Introduction

Bovine respiratory disease continues to cause significant financial losses throughout the world. Morbidity, mortality, lower performance and condemnation of material at marketing all contribute to the economic loss. Prophylaxis and metaphalaxis costs also contribute to the overall effect.

Bovine respiratory disease is of multi-factorial origin. Onset of disease depends on a complex interaction between viruses, bacteria, the environment, and the host. Disease onset is thought to begin by reduced host resistance and infection with one or more viral agents such as Infectious Bovine Rhinotracheitis (IBR).

Bovine Virus Diarrhoea (BVD), or Bovine Respiratory Syncytial Virus (BRSV). Viral infection compromises resistance to bacterial infection in a variety of ways, facilitating subsequent infection with pathogenic bacteria.

BRSV can be considered as a primary BRD pathogen and is also a component of the bovine respiratory disease complex.

This article reviews briefly the role of Bovine Respiratory Syncytial Virus (BRSV) in cattle.

Aetiology:

BRSV is classified as a pneumovirus in the Paramyxovirus family, is enveloped and contains a negative sense, single-stranded RNA genome of approximately 15.2 kb. The RSV viral RNA is transcribed into 10 major sub-genomic mRNAs encoding 11 proteins, because the M2 gene encodes 2 proteins.

Sacco et al outline the virus as follows: Associated with the genomic RNA are nucleocapsid (N), phosphoprotein, large polymerase, and associated proteins, as well as transcriptional anti-termination factor
M2-1 and RNA regulatory protein M2-2. There are 3 transmembrane surface glycoproteins: attachment (G), fusion (F), and a small hydrophobic. A non-glycosylated matrix or membrane protein (M) is associated with the inner face of the envelope. Finally, there are 2 non-structural (NS) proteins that accumulate in infected cells: NS1, NS2.

RSV has been classified into 2 subgroups, A and B, based on antigenic and genetic differences and BRSV isolates can be classified into subgroups based on reactivity of mAb to the G protein, although these may represent variants of a single major antigenic group. The F protein is a type I viral fusion protein synthesized as a precursor that is proteolytically cleaved by a furin into disulfide-linked fragments.

The F and G glycoproteins contain the main neutralization and protective epitopes and the attachment protein is a major target of the host anti-RSV antibody response.

The virus replicates predominantly in ciliated respiratory epithelial cells but also in type II pneumocytes and BRS virus was named for its characteristic cytopathic effect the formation of syncytial cells which are giant multinuclear cells formed by the fusion of several cells.

Epidemiology:
Although cattle are the natural host of BRSV, it is possible that other species such as sheep and goats may play an epidemiological role in certain circumstances. The distribution of BRSV is worldwide and the virus has been isolated from cattle in Europe, America and Asia. In a serological survey published in 1990 Van Vuuren reported a prevalence of 43% seropositive animals in South African feedlots.

According to Sarmiento-Silva et al BRSV infection is most likely as a direct result of the movement of cattle with infectivity rates usually rather high, suggesting that viral transmission is a common event among herds. Intra-herd transmission usually occurs by aero-

solns, allowing the virus to enter susceptible cattle via the respiratory tract. However, local spread and airborne transmission between herds are not of great importance for inter-herd transmission despite the circulation of BRSV in a given geographical region.

Direct transmission between herds is frequently a consequence of the introduction of new infected animals and indirect transmission may occur by individuals visiting farms. Some of the main risk factors for BRSV transmission include large herd size and inadequate biosecurity.

BRSV outbreaks commonly occur during winter and clinical disease is commonly diagnosed during autumn and winter but infection can also be observed during summer. The sero-prevalence of BRSV infection varies greatly across different geographical regions and the distribution of BRSV is most likely affected by the movement of cattle, as insect vectors are not believed to play a role in viral transmission.

The morbidity of the disease is quite high, and in some instances, it has been responsible for up to 60% of the clinical respiratory diseases among dairy herds. In general, the frequency of BRSV is strongly associated with cattle population density in the region and with the age of the host. Interestingly, BRSV infection is also associated with a high morbidity of up to 80% and with mortality that can reach up to 20% in some outbreaks.

BRSV outbreaks can become epidemics affecting animals in all age groups. However, the age distribution of BRSV infection seems to be a function of exposure. In other words, herds that have been previously exposed to the virus tend to experience infections that are limited to younger, more susceptible animals. In consequence, morbidity is commonly high during the occurrence of outbreaks.

Importantly, natural infection affects both beef and dairy cattle, although management practices can significantly impact the infectivity rates. Climate also favours the dissemination of the virus during winter, after sudden drops in temperature, although infection can occur throughout the year.

According to Sarmiento-Silva et al the mechanisms that are responsible for the survival of the virus within a given population are not fully understood. Controversial information has been reported about viral persistence and chronicity has been proposed as a mechanism that might play a role in spread of disease. BRSV can be isolated from asymptomatic animals and can persist for several months therefore, latent infection among herds might occur, providing a possible explanation for the occurrence of outbreaks among relatively isolated calves. Some
reports have suggested that subclinical infection in cattle is not a plausible mechanism for the persistence of BRSV in dairy herds and clinically ill animals are believed to be the most likely sources of infection, and therefore, the most likely explanation for recurrent infections is the reintroduction of the virus into the herd before the occurrence of a new outbreak. Larsen, Tjornehoj and Viuff investigated BRS virus isolated from recurrent outbreaks in closed and other herds in Denmark.

The isolates included viruses from the same herd (closed dairy farms and veal calf production units) in different years and from all confirmed outbreaks in Denmark within a short period. The nucleotides coding for the extracellular part of the G glycoprotein and the full SH protein of bovine respiratory syncytial virus (BRSV) were sequenced and results showed that identical viruses were isolated within a herd during outbreaks and that viruses from recurrent infections varied by up to 11% in sequence even in closed herds. They suggested that it may be possible that a quasi-species variant swarm of BRSV persisted in some of the calves in each herd and that a new and different highly fit virus type (master and consensus sequence) became dominant and spread from a single animal in connection with each new outbreak. Based on the high level of diversity, however, they suggested that the most likely explanation was that BRSV was (re)introduced into the herd prior to each new outbreak.

According to Merck maternally derived antibodies provide at least partial protection against clinical signs after natural and experimental BRSV infection. Virus shedding has occasionally been reported after experimental BRSV re-infection but little or no clinical disease is usually observed in re-infected animals. BRSV is mainly transmitted by direct contact between infected animals or by aerosol but it cannot be excluded that it might also be spread by humans acting as passive vectors.

**Pathogenesis**

BRSV initially infects the upper respiratory tract (nasal, tracheal, and bronchial epithelium) by attaching to respiratory epithelium via G glycoprotein after which the virus spreads cell to cell forming syncytia. Syncytia result from fusion of cells, mediated by fusion (F) glycoprotein until eventually, bronchiolar and alveolar epithelium becomes infected.

Activation of complement causes degranulation of mast cells throughout the lung which cause diffuse bronchoconstriction leading to airway obstruction by cellular debris and emphysema. Infected cells undergo apoptosis and are phagocytized by neighbouring epithelial cells while virions within the lumen of the respiratory tract are cleared by neutrophils. BRSV replication is limited to respiratory epithelium and primarily infects type I and II pneumocytes where viral nucleocapsid material may be found in the cytoplasm. There is apparently no evidence of a viremic phase.

Sacco et al published an excellent article and the readers interested in the technical side of the immune response to BRSV are encouraged to read it. The following is a brief summary of some of the points that they make:

Firstly the initiation of an innate immune response is dependent on the recognition and binding of evolutionarily conserved pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors (PRRs). Recognition of viral PAMPs involves at least 3 distinct classes of PRRs: toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors, and nucleotide-binding oligomerization domain-like receptors.

Recognition of RSV infection involves several TLRs including TLR2, 3, 4, 7, and 8. TLR3 which recognizes double stranded RNA, and TLR7, which recognizes single-stranded RNA are endosomal receptors that are key for the innate recognition of RNA viruses.

Recognition of viral infection through PRRs results in production of type I IFN and induction of IFN-stimulated genes, Paramyxoviruses are known to utilize different mechanisms to affect IFN signalling and BRSV has evolved strategies to inhibit the IFN-induced cellular response that are dependent on NS proteins.

Pneumoviruses, characteristically, have 2 genes that encode for NS proteins. It has been shown that NS1 and NS2 proteins cooperatively mediate resistance of BRSV to IFN stimulated responses in a species-specific manner. NS proteins block phosphorylation and activation of IRF3.

In addition to induction of the IFN response, ligation of PRRs by viral PAMPs stimulates the early release of inflammatory chemokines and cytokines that are important in the initiation of adaptive immunity. In particular, activation of antigen-presenting cells such as dendritic cells and macrophages is critical for this function. Sacco et al have demonstrated that BRSV infection also induces inflammatory cytokine production by alveolar macrophages. Cytokines are cell signalling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma.

Secondly the development of an adaptive immune response is required for the control and clearance of established RSV infections. Following infection, cattle
mount virus-specific antibody and T-cell responses; however, these responses are weak and transient, as animals can be continuously re-infected throughout life. T-cell responses are directed at epitopes within several RSV proteins, including the N, M, NS2, M2-1, F, and G. The F and G proteins are the major histocompatibility antigen class II-restricted targets in cattle.

Calves infected with BRSV develop a mixed cytokine response but favour the development of a Th2-type immune response following infection. They mount antibody responses to several BRSV antigens, but the primary targets for protective humoral responses are the F, G, and NP proteins. BRSV-specific IgM and IgA can be detected in the nasal secretions and serum of BRSV-infected calves as early as 8 days post-infection. BRSV-specific IgG2 is not detected in the serum until 1 to 3 months post-infection, while virus-specific IgG1 is detectable starting at 13 days post-infection.

Calves infected with BRSV exhibit limited BRSV-specific cytotoxicity during primary infections and impaired memory responses following challenge or vaccination. This may be due to the strong Th2 skewing that occurs during RSV infection, which acts to inhibit the development of an efficient CD8 T-cell response and prevent the establishment of a long-lived memory.

Virus-specific IgE, antibody associated with Th2 skewing and airway hyper-responsiveness has been detected in the serum concurrent with the development of clinical signs.

Interestingly, according to Sacco BRSV infection in calves has also been shown to predispose to allergic sensitization.

Humoral immunity plays an important role in defending the host from RSV infection. While not fully effective, maternal antibodies may provide some level of protection from severe BRSV infection; however, their presence has also been described to suppress the development of antibody and T-cell responses during acute infection.

**Symptoms:**
Sacco et al state that the incubation time for BRSV is estimated to be 2 to 5 days. Infection can be subclinical and involve the upper respiratory tract or both the upper and lower regions of the respiratory tree. Clinical signs of BRSV infections may range from minimal to severe with dyspnoea and death. Affected calves can show tachypnea, ocular serous secretions, dry muzzle, reduced activity, anorexia, and fevers up to 40 C. With severe infections, dyspnoea can be pronounced and marked. Dyspnoea, possibly with open-mouthed breathing, may become pronounced in the later stages of the disease. Subcutaneous emphysema may occur and secondary bacterial pneumonia is a frequent occurrence. These signs coincide with BRSV infection, during which viral replication is detectable, beginning at 2 to 3 days post infection and continues until 7 to 10 days post infection. There has been a biphasic pattern of clinical signs noted following some BRSV infections. However, the mechanistic basis underlying this phenomenon is not fully understood. Pathogenesis is not completely understood but immune-mediated host response is proposed to play a role. Cattle with higher levels of IgE apparently have more severe disease.

In grouped calves, individual animals can become infected, but there can be spread involving multiple calves. With subclinical infections, animals may...
have some loss of feed intake and, thereby, a subtle reduction in weight gain and activity. With severe infections, however, anorexia and reduced water consumption can result in weight loss and dehydration in just a few days. Those animals that do survive severe infection lag to varying degrees in weight gain and growth. The virus alters host defence mechanisms of the respiratory tract, allowing secondary bacterial infection most often by *M. haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*.

Grisset et al reviewed a number of clinical trials where calves were challenged by aerosolisation, intranasal challenge or combined intranasal and intra-tracheal methods. They reported that the median time to clinical symptoms began on day 3 (range 1–6 days) post inoculation with time to peak median outbreak occurring on day 6 (range 2–11 days). Median time to recovery did not occur until day 12 (range 7–17 days) post inoculation. Median time rectal temperatures exceeded 40°C was day 5 (range 1–7 days) and median time to maximum rectal temperature occurred on day 6 (range 5–8 days). The median time rectal temperatures returned to less than 40°C was trial day 8 (range 7–10 days) post challenge.

Median time to seroconversion for BRSV was day 9 (range 5–21 days) post challenge using serum neutralization. Time to maximum median antibody response occurred on post challenge day 23 (range 9–32 days). Median time to BRSV shedding began 3 (range 1–5 days) days after challenge with median time to peak shedding on day 5 (range 3–8 days) and median time to resolution on day 9 (range 7–14 days).

These measures may be useful in developing predictive epidemic curves for naturally occurring BRSV outbreaks where uncertainty regarding the particular variant of antigen, environment factors predisposing to spread and immune status of animals exposed can be taken into account. Unfortunately Grisset et al do not report on possible associations or correlations between the periods reported.

Merck veterinary manual reports that severe clinical signs are mainly observed in calves, but might also be observed in adult cattle. The higher frequency of clinical signs induced by BRSV in young calves compared with adults can be explained by the level of specific immunity following frequent exposure to the virus.

They state that clinical signs may be observed in cattle of all ages when BRSV is introduced in herds where most of the animals are naïve to the virus and are observed only in calves when the virus circulates regularly in the herd...

Merck also reports that exacerbated clinical signs have been observed following a natural BRSV infection in animals immunised with inactivated vaccines. A number of authors suggest that concurrent exposure to Bovine Viral Diarrhoea Virus and/or 3-methylindole increases severity of clinical respiratory disease.
**Pathology and diagnosis:**

Macroscopically, cranioventral consolidation and atelectasis with interstitial emphysema and mucopurulent exudate in bronchi is seen. Other gross lesions include a diffuse interstitial pneumonia with sub pleural and interstitial emphysema along with interstitial oedema.

These lesions are similar to and must be differentiated from other causes of interstitial pneumonia. Bronchopneumonia of bacterial origin is usually also present.

Histopathological reports typically describe multifocal lesions with alveolar lumina, bronchioles and bronchi being filled and expanded by moderate numbers of degenerate and non-degenerate neutrophils, foamy macrophages, an eosinophilic fibrillar material (fibrin), necrosis (eosinophilic cellular and karyorrhectic debris), haemorrhage, and oedema. Alveolar septa may be expanded by fibrin, macrophages, congestion, and some haemorrhage.

Multifocally bronchiolar and bronchial epithelium may show one or more of the following changes: ciliary loss, epithelial loss, attenuation, hyperplasia, and syncytial cell formation with up to 10 nuclei.

Moderate numbers of epithelial cells and syncytial cells may contain eosinophilic, 3-6µm round to oval intracytoplasmic inclusions. There may be bronchial associated lymphoid tissue hyperplasia.

Interlobular septa and pleura may be moderately expanded by lymphocytes, macrophages, neutrophils, hemorrhage, clear space and ectatic lymphatics (edema).

A diagnosis of BRSV requires laboratory confirmation. BRSV is a difficult virus to detect, although chances of isolation may improve when sampling animals in the incubation or acute phases of infection. Although virus isolation is difficult due to the fragility of the virus, PCR is a useful and rapid method commonly used to detect the antigen. Other procedures that have proved useful in detection of BRSV antigen are fluorescent antibody and immune-peroxidase staining.

Paired serum samples can be used to establish a diagnosis. However, the antibody titer of animals with well-developed clinical disease may be higher in the acute sample than in the sample taken 2–3 wk later, because the antibody response often develops rapidly, and clinical signs follow virus infection by up to 7–10 days.

Single serum samples with high antibody titers from a number of animals in a respiratory outbreak may help diagnosis if coupled with clinical signs. Calves that become infected with BRSV in the presence of passively derived antibody may not seroconvert.

Immunofluorescence stains have been used to identify BRSV antigens in frozen lung samples; however, cellular and structural resolution is difficult with fluorescent images, and tissues can have endogenous (background) fluorescence.

In many laboratories, the virus is detected with immunohistochemistry (IHC) of lung tissue fixed in formalin and processed IHC sections allow excellent assessment of cellular structures and lesions compared to immunofluorescence, although in practical terms, many samples submitted to diagnostic laboratories have some degree of autolysis due to the time that it takes for samples to be collected from cattle in the field or in feedlots and for immersion into formalin.

Autolysis can affect fluorescence and IHC staining and assessment of histopathologic lesions. Additional diagnostic tests include polymerase chain reaction (PCR), to identify the simple presence of viral genome, or real-time reverse transcription PCR (RT-PCR).

**Differential Diagnosis:**

1. Parainfluenza type-3 (PI-3) (paramyxovirus) - very similar histologically with syncytial cells and eosinophilic intracytoplasmic inclusion bodies.
2. Bovine adenovirus - milder clinical disease with lack of syncytila and basophilic intra-nuclear inclusion bodies.
3. Infectious bovine rhinotracheitis (Bovine herpesvirus-1) - eosinophilic intra-nuclear inclusion bodies.
4. Atypical interstitial pneumonia (fog fever) no inclusions, differentiate multinucleated giant cells from syncytial cells at the level of the bronchioles and alveoli

**Treatment and control:**

Treatment focuses on antimicrobial therapy to control secondary bacterial pneumonia. There is no specific treatment for the viral interstitial pneumonia but the data from Grisset et al (median time to recovery being 12 days) suggests that antibiotic course of longer duration may be necessary to minimise the secondary pathogen invasions of affected tissue.

Supportive therapy and correction of dehydration may be necessary in individual clinical cases. There are apparently anecdotal reports of treatment with antihistamines and/or corticosteroids being of benefit although most animals with uncomplicated infections will recover in several days without treatment.

Inactivated and modified-live vaccines are available and may serve to reduce losses associated with BRSV;
There are multiple vaccines currently marketed for BRSV (killed and modified live) that are generally provided as part of multivalent products.

Although there is widespread use of BRSV vaccines in calves, their efficacy may not be guaranteed, and given the significant disease burden associated with BRSV infection, there is a definite need for improved technologies. BRSV and its target population present the need to vaccinate calves with immature or compromised immune systems or in the face of maternal antibodies, and effect an appropriate, robust, and long-lasting immune response.

Lasen, Tegtmeier and Pedersen reported Danish beef herds experiencing outbreaks of pneumonia in calves that had been vaccinated with an inactivated bovine respiratory syncytial virus (BRSV) vaccine 2 months prior to the outbreak. Their laboratory investigations revealed that BRSV was involved and probably initiated the outbreaks.

Furthermore, the serological results suggested that the vaccine induced only sparse levels of antibodies probably due to the presence of maternally derived antibodies at the time of vaccination.

Sacco reports similar vaccine enhanced disease from 2 cases of natural BRSV infections in calves. In one case, an outbreak of respiratory tract disease among 5- to 7-month-old calves on a beef-finishing farm in the Netherlands started 2 days after administration of a modified-live BRSV vaccine. The disease was severe among vaccinates but absent in non-vaccinated calves 8 months of age or older.

In another case, 30% of 8-month-old Belgian blue calves vaccinated with a b-propiolactone inactivated BRSV vaccine died during a naturally occurring BRSV outbreak. Interestingly, no deaths were recorded among younger calves not vaccinated. Experimentally, vaccine-enhanced disease has been reproduced in some studies of calves vaccinated with formalin-inactivated BRSV preparations but not in others. Researchers are currently studying the use of subunit-based vaccines and live attenuated BRSV. One promising example reported by Valarcher et al was the success of 2 BRSV strains, one devoid of NS1 and the other lacking NS2. Calves vaccinated with either deletion mutant exhibited a robust virus specific antibody and CD4 T-cell response and were protected against virus challenge.

Interestingly, the NS2 mutant was more effective than its counterpart. Another group used a subunit approach by immunizing calves with nanoparticles encapsulating the N protein of HRSV (Human RSV). In this study, investigators observed N-specific antibodies and cellular responses, as well as reduced viral shedding and lung pathology in vaccinated calves. Furthermore, the calves did not exhibit vaccine enhanced disease.

An additional area being extensively pursued against BRSV is the use of new adjuvants coupled with inactivated BRSV or subunit vaccines. Among those showing promise is the use of CpG containing oligodeoxynucleotides and immune-stimulating complexes, both of which induce a robust Th1 skewing.

Immuno-stimulating complexes are multimers composed of cholesterol, phospholipids, proteins, and Quillaja saponins. Recent studies reported in the Sacco article have described the ability of BRSV immune-stimulating complexes to successfully induce BRSV-specific cellular and humoral responses and protect from virulent BRSV challenge in neonatal calves aged 3 to 8 weeks. Interestingly, this protection was robust despite the presence of significant levels of maternally derived antibodies.

**Conclusion:**
The presence of respiratory disease outbreaks in beef cow calf operations in South Africa presents some control challenges to veterinary practitioners particularly if BRSV happens to be present or endemic in such herds. The accurate diagnosis of these challenges is difficult due to the common secondary complications and it is hoped that this article may stimulate interest in and more specific diagnosis and better control of respiratory disease at farm level.

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