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Review Article

Review on Bovine Respiratory Syncytial Virus Characteristic, Pathogenesis and Control Methods Applied for the Disease

Zewde D[1]*, Gemeda G[2] and Ashagrie T[1]

[1]Department of Veterinary Medicine, Animal Health Institute, Sebeta, Ethiopia

[2]Wollega University, Nekemte, Ethiopia

***Corresponding author:** Demeke Zewde, Department of Veterinary Medicine, Animal Health Institute, P.O. BOX: 04, Sebeta, Ethiopia

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Abstract

Bovine Respiratory Syncytial Virus (BRSV) is an envelope, non-segmented, negative-stranded RNA virus belonging to the *Pneumovirus* genus within the subfamily *Pneumovirinae*, family *Paramyxoviridae*. The virus virion consists of a lipid envelope, derived from the host plasma membrane, containing three virally encoded trans-membrane surface glycoproteins, which are organized separately into spikes on the surface of the virion. The virus causes disease in cattle, sheep, goats and camelids where cattle are supposed to be a natural host and all other animal species listed may play as an epidemiological role in certain circumstances. Human Respiratory Syncytial Virus (HRSV) is also an important respiratory pathogen in infants and young children. The distribution of BRSV is worldwide and the virus has been isolated from cattle in Europe, America and Asia. The virus causes regular winter outbreaks of respiratory disease in cattle and a sero prevalence of 30–70% has been detected in this species. BRSV is one of the main causes of severe pneumonia, interstitial edema, and emphysema in cattle which spreads by infected animal aerosols, direct contact and transmission through objects (fomites). It can vary in its ability to cause disease, ranging from fatal to no clinical signs shown. BRSV can also make the animal more vulnerable to secondary infections, where the virus weakens the immune system so that bacteria that are usually harmless can cause disease. BRSV replicates primarily in the superficial layer of the respiratory ciliated epithelium and replication can also be detected in type II pneumocytes. Various types of proteins of this virus can contribute for its pathogenicity. Since the peak incidence of severe BRSV disease is between 2 and 6 months, an effective BRSV vaccine must be capable of stimulating an effective immune response within the first months of life. However, the presence of maternally-derived antibodies poses a major obstacle and deletion of non-essential genes represents an attractive option for production of a live, attenuated virus vaccine.

Keywords: BRSV; Control methods; Pathogenesis; Review; Viral Characteristic

Introduction

Bovine Respiratory Syncytial Virus (BRSV) infection is widely spread around the world, most likely as a direct result of the movement of cattle [1]. Regardless of the geographical location, infectivity rates are usually rather high, suggesting that viral transmission is a common event among herds. Cattle are the principal reservoir of infection; however, sheep can also become infected [2]. Intra-herd transmission usually occurs by aerosols, 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 [3]. On the other hand, direct transmission between herds is frequently a consequence of the introduction of new infected animals, while indirect transmission occurs by individuals visiting farms. Some of the main risk factors for BRSV transmission include large herd size and common farm practices such as not providing boots to visitors and dual-purpose farms [3,4]. Additionally, it has also been proposed that good management and better hygienic routines have a direct impact on overall health status [3].

Bovine Respiratory Syncytial Virus (BRSV) outbreaks commonly occur during winter [5]. Thus, clinical disease is commonly diagnosed during autumn and winter in temperate regions. Nevertheless, infection can also be observed during summer [6]. The sero-prevalence of BRSV infection varies greatly across different geographical regions [4,7]. 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 [8].

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,

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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 favors the dissemination of the virus during winter, after the sudden drop in temperature, although infection can occur throughout the year [1,9].

In Africa, Ethiopia and South Africa have also been shown to have high incidences of BRSV infection. Other countries, in different regions, such as Turkey, have also been shown to have high sero-prevalence, which can reach up to 43% [10]. Unsurprisingly, high sero-prevalence has also been associated with large-capacity facilities, rather than with small farms. Interestingly, organic farms have been shown to exhibit lower antibody prevalence when compared to conventional farms [9].

A serological study was done to establish the occurrence and determine the prevalence of important respiratory tract pathogens, Bovine Respiratory Syncytial Virus (BRSV) in Ethiopia. Prevalence rates for specific antibodies of 92.5% were recorded for BRSV. The presence of antibodies against this virus in cattle from Ethiopia is recorded for the first time in this report. That data suggests disease caused by this virus occur in Ethiopia but, perhaps because disease signs are not specific, they have not been recognized in the past [7]. Bovine Respiratory Syncytial Virus (BRSV) is an economically significant pathogen in cattle production as it is one of the most important causes of lower respiratory tract infections in calves [7]. Therefore, the objective of this review is to assess the characteristic of BRSV with its pathogenesis and control methods applied for the disease.

Disease and Characteristic of Bovine Respiratory Syncytial Virus

Etiology

Bovine Respiratory Syncytial Virus (BRSV) is an envelope, non-segmented, negative-stranded RNA virus belonging to the *Pneumovirus* genus within the subfamily *Pneumovirinae*, family *Paramyxoviridae* [11]. This virus was named for its characteristic cytopathic effect—the formation of syncytial cells. Cattle, sheep and goats can be infected by bovine respiratory syncytial viruses. Human Respiratory Syncytial Virus (HRSV) is an important respiratory pathogen in infants and young children. Antigenic subtypes are known to exist for HRSV, and preliminary evidence suggests there may be antigenic subtypes of BRSV. BRSV is distributed worldwide, and the virus is indigenous in the cattle population [12].

Virion Structure

The BRSV virion consists of a lipid envelope, derived from the host plasma membrane, containing three virally encoded trans-membrane surface glycoproteins, which are organized separately into spikes on the surface of the virion (Figure 1). These glycoproteins are the large glycoprotein (G), the fusion protein (F) and the small hydrophobic protein (SH) [13]. The envelope encloses a helical nucleocapsid, which consists of the nucleoprotein (N), phosphoprotein (P), the viral RNA-dependent polymerase protein (L) and a genomic RNA of around 15000 nucleotides. In addition, there is a matrix M protein that is thought to form a layer on the inner face of the envelope and a transcriptional anti-termination factor M2-1. The genome also encodes an RNA regulatory protein M2-2 and two non-structural proteins, NS1 and NS2 [13] (Figure 1). Thus, BRSV propagated in bovine cells can be neutralized by monoclonal antibodies specific for bovine MHC class I.

The genomic RNA is the template for replication and transcription. The genomic RNA, which is transcribed in a sequential fashion from the 3' end, encodes ten mRNA. There is a polar transcription gradient such that 3'-terminal genes are transcribed more frequently than those at the 5'. The 10 mRNA are then translated into 11 viral proteins. The *Pneumovirus* genome is characterized by the existence of two non-structural proteins, NS1 and NS2, and a transcriptional overlap between M2 and L that lead to the synthesis of M2-1 and M2-2 proteins.

BRSV Proteins

Non-structural proteins NS1 and NS2: One of the major differences between the pneumoviruses and the other *Paramyxoviridae* is the presence of two Nonstructural (NS) proteins, NS1 and NS2, which have 136 AA and 124 AA, respectively [14]. The genes encoding these two proteins are abundantly transcribed in virus-infected cells; however, the proteins are detected only in trace amounts in purified virions. There is evidence that the HRSV NS1 protein co precipitates with the M protein [15], and is a strong inhibitor of viral RNA transcription and replication [16]. The NS2 protein also appears to be a transcriptional inhibitor but at a lower level than the NS1 protein [16]. The NS2 protein co localizes with the P and N proteins in infected cells but does not co precipitate with any viral protein [15]. These proteins are not essential for virus replication in vitro, although the growth of recombinant HRSV and BRSV lacking one or other of these proteins is attenuated in cell culture [17]. The NS1 and NS2 proteins play an important role in regulating IFNα/β and their role in the pathogenesis and host-range restriction of BRSV.

Small hydrophobic SH protein: The SH protein is a short integral membrane protein and is not essential for virus replication in vitro or in vivo and its function is not well defined [19]. This protein has 81 AA and varies by up to 13% between different BRSV isolates. There is evidence that the SH protein may play a role in virus mediated cell fusion by interacting with the F protein [20].

Glycoprotein G: The G glycoprotein is 257AA or 263AA depending upon the BRSV isolate and it is a type II glycoprotein with a signal/anchor domain between AA residues 38 and 66. The G protein was identified as the major attachment protein because antibodies specific to the G protein blocked the binding of virus to cells and it is a major protective antigen of BRSV [21]. However, the G protein is structurally different from its counterparts in other paramyxoviruses (HN and H proteins) [22] and is a heavily glycosylated non globular protein similar to cellular mucins. The G protein is synthesized as two forms, a membrane-anchored form and a secreted form which arises from translational initiation at a second AUG in the ORF. Around 80% of the G protein is produced as the secreted form 24 h after infection [23].

The BRSV G protein appears as a trimer at the surface of the virion and contains several independently folded regions, in which the ectodomain consists of a conserved central hydrophobic region located between two polymeric mucin-like regions [22]. This

conserved central hydrophobic region contains four conserved cysteine residues, which form two disulphide bridges. However, in some field isolates of BRSV, one or several of the cysteines is mutated to either an alanine or an asparagine's [24,25]. The major epitope of this region is located at the tip of a loop, overlapping a relatively flat surface formed by the double disulphide bonded cysteine noose and lined by highly conserved residues. This epitope appears to be immune dominant [21] and the effect of the loss of one or more cysteines on antibody recognition has not been determined.

The G protein of HRSV and BRSV probably favours the attachment of the virion at the cell surface by the interaction of its heparin-binding domains with glycosaminoglycans on cell membranes [26]. In addition, the G is suspected to have other roles by interacting with the immune system. It has been proposed that the secreted form might act as a decoy by binding to neutralizing antibodies [27]. The G protein may also interact with L-select in, annexin II, and surfactant proteins [28]. The role of the G protein in the pathogenesis of BRSV infection will be discussed below.

Fusion F protein: The F protein mediates binding of virus to cells and is responsible for virus penetration by fusion between the viral and host cell membranes, delivering the nucleocapsid to the cytoplasm. It is also responsible for the fusion of the cellular membranes between infected and non-infected cells that give rise to syncytia or multinucleated giant cells. The F protein induces neutralizing antibodies and confers resistance to BRSV infection [29]. The protein F of BRSV is 574 AA and is highly conserved between different BRSV isolates. Partial sequence analysis of a number of different isolates has demonstrated amino acid and nucleotide variation of 1.8% and 0.8% respectively [25,29]. The F protein, which is indispensable to virus replication, is synthesized as an inactive precursor, F0, which has to be proteolytically cleaved at two furin consensus sequences to yield a fusion-active, disulphide-linked heterodimer composed of the F2 and F1 subunits [30]. Cleavage is accompanied by the release of a small peptide composed of 27 amino acids originally located between the two cleavage sites which have homology with tachykinins, a family of bioactive peptides [30,31].

Nucleocapsid proteins: The nucleocapsid is constituted by the nucleoprotein (N), the phosphoprotein (P) and the polymerase (L). The nucleoprotein has a length of 391 AA and variation between different BRSV isolates is 1.5% and 0.7% at the nucleotide and AA levels respectively. Vaccination with recombinant vaccine virus expressing the N protein can induce some protective immunity against BRSV. Since this protein is recognized by BRSV-specific bovine CD8+ T cells, protection induced by this protein may be mediated by cytotoxic T cells. The N protein is present in large amounts in the virion and in infected cells and has several functions. In combination with P, L and possibly M2-2, the N protein is a major element of the nucleocapsid and protects the viral genome RNA from RNAse. The N protein seems to play a role in the transition between the transcription and the replication phases of the viral RNA [32,33]. The P protein has 241 AA and appears to act as a chaperone for soluble N and is implicated as a regulation factor for viral transcription and replication. The polymerase L of BRSV has a size of 2162 AA and is an RNA-dependant RNA polymerase. This protein is responsible for the viral transcription and replication [34,35].

Matrix proteins: In contrast to the *Paramyxoviridae,* Respiratory Synctial Virus (RSV) has 3 matrix proteins. The M protein is 256 AA in length and has little sequence relatedness with other paramyxovirus M proteins. This protein is located on the inner surface of the viral envelope and plays an important role in the formation of virus-like particles. The M2-1 and M2-2 proteins are both encoded by the M2 mRNA, which contains two overlapping translational ORF [36]. The upstream ORF1 encodes the M2-1 protein (194 AA) and the downstream ORF2 encodes the M2-2 protein (83-90 AA). The M2-1 protein is an anti-termination factor that promotes transcriptional chain elongation and increases the frequency of read through at gene junctions. The M2-2 protein mediates a regulatory switch from transcription to RNA replication [37,38].

Virus Replication

Following fusion of the viral envelope and the cell membrane, the Ribonucleoprotein (RNP) complex is released into the cytoplasm and transcription of the viral RNA by the polymerase begins. Transcription involves a sequential start-stop mechanism that produces sub-genomic RNA. It is guided by short conserved signals that flank each mRNA coding unit, namely a transcription Gene Start (GS) signal and a termination/polyadenylation Gene End (GE) signal [39]. There is a polar transcription gradient with promoter-proximal genes being transcribed more frequently than downstream genes. RNA replication occurs when the polymerase switches to a read through mode resulting in the synthesis of a positive-sense replicative intermediate which acts as a template for replicating the negative strand genomic RNA. Both genomic and anti-genomic RNA are packaged. The nucleocapsids are assembled in the cytoplasm and then migrate with the M protein toward the cellular membrane in which viral glycoproteins are present. The viral budding might occur directly at the surface of the cellular membrane or into cytoplasmic vesicles. In polarized airway epithelial cells, budding of HRSV occurs at the apical surface and this is also true for BRSV infection of polarized bovine airway epithelial cells [40].

Antigenic and Genetic Subgroups of BRSV

Antigenic and genetic subtypes have been defined for BRSV through several studies [41]. Using monoclonal antibodies (mAb) directed against the G protein, a classification into four antigenic subgroups, A, B, AB and untyped, has been established for BRSV. Until recently, isolates of subgroup B BRSV had not been detected since 1976 [24]. The existence of six genetic subgroups based on G and of five based on F or N has also been established. This classification showed a spatial clustering of BRSV isolates that has been confirmed by studies including isolates collected in many countries [42]. The degree of genetic variability of BRSV is limited being less than 15%, which is less than that observed within one subgroup of HRSV. The evolution of BRSV appears to be continuous and it has been proposed that evolution may be driven by selective pressure as a result of the immune response induced by vaccination [25]. The biological significance of these subgroups is not known. However, polyclonal sera obtained from calves vaccinated with the BRSV G protein from subgroup A virus recognized a different subgroup A BRSV but not a subgroup B or an untyped isolate. Furthermore, recognition of a subgroup AB virus was less than that of the subgroup A isolate. Thus, mutations in the immune dominant region (AA 174–188) of

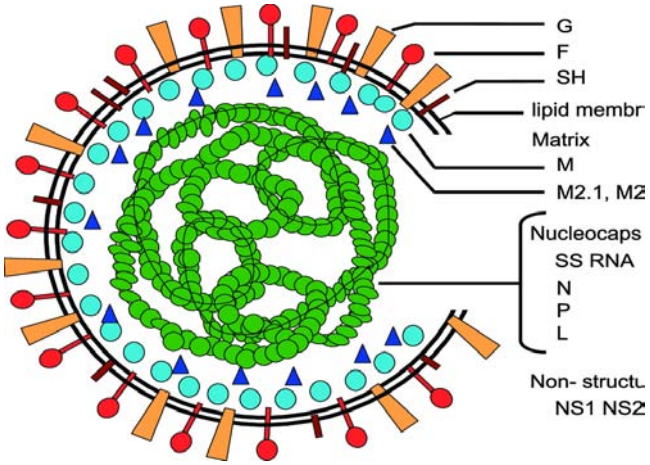

Figure 1: Bovine respiratory syncytial virus structural configuration Source [18].

the G protein may contribute to the lack of cross-protection between vaccine and field isolates. This might be relevant for the development of subunit vaccines [43].

Epidemiology and Clinical Signs of the Disease

Epidemiology

Although cattle are the natural host of BRSV, it is possible that other species such as ovine, caprine, or camelids 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. The virus causes regular winter outbreaks of respiratory disease in cattle. A sero prevalence of 30–70% has been detected in cattle. The frequency of BRSV infections is very high and the virus might be responsible for more than 60% of the epizootic respiratory diseases observed in dairy herds and up to 70% in beef herds [44]. The frequency of BRSV infections is correlated to the density of the cattle population in an area and the age of the animal. Indeed, more than 70% of beef calves were infected with BRSV by the age of nine months in England and in cattle less than one year old in the Netherlands. BRSV antibodies in calves between 5 and 11 months of age were detected in 35% of dairy herds [8].

The frequency of infection in adults is difficult to assess because of the high BRSV sero prevalence in this category of animals. 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. Indeed, clinical signs are usually 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. Maternally derived antibodies provide at least partial protection against clinical signs after natural and experimental BRSV infection. Although virus shedding has occasionally been detected upon experimental BRSV re-infection, little or no clinical disease is observed in re-infected animals [45]. Similar to observations made for HRSV, exacerbated clinical signs have been observed following a natural BRSV infection in animals immunized with inactivated vaccines. BRSV infection is associated with a high morbidity (60 to 80%) and mortality can reach up to 20% in some outbreaks [46].

Clinical disease caused by BRSV is mainly diagnosed in the autumn and winter in temperate climate zones. Although BRSV infection occurs mainly in these seasons, it might also occur in the summer. 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 a passive vector as observed for HRSV. Some data indicate that BRSV may persist in infected animals [47]. However, attempts to demonstrate re-excretion of BRSV from previously infected animals by treatment with 3-methyl indol, BVDV, BHV1 or dexamethasone have failed [48] and transmission of virus from carriers to susceptible animals has not been proven.

Bovine Respiratory Syncytial Virus (BRSV) infections associated with respiratory disease occur predominantly in young beef and dairy cattle. BRSV can be considered as a primary Bovine Respiratory Disease (BRD) pathogen and is also a component of the bovine respiratory disease complex. Passively derived immunity does not appear to prevent BRSV infections but reduces the severity of disease. Initial exposures to the virus are associated with severe respiratory disease; subsequent exposures result in mild to subclinical disease. BRSV is an important virus in the bovine respiratory disease complex because of its frequency of occurrence, predilection for the lower respiratory tract, and ability to predispose the respiratory tract to secondary bacterial infection. In outbreaks, morbidity tends to be high, and the case fatality rate can be 0–20% [49].

Clinical Signs of Disease

BRSV is one of the main causes of severe pneumonia, interstitial edema, and emphysema in cattle [50]. However, the disease is spread by infected animal aerosols, direct contact and transmission through objects (fomites). It can vary in its ability to cause disease, ranging from fatal to no clinical signs shown. BRSV can also make the animal more vulnerable to secondary infections, where the virus weakens the immune system so that bacteria that are usually harmless can cause disease. BRSV infection leads clinical signs watery to thick mucus from nose/eyes, increased temperature, increased breathing rate with open mouth, decreased appetite, appear depressed, reduced

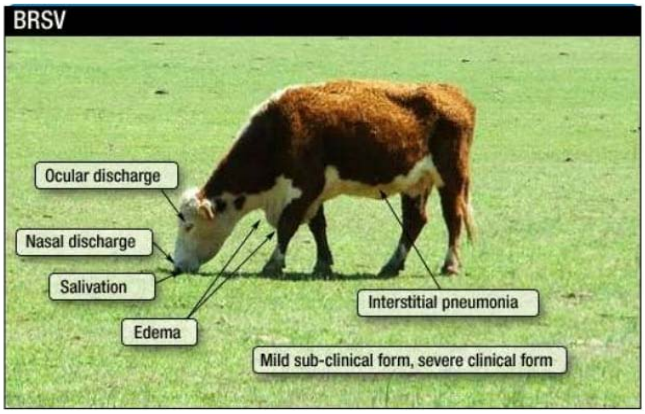

Figure 2: Clinical signs of BRSV on calve Source [18].

milk production, dry cough and difficulty breathing, frothy saliva, trouble drinking, sudden fever, rhinitis, cough, respiratory distress and abdominal breathing, [51].

The incubation period for BRSV is estimated to be between 2 and 5 days. BRSV infection may either be asymptomatic, limited to the upper airways or involve both the upper (URT) and lower respiratory tracts (LRT). URT disease is characterized by a cough with a seromucoid nasal and ocular discharge. In more severe infections, there is slight depression and anorexia, a decrease in milk yield in lactating cows, hyperthermia, polypnea (respiratory rate ≥ 60 rates per min) and an abdominal dyspnea. On auscultation of the lung abnormal breathing sounds caused by bronchopneumonia or bronchiolitis might be detected. Animals may develop severe respiratory distress with a grunting expiration and breathing through an open mouth with the neck stretched and the head down, with saliva poring on the floor and with the tongue out. In these animals, pulmonary emphysema and oedema with some crackles and wheezes may be detected and, in some cases, subcutaneous emphysema might occur [52].

At necropsy, a broncho-interstitial pneumonia may be observed. Areas of the cranio-ventral parts of the lung are consolidated and a muco-purulent discharge may be seen from the bronchus and small bronchi. The caudo-dorsal parts of the lungs are often distended because of interlobular, lobular and sub-pleural emphysematic lesions. Tracheobronchial and mediastinal lymph nodes may be enlarged, oedematous and sometimes haemorrhagic. If bacterial super-infections occur, the lung parenchyma is more swollen and consolidated and fibrin or suppurative bronchopneumonia may be observed. Microscopic lesions are characterized by a proliferative and exudative bronchiolitis with accompanying alveolar collapse and a peribronchiolar infiltration by mononuclear cells [53].

Necrosis of the epithelium and apoptotic epithelial cells, which may be phagocytized by neigh boring cells, can be seen. Giant cells or syncytia may be present, either free in the bronchi lumen, in the bronchiolar epithelium or in the alveolar walls and lumina. The lumen of bronchi, bronchioles and the alveoli are often obstructed by cellular debris consisting mostly of neutrophils, desquamated epithelial cells, macrophages and sometimes eosinophils and may be aggravated by bronchiolar repair and reorganization. Eosinophils and lymphocytes (CD4+, CD8+ and WC1+γ/Δ T cells) are also observed in the lamina propria. Alveolar changes are marked by an interstitial pneumonia and atelectasis in the consolidated areas and there may be severe emphysema and oedema with a rupture of alveolar walls in the caudo-dorsal area of the lung. The presence of microscopic changes in the caudo-dorsal area is rarely associated with the presence of BRSV antigen, syncytia or bronchiolitis. An alveolar epithelization with a pneumocyte hyperplasia contributes to the enlargement of the alveolar septa with the cell infiltration. Hyaline membranes may be present in the alveoli following inflammation and pneumocyte necrosis [54].

Pathogenesis

BRSV replicates primarily in the superficial layer of the respiratory ciliated epithelium and replication can also be detected in type II pneumocytes. Although BRSV is cytopathic in tissue culture, little or no cytopathic effects are seen following infection of differentiated bovine airway epithelial cell cultures. A similar lack of obvious cytopathology has been observed in human airway epithelial cell cultures infected with HRSV, suggesting that the host response to virus infection plays a major role in RSV pathogenesis. Although less well studied, BRSV infection induces an up-regulation of pro-inflammatory chemokines and cytokines in the bovine lung. Thus, increased levels of mRNA for IL-12, IFNγ, TNFα, IL-6, IL-18, IL-8, RANTES, MCP-1, MIP-1α, IFNα and IFNβ have been detected in pneumonic lesions from BRSV-infected gnotobiotic calves [55].

The recovery of Respiratory Synchcial Virus (RSV) from cDNA requires co-expression in cell culture of a complete copy of the viral RNA genome and the N, P, M2-1 and L proteins engineered to be expressed by bacteriophage T7 RNA polymerase. As described above, these are the constituents of the nucleocapsid and polymerase complex, which is the minimum unit of infectivity for mononega viruses. The expressed viral components assemble and result in a productive infection. The recombinant virus produced in this way is identical to the biologically-derived virus except for whatever mutations have been introduced into the cDNA. The role of different proteins in the pathogenesis of BRSV has been investigated by analyzing the effects of deleting genes or introducing mutations in the cDNA copy of the virus genome.

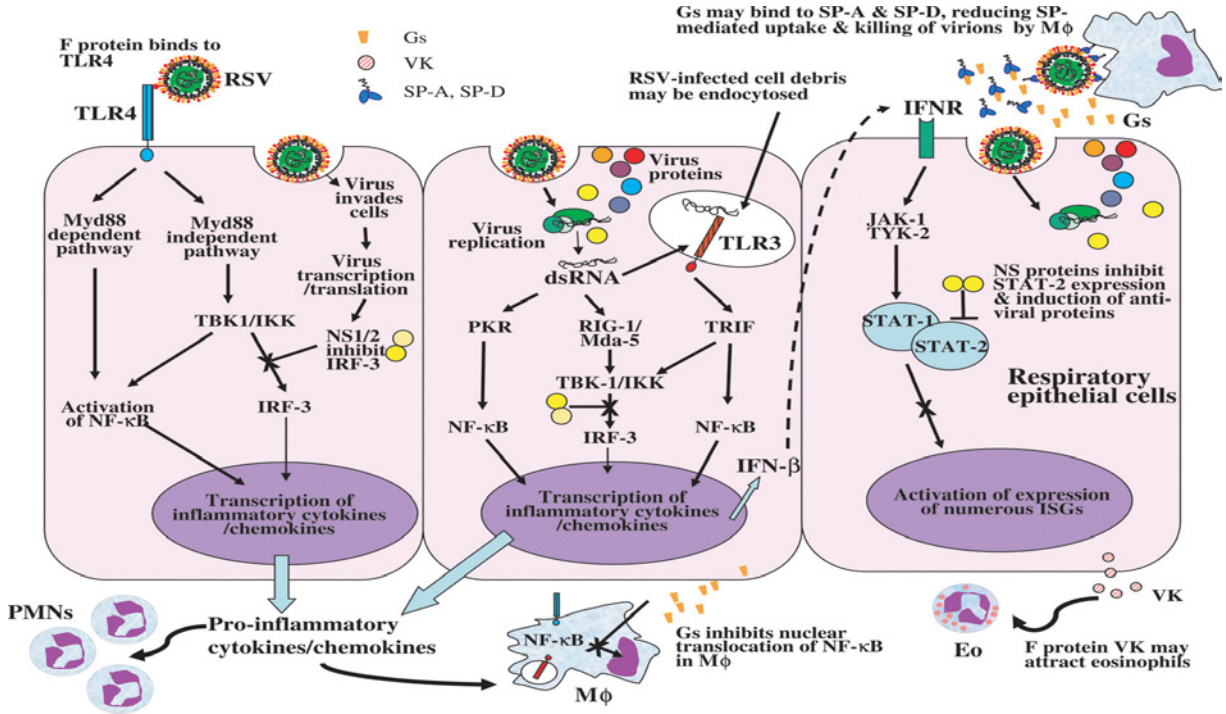

Figure 3: Molecular mechanisms involved in the initiation of the innate response to RSV Source [18].

BRSV attaches to respiratory epithelial cells via glycosaminoglycans and possibly other receptors. The interaction of the F protein with TLR4 leads to activation of NF-κB via the Myd88-dependent pathway. Double-stranded (ds) RNA, a by-product of virus replication, leads to activation of the transcription factors NF-κB, IRF-3 and AP-1 (not shown), which act cooperatively to fully activate the IFNβ promoter. The BRSV NS proteins block activation of IRF-3, inhibiting the induction of IFNβ. Any IFN produced as a result of BRSV infection binds to the type I interferon receptor (IFNR) and activates the expression of numerous Interferon stimulated genes (ISG) via the JAK/STAT pathway, which establishes an antiviral state and the activation of IRF-7 which amplifies the IFN response. The NS proteins decrease STAT2 expression and inhibit IFN signaling. Activation of NF-κB via TLR4 and TLR3 leads to the induction of pro-inflammatory cytokines and chemokines and recruitment of Polymorphonuclear Neutrophils (PMN), macrophages (Mφ) and NK cells to the sites of infection. The soluble form of the G protein (Gs) may compete with the virus for binding to surfactant proteins (SP-A and SP-D) and may act to inhibit the early innate response by suppressing the activation of NF-κB. The virokinin (VK) released following cleavage of the BRSV F protein may contribute to eosinophil (Eo) recruitment or to bronchoconstriction.

The Role of the NS Proteins in the Pathogenesis of BRSV

Interferon (IFN)α/β are produced by most eukaryotic cells in response to virus infection and establish a first line of defence. Transcription of IFNα/β is mediated by the transcription factors IRF-3, NF-κB and AP-1. Once secreted, IFNα/β bind to cell surface receptors and activate the JAK/STAT signaling pathway which induces further production of IFN and an array of IFN-stimulated genes, including ones that establish an antiviral state [56]. In order to establish infection, viruses have evolved a variety of mechanisms to counteract the IFNα/β response. BRSV and HRSV are poor inducers of IFNα/β and are resistant to the antiviral effects of IFNα/β. The ability to regulate the IFNα/β response is mediated by the NS proteins of BRSV and HRSV. The BRSV NS2 protein appears to have a greater inhibitory effect on IFNα/β than the NS1 protein, which is the converse of that described for HRSV. The BRSV and HRSV NS proteins prevent induction of IFNα/β and the establishment of an antiviral state by interfering with the activation of IRF-3 (Figure 2). In addition, the HRSV NS proteins inhibit IFNα/β signaling by inducing a decrease in Stat2 expression (Figure 2). Further studies have demonstrated that the NS genes may also play a role in activation of NF-κB. Thus, activation of NF-κB in Vero cells, which lack IFNα/β structural genes, infected with rHRSV lacking NS2 (ΔNS2) or lacking both NS1 and NS2 (ΔNS1/2) was significantly lower than that in cells infected with wild-type HRSV [57,58].

The role of the BRSV NS proteins in NF-κB activation is not known. Although the NS proteins are not essential for virus replication in vitro, growth of recombinant BRSV lacking one or the other of these proteins is attenuated in cell culture. Furthermore, replication of NS deletion mutants of BRSV in young calves is highly attenuated. Thus, following intranasal (i.n.) and intratracheal (i.t.) inoculation of 2-week-old, gnotobiotic calves with rBRSVΔNS1 or rBRSVΔNS2, only low titres of virus could be isolated from the nasopharynx for only 1 to 2 days and virus could not be detected in the lungs at post-mortem, 6 to 7 days after infection. BRSV lacking both NS1 and NS2 was even more attenuated and virus could not be recovered from calves at any time post-infection. In contrast to calves infected with wild-type rBRSV, neither macroscopic nor microscopic lung lesions could be detected in any of the calves inoculated with any

of the NS deletion mutants. These observations highlight the critical role of IFNα/β in the innate response of the bovine respiratory tract against BRSV infection [17].

The Role of the F Protein in the Pathogenesis of BRSV

Cleavage of the BRSV F0 protein by a furin endoprotease occurs at two sites, FCS-1 (RKRR136) and FCS-2 (RAR/KR109), and results in the formation of F1 and F2 subunits linked by a disulphide bridge and in the release of an N-glycosylated peptide of 27 amino-acids. Cleavage at both sites is required for efficient syncytium formation. In BRSV-infected cells, is further subjected to post-translational modifications and is converted into virokinin, a member of the tachykinin family, which includes substance P, neurokinins A and B, hemokinin and endokinin A and B. Virokinin induces smooth muscle contraction, in vitro, and may therefore contribute to bronchoconstriction in vivo. Using recombinant (r)BRSV with mutations in FCS-2 (K108N/K109/N) that abolished cleavage at this site or in which was deleted, neither FCS-2 nor was found to be essential for virus replication in vitro. However, mutant BRSV in which cleavage was abolished at FCS-2 did not grow as efficiently as the parental wild-type virus during early replication cycles [59].

BRSV infection is associated with a reduction in mitogen-induced lymphocyte proliferation in both calves and lambs. BRSV-infected cells are able to inhibit mitogen-induced lymphocyte proliferation, in vitro, and this effect was found to be mediated by direct contact of the lymphocytes with the F protein. The precise mechanisms responsible for this inhibition are not known, however, contact with the F protein resulted in a defect or delay in the transit of lymphocytes from G0/G1 to S-phase. Although the implications of this suppressive effect for the pathogenesis of BRSV infection are not clear, it may contribute to a decrease in effect or function of CD8+ T cells in the respiratory tract, which has been reported in HRSV-infected mice and/or may influence the generation of BRSV-specific memory T cells [17].

The Role of the G Protein in the Pathogenesis of BRSV

As mentioned above, the G protein was thought to be the major attachment protein of RSV. However, recombinant BRSV and HRSV lacking the G protein (ΔG), and or the SH protein, which is the other surface glycoprotein, can be rescued and such viruses replicate efficiently in cell culture. Thus, the F protein alone is sufficient to mediate attachment and fusion in the absence of G and SH. Whereas there is evidence that for HRSV, expression of the G protein enhances binding of virus to tissue culture cells, cell-to-cell fusion and virion assembly and release, this was dependent upon the tissue culture cells and a similar effect on BRSV replication in MDBK cells could not be demonstrated. Analysis of the replication of rBRSVΔG in differentiated, bovine ciliated airway epithelial cell cultures has shown that although the virus can infect these cells, replication is slightly attenuated when compared with that of wild-type rBRSV5 [60].

In contrast to the limited effects of the G protein on virus replication in vitro, expression of the G protein is essential for significant replication of BRSV and HRSV in vivo. Thus, following i.n. inoculation of calves within the first week of life with ΔG rBRSV, virus could not be re-isolated from the nasopharynx, although some virus replication was detected by RT-PCR, whereas the parental wild-type virus reached peak titres of approximately 104 pfu/ml. In these studies, the effects of ΔG virus on lower respiratory tract infection were not investigated. However, in mice infected with ΔG HRSV, although virus could not be recovered from the nasal turbinate's, it could be isolated from the lungs of about 60% of the mice at titres that were 1 000-fold less than that from mice infected with wild-type HRSV. In order to understand the role of the G protein in the pathogenesis of RSV infection, further recombinant viruses expressing only the membrane-anchored (Gm) or only the secreted form of the G (Gs) protein have been studied. Viruses expressing Gm were produced by introducing a point mutation in the second ATG of the viral G ORF encoding Met-48 to ATC encoding Ile-48 and viruses expressing Gs were made by deleting the first 141 nucleotide segment encoding the cytoplasmic and part of the transmembrane domains of the G protein [61].

The mechanisms by which Gsmediates the establishment of LRT infection are not known, but it may be that this form of G binds to surfactant proteins in the lower respiratory tract, reducing their effects on the virion itself. Support for this suggestion comes from the observations that susceptibility to severe HRSV infection in infants is linked to polymorphisms in SP-A and SP-D genes and SP-A deficient mice have more severe HRSV infection than their wild-type littermates. Furthermore, there is evidence that the HRSV G protein can suppress TLR4-mediated cytokine production by monocytes and macrophages by inhibiting nuclear translocation of NF-κB. The mechanisms by which the G protein mediates this effect are not known. However, the conserved cysteine-rich region of the G protein has homology with the fourth domain of the TNF receptor and may inhibit components of the innate response by binding to TNFα or an unknown TNF homologue. Studies with rHRSV lacking the cysteine-rich region of the G protein suggest that although this region is not required for efficient viral replication in mice, it may play a role in suppressing the anti-viral T-cell response. As mentioned previously, field isolates of BRSV have been identified that lack one or more of the cysteines in the central conserved region, but there is no information on the virulence of these isolates. Studies are in progress to determine the role of the central conserved cysteine-rich region in the pathogenesis of BRSV in calves [61].

The Role of the Small Hydrophobic Protein

The role played by the SH protein during RSV replication is unclear. BRSV lacking the SH protein replicates as efficiently as the wild-type virus in cell culture However, there is some evidence from studies on HRSV that the SH protein may have a negative effect on virus fusion in cell culture. When inoculated into mice, ΔSH HRSV resembled the parental wild-type virus in the efficiency of its replication in the lungs, whereas it replicated 10-fold less efficiently in the nasal turbinate's. These observations contrast with those of rBRSVΔSH in chimpanzees where virus replication was similar to that of the wild-type virus in the nose but was reduced 40-fold in tracheal lavage. Preliminary studies in calves infected with rBRSVΔSH indicate that although the SH protein does not influence virus replication in the nasopharynx, it is important in establishing lower respiratory tract infection. Thus, like Gs, the SH protein may suppress some component(s) of the innate response important in mediating resistance of the lung to BRSV [60].

The Role of Viral Proteins in Determining Host-Range Specificity

Although closely related, BRSV and HRSV display a highly

restricted host range in vivo. Thus, there are no reports of BRSV infection in humans and there is little or no replication of BRSV in chimpanzees following experimental infection. In contrast, although HRSV does not replicate very efficiently in the bovine nasopharynx, it replicates moderately well in the lungs and induces some pneumonic lesions following simultaneous i.n. and i.t. inoculation of young gnotobiotic calves. Studies on the role of different viral proteins in determining host range restriction have demonstrated that the F and G proteins contribute to host range restriction but are not the major determinants. Thus, whereas HRSV and BRSV replicated more efficiently in human and bovine cells respectively, rBRSV in which the F and G proteins had been replaced with those from HRSV exhibited intermediate growth characteristics in a human cell line and grew better than either parent in a bovine cell line. Furthermore, the chimaeric virus was more competent than BRSV for replication in chimpanzees, but remained highly restricted compared with HRSV [62].

Studies of HRSV and BRSV infection of differentiated respiratory epithelial cells, peripheral blood lymphocytes and macrophages also showed a pronounced host-range restriction. Using recombinant HRSV and BRSV expressing chimaeric F proteins assembled from BRSV or HRSV F1 and F2 subunits, the species specificity correlated with the origin of the F2 subunit. Although the HRSV and BRSV G proteins have only 30% amino acid identity, the G protein did not appear to contribute to host-range restriction. The NS proteins also appear to contribute to the host-range restriction of HRSV and BRSV. In a recombinant BRSV in which the NS genes were replaced with those from HRSV, the exchanged genes could fully substitute for BRSV NS1/NS2 in IFNα/β-negative cells. However, in IFN-competent bovine cells, replication of rBRSV expressing HRSV NS1 and NS2 was attenuated. Taken together, these studies indicate that host range restriction of HRSV and BRSV is dependent upon the actions of several proteins, including the F and NS proteins [63].

Diagnosis

Diagnosis of BRSV can be confirmed through different laboratory tests including virus isolation and antibody detection in serum and milk. Serological investigations such as serum neutralization test, complement fixation test, immune precipitation, and Enzyme-Linked Immune Sorbent Assay (ELISA) are commonly used methods for BRSV diagnosis [64,65]. BRSV can also be accurately diagnosed using reverse transcriptase polymerase chain reaction [66].

Prevention and Control of the Disease

Since the peak incidence of severe BRSV disease is between 2 and 6 months, an effective BRSV vaccine must be capable of stimulating an effective immune response within the first months of life. The presence of maternally-derived, RSV neutralizing, serum antibodies poses a major obstacle to successful vaccination at this time. Furthermore, there is evidence from studies in man that vaccination can exacerbate RSV disease. Thus, a formalin-inactivated (FI)-HRSV vaccine not only failed to protect infants against HRSV infection but increased the severity of respiratory disease when they became infected. Vaccine-augmented BRSV respiratory disease has been reproduced experimentally in calves and severe BRSV disease has been reported in calves vaccinated with a β-propiolactone-inactivated virus. It is likely that a parent rally-administered, inactivated virus vaccine would not be effective in inducing a mucosal IgA antibody response, which would help to limit infection of the respiratory tract, and would not be effective in priming BRSV-specific CD8+ T cells, which are important in eliminating virus [67].

Furthermore, we have increased the virulence of a BRSV isolate by sequential passage in gnotobiotic calves. 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 a live, attenuated virus vaccine, since they should be particularly refractory to reversion and may be suitable as marker vaccines [46].

NS Deletion Mutants as Live Vaccines

As described above, the replication of NS deletion mutants of BRSV in the bovine respiratory tract is highly attenuated and does not result in the development of a pulmonary inflammatory response. Despite the poor replication of the NS deletion mutants, infection with either the ΔNS1 or the ΔNS2 mutant induced a BRSV-specific antibody response, primed BRSV-specific CD4+ T cells and induced protection against a subsequent challenge with a virulent strain of BRSV. Although there were no detectable differences in the ability of the ΔNS1 or the ΔNS2 mutants to replicate in the bovine respiratory tract, the ΔNS2 mutant induced higher titers of neutralizing serum antibodies, higher titers of BRSV specific IgG2 antibodies, greater priming of BRSV-specific IFNγ-producing CD4+ T cells and greater protection against a subsequent BRSV infection than the ΔNS1 mutant. Since IFNα/β have profound immune modulatory effects and can enhance the adaptive immune response, it has been suggested that the greater immunogenicity of the ΔNS2 mutant is related to the greater ability of this virus to induce IFNα/β compared with the ΔNS1 virus. Although the ΔNS2 mutant was highly attenuated and immunogenic, it has not yet been evaluated in calves with maternal antibodies and it is possible that it will be too attenuated to induce an effective immune response in such animals [68].

FCS-2 Cleavage Mutants or Mutants as Live Vaccines

Although disruption of furin-mediated cleavage at FCS-2 or deletion of did not affect virus replication in the bovine respiratory tract, viruses with these mutations induced little or no pulmonary inflammation, suggesting that they may be ideal live vaccine candidates. However, tachykinins are potent immune modulators and it is possible that loss of expression of the virokinin may affect the induction of immunity. Studies in calves inoculated i.n. and i.t. demonstrated that neither disruption of furin-mediated cleavage at FCS-2 nor the loss of influenced the induction of BRSV-specific serum antibodies, as detected by ELISA, priming of BRSV-specific T cells, or the induction of a protective immune response in young calves, 6 weeks after mucosal vaccination. However, disruption of furin mediated cleavage at FCS-2 did appear to influence the induction of BRSV-specific neutralizing antibodies, which were 10-fold lower than those induced by either or wild-type rBRSV. Thus, incomplete cleavage of the BRSV F protein appears to influence both the magnitude and the duration of neutralizing antibodies [69].

G Protein Mutants as Live Vaccines

Recombinant BRSV lacking the G protein (ΔG) appears to be highly attenuated in calves inoculated via the i.n. route. Nevertheless,

mucosal immunization with the ΔG virus induced serum neutralizing antibodies, although the titres were 4 to 32-fold lower than those induced by the parental wild-type virus. Following challenge with a virulent strain of BRSV, there was a significant reduction in virus titres in both the nasopharynx and the lungs of calves previously infected with the ΔG virus. However, protection against challenge was not as great as that induced by the parental wild-type virus. These studies suggest that the ΔG virus may be too attenuated to induce a fully protective immune response. Furthermore, the G protein is a major protective antigen and should ideally be present in a BRSV vaccine [61].

In contrast to the ΔG rBRSV, virus expressing only the membrane-anchored form of the G protein replicated as efficiently as the wild-type virus in the nasopharynx of calves but was attenuated in the lungs. Inoculation of calves by the i.n. and i.t. routes with rBRSVGm induced a serum antibody response and T cell response indistinguishable from that induced by wild-type rBRSV6. Furthermore, the Gm virus induced complete protection against subsequent challenge with a virulent strain of BRSV. These findings suggest that the rBRSVGm has promise as a live, attenuated virus vaccine candidate. However, it differs from wild-type BRSV by a single point mutation and the ability of this virus to revert to virulence on repeated passages in calves is not known [61].

SH Deletion Mutants as Live Vaccines

As described previously, rBRSVΔSH replicates as efficiently as wild-type virus in the bovine nasopharynx but is attenuated in the lungs making this virus a suitable vaccine candidate. Although the immunogenic potential of ΔSH virus has not yet been evaluated in calves, inoculation of chimpanzees by the i.n. and i.t. routes with HRSV ΔSH induced serum neutralizing antibodies comparable to those induced by wild-type HRSV. However, in this study the chimpanzees were not challenged with virulent HRSV [69].

Other Strategies for the Development of Live Attenuated BRSV Vaccine Candidates

Deletion of M2-2 in HRSV produced a virus that was attenuated and immunogenic in chimpanzees and a similar deletion mutant of rBRSV may also be a suitable vaccine candidate for calves. Apart from deleting non-essential genes, it is also possible to target a specific protein and replace charged amino acids with noncharged ones. This has been done with the HRSV L protein and a number of the mutations were attenuating [70]. Another strategy is to alter the order of the viral genes. Gene transcription in RSV has a polar gradient such that genes proximal to the 3' promoter are expressed more efficiently than downstream genes. Rearrangement of the gene order might yield sub-optimal ratios of proteins and attenuate the virus. It may also be possible to introduce a gene encoding for a cytokine such as IL-2, IFNγ or GMCSF into the BRSV genome. This has been done for HRSV and although such viruses were attenuated in mice, their ability to induce antibodies and/or prime T cells was similar to that of wild-type HRSV [70].

Conclusion

The infection rate of the BRSV was high at the earliest age and animals with confined state and it is better to manipulate prevention methods based on these factors. Passively derived immunity does not appear to prevent BRSV infections but reduces the severity of disease. Although vaccination at the earliest age was not recommended due to neutralization by maternal derived antibodies it is possible to vaccinate young animals using non-essential gene deleted vaccines.

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