bidokhti
Division of Ruminant Medicine and Veterinary Epidemiology
Department of Clinical Sciences, P.O. Box 7054
Faculty of Veterinary Medicine and Animal Science
Swedish University of Agricultural Sciences (SLU)
SE-750 07 Uppsala, Sweden

To my parents and brothers,



Literal:
The caravan of life shall always pass
Beware that is fresh as sweet young grass
Let's not worry about what tomorrow will amass
Fill my cup again, this night will pass, alas.

Meaning:
To be aware of each moment spent
Is to live in the now, and be present
Worry for morrow shalln't make a dent
Caring for the now, your mind must be bent.

Fitzgerald:
One Moment in Annihilation's Waste,
One moment, of the Well of Life to taste-
The Stars are setting, and the Caravan
Starts for the dawn of Nothing-Oh, make haste!

Omar **Khayyam** Nishapouri

1048- 1123 CE
Nishapour, Persia (Iran)

http://www.okonlife.com

Abstract

Bidokhti, M. R. M., 2007. A Study of Bovine Coronavirus (BCV) and Bovine Respiratory Syncytial Virus (BRSV) Infections in Dairy Herds in Sweden. Master of Science thesis.

Bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) infections are spread in cattle herds worldwide. When introduced in to a susceptible herd, both infections cause respiratory disease. BCV is also associated with diarrhea in both calves and adult cattle.

The purpose of this study was to investigate the seroprevalence of BCV and BRSV in dairy cattle herds in an area of Sweden. Specific goals were to determine the immunity against these infections in both conventional and organic dairy herds during two sampling occasions, and if the prevalence of these infections in conventional herds differ from organic herds. The influence of different risk factors for seropositivity was also investigated.

Around 700 serum samples, taken from 20 conventional and 20 organic dairy herds in south eastern Sweden on two sampling occasions with one year interval, were tested by ELISA for presence of antibodies to BCV and BRSV. On individual level, the seroprevalence at both occasions varied between 82-86% to BCV and 79-82% BRSV. Analyzing the data on herd-level revealed that the conventional herds had a significantly higher mean seroprevalence to BCV and BRSV than the organic ($P< 0.001$). A significant association was found between age and seroprevalence ($P< 0.001$). Cows younger than 5 years old in conventional herds had significantly higher mean seroprevalence than the organic ($P< 0.001$). Only 30% of the youngest cows in organic herds compared to 70% in conventional herds were positive to both BCV and BRSV which increased up to 100% in the oldest cows. The mean absorbance value in positive cows older than 5 years was significantly higher compared with those younger than 5 years ($P<0.001$). Higher mean titres of antibody in the oldest cows most likely make their enriched colostrum a valuable tool in herds with neonatal infection problems.

This study suggests that organic management may be associated with a lower incidence of BCV and BRSV infections compared with conventional management.

Key words: BCV; BRSV; Organic; Dairy herds; seroprevalence

Contents

List of Abbreviations

BCV	bovine coronavirus
BRD	bovine respiratory disease
BRSV	bovine respiratory syncytial virus
BVDV	bovine viral diarrhea virus
cDNA	complementary deoxyribonucleic acid
COD	corrected optical density
ELISA	enzyme-linked immunosorbant assay
EM	electron microscopy
F protein	fusion protein
fRT	fluorogenic reverse transcription
HRSV	human respiratory syncytial virus
Ig	Immunoglobulin
ISCOMs	Immunostimulating complexes
MAbs	monoclonal antibodies
MDBK	Madin Darby bovine kidney
MHV	mouse hepatitis virus
mRNAs	messenger ribonucleic acids
N protein	nucleocapsid protein
ORFs	open reading frames
PCR	polymerase chain reaction
RDE	receptor destroying enzyme
RT	reverse transcription
S glycoprotein	spike glycoprotein
SARSCoV	severe acute respiratory syndrome coronavirus
SOMRS	Swedish official milk recording scheme
VN	virus-neutralization
WD	winter dysentery

Introduction

Bovine coronavirus (BCV) and also *bovine respiratory syncytial virus (BRSV)* are viral infections that cause financial losses to the dairy industry all over the world (Larsen, 2000; Saif, 2004). BCV causes both respiratory and enteric disease, including calf diarrhea, winter dysentery in adults, and respiratory infections in cattle of all ages. Epidemiological studies suggest that serum antibody correlates with immunity (Saif, 2004). BRSV is a major cause of respiratory disease in young cattle less than 6 months old and is frequently associated with severe respiratory signs and sometimes death. The epidemiology of HRSV and BRSV infections is very similar (Van der Poel *et al.*, 1993; Valarcher & Taylor, 2007). The disease may be observed in young individuals despite the presence of maternal antibodies. Outbreaks usually occur in autumn and winter. Recurrent infections in the same individuals are common (Elvander, 1996a; Larsen, 2000). There are currently no effective vaccines available to prevent BCV and BRSV-associated disease**.**

These two viruses frequently precede bacterial invasion of the lung which cause a considerable consumption of antibiotics for the treatment. However, one of the aims of organic production is to reduce the use of antibiotics. Furthermore, animals treated with such restricted substances are subject to doubled withdrawal periods before milk may be sold to the dairy. To be able to reduce use of antibiotics it is important to keep the animals healthy by providing optimal care, feed and housing. This study was initiated to increase the knowledge of BCV and BRSV infections in dairy herds of Sweden and to find the effects of management on the prevalence of these infections in organic dairy herds in comparison with conventional herds.

Aims of the Investigation

The general aim of this study was to increase the knowledge about BCV and BRSV infections in dairy cattle farms in Sweden.

The specific reasons were to investigate:

- The seroprevalence to these viruses in both conventional and organic dairy herds during two sampling occasions.
- If there is a difference in the prevalence of these infections between conventional and organic dairy herds.
- The influences of different risk factors for seropositivity, such as age, stall type, and farm visitors.

Study of Literature

1. Bovine Coronavirus

1.1. The virus

Bovine coronavirus (BCV) belongs to the family *Coronaviridae*, order *Nidovirales* (Van Regenmortel *et al.*, 2000), and possesses a single-stranded, non-segmented, positive sense RNA genome of 32 kb plus poly (A) tail in length (de Vries *et al.*, 1997). The enveloped viral particles are pleomorphic to spherical in shape, helical in symmetry, varying in diameter with a mean of about 120 nm. Its genome includes 13 open reading frames (ORFs) flanked by 5' and 3' untranslated regions. Five major structural proteins are encoded within the genomic RNA: spike (S) glycoprotein (ORF4), transmembrane (M) protein (ORF9), nucleocapsid (N) protein (ORF10), hemagglutinin-esterase (HE) protein (ORF3), and small membrane (E) protein (ORF8) (Clark, 1993; Chouljenko *et al.*, 2001; Masters, 2006).

The HE glycoprotein, with 120-140 kDa in weight, has receptor binding and detachment functions mediated by an acetylesterase (AE) which is a receptor destroying enzyme (RDE). Its targets can be receptors of erythrocytes and susceptible cells (Schultze *et al.*, 1991).

The S glycoprotein also recognizes receptors on the surface of erythrocytes (de Groot, 2006) and has been determined to be the major hemagglutinin of BCV (Schultze *et al.*, 1991). The variation in host range and tissue tropism of coronaviruses is largely attributable to variations in the S glycoprotein. The S glycoprotein facilitates viral attachment to susceptible cells and causes cell fusion. It carries distinct functional domains near the amino (S1) and carboxy (S2) termini (Gallagher & Buchmeier, 2001). The S1 subunit is peripheral and is associated with receptor binding functions whereas the S2 subunit is a transmembrane protein mediating fusion of viral and cellular membranes (Saeki, Ohtsuka & Taguchi, 1997). In addition, the S glycoprotein induces neutralizing antibodies. Although S1 and S2 both contain several antigenic domains, S1 more efficiently elicits monoclonal antibodies (MAbs) with higher neutralizing activity (Vautherot, Laporte & Boireau, 1992; Popova & Zhang, 2002). The BCV HE protein alone is not sufficient for BCV infection. The S protein but not the HE protein of BCV is necessary and sufficient for infection of the virus in HRT-18 cells, suggesting that BCV likely uses the S protein as a primary vehicle to infect permissive cells (Popova & Zhang, 2002).

The BCV N protein is a 50-kd phosphoprotein that binds viral genomic RNA to form the helical nucleocapsid. The N protein may play a role in replication of viral RNA (Smith, Hogan & Hogue, 1998). The N protein of BCV shows significantly higher overall amino acid sequence identity with the other coronaviruses in

serogroup 2 than with the members of serogroups 1 and 3 (Lapps, Hogue & Brian, 1987).

Based on antigenic relationship and sequence similarity, coronaviruses are classified into three groups. The best studied representative of group 1 coronaviruses is porcine transmissible gastroenteritis virus (TGEV). BCV belongs to the group 2 coronaviruses. In this group, however, most information in molecular terms is available for mouse hepatitis virus (MHV). The human coronavirus associated with severe acute respiratory syndrome (SARSCoV) is only distantly related to the other members of this family and has not yet been classified. The third group within the *Coronavirus* genus is represented by avian infectious bronchitis virus (IBV) (Weiss & Navas-Martin, 2005; Schwegmann-Wessels & Herrler, 2006). The evolutionary relationships between BCV and HCoV-OC43 indicated relatively recent common ancestors for these species-specific coronaviruses (Vijgen *et al*., 2006).

1.2. Epidemiology of BCV

Bovine coronavirus has been reported in many countries and is widespread with high seroprevalence in adult dairy cattle (Clark, 1993; Paton *et al.*, 1998; Vijgen *et al.*, 2006). At present the latest BCV reports are from Iran (Khalili & Morshedi, 2006), Cuba (Valle *et al*., 2006), Brazil (Jerez *et al*., 2005) and South Korea (Jeong *et al*., 2005).

1.2.1. BCV in Scandinavia

The first report of winter dysentery in Sweden was published in 1951 (Hedström & Isaksson, 1951) and BCV was reported for the first time in Sweden as the causative agent of winter dysentery in a serological study of 9 dairy herds with epizootic enteritis (Alenius *et al*., 1991).

In Sweden, a nationwide survey of antibodies in bulk tank milk from Swedish dairy herds showed that 89% of the samples were antibody positive and half of them had high levels of antibodies to BCV (COD> 0.7) (Tråvén, Bjornerot & Larsson, 1999). 34% seropositivity and 50% seroconversion to BCV were found in a study of dairy herds in south western Sweden (Hägglund *et al.*, 2006). BCV infections were also detected in all years, with a peak incidence in November, in a six-year study of a bull testing station (Hägglund *et al.*, 2007).

A serological investigation showed that BCV is a common pathogen in Finnish cattle with respiratory problems (Hartel *et al*., 2004). In an etiological study of 40 Finnish dairy farms, 45% of herds were serologically positive. In 33% of studied herds, BCV was detected in fecal samples using real-time PCR (Autio *et al*., 2007).

In a pathobiological survey of lungs from 72 calves with respiratory problems among 68 Danish herds, it was concluded that coronavirus seem to be of less importance than BRSV associated with severe outbreaks of calf pneumonia in Denmark (Tegtmeier *et al*., 1999).

Nucleotide sequence alignments of the first 624 bp amplified from the S gene in a molecular epidemiological study of BCV showed a high degree of sequence identity (96% to 100%) among field isolates from Denmark and Sweden, indicating that these isolates were genetically similar to each other (Liu *et al.*, 2006).

1.2.2. Transmission of BCV

The fecal-oral route and aerosol-nasal route are the presumed methods of transmission of BCV. Feces from clinical cases or clinically normal carriers is a source of infection, and contamination of feed or drinking water (Heckert, Saif & Myers, 1989; Tråvén, Bjornerot & Larsson, 1999; Radostits *et al.*, 2007). Aerosol is probably the major route of rapid within-herd spread of BCV causing the WD outbreak (Tråvén, 2000).

1.3. Clinical manifestation of BCV

BCV was first reported by (Mebus *et al.*, 1972; Mebus *et al.*, 1973), and is now associated with diarrhea in newborn calves (CD), winter dysentery (WD) in adult dairy cattle and respiratory tract infections in calves and feedlot cattle (Saif *et al.*, 1991; Storz *et al.*, 2000). BCV has tropism for both the intestinal and respiratory tracts (Clark, 1993). The coronavirus strains causing WD in adult cattle have a close antigenic and genetic relationship with those causing CD and small differences detected have not been clearly linked to differences in host age (Tsunemitsu & Saif, 1995; Gelinas *et al.*, 2001).

1.3.1. Calf Diarrhea

BCV causes diarrhea in dairy calves worldwide, ranging in age from 1 day to 3 months but mostly between 1 and 2 weeks of age. CD is more common during the winter months, which may reflect the high ability of the virus to survive in a cool, moist environment. The virus replicates and destroys in mature enterocytes of the small intestine and colon, resulting in a malabsorptive diarrhea (Quinn *et al.*, 2002).

1.3.2. Winter Dysentery

WD is a highly contagious disease which is most common in adult lactating dairy cows. Young cattle may be affected but with only mild clinical signs. The disease is most common in northern climates when the cattle are housed. After incubation period of 3-7 days an outbreak of diarrhea affects the dairy cattle. There is a marked drop in milk yield which lasts for up to 1 week, moderate fever, anorexia, and loss of body condition. The feces are liquid with changing the color to dark green or black. Nasal discharge may be observed. In most animals the duration is short and in 2-3 days the consistency of feces returns to normal. Occasionally, the disease becomes more severe; dehydration and weakness are apparent, and dysentery with blood in feces occurs. The morbidity rate may be 30-50% within a few days after the first case is observed, and up to 100% after a week. The case-fatality rate is less than 1%. A typical outbreak may last for 1-2 weeks and in

Sweden, commonly occur between November and January (Tråvén *et al.*, 2001; Radostits *et al.*, 2007).

In mild epidemics of BCV, the maximum decrease in milk production ranges around 10% and may last for 1-2 weeks, after which time milk production levels are regained. In severe epidemics, this decrease in milk production may be around 30% and may last longer for up to 1 month (Radostits *et al.*, 2007).

1.3.3. Respiratory infection

Although diarrhea is recognized as the main clinical feature of BCV infections, BCV also causes respiratory tract infections in calves (Saif *et al.*, 1986). BCV can replicate in epithelium of the upper respiratory tract and clinical signs of respiratory disease occur but are usually mild. Nasolacrimal discharge and cough are often present. So called "respiratory BCV" strains have been isolated from nasal swab samples or lungs of feedlot cattle with respiratory tract disease after shipping (da Silva *et al.*, 1999; Lathrop *et al.*, 2000; Storz *et al.*, 2000; Cho *et al.*, 2001). It is still unclear whether respiratory and enteric BCV isolates are distinctive in biological, antigenic and genetic characteristics, whether these isolates differ in their virulence and tropism for the respiratory and digestive tracts (Gelinas *et al.*, 2001; Hasoksuz *et al.*, 2002b).

1.4. Risk factors of BCV Infection

Several risk factors for WD have been reported. Especially, stress factors, such as dietary changes, parturition, close confinement, lactation, cold weather, and wide fluctuations in temperature might play an important role in initiating the disease (Campbell & Cookingham, 1978; White, Schukken & Tanksley, 1989; Smith *et al.*, 1998a). Large herds with a history of an outbreak in the previous years were at increased risk of an outbreak (Radostits *et al.*, 2007). Also, the immune status of cows and mixed infections of BCV with other microorganisms might be contributory factors to the disease (Quinn *et al.*, 2002).

1.5. Laboratory diagnosis of BCV infections

1.5.1. Sampling

Nasal swab samples can be used for the detection of respiratory infection. However, enteric BCV infections are generally diagnosed by examination of fecal samples for the virus (Clark, 1993).

1.5.2. Detection of virus

Typically, corona virus particles can be demonstrated in fecal samples by direct electron microscopy (EM) and immune EM (Saif *et al.*, 1986; Heckert, Saif & Myers, 1989). Isolation of virus in tissue culture is rarely used as a means of diagnosis as BCV is difficult to isolate. However, BCV has been grown in tracheal and gut organ cultures (Clark, 1993) and also in a large number of cell lines including Human rectal tumour-18 (HRT-18), Vero (African green monkey kidney), BEK-1 (Bovine embryonic kidney), D2BFS (Bovine fetal spleen), BEL

(Bovine embryonic lung), Madin Darby bovine kidney (MDBK) and Madin Darby canine kidney 1 (MDCK1) (Benfield & Saif, 1990; Tsunemitsu & Saif, 1995). HRT-18 is the most sensitive lineage to be used in primary isolation, mainly in the presence of trypsin (Benfield & Saif, 1990; Tsunemitsu *et al.*, 1991). The monolayers of Hamster lung-1 (HmLu-1) also show a high permissivity to BCV (Jerez *et al.*, 2005).

1.5.3. Detection of antibodies

Enzyme-linked immunosorbant assay (ELISA) is probably the most widely used diagnostic test for BCV serological surveys as it can be applied for mass screening and give reliable results quickly (Radostits *et al.*, 2007).

Isotype-capture ELISA tests for BCV-specific IgA and IgM in milk and serum have been developed and are useful for discriminating between primary infection and reinfection (Smith *et al*., 1998b; Näslund *et al*., 2000).

1.5.4. Nucleic acid based detection methods

The 1-step RT-PCR and nested PCR assays are sensitive methods to detect BCV in nasal and fecal specimens (Cho *et al.*, 2001), although the risk of false negative results in the clinical samples needs to be considered (Tråvén *et al.*, 2006). Molecular epidemiology of BCV infections can be investigated using PCR amplification and sequencing of S1 subunit in fecal and nasal samples (Liu *et al.*, 2006). The region spanning amino acid residues 146-179 and 458– 531 of the S1 subunit has been identified as hyper variable regions (Rekik & Dea, 1994; Hasoksuz *et al.*, 2002b). Small genetic differences detected by molecular analysis of the S1 subunit have not been clearly linked to differences in tissue tropism (Hasoksuz *et al.*, 2002b). Nested and semi-nested PCR (SN-PCR) of the N gene of BCV have been developed to detect BCV in fecal and nasal samples as the N gene is highly conserved among BCV strains (Hasoksuz *et al.*, 2002a; Jeong *et al.*, 2005).

Real-time PCR can provide a sensitive method, which further reduces working time and decreases the risk of contamination of samples (Boxus, Letellier & Kerkhofs, 2005). Several different systems are available to detect amplicons in real-time (e.g. TaqMan®, molecular beacon, SYBR Green and Primer-Probe energy transfer). Recently, a one-step real-time RT-PCR was developed based on SYBR Green detection as a diagnostic method on a wide range of coronavirus strains in clinical samples. The assay showed a high sensitivity and specificity for coronaviruses from different animal species (Escutenaire *et al.*, 2007).

1.6. Control of BCV

Protection against BCV diarrhea in the neonatal calf is dependent on the presence of adequate levels of specific antibodies in the gut lumen which is passively acquired from the dam via the colostrum and milk (Radostits *et al.*, 2007). BCV may be introduced to herds either by bought-in cattle or by indirect spread, by humans (Liu *et al.*, 2006). Because of the high contagiousness of BCV and the

lack of completely effective control measures, every management effort must be made to avoid the spread of infection on inanimate objects such as boots and equipments between herds (Radostits *et al.*, 2007).

Treatment is of doubtful value because affected dairy cattle usually recover spontaneously in 24-36 hours. Occasionally dehydration will become severe and the aim of treatment is to replace the loss of fluids and balanced electrolytes which can otherwise lead to acidosis (Clark, 1993; Radostits *et al.*, 2007).

1.7. Vaccination

The immune status of susceptible calves may be raised either by vaccination of pregnant cows to increase the level of passively acquired immunity or by vaccination of neonatal calves to stimulate active immunity (Clark, 1993).

Some preliminary studies have tested the potential of BCV vaccine to induce serum antibodies. In a field study, 2-10-year-old cows injected twice intramuscularly with a vaccine; prepared by solubilizing cells infected with bovine coronavirus showed a high hemagglutination inhibition antibody titer which persisted for several months. This confirms the safety and high antibody-response induced by this prototype vaccine. Therefore, this vaccine may be useful for the prevention of winter dysentery caused by bovine coronavirus infection (Takamura, Matsumoto & Shimizu, 2002).

Intranasal vaccination with a modified-live vaccine against BCV in one investigation also reduced the risk of treatment for bovine respiratory disease (BRD) in calves entering a feedlot (Plummer *et al*., 2004).

2. Bovine Respiratory Syncytial Virus

2.1. The virus

Bovine respiratory syncytial virus (BRSV), like its human counterpart HRSV, is a RNA virus classified in the *Pneumovirus* genus of the *Paramyxoviridae* family, subfamily *Pneumovirinae*, order *Mononegavirales* (Van der Poel *et al.*, 1994; Pringle, 1996). Its helical nucleocapsid is located within the M-protein layer, and includes the 13 to 15-kb single-stranded, negative sense, non-segmented RNA genome (Van der Poel *et al.*, 1994; Valarcher, Schelcher & Bourhy, 2000). Virions are pleomorphic, typically spherical (diameters of 150 to 200 nm), although filamentous particles of up to 400 nm in length have been described (Belanger *et al.*, 1988). The BRSV genome encodes at least 10 proteins which are expressed by transcription of 10 mRNAs. They include two nonstructural proteins (NS1 and NS2); four RNA-associated proteins to form the ribonucleoprotein (RNP) complex, namely, the nucleoprotein N, the phosphoprotein P, the subunit L of the RNA polymerase, and the membrane protein M; a transcription elongation factor is also encoded by the M2 gene (Mallipeddi, Samal & Mohanty, 1990; Samal *et al.*, 1993); and three envelope-associated proteins, namely, the fusion protein F, the attachment protein G, and the small hydrophobic protein SH. The order of transcription is 3' NS1 NS2 N P M SH G F0 M2 L 5'. Viral polymerases

are incorporated in the nucleocapsides and transcription as well as replication takes place in the cytoplasm of the cell. Virus assembly and spread occur by budding from the cell membrane, by fusion and by lysis of cells (Kingsbury, 1990). The major target cells for replication are epithelial cells in the respiratory tract and pneumocytes (Viuff *et al.*, 2002).

BRSV is a sensitive virus and extremely fragile, whereas below -50°C it remains stable for several months (Smith, Lehmkuhl & Phillips, 1975). BRSV is most closely related to the caprine RSV and ovine RSV, indicating inter-species transmission (Grubbs, Kania & Potgieter, 2001a), and even BRSV has been shown in wild ruminants like deer (Gaffuri *et al*., 2006).

The G glycoprotein of BRSV mediates attachment of the virus to cells and is unique among *Paramyxoviridae* since it lacks both neuraminidase and haemagglutinating activity (Levine, Klaiberfranco & Paradiso, 1987). Antigenic variations in the major surface glycoprotein of the G attachment protein may have important implications in pathogenesis of BRSV infections (Prozzi *et al.*, 1997). The use of G protein-specific MAb typing was an accurate method for discriminating BRSV strains (Furze *et al.*, 1994).

The F protein of BRSV is responsible for fusion of the viral and host cell membranes and for syncytia formation between infected cells (Matheise *et al.*, 1995). The results of the genetic studies indicate that the F gene region of BRSV is less variable than the G gene region (Lerch *et al.*, 1991; Elvander *et al.*, 1998). Furin-mediated cleavage of the F protein was shown to result in the release of a peptide that is converted into a biologically active tachykinin, called virokinin. Recent studies suggest that virokinin secreted by BRSV-infected cells may cause bronchoconstriction, since it induces smooth muscle contraction (Zimmer *et al.*, 2003; Valarcher *et al.*, 2006).

BRSV exists as a single serotype and antibody cross reactivity occurs between bovine, human, caprine and ovine RS viruses. According to reactions with MAbs, BRSV has been divided into three antigenic subgroups (A, AB and B) (Furze *et al.*, 1994; Grubbs, Kania & Potgieter, 2001b).

2.2. Epidemiology of BRSV

BRSV appears to be spread worldwide (Baker, Ames & Markham, 1986; Van der Poel *et al.*, 1994; Almeida *et al.*, 2005). The high prevalence of antibodies to BRSV suggests that the infection is endemic in most areas (Baker, Ames & Markham, 1986; Uttenthal, Jensen & Blom, 1996). Epidemiological studies have shown a seasonal periodicity in BRSV and new infections occur most commonly in autumn and winter (Ames, 1993; Van der Poel *et al.*, 1993). It is not known how the virus survives during interepidemic periods (Van der Poel *et al.*, 1994). The virus could persist and replicate in bovine B-lymphocyte cell of local lymph nodes 6 months after BRSV infection. This may explain the viral circulation in the herds and inapparent reinfection of adults (Valarcher *et al.*, 2001).

2.2.1. BRSV in Scandinavia

In Sweden, in the winter of 1988/89, epizootics of respiratory disease caused by BRSV were diagnosed in dairy herds (Elvander, Alenius & Jacobsson, 1991). Since then the respiratory infections due to BRSV occur annually in the country. A nationwide study on bulk tank milk showed that 41-89% of herds were antibody positive in 1990 in Sweden (n=2237) (Elvander, 1996a). In addition, a serological survey in south western Sweden showed high seropositivity rate to BRSV among dairy herds. The prevalence of BRSV was highest in areas with the highest population of dairy cattle (Hägglund *et al.*, 2006).

BRSV is a common pathogen in Finnish cattle with respiratory problems (Hartel *et al.*, 2004). In an etiological study of 40 Finnish dairy herds, BRSV was detected in 10% of respiratory lavage samples using real-time PCR. Serological test showed a seropositivity of 40% in studied herds (Autio *et al.*, 2007).

BRSV has also been demonstrated in Danish dairy herds, based on viral isolation and serological surveys. The findings suggest that BRSV is an important causative agent in calf respiratory disease in Denmark, even in very young calves (Uttenthal, Jensen & Blom, 1996). Therefore, the disease causes substantial losses for the calf rearing industry. Denmark experienced a major BRSV epidemic in 1999 (Larsen, Tjornehoj & Viuff, 2000). Testing 50 Danish dairy herds (453 samples) for IgG1, IgG2 and IgM showed that more than half of the samples had BRSV antibodies to both IgG1 and IgG2 isotypes indicating a high herd prevalence to BRSV (Uttenthal *et al.*, 2000).

The first epidemic respiratory disease associated with BRSV was reported in Norway in 1976/77. Then only sporadic outbreaks occurred until 1995, when a new outbreak of acute respiratory disease associated with BRSV occurred in many cattle herds in central Norway. It was assumed that the infection was introduced with beef cattle imported from Denmark (Norström, Pfeiffer & Jarp, 2000).

2.2.2. Transmission of BRSV

The main route for transmission of BRSV is thought to occur via direct contact with respiratory secretions (Van der Poel *et al.*, 1994; Easton, Domachowske & Rosenberg, 2004). Also airborne transmission of BRSV has been confirmed experimentally, whereas it is not the main route (Mars, Bruschke & Van Oirschot, 1999).

2.3. Clinical manifestation of BRSV

BRSV was first isolated from cattle with respiratory disease in Switzerland (Paccaud & Jacquier, 1970), and now is undoubtedly an important cause of severe respiratory disease in calves under six months of age and also associated with BRD in yearlings and dairy cattle (Elvander, 1996a; Baker, Ellis & Clark, 1997; Larsen, 2000). In Sweden, BRSV was demonstrated in nasal swab samples collected in 1992 from dairy cows in the acute stage of the respiratory disease by a nested PCR assay and culture (Elvander, 1996a).

Similar to HRSV, BRSV results in seasonal infections among domestic cattle herds with a short incubation period followed by symptoms from the respiratory tract. Interestingly, and unlike HRSV infection, natural BRSV infection is often accompanied by concomitant infection (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*), resulting in what has been defined as BRD complex (Easton, Domachowske & Rosenberg, 2004). Immune-mediated mechanisms may play a role in the phathogenesis of BRSV infection as part of a hypersensitivity reaction (Radostits *et al.*, 2007). The virus causes rhinitis, tracheitis, bronchitis, bronchiolitis, and mild interstitial pneumonia particularly involving the cranio-ventral portions of the lungs combined with widespread emphysema and edema throughout the consolidated lungs (Collins *et al.*, 1988; Baker, Ellis & Clark, 1997; Larsen, 2000). The BRSV-related pneumonia shows formation of syncytial cells, hyperplasia of bronchial epithelium with loss of cilia, and influx of neutrophils. In the alveoli, the virus infection results in necrosis of type I pneumocytes and hypertrophy of type II pneumocytes (Tjornehoj *et al.*, 2003). In severe cases BRSV replicates in alveolar macrophages which are important specific defence cells in the lower respiratory tract. These cells produced significantly less nitric oxide (which has a bactericidal effect) than uninfected macrophages (Schrijver, 1998).

The severe highly fatal form of the disease, also known as the "malignant form" or the paroxystic respiratory distress syndrome (PRDS), is associated with extensive pulmonary mast cell degranulation (Jolly, Detilleux & Desmecht, 2004). In acute cases of BRSV infection, there is polypnea and dyspnea which in a few days become worse with mouth-breathing, coughing, nasal secretion, anorexia and emaciation. Loud abnormal breath and crackling sounds, due to consolidation and emphysema, are audible over the anterior lobes of the lung (Elvander, 1996b; Radostits *et al.*, 2007). In large dairy herds, episodes of infection may be mild and unnoticed, despite cattle having a fever of 40ºC, slight inappetence, and a corresponding drop in milk production which lasts 3-5 days (Elvander, Alenius & Jacobsson, 1991; Ferguson, Galligan & Cortese, 1997). The morbidity rate in herd epidemics of clinical BRSV disease can vary from 30 to 50% or higher, up to 100% in severe infections. The case fatality rate is usually low, 3-5%, but may be higher (Baker, Ellis & Clark, 1997).

2.4. Risk factors of BRSV Infection

Outbreaks have been associated with high population of cattle in areas and with changes in weather, especially declining so much temperatures and atmospheric pressure. Also housing conditions, lactation and conception are considerable as risk factors (Baker, Ames & Markham, 1986; Larsen, 2000). Antigenic subtypes may have relevance in explaining different virulence between BRSV isolates (Radostits *et al.*, 2007). Outbreaks of acute respiratory disease associated with BRSV infection can occur in older cattle if the virus is introduced into a previously non-exposed population (Elvander, 1996a). In areas with such cattle population, the BRSV infection often spreads very rapidly from farm to farm. This may lead to the disease becoming endemic and affecting the same herds almost

every year (Wellemans, 1990). The age distribution in the herds also affect the severity of infection; a large proportion of susceptible calves and young cattle increases the risk for a herd outbreak (Norström, Skjerve & Jarp, 2000).

2.5. Laboratory diagnosis of BRSV infections

2.5.1. Sampling

The samples for BRSV detection can be obtained by the use of lung lavage, transtracheal aspirate and nasal swab. However, nasal swabs collect virus present in the upper respiratory tract only in the very early stages of the disease. Thus, during later stages of BRSV infection virus may be present in the lungs not in the nasal cavity (Larsen, 2000).

2.5.2. Detection of virus

The isolation of BRSV is not commonly used for diagnosis since it is time consuming and difficult to culture despite of high concentration of BRSV antigen in tissue samples. However, BRSV has been propagated on primary bovine embryonic turbinate cells, lung cells, and Madin Darby kidney (MDBK) cells (West *et al.*, 1998; Arns *et al.*, 2003). Recently, chicken embryo related cells (CER), hamster kidney hybrid cells showed to be permissive cell lines for multiplication of BRSV (Spilki *et al*., 2006).

The transport of clinical specimens from the field greatly reduces the sensitivity of virus isolation. The immunofluorescent antibody (IFA) staining is one of the most rapid, reliable, and sensitive tests for the BRSV antigens diagnosis in clinical samples (Larsen, 2000).

2.5.3. Detection of antibodies

The virus-neutralization (VN) assay and ELISA can be used for antibody detection in paired acute and convalescent samples (West *et al.*, 1998). The indirect ELISA, microneutralization ELISA and capture ELISA are used for detecting antibodies to BRSV in milk, bulk milk, and serum (Ellis, Hassard & Morley, 1995; Elvander *et al.*, 1995; Uttenthal *et al.*, 2000).

Maternal antibodies of the IgG1 isotype have a half-life of 23 days. They are not actively transported to mucosal surfaces. Maternal antibodies, acquired from colostrum, are predominantly directed against the F and N proteins. Maternal antibodies suppress serum and mucosal antibody responses of all isotypes, despite extensive replication of the virus (Kimman *et al.*, 1987).

2.5.4. Nucleic acid based detection methods

Several RT-PCR assays including real-time quantitative RT-PCR and quantitative competitive RT-PCR have been developed to detect and quantify the BRSV in cell cultures and clinical samples (Achenbach *et al.*, 2004).

A fluorogenic reverse transcription-PCR (fRT-PCR) assay of the F gene, based on TaqMan principle, also provides a rapid and valuable tool in BRSV research and routine virus detection in clinical specimens (Hakhverdyan *et al.*, 2005).

2.6. Control of BRSV

The disease has often been introduced in to a naive herd by the purchase of animals form BRSV infected areas, thus showing the risk of transporting animals from infected areas in to susceptible farm areas (Elvander, 1996a).

Calves with colostral BRSV antibodies are not protected from infection but the incidence and severity of clinical disease is inversely related to the level of maternal antibodies in calves younger than 3 months (Nettleton *et al.*, 2003). Reliable control measures for BRSV are unavailable. The ubiquitous nature of the virus, the circulation of infection in herds, the purchase of infected cattle, the expansion of herds and recurrent infections make control difficult. Bought-in cows should be tested and quarantined for at least 2-3 weeks before mixing with the remainder of the herd. Minimizing stressors and high-quality management can reduce the negative effects of the infection (Radostits *et al.*, 2007).

2.7. Vaccination

Both live and inactivated vaccines are widely used especially in young calves in fattening production units (Schreiber *et al.*, 2000). The relative immaturity of the immune system and the immunosuppressive effects of maternal antibody in the neonatal period constitute main obstacles to successful BRSV vaccination. However, recently an experimental ISCOMs vaccine overcame the suppressive effect of colostral antibodies (Hägglund *et al.*, 2004).

There are two major problems that have hampered the development of effective BRSV vaccines. First, prior vaccination can enhance the severity of disease following infection; and second, natural infection does not provide long-term solid protection against reinfection (Taylor, 2001).

Immunization with formalin-inactivated BRSV vaccine mainly primes an eosinophilic inflammatory response in lung tissues and induce high levels of IgE serum antibodies (Antonis *et al.*, 2003).

The ability to recover infectious recombinant BRSV from cDNA has greatly facilitated the production of live, attenuated, genetically stable vaccine candidates. Deletion of non-essential genes represents an attractive option for production of these kinds of vaccines (Buchholz, Finke & Conzelmann, 1999; Valarcher & Taylor, 2007).

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Research report

Reduced risk for BCV and BRSV infections in organic dairy herds compared with conventionally managed dairy herds in Sweden

Mehdi R.M. Bidokhti [a], Madeleine Tråvén [a*], Nils Fall [a], Ulf Emanuelson [a], Stefan Alenius [a]

[a] Division of Ruminant Medicine and Veterinary Epidemiology, Department of Clinical Science, Swedish University of Agricultural Sciences, Box 7019, SE-750 07 Uppsala, Sweden

* Corresponding author. Tel.: +46 18 67 17 82; fax: +46 18 67 35 45.
E-mail address: madelein.traven@kv.slu.se (M. Tråvén)

Abstract

The seroprevalence to bovine corona virus (BCV) and bovine respiratory syncytial virus (BRSV) infections was studied in 20 conventional and 20 organic dairy herds in Sweden. The enrolled organic farms had produced certified milk for at least two years. On two sampling occasions, with a one-year interval, 699 serum samples were taken from 624 periparturient cows, 5 to 24 cows per herd, and tested by ELISA for antibodies to BCV and BRSV. Descriptive data of sampled animals and herds were collected to study risk factors for high seroprevalence to the infections, hence reflecting risk factors of the infections. The results showed high seroprevalence at both occasions; approximately 85% were positive to BCV and 80% to BRSV. Herd-level analysis implied that the conventional herds had a significantly higher mean seroprevalence to BCV and BRSV than the organic ($P<0.001$). Age was significantly associated to seroprevalence ($P<0.001$). This study suggests that organic management may be more effective in reducing the seroprevalence to BCV and BRSV compared with conventional management.

Introduction

Bovine corona virus (BCV) and bovine respiratory syncytial virus (BRSV) are widespread viral infections with a high seroprevalence in cattle herds (Van der Poel et al., 1995; Saif, 2004). BCV is an enveloped, positive-stranded RNA virus classified as an antigenic group II member of the family *Coronaviridae* (Spaan et al., 1988). BCV causes both respiratory and enteric disease, including calf diarrhoea, winter dysentery in adult dairy cows, and respiratory infections in cattle of all ages (Alenius et al., 1991; Saif, 2004). BRSV, like its human counterpart HRSV, is an enveloped, negative-stranded RNA virus classified in the *Pneumovirus* genus of the *Paramyxoviridae* family (Stott and Taylor, 1985). As a major viral cause of respiratory disease in young calves, BRSV has considerable economic impact (Van der Poel et al., 1994; Valarcher and Taylor, 2007).

However, cattle of all ages can be infected, and severe symptoms and mortality can be seen in adult cattle (Elvander, 1996).

Outbreaks of both infections usually occur in autumn and winter. Long-lasting humoral immunity, remaining detectable for at least one year, has been found after natural or experimental infections with BCV and BRSV. Epidemiological and experimental studies suggest that serum antibody correlates with immunity (Alenius et al., 1991; Schrijver et al., 1996; Tråvén et al., 2001).

These two viruses frequently precede bacterial invasion of the lung, causing a need for considerable amounts of antibiotics for the treatment. One of the aims of organic production is to reduce the use of antibiotics by providing optimal care, feed, housing and a good biosecurity. However, little is known about the precise transmission routes for BCV and BRSV between herds and management risk factors for transmission and, especially, if organic herds in general are more successful than conventional herds in reducing the risk for infection by these viruses. The aim of this study was therefore to investigate the prevalence of BCV and BRSV infections in dairy cows and to explore the association of these infections with selected herd factors, including organic management.

Materials and methods

Selection of herds and animals

The selection of herds for the study was done among herds that had more than 40 cows, were enrolled in the Swedish Official Milk Recording Scheme (SOMRS) and were geographically located in a region in south-east Sweden, based on postcode numbers (Uppland, Södermanland, Östergötland, and Småland). We only enrolled organic farms that had produced milk according to the organic standards for at least two years. From 52 eligible organic farms, 24 farmers were willing to participate in the study and 20 of these were randomly selected. From 156 conventionally managed farms, 32 farmers were willing to participate and we randomly selected 20. All 40 study herds were free from bovine viral diarrhoea virus (BVDV) according to the national control program (Lindberg and Alenius, 1999).

The herds were all visited once in spring 2005 and once in spring 2006. The inclusion criteria for individuals at each visit were that they were from 7 days before their predicted calving date to 42 days after calving, because the original purpose of the sampling was to study the variation in metabolic parameters of periparturient cows (Fall et al., 2008). If the number of such cows in a herd was less than 12, all of them were sampled. If more than 12 eligible cows were in a herd, 12 were randomly selected for sampling using a random number table.

A total of 699 serum samples were taken from 624 cows in 40 dairy herds. In spring 2005, 169 cows were sampled on the conventional farms and 169 on the organic farms. In spring 2006, 189 cows were sampled in the conventional herds and 172 in the organic herds. By chance 75 cows were sampled at both occasions.

Herd and animal data were collected from the SOMRS, and from questionnaires presented to the farmers at each visit. Data on disorders treated by veterinarians were retrieved from the national animal disease recording database.

Sample collection and antibody testing

A blood sample was drawn from the tail vein of all selected cows using 10-ml evacuated tubes without anticoagulant. Within 4 h the tubes were transported to a lab at room temperature. Sera was then prepared by centrifugation (2000 x g for 10 minutes) of clotted blood and stored at -20 ºC.

All sera were tested using commercially available indirect ELISAs (SVANOVA Biotech) to detect BCV- and BRSV-specific IgG. Sera were diluted 1:25 and analysis performed according to the manufacturer's instructions. Serum samples that generated a corrected optical density (COD) value of ≥ 0.2 at 450nm were regarded as positive in both BCV and BRSV tests, whereas samples generating a value below this cut-off point were regarded as negative (Alenius et al., 1991; Elvander et al., 1995). Both positive and negative control sera were included in each assay. Seroconversion was defined as a negative COD value converting to a positive in paired sera.

Data analysis and statistical methods

The Fisher's exact test and t-test were used to investigate possible associations between serological status and some characteristics such as age of the cow at sampling and milk yield. Age was categorized as 1-3 years (12-36 months), 3-5 years (37-60 months), 5-7 years (61-84 months), and >7 years (>85 months), respectively. Herd level prevalence of animals positive to BCV or BRSV or both was analysed with logistic regression models. Explanatory variables included in the initial model were organic management (yes/no), AI performed by farm personnel (AIF; yes/no) instead of using professional AI technicians, stall type (tied/free), herd average milk yield (above/below median of the study herds), number of cows (above/below median), number of visits by veterinarians during 2005 (above/below median). Data on herd size, AI strategy, stall type and milk yield for September 2005 to August 2006 were used. The initial multivariate model was reduced using a backward stepwise procedure, with $P<0.05$ as the exclusion and re-entering criterion. Herd level prevalence of "double-positive" animals (i.e. antibody positive to both BRSV and BCV) was modelled in the same way. Univariable analyses were done with Minitab (Release 14.2; Minitab, PA, USA), and multivariable analyses with SAS (Release 9.1; SAS Institute Inc., Cary, NC, USA).

Results

Study herds

Descriptive data about the organic and conventional herds are given in Table 1. The mean milk yield of the conventional herds was significantly higher than that in the organic ($P<0.01$), but there was no difference in the mean age of sampled

cows, herd size, or number of samples per herd between the conventional and the organic herds on either sampling occasion.

Seroprevalence in the sampled population

The seroprevalence to BCV on individual level for all sampled cows was 86% (292/338) in 2005 and 84% (304/361) in 2006; the seroprevalence to BRSV, 79% (267/338) and 82% (297/361). Seroprevalence to either BCV or BRSV did not differ significantly between the two sampling occasions.

Seroprevalence in the conventional and organic herds

Analysis at herd level showed that the conventional herds had a significantly higher mean seroprevalence to BCV and BRSV than the organic both in 2005 and 2006. The herd-level seroprevalence to the viruses in 2005 was almost the same as that in 2006 (Table 2). The mean prevalence of cows positive to both BCV and BRSV was 85% in 2005 and 89% in 2006 for the cows in the conventional herds, in contrast to 54% in 2005 and 59% in 2006 for the cows in the organic herds ($P<0.001$). There was no herd in which all sampled cows were seronegative to BCV and also no conventional herd with all cows seronegative to BRSV. However, in two organic herds all sampled cows were seronegative to BRSV (Table 2). In organic herds 32% of the youngest cows (1-3 years old) were positive to both BCV and BRSV, in contrast to 70% in conventional herds ($P<0.001$, Fig. 1). In 10 conventional herds, but only 1 organic herd, all sampled cows were seropositive to both BCV and BRSV.

Age-related seroprevalence to BCV and BRSV

The seroprevalence to both BCV and BRSV increased significantly with increasing age of the cows (Fig. 1). The youngest age group (1-3 years old) had significantly lower seroprevalence than the older cows (3-5 years old) to both infections ($P<0.001$). The mean age in the four different clusters of age was 28, 46, 69, and 98 months, respectively.

Seroconversion in paired serum samples

Seventy-five cows in 15 conventional and 17 organic herds were sampled in both 2005 and 2006. Among samples taken in 2005 92% (69/75) were seropositive to BCV and 79% (59/75) were seropositive to BRSV. Mean COD was 1.7 (0.53-2.62) for BCV and 1.1 (0.34-1.77) for BRSV in the positive samples. All the seropositive cows remained seropositive at the second sampling (mean COD was 1.6 (0.85 -2.37) for BCV and 1.2 (0.22 -2.25) for BRSV). All 6 animals, which were seronegative to BCV in 2005, remained seronegative in 2006. Out of 16 animals, which were seronegative to BRSV in 2005, 10 had seroconverted in 2006. The seroconverting animals belonged to 7 different herds (5 organic and 2 conventional) in which all (60/60) samples taken at the second sampling occasion were seropositive to BRSV.

Models

Results of the logistic regression modelling of serological status and potential risk factors are presented in Table 3 and 4. The probability for cows being seropositive to BCV or BRSV or both was significantly lower in organic herds compared with conventional herds, and significantly higher in herds using AIF. The probability for cows being seropositive to both BCV and BRSV was significantly higher in tie-stall herds, and in herds with below-median milk production, in addition to the risks associated with management system and AIF.

Discussion

The results of this study showed high seroprevalence to BCV and BRSV both in 2005 and 2006 (Table 2). This is in agreement with previous reports that these two viruses are endemic in southern Sweden (Elvander, 1996; Tråvén et al., 1999; Hägglund et al., 2006). In fact, these virus infections are probably endemic in most countries with an intensive milk production. In a nationwide study in UK for BCV- and BRSV-antibodies in bulk milk samples, all herds were antibody positive (Paton et al., 1998).

Although the organic and conventional herds included in this study were similar in many aspects (Table 1), the organic herds showed significantly lower seroprevalence to BCV and BRSV at both cow level and herd level, compared with the conventional herds (Table 2, 3 and 4). A possible explanation for the higher risk for conventional herds may be that these herds were managed in ways that lowered their level of biosecurity, compared with the organic herds. Housing conditions, such as close confinement, climate, feed and pregnancy have been proposed as risk factors for BCV and BRSV infections (Baker et al., 1986; Clark, 1993; Smith et al., 1998). Trading of animals is regulated for organic farms; hence, farms are permitted to recruit animals only from other organic farms and strongly recommended to purchase from only one farm and to use a quarantine (KRAV, 2007). Such restrictions could reduce the risk of direct transmission of BCV and BRSV because these viruses may be introduced by purchasing animals (Wellemans, 1990; Saif, 2004). However, previous studies have shown indirect contacts to be more important for both BCV and BRSV transmission (Norström et al., 2000; Tråvén, 2000), and further studies are thus needed to identify causes of the differences between the farming systems.

Veterinarians and AI technicians have been considered to play a role as potential carriers of the viruses in the transmission of BCV and BRSV, especially in large herds which probably receive more frequent visits (Wellemans, 1990; Tråvén et al., 1993; Norström et al., 2000). Our study did not show that the seroprevalence to BCV and BRSV was related to the frequency of visits by a veterinarian and, in fact, herds using AIF were at higher risk than herds using professional AI technicians. A lack of association with total number of visitors was also reported in a previous study (Hägglund et al., 2006). One reason might be that well-educated AI technicians undertake careful biosecurity measures, including disinfection of hands and equipment, and change of clothing/boots between herds

and also educate the farmers about the importance of biosecurity. Systematic data on other contacts were not available. One herd had a high number of community visitors, such as school classes. This organic herd had low seroprevalence to BRSV (1/15), only one positive cow at the age of 59 months, suggesting that contacts unrelated to the cattle industry are not a transmission risk.

Housing cattle in tie stall facilities appeared to increase a herd's risk for BCV and BRSV compared with free stalls. This accords with observations previously reported for BCV (Smith et al., 1998; Hägglund et al., 2006). In tie stalls, cattle are confined and the stocking density is high. Such conditions may lead to increased handling of cattle and a closer contact between cattle and visitors related to the cattle industry, facilitating indirect inter-herd transmission. Higher seroprevalence to both viruses was also associated with a lower milk yield. Outbreaks of these infections have a considerable economic impact on the affected herds (Van der Poel et al., 1995; Larsen, 2000). However, another explanation could be that herds that have a milk production above the average are generally well managed, including but not limited to a higher biosecurity.

Seropositive animals remained positive also at the second sampling and no conversion to negative was observed, confirming earlier data that the antibodies are detectable for several years after these infections (Elvander, 1996; Tråvén, 2000). Seroconversion to BRSV occurred in some herds, in which 100% of the cows sampled on the second occasion were seropositive. This is in agreement with previous observations for both BCV and BRSV that if these viruses are introduced to herds most of the susceptible animals seroconvert within a short time period (Alenius et al., 1991; Tråvén, 2000; Hägglund et al., 2006). In five organic herds, only cows in their fourth lactation and older were seropositive to BRSV, indicating that herds can stay free from this infection for several years.

Seroprevalence to BCV and BRSV in the 1- to 3-year-old animals was significantly lower than in the 3- to 5-year-old animals (Fig. 1), demonstrating the age-dependent seroprevalence to these infections. The probability to be seropositive to these two viruses in either conventional or organic herds gradually increased with age till 100% of the cows in the oldest age-cluster (older than 7 years old) were seropositive (Fig. 1). The mean antibody levels against BCV and BRSV in the oldest age cluster were also the highest compared with the other clusters of age ($P<0.001$; data not shown). Thus, the oldest cows most likely have the highest titres of antibodies against BCV and BRSV in colostrum, making colostrum from the oldest cows a valuable tool in herds with neonatal infection problems.

The seroprevalence of all cows sampled corresponds to the incidence of infection over a longer time period, i.e. several years, whereas the risk factor information may be applicable only to the limited time period when they were collected. However, a less precise definition of risk factors is most likely to decrease the chance of finding significant associations because such misclassifications are likely to be non differential. An alternative would be to perform a risk factor analysis based only on young cows, where risk factors and seroprevalence are

more well-timed. However, the small sample size, especially of young animals due to the sampling strategy, did not allow for such an analysis.

Using young animals as sentinels, to determine the sero-status of herds has been shown to be effective for BVDV and is the method of choice in the Swedish national control programme (Lindberg and Alenius, 1999). It has also been shown that repeated sampling of only three calves per herd can monitor the herd incidence of BCV and BRSV infections over the sampling period (Hägglund et al., 2006). Among the cows born on the farms in our study, there was a clear border between younger seronegative cows and older seropositive ones. Thus the serological results of only the 2 or 3 youngest cows correspond to the herd incidence of these infections during the last two years. Such a sampling strategy may be sufficient to determine whether a large part of the herd is sensitive to infection, and can be applied in an economical, rapid and less laborious screening programme for future investigations.

Conclusion

This study is the first survey, to our knowledge, comparing seroprevalence to BCV and BRSV between organic and conventional dairy farms. It indicates that organic management may have better biosecurity leading to reduced seroprevalence to BCV and BRSV infections. The results also imply that the seroprevalence to BCV and BRSV increases with age. Because the epidemiology of these viruses is still unclear, further research is needed to determine which aspects of the organic management reduce the inter-herd transmission of these infections.

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Table 1. *Descriptive data from 20 conventional and 20 organic Swedish dairy herds studied to determine BCV and BRSV prevalence*

	Herd system	
	Conventional	Organic
Age (months) of cows at sampling Mean (range)	44 (22-136)	46 (23-138)
Herd size Mean (range)	61 (40 - 122)	63 (41 -105)
Herd average milk yield (kg/cow/year) Mean (range)	9171 (7850 -10714)	8222 (5359 -11233)
Number of herds according to stall type Tie, Free	13, 7	6, 14
Number of veterinary visits per year Mean (range)	16 (0 – 39)	21 (3 – 44)
Number of herds with AIF [a]	10	14
Number of samples per herd Mean (range)	18 (8 - 24)	17 (5 - 24)
Number of samples Total (Spring2005, Spring2006)	358 (169, 189)	341 (169, 172)

[a] AI performed by farm personnel instead of using professional AI technicians.

Table 2. *Prevalence of antibodies to BCV and BRSV at herd-level in 20 conventional and 20 organic Swedish herds sampled on two occasions*

Sampling Time	Mean % BCV-positive sampled cows (range)		Mean % BRSV-positive sampled cows (range)		Mean % BCV and BRSV-positive sampled cows (range)	
	Conventional	Organic	Conventional	Organic	Conventional	Organic
2005	96[a] (42-100)	79[a] (30-100)	89[a] (25-100)	70[a] (0-100)	85[a] (25-100)	54[a] (0-100)
2006	95[b] (30-100)	77[b] (9-100)	93[b] (50-100)	74[b] (0-100)	89[b] (34-100)	59[b] (0-100)

[a, b] Numbers within pairs of columns with the same letter are significantly different ($P < 0.001$).

Table 3. *Association between herd level prevalence of animals seropositive to BCV or BRSV or both and herd characteristics as estimated with a logistic regression model*

Variable	Level	Odds ratio (OR)	95% confidence interval of OR	p-value
Organic	Yes	0.07	0.02;0.25	<0.0001
	No	1	n.a.	
AIF [a]	Yes	5.47	2.39;12.50	<0.0001
	No	1	n.a.	

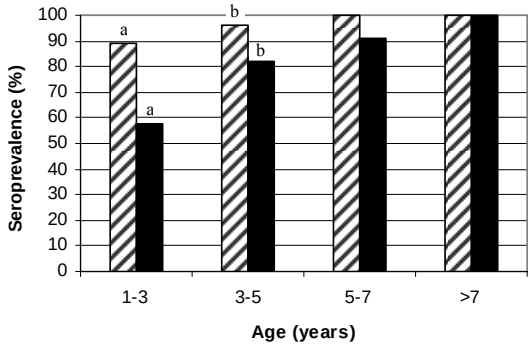
[a] AI was performed by farm personnel instead of using professional AI technicians.

Table 4. *Association between herd level prevalence of animals seropositive to both BCV and BRSV and herd characteristics as estimated with a logistic regression model*

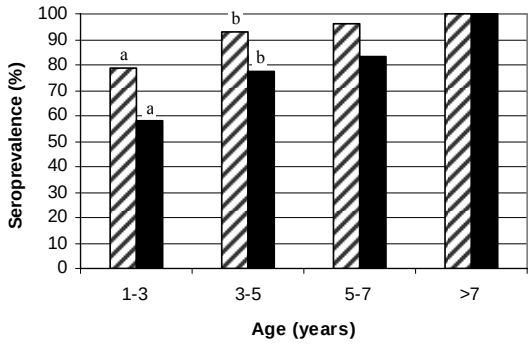
Variable	Level	Odds ratio (OR)	95% confidence interval of OR	p-value
Organic	Yes	0.18	0.12;0.29	<0.0001
	No	1	n.a.	
AIF [a]	Yes	2.40	1.60;3.62	<0.0001
	No	1	n.a.	
Stall type	Tied	2.00	1.33;3.01	0.0009
	Free	1	n.a.	
Milk yield	Above median [b]	0.51	0.34;0.78	0.0021
	Below median [b]	1	n.a.	

[a] AI was performed by farm personnel instead of using professional AI technicians.
[b] The median was 8903 kg/cow/year.

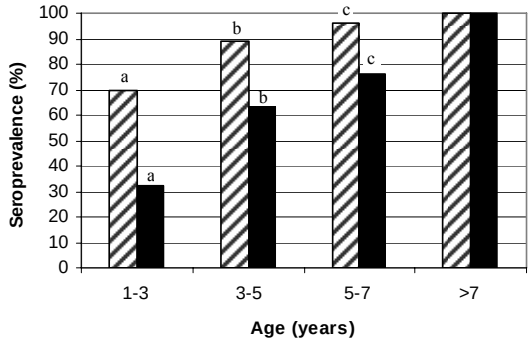
A) BCV



B) BRSV



C) Both BCV and BRSV



Conventional Herds **Organic Herds**

Fig. 1. Age-specific seroprevalence to bovine corona virus (BCV) (A), bovine respiratory syncytial virus (BRSV) (B), both BCV and BRSV (C) in relation to herd management system. Clusters of age in years (months) are 1-3 (12-36), 3-5 (37-60), 5-7 (61-84), and >7 (>85). Number of serum samples per cluster is 120, 147, 58, and 16, respectively, from the organic herds and 143, 149, 54, and 12, from the conventional herds. The a-c seroprevalences with the same letter differ significantly between the conventional and organic herds ($P<0.001$).

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