ANIMAL MODELS OF HUMAN RESPIRATORY SYNCYTIAL VIRUS DISEASE

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Running head: Animal models of RSV disease

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Abstract
Infection with the human pneumovirus pathogen, respiratory syncytial virus (hRSV), causes a wide spectrum of respiratory disease, notably among infants and the elderly. Laboratory animal studies permit detailed experimental modeling of hRSV disease and are therefore indispensable in the search for novel therapies and preventative strategies. Current animal models include several target species for hRSV, including chimpanzees, cattle, sheep, cotton rats and mice, as well as alternative animal pneumovirus models, such as bovine RSV (bRSV) and pneumonia virus of mice (PVM). These diverse animal models reproduce different features of hRSV disease, and their utilization should therefore be based on the scientific hypothesis under investigation. The purpose of this review is to summarize the strengths and limitations of each of these animal models. Our intent is to provide a resource for investigators and an impetus for future research.

Keywords: respiratory virus, pneumovirus, rodent, inflammation
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ALI/ARDS</td>
<td>acute lung injury/acute respiratory distress syndrome</td>
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<td>BALF</td>
<td>broncho-alveolar lavage fluid</td>
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<td>bRSV</td>
<td>bovine respiratory syncytial virus</td>
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<td>IFN</td>
<td>interferon</td>
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<td>LRTD</td>
<td>lower respiratory tract disease</td>
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<td>oRSV</td>
<td>ovine respiratory syncytial virus</td>
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<tr>
<td>pfu</td>
<td>plaque forming units</td>
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<tr>
<td>PMN</td>
<td>polymorphonuclear</td>
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<td>PVM</td>
<td>pneumonia virus of mice</td>
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<tr>
<td>hRSV</td>
<td>human respiratory syncytial virus</td>
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<td>URTD</td>
<td>upper respiratory tract disease</td>
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</table>
Article outline

Human RSV disease: why model?

Virus phylogeny and structure

Human RSV disease: what to model?

1. Respiratory disease symptoms and pathophysiology
2. Lung histopathology
3. Lung inflammatory response
4. Virus replication

Animal models of RSV infection

Heterologous (non human) hRSV models:
1. Chimpanzees
2. Sheep
3. Cotton rats
4. Mice

Cognate host – pneumovirus models:
5. Cattle – bovine RSV
6. Mice – pneumonia virus of mice (PVM)

Summary: making the choice
Human RSV disease: why model?

Human respiratory syncytial virus (hRSV) is among the most important respiratory pathogens in young children worldwide. hRSV infection and transmission results in community outbreaks with peak activity during the winter months in areas with temperate climates, while hRSV disease activity is observed on a more continuous basis throughout the year in tropical regions (119). In a recent evaluation of the global burden of hRSV-associated acute lower respiratory tract disease (LRTD), Nair and colleagues noted that each year, over 33 million children under the age of five years are affected by this disease, leading to over three million hospitalizations, and almost 200,000 deaths (82). The annual costs of health care associated with hRSV infection among children in the US alone are over $600 million (86). In addition, a growing body of evidence suggests that hRSV infection results in substantial morbidity among the elderly and in adults with underlying chronic illnesses, further highlighting both the health-care related and economic burden of hRSV disease (44).

At this writing, there is no licensed vaccine for hRSV, and there are no specific treatment options for severe disease. There is currently no clear evidence supporting the routine use of the antiviral ribavirin (114), inhaled or systemic corticosteroids (12; 87), or bronchodilators (47) as mainstays of acute therapy in non-immunosuppressed infants and children. The humanized monoclonal anti-RSV F protein, palivizumab, is approved for prophylactic use in high-risk infants only (41; 72) and thus this modality does not currently address the burden of disease among those with no apparent risk factors (55).

Modeling human hRSV disease in vivo is an indispensable step in the search for novel therapies and preventive measures for hRSV disease. Animal models provide for the essential means to test pathophysiologic hypotheses in vivo, and they are the crucial link between mechanistic tissue culture studies and phase I human trials. In addition, they contribute to essential pre-clinical safety testing of new pharmacological and vaccine approaches. Needless to say, animal studies are expensive, and are accompanied by significant ethical concerns and costs. A thorough understanding of the strengths and limitations of the numerous available animal models and specific experimental choices is therefore of utmost importance. The purpose of this review is to provide an overview of the existing, most commonly-used animal models of hRSV disease, thereby to assist investigators in navigating among these options.
Virus phylogeny and structure

The human RSV pathogen belongs to the family *Paramyxoviridae*, subfamily Pneumovirinae, and genus Pneumovirus. There are several additional closely-related, species-limited pneumoviruses including bovine respiratory syncytial virus (bRSV), ovine respiratory syncytial virus (oRSV) and pneumonia virus of mice (PVM). Pneumoviruses are enveloped viruses with a negative-sense, non-segmented RNA genome approximately 15,000 nucleotides in length (reviewed by Easton *et al.* (38)). The virions are structurally similar to one another, consisting of a nucleocapsid within a lipid envelope, and three transmembrane surface glycoproteins: the attachment (G), fusion (F), and small hydrophobic (SH) proteins. The G and F proteins are involved in the attachment and entry of hRSV and bRSV into target cells. Although the exact function(s) of hRSV-SH are unknown, this surface protein has recently been shown to have strong anti-apoptotic properties (45). The pneumovirus genome encodes eight structural proteins (L, G, F, N, P, M, M2-1 and SH) and two unique nonstructural proteins (NS1 and NS2). Although pneumoviruses share this overall genomic structure, the direct amino acid sequence homology is limited, in particular that of the G protein (23; 40; 95; 120). The resulting differences in specific antigenicity of hRSV, bRSV and PVM, may have substantial impact on what research questions can be asked in the individual animal models.
Human RSV disease: *what to model?*

Prior to any discussion of the features of a given animal model, one needs to specify what characteristics of human disease are crucial for focus and attention. The following section contains a description of several specific features of hRSV disease in humans that could be modeled in animal studies. However, it is important to realize that many of the findings from humans are directly affected by factors such as co-morbidity (underlying illness, bacterial and viral co-infections) and treatment (e.g. oxygen therapy and mechanical ventilation). In addition, our understanding of disease pathogenesis in humans is limited for ethical and practical reasons, and autopsy/biopsy findings from patients with severe hRSV disease are scarce. As such, these issues limit the extent to which direct comparisons can be made between hRSV disease in humans and the animal models available.

1. **Respiratory disease symptoms and pathophysiology**

   Human infection with hRSV is associated with a large range of disease symptoms. Many of these symptoms are highly dependent on age at infection. Whereas neonatal hRSV infection is often associated with non-specific symptoms such as failure to thrive, periodic breathing or apnea, and feeding difficulties, most hRSV-infected older infants experience mild upper respiratory tract disease (URTD) symptoms such as coryza and cough. However, this latter age group is particularly prone to develop LRTD (bronchiolitis and broncho-pneumonia) with tachypnea and crackles, inspiratory rhonchi and wheezing on auscultation, which can result in mild hypercapnia and hypoxemia. Some of these findings, such as bronchiolitis involving alveolar hypoventilation by obstructive atelectasis and air trapping may be explained by structural features of the infant lung, such as relatively small airway caliber and poor collateral ventilation. hRSV-infected infants are at risk for respiratory failure, a feature that results from the development of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), which are acute-onset inflammatory conditions characterized by severe hypoxemia (PaO₂/FiO₂ ratio < 300mmHg for ALI; < 200mmHg for ARDS) and bilateral lung infiltrates in the setting of a normal cardiac preload (30; 50; 106). In addition to these acute symptoms, young children may also experience delayed sequelae of hRSV disease, specifically, recurrent wheezing with airway hyperreactivity in later childhood (88). Interestingly, Wu and colleagues have reported that infant age at time of initial infection is associated with a high risk for developing childhood asthma (118). Finally, throughout adolescence and adulthood, and in particularly in elderly
adults, hRSV-associated URTD and LRTD can promote exacerbations of asthma and/or COPD (43; 44).

2. Lung histopathology
As stated above, there are only a few published histopathology studies of acute hRSV disease available for general consideration (2; 62; 96; 116; 117). In the rare fatal cases of bronchiolitis, small airway entrapment by dense plugs composed of mucus, fibrin and cellular debris from leukocytes and dead bronchial epithelial cells has been observed (2; 62; 96; 116). Submucosal edema and peribronchiolar infiltrates composed of mixed mono- and polymorphonuclear cells (PMNs) further contribute to the small airway narrowing and occlusion. In cases with hRSV-associated interstitial pneumonia, there is more prominent alveolar involvement with air-space edema and cellular infiltrates, as well as injury to the alveolar epithelium. Lung epithelial cell death in hRSV LRTD involves both extensive necrosis and classical apoptosis (2; 62; 96; 116). At the severe end of the spectrum, diffuse alveolar damage with the formation of hyaline membranes, characteristic of ALI/ARDS, is observed (2). Similarly, histopathology studies in high risk adults and elderly individuals infected by hRSV are similarly scarce, but findings include bronchiolitis, diffuse alveolar damage with hyaline membranes and organizing pneumonia (39).

3. Lung inflammatory response
Natural hRSV infection in children and experimental hRSV inoculation in adults results in prominent local secretion of pro-inflammatory cytokines, such as TNF-alpha, interleukin (IL)-6, and CXC/CC chemokines, including IL-8, MIP-1alpha/CCL3, RANTES/CCL5 and MCP-1/CCL2 (33; 57; 70; 73; 110). The coordinated actions of several of these cytokines such as TNF-alpha, MIP-1alpha/CCL3 and IL-8 strongly promote the recruitment and activation of neutrophilic PMNs (neutrophils). Studies in children with hRSV disease have shown that neutrophils are by far the most abundant cells (> 80%) in bronchoalveolar lavage fluid (BALF), followed by monocytes/macrophages (8; 42; 71). Eosinophils and effector lymphocytes (natural killer cells, cytotoxic CD8+ T-cells and CD4+ T-cells) represent only a small fraction of the total cells in the BALF of children with hRSV LRTD, but despite their low numbers there is evidence that both eosinophils and effector lymphocytes are highly activated (8; 42; 57; 71; 113). Finally, the effector lymphocytes found in hRSV disease secrete interferon (IFN)-gamma, but local levels are relatively low when compared to those detected in several other viral respiratory diseases (22; 63; 73).
4. Virus replication

Transmission of hRSV between individuals occurs via direct contact with respiratory secretions and large droplet particles at short distance. The incubation period is between 2 to 8 days. In the first stage of infection, there is robust replication of hRSV in the nasopharynx, reaching titers of approximately $10^5$ plaque forming units (pfu) (32) or $10^{5-6}$ TCID$_{50}$ per ml nasal secretion (54). Subsequently, hRSV spreads to the lower airways, where the airway epithelium is the primary site of replication. Macrophages in the lungs may be infected as well, and in cases of pneumonia, hRSV immunoreactivity is also found in the alveolar epithelium (76; 117). Although spread of hRSV among host cells may occur directly by formation of syncytia as observed in vitro, optimal viral entry as well as budding is via the epithelial apical surface. Children with severe hRSV LRTD admitted to the intensive care for mechanical ventilation have up to $60 \times 10^9$ viral copies per ml in tracheal aspirate washes, as determined by quantitative PCR (113), with similar virus concentrations in both upper and lower airways (89). DeVincenzo and colleagues have reported that viral load is associated with hRSV disease severity in both children and adults (32; 33). In experimental hRSV URTD in adults, peak virus recovery from nasal washes is attained on days 6 - 7 after inoculation, and the total duration of viral shedding is approximately 7 days (33). In most pediatric cases of hRSV LRTD, virus shedding continues after clinical recovery, and up to approximately 2 weeks after infection (53).
Animal models of RSV infection

Ideally, an animal model of hRSV disease provides the means to study the entire hRSV disease spectrum, consisting of clinical signs of illness with appropriate histopathological and physiological alterations, including relevant pathogenic mechanisms. Understanding that it is unlikely that a single animal model reproduces all these aspects of human disease, one needs to specify what features of hRSV disease are crucial for focus and attention in order to find the most appropriate animal model(s) for a given study. The following sections summarize the major features of the most frequently utilized animal models of hRSV infection (Table 1), with reference to our understanding of hRSV disease as described above.

Heterologous (non human) hRSV models:

1. Chimpanzees

   Human RSV was originally isolated from a group of chimpanzees with URTD at the Walter Reed Army Institute for Research in Washington DC in 1956 (original name: chimpanzee coryza agent) (13), and was later recognized as a human pathogen. Chimpanzees permit hRSV replication in experimental modeling, with peak viral titers per ml of nasal or tracheal secretion limited to one order of magnitude higher than the initial inoculate (28; 108).

   Development of anti-hRSV neutralizing antibodies provides much information on the effectiveness and immunogenicity of vaccines. In addition, URTD disease symptoms, including rhinorrhea, sneezing and coughing, can be monitored in chimpanzees (7). Although there is no documentation of LRTD in experimental hRSV-infected chimpanzees, one case report has described the development of non-experimental, fatal hRSV-associated bronchopneumonia and histopathological alterations characteristic of ALI/ARDS, including neutrophilic inflammation, extensive lung edema and the deposition of hyaline membranes in a captive 14-month old chimpanzee (24).

   Clearly, the major advantage of research with chimpanzees is their genetic and anatomical similarity to humans. However, the economic, logistical, ethical and emotional burden of working with chimpanzees is extremely high. Housing and handling of the animals requires unique expertise. In addition, there are no inbred chimpanzee strains, the experimental sample size is typically small, and there are few specific reagents for
experimental use. Currently, the hRSV chimpanzee model is primarily used in vaccine studies (56; 108).

2. Sheep

Sheep develop respiratory disease due to oRSV in the natural setting, and they are also susceptible to bRSV (75). Lambs are also susceptible to experimental hRSV infection as shown in a recent series of studies characterizing this model (84; 102).

Neonatal lambs inoculated intra-tracheally with hRSV show clinical signs of URTD, including coughing, on day 6 after inoculation (84). Evidence of LRTD include bronchiolitis with airway entrapment by cellular debris and activation of classical apoptotic changes in airway epithelial and alveolar wall cells (84; 102). In these studies there is evidence of mild interstitial pneumonia, resolving after 14 days (84). Neutrophils are recruited to the lungs in hRSV infected lambs, however the magnitude of the macrophage and effector lymphocyte response appears to be more prominent in terms of total cell counts (84; 102). There is an early increase of TNF-alpha levels and strong IL-8 response, but the levels of the chemokines MIP-1alpha/CCL3, RANTES/CCL5 and MCP-1 are relatively low (102). Importantly, hRSV replication in airway epithelium is robust and peak viral load is reached 6 days after virus inoculation (e.g. ~30 fold increase in viral RNA levels in the lungs on day 6 compared to day 3 after challenge with 10^8 pfu), after which there is a rapid decline (84; 102).

The respiratory tracts of sheep and humans, unlike rodents, share many structural features such as the size of the nasal cavity and airways, and organization of local lymphoid tissue (90; 100). Due to their larger size and body weight, pulmonary function tests can be interpreted more reliably in sheep than in rodents, although there are several important differences in physiological measures, such as dead space volume in relation to tidal volume (64). Finally, lung development in sheep occurs in a manner similar to that in humans (e.g. alveolarization starts preterm), in contrast to rodents (primarily postnatal alveolarization), which suggest that sheep may be an advantageous model when considering the role of age in hRSV disease (1). A clear disadvantage of research with sheep is the currently rather limited availability of sophisticated molecular tools (antibodies, genetic sequence). In addition, although not as complex as many other large animal species, the housing and handling of sheep in requires significant physical space and veterinary expertise.
3. Cotton rats

The cotton rat (*Sigmodon hispidus*) has proven to be a very important laboratory animal for studying human infectious diseases. The cotton rat has become a standard model for evaluation of vaccines, antivirals, and neutralizing antibodies (e.g. palivizumab) (91), and has facilitated preclinical testing for anti-hRSV therapeutic modalities.

Cotton rats are semi-permissive for hRSV replication as first described by Dreizin et al. in 1971 (37; 83), reportedly ~100-fold more so than are standard inbred mouse strains. In this model, hRSV virions are detected in both the upper and lower airways after intranasal inoculation with $10^4$ pfu, with viral replication predominantly in the lower airway epithelium reaching peak viral titers per gram of lung tissue of approximately one order of magnitude higher than the initial inoculum (18; 93). Histopathological lesions associated with hRSV challenge in cotton rats consist of mild to moderate bronchiolitis or pneumonia, sloughed epithelial cells and patchy atelectasis (18; 93; 94). The cellular and biochemical responses to virus challenge are relatively limited; primarily lymphocytes associated with moderate increases in expression of MIP-1alpha, MCP-1 and IFN-gamma (18; 19; 94), and no clear evidence of significant clinical signs of URTD of LRTD (18; 93). No specific age-dependency in the susceptibility to hRSV has been reported, although elderly cotton rats exhibit delayed viral clearance (20).

An important disadvantage of the cotton rat model is the limited pool of immunological reagents available as compared to studies conducted in mice, although the set of cotton rat specific reagents is certainly growing (83). Inbred strains of cotton rats are commercially available, but no transgenic or gene-deleted strains have been constructed. Finally, it is crucial to note that special training is needed for care and handling of cotton rats, as they are fragile and easily agitated. The cotton rat model has been reviewed extensively by Boukhvalova et al. (18) and Niewiesk et al. (83).

4. Mice

Inbred laboratory mouse strains (particularly BALB/c) have by far been the most popular animal species for experimental modeling of human hRSV disease. Prince et al. first described hRSV infection in inbred mice in 1979 (92). Wild-type inbred mice are described as semi-permissive hosts for hRSV; in the most permissive mouse strains, such as BALB/c, a very high intranasal inoculum ($10^5$ to $10^7$ pfu per mouse) is administered in order to detect LRTD symptoms and general signs of illness such as weight loss, reduced activity and ruffled fur at later time points (26; 61; 77; 104). Stark and colleagues have
reported peak viral titers of approximately $15 \times 10^3$ pfu per gram lung tissue homogenate in BALB/c mice upon challenge with $10^7$ pfu (104). However, it should be noted that different hRSV antigenic subgroup strains, such as A/long, A2 and clinical isolates (e.g. Line 19, RSV A2-20), have been reported to induce differential clinical and biochemical responses in mice (67; 80; 105). Likewise, specific mouse strains, including New Zealand Black mice (which have inherent impaired macrophage function) (96), and gene-targeted mice deficient for Toll-like receptor (TLR)2, TLR4, TLR6 and TLR7 (65; 68; 81) show increased susceptibility for hRSV. Unlike in humans, young age in mice is not an important risk factor for severe primary LRTD (51), although age at initial infection as been reported to play a critical role in the development of delayed sequelae of pulmonary dysfunction, with younger mice experiencing increased airway hyperreactivity with enhanced mucus production, eosinophilic inflammation and Th2 responses upon re-infection during later life (27; 29; 31).

Lung function analysis, using whole-body plethysmography and methacholine challenge tests, shows that hRSV-infected mice have an increased breathing rate with evidence of airway obstruction and hyperresponsiveness (61; 112). In particular the clinical hRSV isolate Line 19 induces substantial mucus production, associated with airway hyperreactivity in mice (67). Likewise, inoculation with the recently-described RSV 2-20 results in increased mucin expression, bronchiolitis and airway hyperresponsiveness (105). Mouse strains of gene-targeted mice deficient for specific chemokine receptors such as CCR1 and CXCR2 show decreased airway hyperreactivity (78; 79). Interestingly, similar to pediatric hRSV disease, the airway hyperreactivity of hRSV infected mice appears to persist in long-term follow-up (61).

Mice inoculated with high dose hRSV show important increases in the concentrations of several cytokines and CC/CXC chemokines, such as TNF-alpha, IL-6, IFN-gamma, MIP-1alpha/CCL3, RANTES/CCL5 and KC (murine homologue of human IL-8) in BALF (5; 61; 77; 99). Recruitment of neutrophils to the lungs in hRSV-infected mice is limited, whereas the response of effector lymphocytes is more pronounced (59; 99; 101; 112). In the latter, early NK cellular infiltration precedes an increase in CD8$^+$ and CD4$^+$ T-cells in the lungs.

The most prominent histopathological alterations of mice inoculated with high dose hRSV ($10^5$-$10^6$ pfu) include peribronchiolar and perivascular mononuclear cell (lymphocytes and macrophages) infiltration (51; 107). Mice inoculated with an ever higher dose ($10^7$ pfu) of hRSV also show interstitial pneumonia, with scattered neutrophils (61).

Clearly, one of the major strengths of experimental modeling in mice is the extensive experience with gene targeting, which in combination with rapid and easy breeding, has
resulted in a wide variety of transgenic and gene-deleted mice. The commercial availability of numerous iso- and congenic mouse strains and a vast array of mouse specific reagents and molecular tools, makes mice indispensible when attempting to delineate specific pathophysologies and disease patterns. In part due to their small size, housing and handling of mice is relatively easy and cost-effective, making it possible to design studies with a statistical relevant sample size.

A disadvantage of conducting studies in mice is the fact that there are many differences in the innate and adaptive immune responses between humans and mice, such as species differences in the type of cytokines/chemokines (e.g. absence of IL-8 in mice), ratio of neutrophils/lymphocytes in blood (higher in humans), pattern recognition receptors (e.g. TLR signaling), and the leukocyte surface expression of several specific cluster of differentiation markers (74; 97). Furthermore, the lung anatomy of mice differs substantially from humans (60). For example, mice have fewer bronchioles, less complex airway branching, and a relatively large caliber of the airway lumen. Lastly, hRSV does not replicate robustly in mouse lung tissue and is not a true infectious pathogen in rodent species, thus limiting the nature of experimental questions that can be asked regarding inflammation and antiviral host defense.

Cognate host – pneumovirus models:  
5. Cattle – bovine RSV

The first description of bRSV disease in cattle dates from 1970 in a study by M.F. Paccaud and C. Jacquier (85). bRSV is recognized as one of the leading respiratory pathogens in cattle, resulting in a major health and economic burden resulting from seasonal outbreaks (reviewed by Gershwin (49)). Although cattle are also susceptible to hRSV (109), detailed characterization of this animal model comes primarily from experimental bRSV infection. As such, it is important to realize that most of the veterinary studies using the bRSV model have been designed and interpreted in the context of cattle respiratory disease, rather than from a human perspective.

hRSV disease in humans and bRSV disease in cattle share many features. Most importantly, there is a similar age-dependency in the development of bRSV disease: more severe LRTD occurs in young calves, whereas neonatal infection follows a non-specific pattern of disease (4; 52; 111). bRSV infection in calves causes overt clinical signs of URTD and LRTD (peak at day 7 after experimental inoculation), such as nasal discharge, cough, tachypnea with chest retractions, wheezing, hypercapnia and hypoxemia (4; 6; 25;
General symptoms, including fever, anorexia and reduced activity, are also observed. The disease symptoms in bRSV disease in calves is associated with prominent histopathological changes in lung tissue, involving bronchiolitis and interstitial pneumonia with patchy areas of consolidation, atelectasis or air trapping, and in some cases the deposition of hyaline membranes (4; 21; 49; 66; 69; 115). Occasionally emphysematous bullae are found in animals with severe bRSV disease (49; 115). Finally, there is evidence of extensive apoptosis of lung epithelial cells (115). In addition, calves with bRSV LRTD show a strong lung neutrophil and macrophage response, whereas effector lymphocytes are detected in lower numbers in lung tissue or BALF (4; 69; 115). bRSV LRTD is associated with an increase in the levels of IFN-gamma, TNF-alpha, and IL-6 in BALF (4). Specific lung chemokine responses in bRSV LRTD have not been characterized.

Infection and replication of bRSV in the upper respiratory tract of cattle is followed by spread to the lower airways, with peak viral titers at around day 6 post inoculation (~3 orders of magnitude higher compared to day 3), after which there is a rapid decline (4; 49; 111; 115). Shedding of bRSV is detected in the period of day 4 to day 10 post inoculation. The predominant site of bRSV replication is the bronchial/bronchiolar epithelium, but immunoreactive bRSV is also detected in alveolar epithelium and to a lesser extent in lung macrophages (49; 115).

Although bRSV and hRSV are highly similar to one another antigenically, they are distinct viruses and promote unique lung histopathologies in their cognate hosts (reviewed by Bennett et al. (11)). Furthermore, housing and handling of cattle also requires substantial expertise, and the availability of bovine specific materials and reagents remains limited. Finally, a distinct feature of bRSV infection, co-infections with bacteria, such as Pasteurella and Haemophilus species (bovine respiratory disease complex), are quite commonplace (103). In contrast, this is unusual in hRSV infection in humans. An important strength of experimental research with cattle is the high overlap of clinical symptoms of respiratory disease, in part related to shared structural features of the lungs with humans (64). Because of the relative large size and high weight of the animals, both young and adult, lung function testing in cattle may be performed more easily and reliably as compared to rodents, providing an objective measure of respiratory disease and potential treatments (64).

6. Mice – pneumonia virus of mice (PVM)
As noted earlier, hRSV is formally a challenge-clearance model in wild-type inbred mice. In contrast, PVM, a pneumovirus pathogen originally isolated from mouse lung tissue (58), undergoes robust viral replication in the lower airways, with peak titers of up to $10^8$ pfu/g of lung tissue of BALB/c mice in response to a minimal virus inoculum (~200 pfu) (34; 35). The major site of PVM replication is the bronchiolar epithelium (14) (Figure 1A). PVM-infected mice show marked clinical signs of LRTD, including tachypnea and labored breathing, with evidence of airway obstruction and apnea at the time of peak virus recovery (14). In addition, PVM-infected mice experience symptoms of severe disease, including profound weight loss, leading to mortality rates as much as 100% (35; 98). However, the use of a lower inoculum and less susceptible strains of mice (3), also allows for modeling of mild to moderate disease; inocula of 5, 10, 30, or 60 pfu PVM in C57BL/6 mice results in a survival rate of 100%, 95%, 75%, and 10% respectively (14).

PVM-associated histopathology includes primarily neutrophilic infiltration concentrating around the bronchioles at the initial phase, with progression to alveolitis (Figure 1B) with areas of fibrin depositions, intra-alveolar edema and hemorrhage (10; 35; 48; 98). In addition, similar to human hRSV LRTD, there is widespread evidence of apoptosis in alveolar epithelium (9; 10). Cytokine responses in the lungs and airways of PVM-infected mice include prominent expression of TNF-alpha, IL-6, KC (murine homologue of human IL-8), MIP-1alpha/CCL3, RANTES/CCL5, MCP-1/CCL2 and IFN-gamma (9; 14; 15). Mouse strains of gene-targeted mice deficient for chemokine receptors such as CCR1 show decreased lung inflammatory responses, but increased virus recovery and accelerated mortality (36). The PVM model has been extremely useful for the development of antiviral inflammatory responses and anti-inflammatory, immunomodulatory therapeutic and prophylactic strategies (16; 46).

Similar to human hRSV LRTD, age is a critical variable in the PVM mouse model, with differential clinical and pro-inflammatory responses in neonatal, weanling, adult and elderly mice, although the latter age group appears less susceptible for severe PVM LRTD (15; 17).

General strengths and limitations of using mice in experimental modeling for human disease have been discussed above (section on the hRSV mouse model). A specific disadvantage of the PVM mouse model is the obvious difference in antigenicity when one compares PVM to hRSV. In addition, experiments with PVM should be carried out under specific biosafety requirements in order to avoid spread within a general vivarium, and may not be available in all research facilities.
Summary; making the choice

Animal models are essential to our understanding of the pathophysiology of human hRSV disease. They provide for a critical step in pre-clinical testing of the effectiveness and safety of new pharmacological approaches and vaccine strategies. Currently, among the animal models for human hRSV disease there are heterologous (non-human) models of hRSV infection in chimpanzees, sheep, cotton rats and mice, as well as the cognate host-pneumovirus models cattle-bRSV and mice-PVM. Although each of these experimental animal models has provided important scientific progress, none of them fully reproduces all the specific features of hRSV disease in humans. In addition, each experimental system has inherent advantages and disadvantages with respect to laboratory research.

Investigators should understand the basic strengths and weaknesses of the proposed animal models for hRSV disease. The choice between these different animal models is at least in part dependent on several hierarchical organized criteria including the ethical burden, cost-effectiveness, husbandry and availability of host-specific resources, reagents and tools. However, the single most important rule is that the model should be suitable to answer the scientific question derived from the proposed study hypothesis. The most appropriate animal model reproduces those precise features of hRSV disease to which this hypothesis is related. In particular, one needs to consider potential limitations due to species-restriction of the host-pneumovirus interaction, and at the same time potential limitations due to differences in antigenicity between different pneumovirus pathogens. This review has provided an overview of the unique features of the current animal models for hRSV disease, with the intent of assisting investigators in the field of RSV research.
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**Figure legends**

**Figure 1.** A, H&E stained lung tissue from a BALB/c mouse on day 7 after inoculation with PVM strain J3666, showing extensive alveolar inflammation and areas with hemorrhage (magnification 5x) (reprinted with permission from (46)); B, Immunohistochemical detection of PVM antigen in bronchiolar epithelial cells in lung tissue from a C57BL/6 mouse on day 6 after inoculation with PVM strain J3666 (magnification 40x) (reprinted with permission from (14)).
Table 1. Features of experimental animal models for hRSV disease.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Viral replication*</th>
<th>Clinical Signs</th>
<th>Mortality</th>
<th>Histopathology</th>
<th>Predominant leukocyte response</th>
<th>Major References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee-hRSV</td>
<td>Moderate</td>
<td>Mild</td>
<td>No</td>
<td>Unknown</td>
<td>Unknown</td>
<td>28, 56, 108</td>
</tr>
<tr>
<td>Sheep-hRSV</td>
<td>High</td>
<td>Moderate</td>
<td>No</td>
<td>Moderate</td>
<td>Lymphocyte, macrophage</td>
<td>84, 102</td>
</tr>
<tr>
<td>Cotton rat-hRSV</td>
<td>Moderate</td>
<td>Limited</td>
<td>No</td>
<td>Moderate</td>
<td>Lymphocyte</td>
<td>18, 83, 93</td>
</tr>
<tr>
<td>Mouse-hRSV</td>
<td>Limited</td>
<td>Moderate</td>
<td>No</td>
<td>Low to Moderate</td>
<td>Lymphocyte</td>
<td>51, 61, 67, 92, 104, 105, 107</td>
</tr>
<tr>
<td>Cattle-bRSV</td>
<td>High</td>
<td>Severe</td>
<td>Yes</td>
<td>Severe</td>
<td>Neutrophil</td>
<td>49, 69, 115</td>
</tr>
<tr>
<td>Mouse-PVM</td>
<td>High</td>
<td>Severe</td>
<td>Yes</td>
<td>Severe</td>
<td>Neutrophil</td>
<td>14, 35, 98</td>
</tr>
</tbody>
</table>

*See text for specific details
### Table 2. Overview of major advantages and disadvantages of experimental animal models for hRSV disease.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Heterologous host-virus models</strong></td>
</tr>
<tr>
<td>Chimpanzee-hRSV</td>
<td>High genetic and structural similarity with humans</td>
<td>Extensive economic, ethical and logistical burden</td>
</tr>
<tr>
<td>Sheep-hRSV</td>
<td>Large size allowing for lung function testing</td>
<td>Need for extensive veterinary expertise for housing and maintenance</td>
</tr>
<tr>
<td></td>
<td>Lung development similar to humans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Efficient replication of human pathogen</td>
<td>Limited molecular tools available</td>
</tr>
<tr>
<td>Cotton rat-hRSV</td>
<td>Permissive replication of human pathogen</td>
<td>Difficult to handle</td>
</tr>
<tr>
<td></td>
<td>Small size</td>
<td>Limited molecular tools available</td>
</tr>
<tr>
<td>Mouse-hRSV</td>
<td>Possibility of gene targeting</td>
<td>Limited replication of human pathogen</td>
</tr>
<tr>
<td></td>
<td>Small size and relatively low cost</td>
<td>Important genetic and structural differences with humans</td>
</tr>
<tr>
<td></td>
<td>Extensive molecular tools available</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Cognate host-virus models</strong></td>
</tr>
<tr>
<td>Cattle-bRSV</td>
<td>Efficient replication of cognate pathogen</td>
<td>Different virus</td>
</tr>
<tr>
<td></td>
<td>Clear clinical respiratory disease symptoms</td>
<td>Need for extensive veterinary expertise for housing and maintenance</td>
</tr>
<tr>
<td></td>
<td>Large size allowing for lung function testing</td>
<td>Limited molecular tools available</td>
</tr>
<tr>
<td>Mouse-PVM</td>
<td>Efficient replication of cognate pathogen</td>
<td>Different virus</td>
</tr>
<tr>
<td></td>
<td>Possibility of gene targeting</td>
<td>Special biosafety requirements</td>
</tr>
<tr>
<td></td>
<td>Small size and relatively low cost</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extensive molecular tools available</td>
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</table>
Figure 1