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Bovine Respiratory Syncytial Virus (BRSV): A review

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Larsen LE: Bovine respiratory syncytial virus (BRSV): A review. Acta vet. scand. 2000, 41, 1-24. – Bovine respiratory syncytial virus (BRSV) infection is the major cause of respiratory disease in calves during the first year of life. The study of the virus has been difficult because of its lability and very poor growth in cell culture. However, during the last decade, the introduction of new immunological and biotechnological techniques has facilitated a more extensive study of BRSV as illustrated by the increasing number of papers published. Despite this growing focus, many aspects of the pathogenesis, epidemiology, immunology etc. remain obscure. The course and outcome of the infection is very complex and unpredictable which makes the diagnosis and subsequent therapy very difficult. BRSV is closely related to human respiratory syncytial virus (HRSV) which is an important cause of respiratory disease in young children. In contrast to BRSV, the recent knowledge of HRSV is regularly extensively reviewed in several books and journals. The present paper contains an updated review on BRSV covering most aspects of the structure, molecular biology, pathogenesis, pathology, clinical features, epidemiology, diagnosis and immunology based on approximately 140 references from international research journals.

enzootic pneumonia; pathogenesis; epidemiology; calves; disease.

Introduction

Coinciding with the intensification of the agricultural industry the frequency and severity of bovine respiratory diseases have increased and the disease is currently regarded as the principal health problem in calf rearing world-wide (*Lekeux* 1995). In Denmark, the incidence rate of respiratory disease has been estimated to be as high as 0.25 per day at risk in calves between 2 and 24 weeks of age (*Uttenthal et al.* 1996, *Larsen et al.* 1998). This economically important multifactorial health problem is characterised by several different terms including "enzootic pneumonia" (*Uttenthal et al.* 1996). The severity of disease seems to be influenced by the animal itself (immune status and general condition), the environment (housing conditions and climate), the management (number of animals per square meter, commingling of animals from multiple sources, stress factors, prophylactic precautions and management practices) and the presence and spreading of infectious agents such as virus and bacteria (*Lekeux* 1995). Numerous surveys in Denmark (*Bitsch et al.* 1976, *Uttenthal et al.* 1996) and elsewhere (*Verhoeff & Van Nieuwstadt* 1984, *Bryson et al.* 1978) scrutinised the role of different specific pathogens in respiratory distress. Several species of bacteria and virus were found to be involved. Nevertheless, these studies unambiguously showed that bovine respiratory syncytial virus (BRSV) was the agent most often associated with severe epizootics of respiratory disease in especially younger dairy and beef calves. During the last decade increasing numbers of papers regarding infection with BRSV have been published, but many aspects of the pathogene-

sis, epidemiology, immunology etc. remain obscure and the outcome of the infection under different circumstances seems to be very complex and unpredictable. BRSV is closely related to human respiratory syncytial virus (HRSV) (*Lerch et al.* 1989), which is an important cause of respiratory disease in children. The current knowledge of HRSV is regularly extensively reviewed in several books and journals, whereas there seems to be a need for an updated review covering most aspects of the BRSV and the infection induced by this virus. This review represents an attempt to compile the present knowledge of BRSV.

History, taxonomy and host range

During the autumn of 1955 an episode of respiratory illness was recognised in a colony of laboratory chimpanzees and in one laboratory employee in Washington D.C., USA (*Morris et al.* 1956). An apparently new virus, initially named "Chimpanzee Coryza Agent", was isolated from one of the diseased chimpanzees. The virus was later renamed: "Respiratory Syncytial Virus" in recognition of its characteristic cytopathological effect in cell culture (*Chanock et al.* 1957). A series of surveys in the early 1960`s established the importance of HRSV in respiratory disease of children worldwide (*Chanock et al.* 1961). More than 10 years later, a virus closely related to HRSV was isolated from calves during an epizootic of severe respiratory disease in Switzerland. The virus was named BRSV (*Paccaud & Jacquire* 1970). Several surveys in the early 1980`s and onwards confirmed that BRSV is endemic in the calf population throughout the world (*Baker et al.* 1986a, *Stott et al.* 1980, *Thomas et al.* 1980, *Uttenthal et al.* 1996).

BRSV is a member of the pneumovirus genus within the *Pneumovirinae* subfamily of the *Paramyxoviridae* family that belongs to the virus order, *Mononegavirales* (*Murphy et al.* 1995). The *Pneumovirinae* subfamily differs from the other paramyxoviruses by lacking neuraminidase – but having the fusiogenic activity. RSV also lacks haemagglutination activity possessed by most virus of the paramyxovirus genus and the pneumovirus, pneumonia virus of mice (PVM) (*Richman et al.* 1971). All members of the subfamily cause respiratory disease. In addition to the bovine and human respiratory syncytial viruses, antigenically related respiratory syncytial viruses are present in sheep (ORSV) and goats (CRSV) (*Mallipeddi & Samal* 1993, *Smith et al.* 1979). HRSV, BRSV, ORSV and CRSV are structurally and antigenically very similar with the ruminant viruses being closest related (*Mallipeddi & Samal* 1993, *Trudel et al.* 1989). In contrast to the human, bovine and ovine RSV no sequence data are available on the caprine virus. Thus, the presence of a separate caprine virus has been determined on basis of the level of cross-reactivity with BRSV specific antibodies in serum neutralisation test, only (*Smith et al.* 1979).

BRSV can replicate in a wide range of primary bovine cell cultures derived from the testis, turbinate, trachea, aorta, spleen and lung (*Inaba et al.* 1970, *Jacops & Edington* 1971, *Openshaw* 1995, *Bastien et al.* 1997) and may also be adapted to grow in cell types from sheep (*Al Darraji et al.* 1982). BRSV was adapted to grow in different human cells in some studies (*Matumoto et al.* 1974), but not in others (*Paccaud & Jacquire* 1970). HRSV may induce mild respiratory disease with few macroscopic lesions in calves under experimental conditions (*Jacobs & Edington* 1975, *Thomas et al.* 1984), but there is no proof that BRSV can infect humans. Attempts to infect mice and guinea pigs failed (*Matumoto et al.* 1974), whereas sheep were successfully infected with BRSV (*Trigo et al.* 1984). RSV specific antibodies have been detected in sera from roes, cats and swine, but the virus has never been isolated from these

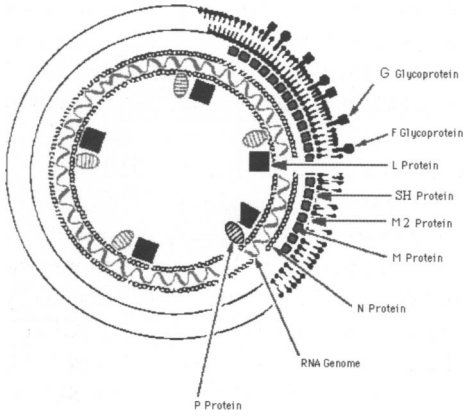

Figure 1. The structure of RSV. The names, sizes, properties and functions of the BRSV proteins are shown in the table (in the order 3'- to '5). [a]Length in number of amino acids deduced from gene sequence; [b]Estimated mass (kDa) of un-processed protein predicted from the amino acid sequence. See text for references.

Protein	**Lenght[a] / weight[b]**	**Localization / Function**
NS1 (Non-structural protein)	136 / 15.2	Nonstructural, gene regulation
NS2 (Non-structural protein)	124 / 14.6	Nonstructural, unknown function (gene regulation ?)
N (Nucleocapsid protein)	391 / 42.6	Nucleocapsid, virus transcription/replication
P (Phosphoprotein)	241 / 27.2	Nucleocapsid, virus replication/transcription
M (Matrix protein)	256 / 28.7	Membrane protein (inner surface), unknown function
SH (Small hydrophobic protein)	73 / 8.4	Transmembrane (surface exposed), fusion of cells
G (Glycoprotein)	257 / 28.6 or 263 / 29.0	Transmembrane (surface exposed), attachment to host cells
F (Fusion protein)	574 / 63.8	Transmembrane (surface exposed), fusion of cells
M2 (Matrix protein 2)	186 / 22 (ORF 1) 95 /11 (ORF 2)	Membrane protein (inner surface with the M protein), transcriptional elongation factor (ORF1)
L (Polymerase protein)	2161 / 200	Nucleocapsid, viral polymerase

species (*Van der Poel et al.* 1995, *Allan et al.* 1998).

Structure and molecular biology

Morphologically, BRSV appears as highly filamentous or pleomorphic virions with an average diameter of 200 nm. A 7-15 nm thick lipid membrane derived from the host cell and covered with cub-shape projections of 7-19 nm in length, encloses the particles. The caprine and bovine virus particles form well-defined bridges linking them in a distinctive and unique network. Interestingly, HRSV apparently lacks these bridges (*Al Darraji et al.* 1982, *Belanger et al.* 1988, *Trudel et al.* 1989). The virus particle encloses the nucleoprotein-core tightly connected to the single-stranded RNA (*Ito et al.* 1973).

BRSV is sensitive to low pH and heating at 56°C for 30 min. Exposure to diethyl ether, chloroform and other lipid solvents destroys infectivity (*Paccaud & Jacquire* 1970, *Smith et*

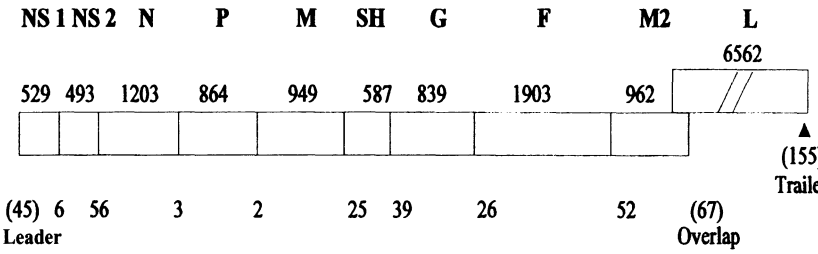

Figure 2. The gene order of the negative single stranded BRSV genome. The bold-face types are the abbreviations of the 10 coding proteins. The numbers shown above the genome states the number of nucleotides of each gene of BRSV, including the poly (A) template. The figures below indicate the size (nucleotides) of the leader and trailer region and of the intergenic junctions. The figures are those determined for the BRSV strain A51908. See text for references.

al. 1975). The virus is extremely fragile, but remains stable below -50 °C for many months. However, repeated freeze-thawing destroys infectivity. BRSV has a density of 1.23 g/ml as determined by sucrose density gradient centrifugation (*Ito et al.* 1973).

The virus attaches to cells through the glycoprotein G, but a specific receptor has not yet been identified (*Levine et al.* 1987). Immunofluorescence studies revealed that the virus specific proteins are located in the cytoplasm only (*Berthiaume et al.* 1974) and that the virus proteins assemble and mature on the apical surfaces of polarised epithelial cells (*Roberts et al.* 1995).

The negative strand RNA of RSV is neither capped nor polyadenylated and consists of approximately 15,222 nucleotides of which more than 85% code for protein (*Huang & Wertz* 1982). The nucleotide sequences of all BRSV genes and intergenic regions have been published (*Lerch et al.* 1990, *Lerch et al.* 1991, *Mallipeddi & Samal* 1992, *Pastey & Samal* 1995, *Samal & Zamora* 1991, *Samal et al.* 1991, *Zamora & Samal* 1992, *Zamora & Samal* 1992, *Buchholz & Conzelmann* 1997, *Buchholz et al.* 1999). The order of transcription of these genes is shown in Fig. 2. All steps in the virus replicative cycles occur in the cytoplasm and probably follow the general schedule for negative strand viruses.

Viral polypeptides and nucleic acids

Ten viral polypeptides, with molecular weights very similar to those described for HRSV, are identified in BRSV infected cells (Fig. 1) (*Cash et al.* 1977, *Lerch et al.* 1989, *Mallipeddi et al.* 1990, *Fisher et al.* 1997).

Attachment (G) glycoprotein

The G glycoprotein of RSV mediates attachment of the virus to cells (*Levine et al.* 1987) and is unique among *Paramyxoviridae* since it lacks both neuraminidase and haemagglutinating activity (*Richman et al.* 1971). Furthermore, the amino acid sequence shows no similarity to any other known RNA virus protein described to date (*Lerch et al.* 1990). The G protein mRNA is for most BRSV strains calculated to 838 nucleotides in length excluding the poly (A) tail. The mRNA contains a single open reading frame (ORF) which encodes a polypeptide of 257 amino acids (*Lerch et al.* 1990, *Larsen et al.* 1998). Some BRSV strains possess a 6 amino acids extension at the C-terminal end (*Furze et al.* 1997, *Mallipeddi & Samal* 1993).

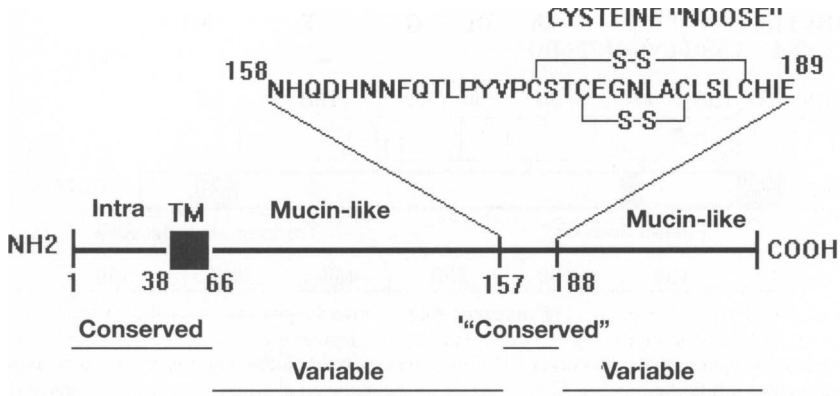

Figure 3. Structure of the RSV G protein. The "intra", "TM" and "mucin like" represent the parts of the protein which are situated intracellularly, transmembranelly and extracellularly, respectively. The amino acid sequence (one letter code) of the cysteine-noose of the reported BRSV strain (391-2) is also indicated. The figures below the structure indicate relatively conserved and variable parts of the protein, respectively. See text for references.

The amino acid sequence predicts a polypeptide with a mass of 28.6 kDa. However, a broad band migrating between 68 and 97 kDa is revealed in SDS PAGE due to extensive glycosylation involving both O and N linked side-chains (*Lerch et al.* 1990). The G proteins of HRSV and BRSV are typical type II integral membrane proteins with a very atypically cysteine noose (Fig. 3). The G protein might exist as a multimer (dimer or trimer).

Fusion (F) glycoprotein

The fusion protein of BRSV is situated at the surface of virions (*Himes & Gershwin* 1992) and is probably responsible for the fusion of the viral and host cell membranes and for the generation of syncytia between infected cells (*Matheise et al.* 1995). RSV fusion proteins are structurally and functionally related to the F proteins of other paramyxovirus (*Richardson et al.* 1986). The BRSV F mRNA comprises 1899 nucleotides excluding a poly (A) tail (Fig. 4). The gene contains a single open reading frame, which predicts a polypeptide of 574 amino acids with an estimated molecular weight of 63.8 kDa (*Lerch et al.* 1991). The F protein is highly conserved among BRSV isolates (97% to 99% homology) (*Himes & Gershwin* 1992). The most variable site is the region preceding the cleavage site. The F protein is synthesized as a precursor (F_0), which consists of the F_2 domain, the cleavage peptide and the F_1 domain (*Lerch et al.* 1991) (Fig. 4). The protein is post-translationally modified by mainly N-linked carbohydrate side-chains. The resulting F protein migrates as 3 bands at 68-kDa (F0), 46 kDa (F1) and 24-kDa (F2) in SDS-PAGE (*Himes & Gershwin* 1992).

Small hydrophobic protein (SH)

The nucleotide sequences corresponding to the SH proteins of 2 different BRSV strains varies between 462 and 566 nucleotides in length. The mRNAs have major reading frames that predict polypeptides of 81 and 73 amino acids, respectively (*Anderson et al.* 1992, *Samal & Zamora* 1991). The nucleotide sequence identity between these 2 BRSV isolates was 85%. The SH

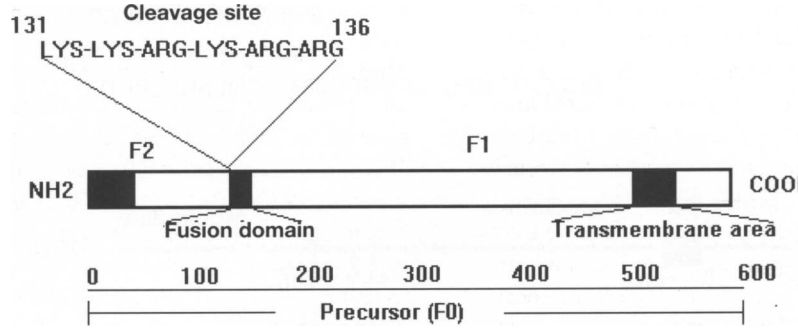

Figure 4. Linear structure of the precursor BRSV F protein. Hydrophobic regions are filled areas within the long rectangle representing the protein. The sequence of the deduced cleavage site is shown above the figure. The F1 and F2 subunits are generated by proteolysis of the cleavage site. This releases the hydrophobic domain at the N terminus of the F1 unit, which is thought to be the fusion domain. See text for references.

protein is an integral membrane protein with the C-terminus at the extra-cellular side of the membrane (*Collins & Mottet* 1993). Examinations of infected cells reveal several forms of the SH protein (*Anderson et al.* 1992, *Olmsted & Collins* 1989). The function of the SH protein is not fully defined, but the protein probably enhances the F protein mediated fusion of membranes and thereby contributes to the formation of syncytia (*Pastey & Samal* 1997).

Matrix protein (M)

The nucleotide sequence corresponding to the BRSV M mRNA constitutes 938 nucleotides excluding the poly (A) tail. The deduced polypeptide is 256 amino acids long and has a calculated molecular weight of 28.7 kDa (*Samal & Zamora* 1991). This agrees closely with the apparent molecular weight of 29 kDa determined in SDS-PAGE (*Mallipeddi et al.* 1990). The protein is moderately basic with a single hydrophobic region (residues 188 through 204). The function of the matrix protein is not clear, but its association with the membrane suggests that it may be involved in the interaction between the viral nucleocapsid and the cell membrane prior to assembly, maturation and budding of the virions. The M protein may also interact with the NS1 protein (*Evans et al.* 1996).

Nucleocapsid complex and associated proteins

The inclusion bodies in the cytoplasm of RSV infected cells apparently lack membranes, contain amorphous material and are exclusively present in the cytoplasm in the vicinity of the cell nuclei.

The BRSV N protein mRNA is 1196 nucleotides long excluding the poly (A) tail (*Samal et al.* 1991). It encodes a polypeptide of 391 amino acids with a calculated molecular weight of 43 kDa identical to that revealed in SDS-PAGE (*Mallipeddi et al.* 1990). Sequence comparison of the nucleocapsid protein among several RNA viruses suggested a high degree of structural homology (*Barr et al.* 1991).

The P protein of BRSV is 860 nucleotides long, excluding the poly (A) tail (*Mallipeddi & Samal* 1992). The sequence contains a single ORF of 723 nucleotides. The predicted protein is 241 amino acids long with a calculated molecular weight of 27 kDa. The high proportion of serine and threonine residues (17%) reflects profound phosphorylation of the P protein of BRSV. Consequently, the P protein migrates as a discrete

band of 34-38 kDa in SDS-PAGE (*Mallipeddi et al.* 1990). The 2 bovine isolates sequenced to date share 97% identity (*Mallipeddi & Samal* 1992). The extent of post-translational modifications (phosphorylation) might contribute to the structural differences between different isolates (*Shadomy et al.* 1997).

The M2 protein does not seem to have a counterpart in other non-segmented negative strand viruses, but is associated with the nucleocapsid complex of the RS viruses (*Garcia et al.* 1993). The nucleotide sequence corresponding to the BRSV M2 mRNA is 958-962 nucleotides long, excluding the poly(A) tail (*Zamora & Samal* 1992). The M2 gene contains 2 overlapping open reading frames (ORFs): The major ORF (ORF1) encodes a protein of 186 amino acids and is recognised in SDS-PAGE of extracts from cells infected with BRSV (*Mallipeddi et al.* 1990). The protein apparently lacks post-translational modifications and consequently migrates corresponding to the predicted molecular weight of 22 kDa. The second ORF (ORF2), which overlaps the ORF1, potentially encodes a polypeptide of 95 amino acids (*Collins et al.* 1990). This ORF lacks a designated protein and it is unknown whether it is expressed in RSV infected cells. The protein product of ORF1 is involved in elongation of transcription whereas the putative ORF2 gene product might be associated with inhibition of RNA synthesis (*Hardy & Wertz* 1998).

The BRSV L gene consists of 6562 nucleotides and contains a single open reading frame of 2161 amino acids. The protein shares 84% identity with the L protein of HRSV (*Buchholz et al.* 1999). Sequence comparison with the published sequences of other non-segmented negative strand viruses revealed the presence of 4 conserved motifs that are common for all RNA-dependent polymerases (*Poch et al.* 1990).

RS viruses are unique among paramyxoviruses in having 2 genes coding for non-structural (NS) proteins preceding the nucleoprotein. These NS proteins are among the most abundant proteins in RSV infected cells due to the polarity of transcription (*Collins & Wertz* 1983). The NS1 (former 1C) gene of BRSV of a representative BRSV strain is 524 bases long, excluding the poly (A) tail. It encodes a protein of 136 amino acids with a predicted molecular weight of 15-kDa (*Pastey & Samal* 1995). The NS2 (former 1B) gene of BRSV is 488 nucleotides long, excluding the poly (A) tail and encodes a protein of 124 amino acids. The protein has a calculated molecular weight of 14.5 kDa consistent with that estimated from SDS-PAGE (*Mallipeddi et al.* 1990). Expression of the NS proteins in a baculovirus system generates multimer forms, probably as a consequence of intra- or inter molecular disulphide bindings involving the 4 and 2 cysteine residues present in the NS1 and NS2 proteins, respectively (*Evans et al.* 1996). The NS1 protein is a potent inhibitor of transcription and RNA replication (*Atreya et al.* 1998). The NS2 protein is not necessary for propagation of the virus in vitro (*Buchholz et al.* 1999).

Pathogenesis and pathology

Experimental infections

Numerous attempts to induce BRSV infection in conventional and colostrum-deprived calves have been published during the last 2 decades. A recent review described the experimental outlines and major results of 19 of these studies (*Belknap et al.* 1995). At least 3 novel important studies were, however, not included in this review (*Otto et al.* 1996, *Taylor et al.* 1995, *Van der Poel et al.* 1996). These experimental inoculations were generally unable to reproduce disease as severe as that seen during natural outbreaks. Furthermore, in the few studies capable of inducing fulminate disease, bacteriological examinations were not done or microbio-

logical contamination was detected that may have influenced the clinical picture. The most successful of these experimental designs included more than one inoculation per day for up to 4 consecutive days which greatly hampered the interpretation of the data. However, recently it has been demonstrated that the clinical symptoms seen in the field repeatedly can be experimentally reproduced by a single BRSV inoculation, which thereby proves its primary etiological role (*Larsen et al.* 1999). Despite the former reservations, most of the experimental studies provided some useful data on the pathogenesis, pathology and immunology of BRSV infections, which are included in the following sections, when appropriate.

Pathogenesis

The pathogenesis of RSV infection is not clear, but accumulated data indicate that immune-mediated mechanisms play a dominating role. Another important aspect of the pathogenesis of respiratory disease caused by BRSV (and other respiratory viruses) is that the virus enhances bacterial colonisation and adherence and alters the specific and non-specific defence mechanism of the respiratory tract. Thus, it has been estimated that 90% of bacterial pneumonias in general develops after a previous viral infection (*Babiuk et al.* 1988).

Macroscopic pathology

The macroscopic changes observed in the respiratory tract of calves, following natural infection with BRSV, are characterised as typical interstitial pneumonia particularly involving the cranio-ventral part of the lungs (*Bryson et al.* 1983, *Collins et al.* 1988, *Kimman et al.* 1989, *Pirie et al.* 1981, *Viuff et al.* 1996). In this region, the consolidated lung areas encompass from a few lobuli to one half of the total lung area. The bronchi and bronchioles are often filled with mucopurulent exudate and haemorrhage and emphysema may be present (*Johnson et al.* 1982). The interlobular septa often appear broad owing to pronounced oedema. The craniodorsal and dorsal portions of the lungs often appear normal, but may also be markedly distended due to oedema and severe alveolar, interstitial and subpleural emphysema. The emphysema appears to be caused by widespread bronchoobstruction and probably results in severe dyspnoea (*Pirie et al.* 1981). The mucosae of the nose, trachea and bronchi may be hyperaemic, especially at early stages of infection, probably reflecting ongoing viral replication (*Bryson et al.* 1983). The bronchial and mediastinal lymph nodes are often markedly enlarged, oedematous and occasionally emphysematous. In experimental studies, only few changes were seen on 2-5 days past infection (PID) whereas profound changes appeared on days 5-8. Some workers observed only minimal changes on day 10-14 (*Bryson et al.* 1983, *Castleman et al.* 1985a, *Mohanty et al.* 1975) in contrast to the moderate to severe changes encountered on days 14-16 by others (*Thomas et al.* 1984).

Microscopic pathology

Histologically, bronchitis and peribronchitis accompanied by a large number of syncytial cells in the nasal and tracheal mucosae and alveolar and bronchiolar epithelium are characteristic findings in calves after natural BRSV infection (*Collins et al.* 1988, *Kimman et al.* 1989, *Thomas & Stott* 1981, *Viuff et al.* 1996). The viral antigen is first detected in the bronchiolar epithelium, later in the alveolar cells and may also be detected in the alveolar macrophages (*Castleman et al.* 1985b). There are conflicting reports on the role of alveolar macrophages in the BRSV pathogenesis, but it seems likely that at least a subset of these cells may be permissive for replicating BRSV and thereby contributes to the pathogenesis, i.e. by decreased functional ability. The activated macrophages may

also release specific cytokines that might contribute to the pathologies (*Midulla et al.* 1993, *Panuska et al.* 1990, *Schrijver et al.* 1995, *Sharma & Woldehiwet* 1996, *Toth & Hesse* 1983). The virus is capable of cell-to-cell spread resulting in the generation of the characteristic syncytial giant-cells. However, the virus may also enter the lower airways by aspiration of virus containing respiratory secretions. A viraemic phase has not been reported and the virus is very seldom found outside the respiratory system (*Van der Poel et al.* 1996). Degeneration, necrosis and hyperplasia of bronchial epithelium and of lymphoid tissue around the bronchi are consistently present. The exudate frequently found in the lumen of bronchi and bronchiole contains mainly epithelial cells, neutrophils and occasionally eosinophils often accompanied by oedema and formation of hyaline membranes (*Baker et al.* 1986, *Bryson et al.* 1983, *Kimman et al.* 1989, *Pirie et al.* 1981). BRSV specific antigens and nuclei acids have been demonstrated in nasal and tracheal mucosae and epithelial cells of the bronchi and bronchiole and in alveolar type I and II cells (*Haines et al.* 1989, *Lokensgard et al.* 1992, *Bryson et al.* 1991, *Viuff et al.* 1996). The findings of hyaline membranes and eosinophils in the caudal lung areas, even in areas where no virus was detected, further indicate that immuno-mediated pathological effects may play an important role in the pathogenesis (*Kimman et al.* 1989).

Clinical features and therapy

Mild respiratory disease is characterised by coughing, mucous to seropurulent nasal discharge, slight to moderate increased respiratory rates and abnormal breathing sounds. Moderately affected calves exhibit respiratory rates above 80/min, tachypnoea, harsh lung sounds across most of the lung wall and profound coughing. The most severely affected calves may be dyspnoeic and have subcutaneous, emphysematous bullae, although emphysema is not consistently found. Generalised symptoms range from slightly elevated rectal temperature, mild CNS depression and anorexia to high fever, deep depression and coma (*Baker et al.* 1986b, *Bryson et al.* 1978, *Kimman et al.* 1988). In experimental studies, the onset and duration of clinical disease varied considerably, but symptoms were generally present between day 2 and day 8 after infection (*Belknap et al.* 1995).

The therapy of BRSV infections is primarily of supportive character. Thus, concentrate and silage should be withdrawn from severely diseased animals and dehydration may be corrected by oral or intravenous administered fluids. Since immune-mediated mechanisms may contribute to the clinical symptoms, corticosteroids or non-steroid anti-inflammatory drugs (NSAIDs) may prove beneficial, but this has not been shown in controlled clinical trials. Antiviral therapy (ribavirin) has been approved for treatment of HRSV infections in children in form of aerosols, but this form of therapy is cumbersome and very expensive which would limit its use in veterinary practice (*Collins et al.* 1996). BRSV infections are often associated with secondary bacterial pneumonia (*Babiuk et al.* 1988, *Larsen et al.* 1999). Therefore, when respiratory disease is first recognised, treatment of all susceptible calves with anti-microbiological agents is often initiated. The use of antibiotics may decrease the incidence of severe respiratory complications, but results in significant economical loss for the farmer and is of growing public concern due to the risk of inducing drug-resistant bacteria.

Diagnosis

In conventional diagnostic work the demonstration of BRSV is often performed only on lung tissues that are available from post mortem samples only. Alternatively, samples for vi-

rus/antigen detection may be obtained by the use of lung lavage, tracheal washes and/or nasal swabs, which can be collected from living calves. It should be emphasized, however, that nasal swabs collect virus present in the upper airways only. Thus, during later stages of active BRSV infections large amounts of BRSV may be present in the lungs even if no virus is present in the nasal cavity (*Pirie et al.* 1981, *Larsen et al.* 1999). The most diseased calves may not be the best candidates for sampling since the amount of virus seems to reach maximum levels 2 to 3 days before the onset of severe clinical symptoms as revealed in several experimental infections (*Belknap et al.* 1995). Most appropriate, samples should be collected from calves exhibiting mild respiratory symptoms such as mild coughing and/or slightly elevated temperature or from pen-mates to severely diseased calves.

The classical method of diagnosing BRSV infection is to isolate the virus in cell culture. This method is, however, time consuming and ineffective when not performed immediately after collection of the sample. The immunofluorescent antibody test (IF) has been the most common used alternative test in the veterinary diagnostic laboratory (*Thomas & Stott* 1981). However, numerous different enzyme-linked immunosorbent assays (ELISAs) have been developed for the detection of BRSV (*Lokensgard et al.* 1992). The reliability and accuracy of these antigen detection tests are dependent of the reagents used and particularly of the sensitivity and specificity of the anti-BRSV antibody applied. In general, the antigen detection tests are fast, reliable and easy to perform. Application of more sensitive nucleic acid detection tests such as polymerase chain reaction (PCR) and probe hybridisation also does not require infectious virus and they are not affected by the presence of antibodies (*Belak & Ballagi Pordany* 1993, *Larsen et al.* 1999). However, these tests are often cumbersome, time consuming and expensive to perform.

The diagnosis of BRSV infection may also be performed indirectly, i.e. by measuring specific antibodies against the virus in acute or convalescent serum. A variety of different assays have been used for detection of BRSV specific antibodies (*Paccaud & Jacquire* 1970, *Potgieter & Aldridge* 1977, *Martin* 1983, *Westenbrink et al.* 1985). Among them, a variety of ELISAs which specifically detect antibodies directed against BRSV specific proteins (*Westenbrink et al.* 1985, *Samal et al.* 1993, *Langedijk et al.* 1996) and the different immunoglobulin isotypes (*Kimman et al.* 1987, *Westenbrink & Kimman* 1987). As with all serological tests used to determine the cause of an outbreak of infectious disease, paired serum samples taken 2 to 3 weeks apart are recommended. Because of the presence of maternal antibodies, these classical sero-diagnostic methods are often useless in the diagnosis of BRSV infections in calves less than 3 months of age, but have proved useful in the diagnosis of BRSV infections in older calves (*Kimman et al.* 1988, *Uttenthal et al.* 1996, *Westenbrink & Kimman* 1987). The major disadvantage of paired serum samples is, however, the long time period from the start of the outbreak to a final diagnosis is provided. The use of isotype-specific ELISAs provides a suitable diagnostic alternative. Maternally transferred antibodies are restricted to the IgG1 isotype in calves (*Kimman et al.* 1987). Consequently, the presence of other isotypes in the serum of calves may therefore be indicative of an active BRSV infection (*Kimman & Westenbrink* 1990, *Kimman et al.* 1987, *Kimman et al.* 1988, *Westenbrink & Kimman* 1987).

In summary, it is obvious that the diagnosis of BRSV infection on herd-basis should be based on samples from more than one acute diseased animal and that most predictive results are ob-

tained if more than one test is performed on each animal. Finally, it is important to evaluate the outcome of the different diagnostic tests on basis of the disease history, age of the animals, previous treatments etc. before a proper diagnostic answer can be reached.

Epidemiology

Infection has been induced in a variety of breeds, but certain breeds may be more susceptible than others (*Baker et al.* 1986b, *Bryson et al.* 1983, *Thomas et al.* 1984, *Inaba et al.* 1970, *Bryson et al.* 1983). During natural outbreaks of BRSV, clinical disease is seldom seen in calves less than 2 weeks of age, and disease is most severe in calves aged one to 5 months and is virtually absent in calves more than 9 months of age (*Baker et al.* 1986). Nevertheless, in certain areas adult cattle may experience severe disease following BRSV infection (*Ellis et al.* 1996, *Elvander* 1996).

The mode of transmission during the cause of natural infection has not been defined, but direct contact is probably required. Experimental data, however, suggest that aerosol transmission may also be possible over short distances (*Mars et al.* 1999, *Smith et al.* 1975). The high prevalence of antibodies against BRSV suggests that the infection is endemic in most areas (*Baker et al.* 1986, *Uttenthal et al.* 1996). In these regions, outbreaks of clinical disease often occur sporadically involving only the most susceptible groups of calves, while outbreaks involving thousands of cattle of all ages were reported in the early seventies (*Inaba et al.* 1972). With a morbidity of this level (80%-100%), the mortality rate seldom exceeds 5%-10%, although it occasionally may be higher (*Bryson et al.* 1978, *Inaba et al.* 1972). To give a precise estimate of the true incidence rate of BRSV is difficult, since other pathogens such as parainfluenza-3 virus, bovine virus diarrhoea virus, bovine corona virus, bovine herpes 1 virus and several bacterial agents may be involved. On the other hand, outbreaks of respiratory disease due to secondary bacterial infections may have been triggered by a previously subclinical BRSV infection (*Larsen et al.* 1999).

In temperate climates most outbreaks caused by BRSV occur in the early winter season (*Van der Poel et al.* 1993). In closed dairy farms, annual reinfections, recognised as clinical respiratory disease, often strike the replacement calves (*Verhoeff & Van Nieuwstadt* 1984).

It is not known how BRSV survives between outbreaks. The virus might circulate at a very low level among seropositive cows from which it can periodically be reactivated (*Van der poel et al.* 1995). The reappearance of virus in closed herds might also be explained by persistence since attempts to reactivate BRSV by corticosteroid treatment of previously infected calves caused a fourfold increase in BRSV specific antibody titers (*Van der Poel et al.* 1997).

Changes in the climate may augment BRSV infections, i.e. sudden change in outdoor temperature, particularly to wet and windy weather, often coincides with the onset of respiratory disease (*Baker et al.* 1986b). Factors affecting mucocilliary action (i.e. high levels of ammonia, high relative humidity, extreme temperature variations) are considered particularly important (*Baker et al.* 1986a, *Bryson et al.* 1978). Several surveys clearly showed that separate housing of calves significantly decreases the incidence of severe respiratory disease (*Kimman et al.* 1988, *Stott et al.* 1980). Nevertheless, even herds with excellent housing conditions and management may experience severe outbreaks, suggesting that BRSV can induce disease without environmentally predisposing factors (*Baker et al.* 1986a).

BRSV subgroups

BRSV isolates have been characterised on the

basis of monoclonal antibody (MAb) reaction patterns against panels of BRSV G-protein specific, monoclonal antibodies (*Furze et al.* 1994, *Furze et al.* 1997, *Schrijver et al.* 1996, *Larsen et al.* 1998) and by nucleotide sequencing of the G protein (*Furze et al.* 1997, *Mallipeddi & Samal* 1993, *Prozzi et al.* 1997, *Stine et al.* 1997, *Larsen et al.* 1998). Based on antigenic reactivity BRSV isolates were divided into 3 subgroups (designated A, B and intermediate or AB) exhibiting considerable differences in reactivity with MAb, but with limited sequence variability (up to 15%). Mapping studies showed that the G specific MAb used for subgrouping each binds to the region between amino acids 174 and 210, which includes the cysteine noose (Fig. 3) (*Furze et al.* 1997, *Langedijk et al.* 1997). Thus, single amino acid changes at certain positions in the cysteine noose have major structural and functional consequences and thereby determine the subgroup distinction. The biological significance of the appearance of different subgroups of BRSV has not yet been assessed, but some degree of cross protection is evident.

Humoral immunity

Despite decades of research on the immunity and the immunopathological mechanism involved in the pathogenesis of RSV infections, several intriguing questions remain to be solved. The age of the animal, animal species, immune status and quality and quantity of serum and local antibodies are believed to influence the level of protection. Nevertheless, so far it has not been possible to prove a clear link between protection and level of actively produced or passively acquired antibodies in natural BRSV infection.

Serum antibodies

Experiments on the kinetics of the antibody responses to BRSV have generated conflicting results, probably due to different sensitivity of the applied tests. Thus, 2 authors (*Castleman et al.* 1985b, *Elazhary et al.* 1984) detected (low level) of antibodies as early as 3 days after inoculation. In several other reports, antibodies were not detected before 7-10 days after inoculation (*Jacobs & Edington* 1975, *Kimman et al.* 1987). In all studies, the neutralising antibodies persisted in serum for months. Antibodies of the IgM class have been detected in sera from gnotobiotic calves on days 8-10 after experimental BRSV infection, peaked on days 10-13 and remained detectable for 7-29 days (*Kimman et al.* 1987, *Thomas et al.* 1984, *Westenbrink et al.* 1985). IgG_1 was detectable from days 13-17, peaked on days 24-38 and remained detectable for up to 8 months after experimental infections (*Kimman et al.* 1988, *Schrijver et al.* 1996). The half-life of acquired BRSV-specific IgG has been calculated to 21-32 days. Expectedly, the IgG_2 isotype did not appear in serum until days 25-86; peaked on days 38-90 and lasted for at least 8 months. Serum IgA may be detected concurrently with the IgM (*Kimman et al.* 1987) or be absent (*Thomas et al.* 1984).

Local immune response

The protective role of mucosal antibodies in the clearance of the virus during BRSV infection is not fully defined, although it has been the subject of several experiments (*Kimman & Westenbrink* 1990, *Kimman et al.* 1987, *Kimman et al.* 1989a, *Kimman et al.* 1987, *Westenbrink et al.* 1989). IgM and IgA were the only classes of antibodies found in secretions from the eye, nose and lungs of colostrum deprived calves 8-10 days after experimental infection (*Kimman et al.* 1987). IgA remained detectable for up to 3 months or longer, while local IgM persisted for 10 to 18 days, only. Local IgG was not detected in this study, while BRSV specific mucosal IgG antibodies were detected up to 42 days

after (3) vaccinations with modified-live or glutaraldehyde inactivated vaccine (*Stott et al.* 1984). In addition, IgG appeared on days 7-12 in nasal secretions from experimentally infected lambs (*Sharma & Woldehiwet* 1996). Virus administered intramuscularly to seronegative calves or infection of calves with maternal antibodies failed to induce a primary mucosal response, but did prime for a local memory response as revealed by a strong, rapid and probably protective response at rechallenge (*Kimman et al.* 1989a). Thus, it appears that protection is not clearly associated with the presence of IgA at the mucosae at the time of challenge but with the ability to mount a rapid and strong response. A peculiarity of the IgA mucosal response in BRSV infected animals is the apparently long duration and course (*Kimman & Westenbrink* 1990).

Maternally acquired antibodies

Remarkably, most cases of severe BRSV mediated disease develop in calves aged 1-3 months, at which time they still possess maternal antibodies in serum (*Kimman et al.* 1988, *Stott et al.* 1980). Nevertheless, maternal antibodies may be protective to a certain degree, since the severity and incidence of disease in calves less than 3 months of age seem to be inversely related to the serum level of (maternally derived) BRSV specific antibodies (*Kimman et al.* 1988, *Stott et al.* 1980).

Cell mediated immunity

Several studies revealed a profound cytotoxic (CD8+) T-cell response in the peripheral blood of calves (*Thomas et al.* 1996) and lambs (*Sharma & Woldehiwet* 1996, *Sharma et al.* 1990) 5 to 10 days after experimental infection with BRSV. Furthermore, significant proliferative responses of CD4+ T-cells and increased IL-2 and INFγ concentrations (the only cytokines measured) were observed in calves following vaccination with modified live or inactivated vaccines (*Ellis et al.* 1992). Depletion of CD8+ T-cells in experimentally infected calves affected neither the serum nor local antibody responses to BRSV, whereas depletion of CD4+ cells suppressed the antibody response (*Taylor et al.* 1995, *Thomas et al.* 1996). Depletion of CD8+ cells, but not CD4+ cells, resulted in delayed viral clearance and increased the severity of lung consolidation. These studies provided evidences for the importance of a strong CD8+ T-cell response in the clearance of virus in calves. Roles of (different subsets of) CD4+ T-cells and other subsets of T-cells (i.e. γδ T-cells) are more uncertain and need further research.

Immunopathology

In the mid-sixties, a formalin-inactivated vaccine against HRSV was tested in children aged 4 months to 9 years. Forty-seven to 97% of the vaccinees developed significant increase in serum neutralising antibody titers (*Fulginiti et al.* 1969). Unexpectedly, much higher ratios of vaccinated children were infected with HRSV during the following winter season. Furthermore, the vaccine not only failed to confer protection, but also induced an exacerbated, altered clinical response. Post-mortem examinations of 2 vaccinated children, who died of pneumonia, revealed high levels of HRSV in the lungs and widespread bronchiolitis with peribronchial infiltration of monocytic cells, mainly eosinophils (*Kim et al.* 1969). Closer examinations of the serum from these children revealed that most of the antibodies directed against the F protein, lacked neutralising and fusion-inhibiting activities. This might have contributed to the potentiation of disease (*Murphy et al.* 1986, *Murphy & Walsh* 1988). The mechanism behind the immunopathological effects of antibodies is not completely elucidated at present. Complement activation (*Kimman et al.* 1989b), type I and II hypersensitivity reactions (*Kimpen*

et al. 1992), type III hypersensitivity reactions (Arthus reaction) (*Kimman & Westenbrink* 1990), antibody-dependent enhancement (*Gimenez et al.* 1996) and delayed virus clearance (*Murphy et al.* 1986) have been suggested as possible explanations. The dose, route of administration and employed adjuvant may also determine the response to administered antigens and vaccines (*Vaux Peretz et al.* 1992).

Resistance to viral infections (and most other intracellular microbes) is believed to be linked to the development of predominant Th1-like responses (*Abbas et al.* 1996). Interestingly, several studies have indicated that predominately Th2 cells are responsible for the immunopathological changes seen after RSV infection or vaccination (*Jackson & Scott* 1996) and that the different RSV proteins activate different subsets of Th cells (*Alwan & Openshaw* 1993, *Gershwin et al.* 1998, *Jackson & Scott* 1996).

Cytokines

It is evident that the outcome of RSV infection is influenced by several factors of the adaptive and non-adaptive immune system. The key signals (or regulators) of the immune response are the cytokines. A variety of different cells (NK cells, T-cells, macrophages, epithelial cells, B-cells etc.) have the potentials of secreting specific cytokines in response to stimulation. The contribution or lack of contribution of some of these subsets of cells may thereby determine the outcome of disease (*Graham* 1996). Previous infection, vaccination, and maternal antibodies are examples of factors which may change the balance of the local cytokine response and thereby trigger either a protective response (Th1-like) or initiate the cascade that subsequently leads to exacerbated disease (Th2-like). In order to understand the immunological mechanisms involved on the pathogenesis of BRSV all these factors have to be considered.

Immunoprophylaxis

There is a great need for protective vaccines against HRSV and BRSV. Appropriate vaccines must be able to confer protection even in the presence of maternal antibodies, must protect against all subtypes and should lack disease exacerbating side effects.

Several inactivated and modified live BRSV vaccines are available, although proper assessment of protection is hampered by the lack of suitable experimental protocols (*Belknap et al.* 1995). The few published field trials, with live or attenuated vaccines, revealed different levels of protection (*Taylor et al.* 1989, *Mohanty et al.* 1981, *Fulton et al.* 1995, *Gershwin et al.* 1998, *Howard et al.* 1987, *Kubota et al.* 1992, *Verhoeff & van Nieuwstadt* 1984) while other reports found that vaccination enhanced disease in calves (*Gershwin et al.* 1998, *Kimman et al.* 1989b). Intramuscular administration, especially in calves that possessed maternal antibodies, seems to be least effective in inducing protection whereas intranasal inoculation of live virus in colostrum deprived calves proved most effective (*Gershwin et al.* 1998, *Kimman et al.* 1989a). DNA based vaccines may prove to be useful in the future, but these are still in the early experimental phase (*Schrijver et al.* 1997).

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Sammendrag

Bovint respiratorisk syncytial virus (BRSV): Review.

Infektion med bovint respiratorisk syncytial virus (BRSV) er den hyppigste årsag til lungebetændelse hos specielt yngre kalve. Anvendelse af nyere molekylærbiologiske metoder har de senere år resulteret i en eksplosiv vækst af publicerede artikler om handlende dette virus og den infektion, det er årsag til.

BRSV er et enkelt-strenget RNA virus, der tilhører genus *Pneumovirus.* Infektionen rammer primært yngre kalve, hvor det giver anledning til alvorlig lungebetændelse med kraftige kliniske symptomer og udbredte lungeforandringer. Erhvervede eller passivt overførte antistoffer synes ikke at beskytte fuldt mod infektion, idet selv kalve med maternel immunitet inficeres ligesom ældre dyr hyppigt gennemgår reinfektion, der dog ikke giver anledning til alvorlig sygdom. Smittevejene er delvis ukendte, men tæt kontakt er formodentlig nødvendig. Immunopatologiske mekanismer synes at bidrage til sygdomsudviklingen, hvilket menes at være medvirkende årsag til, at det er problematisk at vaccinere mod infektionen.

Baseret på cirka 140 referencer præsenteres et opda-

teret overblik omhandlende BRSV, herunder de væsentlige aspekter af virusstruktur, molekylær biologi, patogenese, patologi, kliniske tegn, epidemiologi, diagnose og immunologi.

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