Neutrophils in respiratory syncytial virus disease

Untangling the NET

Cortjens, B.

Creative Commons License (see https://creativecommons.org/use-remix/cc-licenses):
Other

Citation for published version (APA):
Cortjens, B. (2017). Neutrophils in respiratory syncytial virus disease: Untangling the NET.

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 2

Bovine and Human Respiratory Syncytial Virus Infections: comparing Disease Dynamics in the Pre-clinical Calf Model


Submitted
Abstract

Human respiratory syncytial virus (hRSV) is the most important respiratory pathogen in young children worldwide. Experimental modelling of hRSV disease by bovine RSV (bRSV) infection in calves provides an important tool for developing new strategies for prevention and treatment. Depending on the scientific hypothesis under investigation, this cognate host-virus model might have the disadvantage of using a highly related but not genetically identical virus. In this study, we aim to describe viral kinetics and (clinical) disease characteristics in calves inoculated with hRSV, as compared to the established bRSV calf model. Our results show that hRSV infects the upper and, to a lesser extent, the lower respiratory tract of calves, associated only with upper airway clinical disease symptoms. This is in contrast with the overt respiratory distress, related to robust viral replication with neutrophilic inflammation in the lower respiratory tract, of bRSV-infected calves, which mimics hRSV disease in young infants. We conclude that both the bRSV and hRSV model in calves may aid future research involving distinct scientific questions related to hRSV disease in children.

Highlights

- Calves can be effectively infected with both human and bovine RSV in an experimental setting.
- The bRSV calf model closely mimics human lower respiratory tract disease, with prominent neutrophil inflammation.
- hRSV infection in calves produces upper respiratory tract disease.
- Both hRSV and bRSV infection in calves have attractive, but distinct features to study hRSV disease in humans.
Introduction

Human respiratory syncytial virus (hRSV) is one of the most important respiratory pathogens in children worldwide.\(^1\) Clinical symptoms associated with hRSV infection range from mild upper respiratory tract disease (URTD) to potentially life-threatening lower respiratory tract disease (LRTD), mainly in infants. In low and middle income countries, hRSV is responsible for almost a third of the community-acquired pneumonias in young children, making hRSV a global health issue with tremendous socio-economic impact.\(^2\) Acquiring insight into the key processes that influence hRSV disease is essential to develop new strategies for prevention and treatment.

Animal models are an invaluable tool to study hRSV disease. They form the bridge between tissue culture experiments and human randomized controlled trials, and they also serve to evaluate the efficacy and safety of new vaccine candidates and new therapeutic intervention strategies. Different animal models are available to mimic hRSV disease and each model has its distinct advantages and disadvantages (reviewed in Bec et al.\(^3\)). One of the models used to study hRSV disease is the bovine RSV (bRSV) infection model in calves.\(^4\) This natural-host model has distinct advantages over models that are based on a non-natural host-virus interaction e.g. the heterologous hRSV mouse model.\(^3\) There is a striking similarity in age-dependency between bRSV disease in cattle and hRSV disease in humans\(^3,5,6\) and unlike heterologous models, bRSV infection in calves manifests with overt clinical symptoms, such as wheezing, coughing, and tachy- and dyspnoea\(^5,7\), similar to hRSV infections in young infants. Importantly, these clinical signs of bRSV LRTD are accompanied by histopathological evidence of bronchiolitis and pneumonia, including prominent neutrophilic inflammation in the airways and lungs, thereby closely mimicking findings in children.\(^3,5,8-10\)

Despite the similarities between hRSV disease in humans and the bRSV calf model, differences inevitably remain. The most important one being that bRSV and hRSV are not identical viruses, although both are members of the same Pneumoviridae family and thus phylogenetically strongly related. Differences in the genetic composition of viral surface glycoproteins are illustrated by a 38-41% and 81% protein sequence identity between the bRSV and the hRSV G protein and F protein, respectively.\(^11,12\) The use of the bRSV calf model for preclinical testing of specific vaccines and antivirals may be hampered by these inherent genetic differences between bRSV and hRSV.

To overcome the potential drawback of studying different viruses without losing major advantages of the large animal bovine model, we were interested to explore viral dynamics and disease characteristics of experimental hRSV infection in calves. Although it has been shown that bovine cell culture-adapted hRSV can infect calves decades ago\(^13\), this report
The bRSV calf model has offered very limited information on viral dynamics and specific disease characteristics. Here, we aimed to further explore and compare experimental hRSV infection in calves and thereby discuss its potential use in future hRSV research as compared to the established bRSV infection model.

**Methods**

All animal experiments were authorized by the Animal Ethics Committee of the Animal Sciences Group, part of Wageningen University and Research and conducted in accordance with the Dutch law for animal experimentations.

**Animals**

Seven caesarean-derived and colostrum-deprived calves were raised in an isolated environment at Wageningen Bioveterinary Research from birth until the start of the study at approximately 5 weeks of age. All calves were tested free of antibodies against natural occurring bRSV before start of the study by ELISA (Priocheck bRSV, Thermo Scientific, Rockford, USA).

**Virus inoculation**

Intranasal inoculation of virus was performed on day zero using an air-jet nebulizer, as described before. Calves in the already established bRSV model group received $10^3$ TCID$_{50}$ in 3 mL of bRSV field isolate Odijk (subtype A, fourth in vivo passage, $N = 3$) based on titration on embryonic bovine trachea cells. Calves in the hRSV group received $10^5$ TCID$_{50}$ in 2 mL of hRSV clinical isolate Memphis 37 (EV9508, Meridian LifeScience, Memphis, USA, subtype A, seventh in-vitro passage on Vero cells, $N = 4$), based on titration on HEP2 cells.

**Sample procedures**

For assessment of infection of the upper respiratory tract (URT), nasopharyngeal (NP) samples were collected on study days 0 to 9 after inoculation using sterile nylon bristle brushes (MW126, Medical Wire and Equipment Co. Ltd, Corsham, UK). Following sampling, the brushes were agitated in 3 mL transport medium. For assessment of infection of the lower respiratory tract (LRT), broncho-alveolar lavages (BAL) were collected on study days 5 to 9 after inoculation. BAL was performed according to the method described by Fogarty et al., using 100 mL of D-PBS (GIBCO) instillation. After centrifugation, the supernatant of NP and BAL samples were stored at -80°C. In a separate experiment with only bRSV inoculated calves, we were also able to visualize the LRT infection by tracheobronchoscopy using a flexible bronchoscope (PV-G-28, Karl Storz, Tuttingen, Germany) at peak disease on day 7 after inoculation.
Clinical Monitoring

Clinical observations for bRSV-related signs of illness were performed on study days -2 to 12 by a bio technician. Clinical observations were performed according to the scoring system outlined in table 1. Rectal temperature measurements were performed simultaneously.

Viral load detection

Viral load in NP and BAL supernatant samples were measured by RT-PCR. From 200µL BAL or NP, total nucleic acid was isolated using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Hoffmann-La Roche, Basel, Switzerland). The conserved bRSV N-gene was amplified (40 cycli) with a primer/taqman probe mix by a one-step qRT-PCR using the QuantiFast Multiplex RT-PCR Kit (Qiagen, Hilden, Germany) on the ABI 7500 real-time PCR system. The hRSV N-gene was amplified (45 cycli) as described before.\(^\text{17}\) Data are expressed as \(\Delta\text{Ct}\) (total number of PCR cycles minus threshold cycle).

Inflammatory cell responses

Total white blood cell counts in BAL samples were performed using a Coulter Counter (Beckman Coulter, Brea, USA) on days 6 and 9. Cells were centrifuged to a slide in the Shandon cytospin 3 (Thermo Scientific) and stained with a modified Wrights staining for differential counts by microscopic evaluation. For an indication of neutrophil degranulation, we determined myeloperoxidase concentrations (MPO) in BAL supernatant by ELISA (BM0039, Neobiolab, Woburn, USA) following the manufacturer's instructions.

Histopathology

On day 12 and 16 post virus inoculation, calves were euthanized with an overdose of Pentobarbital followed by exsanguination. The macroscopic consolidated lung area was calculated (Image Pro Plus) and the extent of consolidation was rated as a percentage of the total lung tissue area.\(^\text{18}\) Lung tissue samples were collected from 10 pre-determined sites and stored in 10% neutral buffered formalin, followed by microscopic examination of each hematoxylin and eosin stained lung tissue section.

Statistics

Statistical analysis was performed using Graphpad Prism 6 (Graphpad Software Inc, La Jolla, USA). Results are presented as median and interquartile range (IQR) or mean and standard error of the mean (SEM) where appropriate. Results between hRSV and bRSV infected calves were compared using the student t-test or 2 way mixed ANOVA where appropriate. A two-sided \(P\) value of < 0.05 was considered significant.
Table 1: Clinical scoring system for disease severity.

<table>
<thead>
<tr>
<th>Score</th>
<th>General Illness</th>
<th>Upper Respiratory Tract Disease</th>
<th>Lower Respiratory Tract Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (absent)</td>
<td>Bright, alert</td>
<td>No nasal discharge</td>
<td>Normal respiration</td>
</tr>
<tr>
<td></td>
<td>Normal appetite</td>
<td>No coughing</td>
<td>RR &lt; 50 (breathings per minute)</td>
</tr>
<tr>
<td></td>
<td>Normal behaviour</td>
<td>No ocular discharge</td>
<td></td>
</tr>
<tr>
<td>1 (mild)</td>
<td>Reduced responsiveness</td>
<td>Nasal or ocular discharge</td>
<td>Increased respiration</td>
</tr>
<tr>
<td></td>
<td>Decreased appetite</td>
<td>Intermittent watery – mucus</td>
<td>RR 51-70 (breathings per minute)</td>
</tr>
<tr>
<td></td>
<td>Otherwise normal behaviour</td>
<td>Occasional spontaneous dry cough – induced cough (unproductive) present</td>
<td></td>
</tr>
<tr>
<td>2 (moderate)</td>
<td>Depressed - lethargic</td>
<td>Increased nasal or ocular discharge</td>
<td>Abnormal respiration obvious abdominal breathing</td>
</tr>
<tr>
<td></td>
<td>Decreased appetite</td>
<td>Persistent mucoid – mucopurulent discharge</td>
<td>RR 71-100 (breathings per minute)</td>
</tr>
<tr>
<td></td>
<td>Retreats</td>
<td>Frequent spontaneous productive cough – induced productive cough present</td>
<td></td>
</tr>
<tr>
<td>3 (severe)</td>
<td>Depressed - soporous</td>
<td>Severe nasal or ocular discharge</td>
<td>Abnormal respiration - severe abdominal breathing</td>
</tr>
<tr>
<td></td>
<td>Anorexia</td>
<td>Persistent purulent – haemorrhagic discharge</td>
<td>Dyspnoeic (e.g. mouth breathing / frothing)</td>
</tr>
<tr>
<td></td>
<td>Unable to stand without assistance</td>
<td>Frequent spontaneous productive cough – induced productive cough present, prolonged when induced</td>
<td>RR &gt;100 (breathings per minute)</td>
</tr>
</tbody>
</table>

Results

Inoculation of bRSV and hRSV in calves results in productive infection of the upper and lower respiratory tract

First, we aimed to explore if hRSV inoculation in calves results in virus replication in the upper and lower respiratory tract. We therefore compared the dynamics of viral loads by detection of hRSV and bRSV in NP and BAL respectively (Fig. 1A). In calves inoculated with hRSV, virus was first detected at day 2 in NP samples of the upper respiratory tract and viral loads peaked on day 5. In calves inoculated with bRSV, virus was first detected at day 3 in NP and peaked on day 7. HRSV was also detected in BAL samples collected from the lower respiratory tract, although less pronounced and with a delay in the peak viral load as compared to the upper airways. Throughout the course of infection, both NP and BAL viral loads were lower in the calves inoculated with hRSV inoculum ($10^5$ TCID$_{50}$) as compared to bRSV inoculum ($10^3$ TCID$_{50}$). At necropsy on day 12 or 16 after inoculation (after recovery of the clinical disease), only one bRSV inoculated calf had detectable viral antigen by immunohistochemistry on lung sections (data not shown).
Inoculation of bRSV and hRSV in calves induces distinct clinical respiratory disease symptoms

Consistent with previous findings\textsuperscript{5-19}, bRSV infection was accompanied with clear URTD, starting at day 5 and peaking at day 8 after inoculation (Fig. 1B). The most apparent symptoms included nasal and ocular discharge and productive cough. In contrast, the hRSV infected calves developed URTD at a later stage, between day 8-10 (Fig. 1B), and they had lower disease scores as compared to bRSV infected calves ($P = 0.0014$). Interestingly, none of the hRSV infected calves showed LRTD symptoms or increase in respiratory rate throughout the course of disease (Fig. 1C-D). LRTD symptoms, including tachypnea and abdominal breathing, only occurred in bRSV infected calves, appearing on day 6 and reaching their maximum scores at day 8 after inoculation (Fig. 1C). The LRTD symptoms in bRSV infected calves were accompanied by a significant rise in breathing rate, from $< 40$ before infection up to $83 \pm 12$ breathings per minute at day 8 (Fig. 1D), and tracheobronchial inflammation with mucus and protein-rich exudate, as observed in a separate experiment using bronchoscopy to visualize the respiratory tract (Fig. 2). Finally, rectal temperatures showed a slight elevation throughout
The bRSV calf model

the infection resulting in mild fever (defined as > 39.5°C) in one of the bRSV infected calves on
days 7-8 (data not shown). Regardless of infected with either bRSV or hRSV, all calves started
to recover from 10 days after inoculation, with less severe disease scores and normalization of
breathing rates (Fig. 1B-D).

Inoculation of bRSV and hRSV in calves leads to different lung pro-inflammatory cell responses

To assess the (innate) pro-inflammatory responses in the lungs of hRSV and bRSV inoculated
calves we determined the total and differential white blood cell counts in BAL samples on two
time points after inoculation (days 6 and 9). In calves infected with hRSV, we observed higher
cell concentrations including neutrophils and macrophages on day 6 compared to day 9, while
in calves infected with bRSV, BAL cell concentrations were higher on day 9 (Fig. 3). Overall,
BAL cell concentrations were higher in calves inoculated with bRSV, as compared to hRSV
(day 9: 1.30 × 10^6/mL ± 0.3 × 10^6/mL versus 0.35 × 10^6/mL ± 0.19 × 10^6/mL, P = 0.004, fig. 3A).
In hRSV infected calves, macrophages were the dominant cell-type in BAL (maximal 79 ± 3%
versus 50 ± 5% in the hRSV and bRSV group respectively, P = 0.002, fig. 3B). In contrast,
bRSV infection resulted in a pronounced neutrophilic infiltration (46 ± 3.5% neutrophils
on day 6, versus 18 ± 5% neutrophils in the bRSV and hRSV group respectively, P = 0.004,
fig. 3C). Lymphocyte infiltration appeared at day 9 in both hRSV and bRSV infected calves at

Figure 2: Bronchoscopy images before and after bRSV infection.

Bronchoscopy images of the trachea and a bronchus of a calf before infection (A-B) and after infection with bRSV (C-D). Infection leads to inflammatory responses in the lower respiratory tract with substantial mucus and protein-rich exudate deposition.

Inoculation of bRSV and hRSV in calves leads to different lung pro-inflammatory cell responses

To assess the (innate) pro-inflammatory responses in the lungs of hRSV and bRSV inoculated
calves we determined the total and differential white blood cell counts in BAL samples on two
time points after inoculation (days 6 and 9). In calves infected with hRSV, we observed higher
cell concentrations including neutrophils and macrophages on day 6 compared to day 9, while
in calves infected with bRSV, BAL cell concentrations were higher on day 9 (Fig. 3). Overall,
BAL cell concentrations were higher in calves inoculated with bRSV, as compared to hRSV
(day 9: 1.30 × 10^6/mL ± 0.3 × 10^6/mL versus 0.35 × 10^6/mL ± 0.19 × 10^6/mL, P = 0.004, fig. 3A).
In hRSV infected calves, macrophages were the dominant cell-type in BAL (maximal 79 ± 3%
versus 50 ± 5% in the hRSV and bRSV group respectively, P = 0.002, fig. 3B). In contrast,
bRSV infection resulted in a pronounced neutrophilic infiltration (46 ± 3.5% neutrophils
on day 6, versus 18 ± 5% neutrophils in the bRSV and hRSV group respectively, P = 0.004,
fig. 3C). Lymphocyte infiltration appeared at day 9 in both hRSV and bRSV infected calves at
very low levels (data not shown). We evaluated BAL MPO content as a marker of neutrophil activation and found a clear increase of MPO content during peak disease in bRSV infected calves, but not in hRSV infected calves (day 8, fig. 3D, \( P = 0.01 \)).

**Figure 3: Inflammatory response during HRSV and bRSV infection in calves.**

(A) Broncho-alveolar lavage (BAL) total cells during hRSV infection (white bars) and bRSV infection (black bars) on day 6 and 9 after infection. ** \( P = 0.004 \). (B) BAL macrophage percentages. *** \( P = 0.0002 \). (C) BAL neutrophil percentages. *** \( P = 0.0004 \). (D) BAL MPO concentration from day 6 until day 9. BRSV infected calves have peak MPO levels at the top of disease severity (day 8, \( P = 0.01 \)). Data are presented as mean ± SEM.
The bRSV calf model

**Lung pathology after recovery from hRSV or bRSV disease is not distinctive**

Lung histopathology data were only available after full clinical recovery from hRSV and bRSV disease (day 12 and 16 after inoculation). In bRSV infected calves, small areas with macroscopic lung consolidation were still present varying from 0.5 to 2.1% (mean 1.4%, data not shown). In hRSV infected calves, macroscopic lung consolidation was not observed. However, microscopically similar changes consisting of mild bronchiolitis and bronchopneumonia remained in both hRSV and bRSV infected animals (Fig. 4).

![Figure 4: Histopathological lung injury after clinical recovery from hRSV and bRSV infection.](image)

Representative lung tissue section with haematoxylin and eosin staining, showing mild peri-bronchiolar and alveolar cellular infiltrates with areas of alveolar wall thickening, adjacent to areas with normal alveolar architecture in hRSV infected (A) and bRSV infected (B) animals, on day 12 after virus inoculation. Magnification 40×.
Discussion

The goal of this study was to describe the viral kinetics and (clinical) disease characteristics of an experimental hRSV infection in calves, as compared to the established bRSV model. Our results show that hRSV and bRSV inoculation in calves results in differential responses of viral infection, clinical disease and inflammation in the URT and LRT. Both models may aid future research related to hRSV disease in children.

Since its discovery in 1970, bRSV has been recognized as a leading pathogen in outbreaks of respiratory disease in dairy cattle. The calf has been used to study bRSV disease epidemiology, pathogenesis and prevention in an experimental setting. More recently bRSV-infected calves have also been exploited as an age-relevant model of hRSV infection in infants, owing to its striking similarities with human disease. In the present work, we increase our current understanding of RSV animal models, as it is the first structural description of experimental hRSV infection in calves. One report from the 1980’s showed viral shedding between day 3 and 11 after inoculation of hRSV A2 in 2-7 week old calves, which is comparable with our results. However, the animals used were of a different age and were sacrificed at different time points. Furthermore, their viral inoculum was highly passaged in bovine cell cultures prior to inoculation and none of the calves developed any clinical signs of infection. We have elaborated on these experiments and showed hRSV replication in the upper and to a lesser extent in the lower airways, peaking on day 6, combined with clinical symptoms of the upper respiratory tract and inflammatory responses in the lower respiratory tract. Combined, these results show the potential of an experimental hRSV infection model in calves to study RSV intervention strategies within the context of pre-clinical research.

Similar to other heterologous models, hRSV infection in calves resulted in a respiratory tract infection, albeit of a moderate intensity and with clinical symptoms limited to the upper respiratory tract. This is in contrast to the high disease severity, involving the LRT, after bRSV infection. Due to the high inoculation doses in other hRSV models, for example in lambs or mice, the dose of the hRSV inoculum in calves was more in the range of the viral inoculum needed to establish productive infection in humans, but in this way hRSV infection in calves may provide a more physiological model to mimic primary hRSV infection compared to other heterologous animal models.

In this experiment, hRSV infection in calves resulted only in mild upper respiratory tract disease and predominant macrophage recruitment to the lungs. The peak of URTD (day 5 after inoculation) appears similar to that of experimental hRSV infection in humans (day 4 after infection), as opposed to day 7 during bRSV infection. The bRSV model, on the other hand, is associated with strong clinical symptoms of lower respiratory disease and prominent
The bRSV calf model

neutrophilic inflammation similar to humans. The peak viral load in NP and BAL samples (day 6-7) slightly preceded the peak in clinical symptoms (day 8), which is comparable with data from hRSV infected children.

Previously, it has been reported that bRSV disease in calves is associated with prominent histopathological changes in lung tissue, including bronchiolitis with extensive airway mucus obstruction and atelectasis, and interstitial pneumonia at peak disease. As we aimed to investigate the dynamics of viral replication and disease characteristics over the whole course of experimental hRSV infection as compared to bRSV infection, we only studied lung histopathology after full recovery of the animals. The remaining mild histopathological changes were comparable between bRSV and hRSV. However, given the absence of LRTD symptoms in hRSV-infected calves it is unlikely that the severity of small airway and alveolar changes at peak disease are comparable to the findings in bRSV infection.

Despite the dissimilarities between hRSV infection in calves and humans which seem to hamper the detailed study of hRSV pathogenesis, a hRSV model in calves may still be beneficial over other heterologous RSV models (e.g. mice, cotton rats, ferrets, lambs) depending on the research question under investigation. The age-dependency of hRSV is less present in mice, and anatomical differences between rodents and children are significant. The calf model is better suited to overcome these differences and is further useful for its ability to deprive animals from colostrum, which contains all maternal antibodies as there is no trans-placental transfer of antibodies in the bovine species. Depriving new born calves from colostrum results in maternally derived antibody (MDA)-deficient calves, which allows for controlled vaccine studies without interference of the maternal immune system. The hRSV calf model may also be advantageous in testing therapeutics that require specific hRSV epitopes (e.g. vaccines or monoclonal antibodies). For example, mucosal hRSV vaccination has recently received great interest and showed preliminary efficacy in mouse and cotton rat models. However, rodents are difficult to sample longitudinally, lack mucosal immune reactive areas (e.g. tonsils), have less submucosal glands and have large areas of the airways which are not lined with ciliated cells. Similar to humans, calves have tonsils and submucosal glands, which play key roles in the regulation of immune responses to respiratory pathogens. Furthermore, this large animal model allows in-depth longitudinal analysis of the immunological pathways involved in vaccination response and efficacy at the mucosal level. This includes the possibility to test cross-reactive compounds in both models (hRSV and bRSV). The bRSV calf model has recently shown to reproduce the same therapeutic effects of early administration of anti-viral fusion-inhibitors as reported in human studies. Additionally, the model has shown its ability to evaluate cross-reactive antibodies upon vaccination with human RSV vaccines. The combination of a host-specific virus and a human virus in the same animal is unique for the bovine RSV model, and is not available in other
animal models. Additionally, mice are less susceptible to hRSV re-infection⁴, which contrasts the frequent re-infection of calves and humans by bRSV and hRSV respectively.³⁴ Finally, as calves have a longer lifespan compared to rodents, immunological memory responses to (re)infection can be investigated over longer periods.

In conclusion, in this study we explored the viral kinetics during primary hRSV infection in MDA deficient calves. HRSV infection in calves was associated with clinical URTD and a macrophage-dominant inflammatory response in the lung. Although certainly less virulent and pathogenic as compared to the established host-specific bRSV model, experimental infection of calves with hRSV may serve as a tool for tailored research questions, in particular related to the evaluation of (mucosal) vaccines and antiviral therapies against hRSV disease in humans that are not cross-reacting with bRSV.

Acknowledgements

We would like to thank Frank Coenjaerts for his help with the hRSV viral load detection. This work was supported by Steun Emma Children’s Hospital [CC200001], the Netherlands and the Amsterdam Economic Board [EZ1311 ALOHA RSV].

Statement of contribution

BC, JW, RJ, AA and RB designed the study. BC, RJ, JB and AA conceived and carried out the animals experiments. BC, RJ and JB carried out the BAL measurements and analysed the data. BC, RJ, AA, JW and RB interpreted the results. All authors were involved in writing the paper and had final approval of the submitted and published versions.
The bRSV calf model

References


