

Studying human respiratory disease in animals – Role of induced and naturally-occurring models

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ABSTRACT

Respiratory disorders like asthma, emphysema, and pulmonary fibrosis affect millions of Americans and many more worldwide. Despite advancements in medical research that have lead to improved understanding of the pathophysiology of these conditions and sometimes to new therapeutic interventions, these disorders are for the most part chronic and progressive; current interventions are not curative and do not halt disease progression. A major obstacle to further advancements relates to the absence of animal models that exactly resemble the human condition, which delays the elucidation of relevant mechanisms of action, the unveiling of biomarkers of disease progression, and identification of new targets for intervention in patients. There are currently many induced animal models of human respiratory disease available for study, and even though they mimic features of human disease, discoveries in these models have not always translated into safe and effective treatments in humans. A major obstacle relates to the genetic, anatomical, and functional variations amongst species, which represents the major challenge to overcome when searching for appropriate models of respiratory disease. Nevertheless, rodents, in particular mice, have become the most common species used for experimentation due to their relatively low cost, size, and adequate understanding of murine genetics, among other advantages. Less well known is the fact that domestic animals also suffer from respiratory illnesses similar to those found in humans. Asthma, bronchitis, pneumonia and pulmonary fibrosis are among the many disorders occurring naturally in dogs, cats, and horses, among other species. These models might better resemble the human condition and are emphasized here, but further investigations are needed to determine their relevance.

Keywords: Animal models, lung, pulmonary disease, pulmonary fibrosis, asthma

Modelling human disease in animals – an historical perspective

Respiratory disorders, such as chronic obstructive pulmonary disease, lung cancer and asthma, account for a high percentage of morbidity and mortality in the United States [1] and around the world. With millions suffering from these disorders, in addition to millions more afflicted with pulmonary fibrosis, pneumonia, sepsis, and sleep disordered-breathing, respiratory illnesses will remain a major cause of morbidity and mortality for the foreseeable future. In addition, tobacco exposure continues to exacerbate the problem [1]. The World Health Organization estimates that chronic obstructive pulmonary disease is responsible for the deaths of 3 million people worldwide each year [2], furthermore, there is an epidemic related to biomass exposure in developing countries which has been linked to obstructive pulmonary disease, cancer, asthma, pneumonia, and chronic fibrosing disorders [3]. Not surprisingly, respiratory disorders affect the poor and disenfranchised disproportionately, thereby representing a major source of health inequality [4]. Considering the above, much is being invested on the development and implementation of strategies capable of reducing the burden of respiratory disease. Advances in this area have however been hampered by our limited understanding of the pathogenesis of many respiratory disorders.

Investigations into lung disease have depended, among other approaches, on the examination of the histology of diseased organs from affected people and on attempting to model human disease in non-human animals. The idea of using animals as models of human disease, as we know it now, is a relatively recent development, whereas the recognition of the commonalities in structure and function between humans and non-human animals is quite ancient. The earliest written records of people drawing inferences about biology and physiology through observation of the similarities in

anatomy between humans and non-human animals extends back to at least the 6th century B.C.E. [5-7].

Modern modelling of human disease in animals – especially since the adoption of mice as the species of choice – has been subdivided into five categories by Hau [8], namely: 1) induced models; 2) spontaneous models; 3) genetically modified models; 4) negative models; and 5) orphan models [8]. Induced animal models utilize healthy animals to attempt to create conditions reflective of a human disease. Such models are common in biomedical research and are widely utilized in pulmonary disease research; ovalbumin sensitization in mice to study allergic asthma in humans is an example of an induced animal model [9]. Spontaneous animal models, as defined by Hau, reflects the modern emphasis on the mouse, referring only to naturally occurring disease in genetic variants (mutant mice) that share similarities with a human disease [8]. This category of animal models is less widely known in pulmonary disease research; an example is the spontaneous development of lung fibrosis associated with elevated levels of tumor necrosis factor-alpha (Tnf) in the viable *motheaten* mouse [10]. Genetically modified animal models – e.g., gene knockout and transgenics - are ubiquitous in mice and have become more common in other species when the mouse does not optimally model the human condition. Recent prominent examples in pulmonary disease are the genetically modified Cystic Fibrosis (CF) pig and ferret [11]. Negative models are those that utilize a lack of response to a predicted outcome based on exposure and genetic background, among other characteristics, relative to what is known in the human disease. This category is especially important in studies of infectious disease, carcinogenicity and toxicity. The final category of animal models – orphan models – describes the discovery of disease in a non-human animal species that has not yet been identified in humans.

Veterinary medicine has provided many important examples of such diseases that are now known to occur in humans. The most famous example is the discovery of

vaccination by Jenner through his work and observation of cowpox viral infections in milk maids [12]; more recent examples include the discovery of oncogenic viruses in chickens by Rous in 1911 [13] and tick-borne hemoparasites by Babes in cattle in Romania in 1888 and Smith and Kilbourne in Texas in 1893 (reviewed in [14]). However, the establishment of Land Grant universities in smaller rural communities in the U.S., while human medical schools stayed in larger urban centres, among other causes, established a divide between the two disciplines leading to a breach in communication between human medical and veterinary medical professionals; this continued until quite recently, improving dramatically over the last decade. Primarily in response to fears of the spread of pandemic infectious zoonotic diseases, a 'one medicine' and 'one health' initiative was initiated for veterinary and human medicine to address shared concerns regarding such diseases [16-20]. Less well known within this initiative has been the growth of shared research on a variety of diseases, especially cancer, cardiovascular disease, and metabolic diseases, that afflict both human and veterinary patients [20]. Notably missing from this initiative is any mention of a comparative medical effort focused on respiratory disease. Toward this end, the authors hope that this review might contribute to beginning a dialogue between human and veterinary medicine regarding the investigation of spontaneous respiratory diseases in veterinary species that might contribute to a better understanding of the pathogenesis and treatment of diseases in humans, that currently have no or inadequate models using conventional (mostly rodent) species. Emphasis in this report will be given to naturally-occurring progressive fibrosing lung diseases in cats, dogs, horses, and donkeys; and reactive airway disease in horses and cats.

The challenge of modeling human respiratory disease

The spectrum of respiratory disease in humans in some respects likely reflects conditions unique to being human that may not be readily recreated in most conventional laboratory animal models. We are a long-lived species with comparatively little genetic diversity across populations [21]. The development of the human lung continues well after birth, with continued alveolar development documented to progress through the first 2-3 years of life [22]. The mostly high-density social structure of contemporary society and the diversity of human culture undoubtedly contributes to a number of important respiratory diseases as we are knowingly or unknowingly exposed to a panoply of chemicals and particles through inhalation, ingestion, and injection whose impact on respiratory health are still mostly not known.

Inter-species variation in the respiratory system is another challenge to modeling human disease in non-human animals. The main function of the respiratory system is gas exchange. Thus, while the general principles underlying the physiological and biological requirements for such function are shared by all mammals there are significant inter-species differences in the structure and cellular composition of lung compartments that may dictate whether or not a particular species develops, either experimentally or naturally, respiratory diseases similar to those in humans. The inability to induce cystic fibrosis (CF) lung disease in mice is an important example of this principle, where the lack of submucosal glands in the mouse may play a role in preventing the induction of cystic fibrosis lung disease in the CF mouse [23].

The overall pattern of the tracheobronchial tree is very similar in mammalian species, being characterized by its dichotomous branching pattern, with the daughter branches becoming progressively smaller, eventually leading to the gas-exchange units. However, the length of the tracheobronchial tree, the angle of the branch points, and the surface area vary greatly amongst species. There are important species differences in the epithelial cells populating the tracheobronchial tree (e.g., number of secretory cells)

as well as in the architecture of the junction between the distal conducting airway and the alveolar parenchyma. Primates (including humans) and carnivores (dogs and cats) have a portion of respiratory bronchiole (Figure 1A) between the terminal bronchiole and alveoli, whereas rodents, equids (horses and donkeys), pigs, and ruminants mostly have alveolar ducts interposed between the terminal bronchiole and the alveoli (Figure 1B) [24].

These differences in anatomy are likely important in the pathogenesis of a variety of diseases in humans and non-human animals. For example, the proximal acinus is considered most susceptible to air pollutants [25, 26] and it is the site for early lesions induced by cigarette smoke in young smokers [27]. The islands of smooth muscle present within the respiratory bronchiole are quite dynamic and capable of significant hypertrophy/hyperplasia in cats with feline asthma and pulmonary fibrosis, contributing significantly to the remodelling of the lung in each disease [28, 29]. This leads one to consider if perhaps, given the anatomic similarity of the distal lung of the cat to the human lung, the development of these diseases in the cat might provide important information on the role that distal lung anatomy plays in the susceptibility of the human lung to the development of asthma and pulmonary fibrosis. Finally, the tissue regenerative capacity of distinct species appears to differ.

Thus, differences in lifestyle, lifespan, anatomy, and genetics, among others, must be taken into consideration when modeling human respiratory disease in non-human animals. Despite these differences, modeling human disease in animals has provided much insight into the pathogenesis of respiratory illnesses.

Induced animal models of respiratory disease

Induced animal models of respiratory disease are used to learn about disease pathogenesis in the hope that they will unveil potential targets for intervention. For the most part, these models mimic some, but not all, features of respiratory disease. Models of Chronic Obstructive Pulmonary Disease (COPD), pneumonia, pulmonary fibrosis, and asthma are commonly examined since these conditions, among several others, represent an overwhelming number of respiratory disease cases in humans.

COPD is a leading cause of death worldwide [30]. This umbrella term encompasses several disorders characterized by airway and parenchymal inflammation as well as tissue destruction (i.e., emphysema, chronic bronchitis, chronic bronchiolitis), all leading to airflow limitation (also call obstruction). Patients with COPD may have one or a combination of these disorders. This not only makes it a complex disorder to treat, but also a very complex disorder to model, and today no model perfectly resembles human COPD.

There are few animal models of COPD induced by air pollution, fumes, dust, and biomass exposure, however most models of COPD use cigarette smoke exposure considering its predominant role in the development of this disease in humans (for an extensive review, see [31]). These studies typically involve small animals; specifically, rodents. Guinea pigs develop emphysema, small airway remodelling, and goblet cell metaplasia in the airways; because of their size, they are useful for pulmonary physiologic testing and cardiovascular instrumentation. However, they are more expensive and their usefulness is limited by the laboratory reagents (e.g., antibodies) available for testing guinea pig tissues [31]. In contrast to guinea pigs, rats develop minimal disease when exposed to cigarette smoke. Considering the above, their relatively low cost, availability, and an ever-expanding library of reagents, the mouse has

emerged as the most commonly used animal to model COPD [32]. There is, however, significant variability in laboratory results, which is likely due to differences in strains obtained through different providers, the use of different smoking delivery systems, and the intensity of exposure, among other factors. In general, the cigarette smoke-induced model of COPD manifests many of the characteristics observed in humans including the development of emphysema, airway and vascular wall remodelling in certain strains, as well as the expected physiological alterations. Interestingly, cigarette-induced emphysema develops earlier in females of certain murine strains (e.g., A/J) when compared to their male counterparts, which is reminiscent of the increased susceptibility observed in women [33]. However, the model does not progress after cessation of exposure and therefore does not produce severe disability as observed in humans. Also, the model requires several months of exposure, which adds to the complexity and costs of experiments.

Emphysema can also be modelled in animals exposed to the elastin-degrading protease elastase [34]. This is important since emphysema is thought to occur from an imbalance between proteinases and anti-proteinases in the lung, especially those related to elastin metabolism. However, less than 1% of patients with COPD have decreased elastin levels as seen in a genetic defect called $\alpha 1$ antitrypsin deficiency suggesting the presence of defects in other proteinase - antiproteinase systems. This, and the nature of the model raise questions about its relevance to human disease. This is also true for the starvation model [35]. Several other models of COPD have been generated in genetically modified animals. For example, transgenic overexpression of collagenase induces histological changes consistent with emphysema [37].

Lower respiratory tract infections represent another leading cause of death in humans. As is the case for other disorders, rodents are the species most commonly used to study respiratory tract infection even though larger mammalian species like rabbits, dogs and

monkeys allow for physiological monitoring [36]. Infection of animals can be achieved by multiple methods including co-habitation of animals, aerosolization, or the use of nebulizers; each method having advantages and disadvantages [36]. Direct endotracheal or endobronchial instillation can also be used to infect mice, but these methods require anaesthesia [37]. On the other hand, they model oropharyngeal aspiration and thus allow for more precise delivery of inoculum, and biocontainment is easier and equipment is less expensive. Intranasal instillation requires anesthetized mice to be held in a vertical position while the inoculum is deposited in the nares and is aspirated into the lungs. Overall, these models are useful but limited considering the differences in pathogenicity of microbes between animals and humans. Furthermore, because animals are typically housed in controlled environments, their microbiome is distinct from that found in their natural habitat. The latter is highly relevant considering emerging data pointing to the influence of the microbiome in human chronic disorders.

Pulmonary fibrosis affects millions worldwide [38]. It is characterized by inflammation and progressive scarring of the lung resulting in effacement of architecture and ultimately loss of lung function and death. Pulmonary fibrosis can be elicited by environmental exposures (e.g., asbestosis, silicosis, allergens). Chronic tobacco smoke exposure is considered a contributing factor, but the aetiology of lung fibrosis is often unknown. The archetype of pulmonary fibrosing disorders is a condition termed Idiopathic Pulmonary Fibrosis or IPF [39]. The aetiology of IPF is unknown (hence its name) and treatment options are suboptimal. However, both *in vitro* and *in vivo* studies carried out over the past two decades have unveiled key molecules (e.g., growth factors such as transforming growth factor β 1), cellular signals (e.g., Smads, tyrosine kinases), and biological pathways (e.g., regulation of matrix expression) that, if uncontrolled, lead to tissue fibrosis [40]. Genetic mutations have been linked to the development of IPF including mutations in telomerases and in surfactant and mucin proteins [41]. Two new

'anti-fibrotic' drugs are available [42,43], but other than lung transplantation, curative interventions and agents capable of effectively halting disease progression remain elusive.

Many induced animal models are available to study lung fibrosis; importantly, none of these models leads to progressive lung fibrosis and clinical disease similar to that experienced by human IPF patients. Of these models, the most common is the bleomycin-induced lung injury model [44]. Bleomycin belongs to a family of glycopeptide antibiotic drugs initially used in the treatment of cancers and later discovered to induce lung fibrosis in humans. It works, among other mechanisms, by oxidant-mediated DNA damage inducing cellular apoptosis and the release of pro-inflammatory and pro-fibrotic cytokines. Mice treated with intra-tracheal injection of bleomycin typically develop early inflammatory changes that peak at day 7 characterized by epithelial cell apoptosis, oedema, release of pro-inflammatory cytokines such as Tnf and interleukin-1 beta, and neutrophil activation and infiltration. This phase is followed by fibroproliferation and increased deposition of collagen and other extracellular matrices resulting in fibrosis; this latter stage peaks at day 21. There are differences in murine strain susceptibility to bleomycin, which may be influenced by variations in drug metabolism by bleomycin hydroxylase [45]. Bleomycin can also be administered systemically via intravascular or intraperitoneal routes.

Other agents used for the induction of experimental lung fibrosis include hypochlorous acid (HOCL), which has been used to study skin and lung fibrosis in models of progressive systemic fibrosis or scleroderma [46]. Inhalation of inorganic particles such as asbestos, silica, and vanadium pentoxide can also induce lung fibrosis [49]. Models of hapten-induced experimental lung fibrosis have also been studied. Of these, fluorescein isothiocyanate (FITC) is the best characterized, leading to fibrosis within 2 to 4 wk and persisting for several weeks afterwards. Lung irradiation and

infection with viruses (e.g., herpesvirus MHV-68) have also been used. Like the other models, these show strain variability and have distinct advantages and disadvantages [50].

Studies in genetically modified animals allow for control of the expression of the relevant gene, while providing a window into specific pathways. Genes can be targeted to the lung by using lung-specific promoters such as those for surfactant proteins. Using this method, the diphtheria toxin receptor, for example, can be expressed on epithelial cells [47]; through the repetitive injection of diphtheria toxin, epithelial injury is caused and followed by fibrogenesis. Virus-mediated transgene delivery has also been used to induce fibrosis via overexpression of pro-fibrotic growth factors such as transforming growth factor- β 1 [48].

Asthma affects over 15 million Americans and many more around the world. The most frequently used animal models of asthma depend on experimental induction by repeated challenge with allergens. Animals subjected to such challenges develop goblet cell hyperplasia, airway inflammation and hyperresponsiveness as well as remodelling, which are the hallmarks of asthma [49]. Although these fairly acute models show functional improvement in response to glucocorticoids and bronchodilators as seen in humans, translation of findings in these models into the clinical arena has not always been successful [50, 51]. More-chronic models of asthma might be more helpful and are being refined, including those related to 5-7 wk exposure to house dust mite [52]. As in all animal models tested, the choice of species is crucial because humans and guinea pigs show more responsiveness to β 2 agonists when compared to mice, pig, cat and rabbit, which might be explained by differential expression of soluble mediators [53].

Overall, these and related models of induced respiratory disease have provided excellent opportunities for the exploration of biological pathways involved in injury and

repair. However, in general, although they manifest features of human disease, they do not exactly resemble the human condition and interventions successfully tested in these models have not always led to safe and effective therapeutic agents in humans.

Naturally occurring models of respiratory disease

For thousands of years of human history, we have shared our lives with a number of domestic animal species as we moved from hunting societies, to primarily agricultural, to contemporary society where now the majority of people live in urban environments. The dog is the oldest domestic species, dating back to at least 30,000 years [54], while cats have been domesticated for at least 7,000 years [55-57]. The lives of domestic animals, in particular dogs and cats, parallel our own lives more closely than ever, as they breathe the same air as us in our urban, agricultural, and home environments, eat foods formulated by us, and lead increasingly inactive lives. Perhaps we should not be surprised to discover that these animals develop chronic respiratory diseases similar to our own. However, when considering naturally occurring respiratory diseases in domestic animals as models of human disease, it is important to remember that all of these diseases are spontaneous, first recognized by a veterinarian for their respiratory clinical signs, which was then followed by identification of the associated pathology. As veterinary medicine recognized and described these diseases, practitioners looked to human medicine to name the diseases, using the human as the model species for the veterinary patients. This is in stark contrast to most models of human lung disease, e.g. pulmonary fibrosis and models of asthma, where the rodent models typically do not develop clinical signs similar to the corresponding human

disease. Other limitations include the fact that these models have not been tested systematically, and consequently most information available relates to their characterization. Also, the aetiology and pathogenesis of disease in these models is, for the most part, unclear. Thus, they may not resemble the human condition in this regard. Another limitation is that natural models of disease occur in outbred species. This heterogeneity would require that larger cohorts of animals be tested in order to have sufficient power for generating statistically valid results; this may not be feasible in many situations.

Naturally occurring progressive fibrosing lung disease in domestic animals

Dogs, horses, cats, and donkeys spontaneously develop progressive fibrosing lung diseases. Based on the progressive clinical course and predominance of fibrosis within the lung, veterinary scientists have borrowed terminology from human medicine describing these diseases using nomenclature established by physicians and pathologists. Idiopathic pulmonary fibrosis (IPF) is a recognized disease in dogs and cats [58-60], while a variant of pleuroparenchymal fibroelastosis has been described in donkeys [61].

In human medicine, considerable effort has been put forth to classify idiopathic interstitial lung diseases, with particular emphasis given to defining IPF based on the combination of clinical signs, imaging information, and pulmonary histopathological features of the disease [62, 63]. At this time, human IPF is defined histologically as having the usual interstitial pneumonia (UIP) pattern [62, 63], characterized briefly, histologically by subpleural involvement, temporal heterogeneity (affected areas adjacent to normal tissue), myofibroblast accumulation in fibroblastic foci, honeycombing, and scant inflammation [63]. In veterinary medicine there has been no

similar attempt to date to understand the spectrum of fibrosing interstitial lung diseases in domestic species. Thus, though IPF is used to describe idiopathic pulmonary fibrosis in the cat and the dog, while technically correct, this does not necessarily mean these diseases are identical to IPF in humans. Defining progressive fibrosing lung diseases in domestic animals should be a research priority.

Canine pulmonary fibrosis

Pulmonary fibrosis in domestic animals is most well described and characterized in the dog. Canine pulmonary fibrosis is most commonly described in the West Highland White Terrier (WHWT) breed [58, 64-66], although it occurs in a variety of other dog breeds. Pulmonary fibrosis in WHWT is a disease of middle-aged to elderly dogs, reportedly most common between the ages of 8 and 15 yr [64, 67]. Clinical signs in affected dogs include exercise intolerance and cough, which can progress to tachypnea, cyanosis, and respiratory failure [64, 68, 69]. Thoracic radiography of cases of pulmonary fibrosis in WHWT is not specific; findings usually consist of bronchointerstitial to interstitial lung disease patterns [58]. High-resolution computed tomography (HRCT) of the chest is not typically performed in cases of canine pulmonary fibrosis so information is incomplete. The HRCT changes in the lung in WHWT with pulmonary fibrosis are most prevalent in the caudodorsal lung and include findings such as ground glass opacity, parenchymal bands, subpleural lines and interstitial thickening, thickening of the interstitium of bronchovascular bundles, traction bronchiectasis, and honeycombing [70].

The gross and histological pathology of pulmonary fibrosis in WHWT has been described in a small number of studies from Europe and the United States [64, 65, 71]. The most commonly described histologic lesion in the lungs of affected WHWT is mature and uniform thickening of alveolar walls (Figure 2A,2B) [64, 65, 71]. A recent study by

Syrja et al. systematically assessed the lung histopathology from 18 WHWT with pulmonary fibrosis, comparing the spectrum of lung histopathology with lung biopsies from human IPF patients [71]. The authors, consisting of a team of veterinary and human medical professionals, describe changes in the WHWT lung that share features with UIP as well as fibrotic NSIP; importantly there was no evidence of fibroblast foci in canine pulmonary fibrosis [71]. The histopathology of pulmonary fibrosis in non-WHWT is less well described in the literature (71). Cases of PF in these individuals have changes in the lungs similar to UIP in humans, including subpleural fibrosis, temporal heterogeneity and little inflammation.

The cause(s) and pathogenesis of pulmonary fibrosis in dogs is not known at this time. Analysis of gene and protein expression from WHWT with pulmonary fibrosis has identified changes in expression similar to human IPF lung, reflecting the pro-fibrotic environment of the canine pulmonary fibrosis lung. Increased expression of transforming growth factor- β as well as proteins critical to TGF- β signalling including TGF- β receptor I and phospho-Smad 2/3 have been detected immunohistochemically [72,73, 74]. The expression of TGF- β co-localized in the lungs of WHWT with pulmonary fibrosis with TGF- β receptor I and phospho-Smad 2/3 in areas of fibrosis, with increased expression present within epithelial cells in these foci, consistent with a role for TGF- β in the pathogenesis of the lung fibrosis as has been found in human IPF [74-76]. Elevated circulating levels of CXCL8 were detected in the serum of healthy (non-pulmonary fibrosis) WHWT when compared to other breeds of dogs in a recent study [77]; serum CCL2 was also increased in WHWT, but the increase was not restricted to only the WHWT [77]. Both CCL2 and CXCL8 were also increased within the BALF of WHWT with PF compared to healthy control dogs [78].

Feline pulmonary fibrosis

Progressive fibrosing lung disease in the cat is less well understood than the disease in the dog. There are to date no apparent breed predispositions identified for the development of the disease that might facilitate accumulation of patients with which to perform prospective studies as in the WHWT [29, 79]. As with the canine disease, the disease in cats has been named IPF based on its unknown cause, progressive clinical course, and histologic features in common with human IPF [29]. The disease in cats occurs in middle-aged to adult animals; the clinical signs of cats with pulmonary fibrosis include tachypnea and increased inspiratory effort [79]. Imaging of the lungs using radiography may show a combination of a bronchointerstitial and alveolar pattern [80]. Cardiac echocardiography has documented right ventricular hypertrophy in some cats with pulmonary fibrosis, suggesting pulmonary hypertension developing secondary to the fibrotic lung disease [80].

The pathology of feline pulmonary fibrosis shares a number of features with the human UIP pattern [29], including subpleural distribution, temporal heterogeneity, regions of honeycomb lung formation and usually scant inflammation; proliferation of smooth muscle which may arise from the respiratory bronchioles is a prominent feature of feline PF (Figure 3) [29]. Unlike pulmonary fibrosis in the dog, myofibroblasts are reported as a consistent feature within feline pulmonary fibrosis where they are reported to occasionally form foci similar to fibroblast foci as described in human IPF [29].

Equine pulmonary fibrosis

The progressive fibrosing lung disease of horses is termed *Equine Multinodular Pulmonary Fibrosis* (EMPF); this name is based on the characteristic nodular foci of fibrosis that develop within the lung in adult animals (Figure 4A) [81]. EMPF is a disease of adult horses that develops in association with lung infections with the

gammaherpesvirus EHV 5 [81, 82]. Viral infections have been suggested as playing a role in the underlying epithelial injury that is assumed to drive lung fibrosis in humans with IPF [83-91]. The strongest evidence to date implicates EBV in IPF, although this is controversial as the evidence for virus in the lungs of IPF patients is inconsistent [91-93]. The equine gammaherpesvirus EHV 5 is similar to EBV in that evidence of subclinical infection in horses is nearly ubiquitous [94, 95]. In the initial description of EMPF, EHV 5 was detected in 100% of affected horses; this was the first example of a spontaneous progressive fibrosing lung disease being directly associated with lung infection with a gammaherpesvirus [81]. The clinical signs of the disease include tachypnea, weight loss, and low-grade fever; nodular densities are noted within the lungs in thoracic radiographs [96].

The pathology of EMPF is distinct from human UIP and much different from canine or feline pulmonary fibrosis. The distribution of the fibrosis in EMPF is within the alveolar parenchyma where it is organized into discrete to coalescing nodules, which may be multiple centimetres in diameter (Figure 4B) [81]. Histologically the nodules are comprised of abundant interstitial collagen accumulation surrounding alveoli-like spaces that are lined by cuboidal epithelial cells. Within the spaces there are often neutrophils and macrophages, with occasional macrophages containing large intranuclear herpesviral inclusion bodies (Figure 4C) [81].

The pathogenesis of EHV 5 infection and its role in EMPF is not known. In 2013, a study was published that addressed the ability of EHV 5 to induce nodular lung fibrosis similar to EMPF [82]. In that study, EHV 5 was instilled directly into the lungs of clinically normal horses, resulting in the development of nodular lung fibrosis that was histologically similar to EMPF [82]. This study provided strong evidence that EHV 5 alone is capable of inducing lung fibrosis in horses, which is strong 'proof of concept'

that gammaherpesviruses may play a role in driving lung fibrosis in humans and other animals.

Donkey pulmonary fibrosis

Pulmonary fibrosis in donkeys, unlike pulmonary fibrosis in cats, dogs, and horses, is reported to be a relatively common finding within the lungs of elderly donkeys [97]. In spite of the frequency of occurrence of pulmonary fibrosis in donkeys, little was known regarding this disease until recently when it was reported that pulmonary fibrosis in donkeys most likely resembles human pleuroparenchymal fibroelastosis [98], a recently recognized variant of IPF interstitial lung disease (Figure 5) [99-102].

Naturally occurring hyperreactive airway disease in domestic animals

Horses and cats develop hyperreactive airway diseases that have a number of similarities to human allergic asthma. Unlike the various IPF interstitial lung diseases that occur in domestic animals (discussed above), this group of diseases is comparatively well characterized [103-107].

Recurrent airway obstruction (Heaves) in horses

Recurrent airway obstruction (RAO), also known as heaves, is a common and very important inflammatory airway disease of adult horses whose primary clinical expression is the result of bronchoconstriction [108]. Clinical RAO develops, as with many cases of human asthma, following inhalation of airborne antigens; fungal antigens and environmental dusts present within feed and stable environments are commonly associated with induction of clinical 'heaves' in susceptible horses [109, 110]. The clinical signs of 'heaves' reflect the underlying small airway inflammation and mucus

accumulation characteristic of RAO: horses exhibit increased expiratory effort, exercise intolerance, and coughing [103, 104]. Importantly, for the purposes of considering RAO as a model of asthma or inflammation-induced hyperreactive airway diseases, clinical disease can be induced in susceptible horses through exposure of the animal to allergens in their environment [115,116].

The lung pathology of RAO has some similarities to human asthma. Like asthma, RAO is an inflammatory airway disease; unlike human asthma, eosinophils are a minor component of the disease, with the neutrophil being the predominant cell type present within affected airways [111]. Mucus accumulation within the lumen and mucous cell metaplasia accompany the inflammation, and there is evidence of increased distal airway smooth muscle in the lungs of RAO horses (Figure 6A) [112, 113].

Feline asthma

A form of hyperreactive airway disease associated with eosinophilic airway inflammation, known as feline asthma, is relatively common in domestic cats [114]. Feline asthma is usually considered to be a disease of young adult cats, with the median age at diagnosis of 4-5 years. At this time, there are not clear clinical diagnostic criteria for differentiating feline asthma from other diseases, e.g. chronic bronchitis, that can present with clinical signs similar to feline asthma, thus the actual incidence of feline asthma is not known, although it has been estimated that 1-5% of cats in the United States are affected by the disease [106]. Clinically, active feline asthma is expressed as a combination of cough, wheeze, and variable respiratory distress [114]. Thoracic radiographs may be uninformative or there may be evidence of a bronchial to bronchointerstitial lung pattern [115, 116].

The lung pathology of feline asthma (Figure 6B) shares many similarities with human asthma. As noted above, feline asthma is characterized by airway eosinophilic

inflammation. The epithelium lining the bronchi and bronchioles is variably hyperplastic and often infiltrated with eosinophils [115, 116]. Hypertrophy and hyperplasia of smooth muscle at the level of the terminal and respiratory bronchiole is common and hypertrophy of submucosal glands in the bronchi is often prominent. All of these changes are reported to be features of human asthma lungs [117].

As with human asthma, cats with feline asthma develop a Th2 polarized inflammatory environment within the lung in response to allergens [106]. The nature of the offending allergens is usually not identified in spontaneous cases of feline asthma, although given the shared environment between cats and people both may share common allergens [107]. An experimental model of feline asthma has been developed by Reinero et al. using a combination of house dust mite or Bermuda grass allergen, that develops airway hyperreactivity and eosinophilic inflammation, and airway remodelling similar to the natural disease [28]. Given the apparent similarities between feline asthma and human asthma, experimentally-induced feline asthma could be a powerful model for understanding human asthma [AQ: please check that your meaning has been retained].

Conclusions

Animal models allow us to evaluate biological pathways responsible for lung function during health and dysfunction during illness, and to examine the safety and efficacy of interventions within the complex environment of an organ within a live animal. This is not only warranted, but represents an ethical requirement before any new intervention is tested in humans. Currently, there are many models of respiratory disease being used for the above that have a variety of advantages and disadvantages (Tables 1, 2). Most of these are induced models of disease that mimic some, but not all features of respiratory disorders like emphysema, pneumonia, asthma, and pulmonary

fibrosis, among others. Studies performed in these models have been invaluable and induced animal models, especially in mice, continue to represent a commonly used approach to understanding human biology.

Unfortunately, there is no ideal animal model since they do not exactly resemble the human condition due to significant inter-species variations in the anatomy of the respiratory system and genetics, among many other variables. Functional differences are also evident; for example, mice do not have a cough reflex. In fact, the dependence on murine models based on financial considerations and understanding of mouse genetics might be short-sighted since these models often do not resemble the human condition, and spending effort upfront to identify better models could prove more efficient. Combining models of induced lung disease with gene targeting might improve the relevance of the phenotype generated. Also, *in vitro*, *ex vivo*, explant culture, and *in silico* models may serve to complement the *in vivo* models, thereby bringing us closer to the human reality. Choosing the 'right' model requires careful consideration of inter-species variations in addition to the implications of testing acute versus chronic exposures, end points to be evaluated, as well as gender and age of the experimental animals. The drawbacks and advantages of many of these induced models have been reviewed by others [124].

Considering the limitations of induced models of human respiratory disease, attention is moving toward naturally occurring diseases in domestic animals. When considering these models, it is important to remember that these diseases are spontaneous and were first recognized because of their characteristic clinical features; this is in contrast to some murine models of lung disease which do not develop clinical disease similar to the disease being studied. Just as important, the clinical signs that develop in these animals are accompanied by lung pathology that often shares

considerable homology with the corresponding human disease. Ethical considerations related to using such ‘animal patients’ to study ‘human patients’ should be evaluated. Nevertheless, in a 2007 article Hunninghake and Schwarz challenged the research community to make sure that mechanistic data generated in experimental models “pass the test of explaining the clinical histologic features of the disease” before assuming such data are unique to the condition [118]. It is our opinion that by studying an array of spontaneous, naturally occurring respiratory diseases in domestic animals (some of which are highlighted in this review), we may be able to pass this test and provide important information regarding underlying mechanisms that lead to many important respiratory diseases in humans that currently are underserved by conventional laboratory animal models.

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REFERENCES

1. Grimshaw GM, Stanton A. Tobacco cessation interventions for young people. Cochrane Database Syst Rev 2006; CD003289; DOI 10.1002/14651858.CD003289.pub4.
2. Diaz-Guzman E, Mannino DM. Epidemiology and prevalence of chronic obstructive pulmonary disease. *Clin Chest Med* 2014; **35**: 7-16.
3. Gordon SB, Bruce NG, Grigg J, et al. Respiratory risks from household air pollution in low and middle income countries. *Lancet Respir Med* 2014; **2**: 823-860.
4. Celedon JC, Roman J, Schraufnagel DE, et al. Respiratory health equality in the United States. The American thoracic society perspective. *Ann Am Thorac Soc* 2014; **11**: 473-479.
5. Ericsson AC, Crim MJ, Franklin CL. A brief history of animal modeling. *Mo Med* 2013; **110**: 201-205.
6. Lennox JG. *Aristotle: On the Parts of Animals* edn). Oxford University Press: Oxford England, 2001; 52-54.
7. Gotthelf A. Darwin on Aristotle. *Journal of the History of Biology* 1999; **32**: 3-30.
8. Hau J. Animal Models of Human Disease. In *Sourcebook of Models for Biomedical Research*, Conn P (ed). Humana Press: Totowa, NJ, 2008; 3-8.
9. Kumar RK, Herbert C, Foster PS. The "classical" ovalbumin challenge model of asthma in mice. *Curr Drug Targets* 2008; **9**: 485-494.
10. Thrall RS, Vogel SN, Evans R, et al. Role of tumor necrosis factor-alpha in the spontaneous development of pulmonary fibrosis in viable motheaten mutant mice. *Am J Pathol* 1997; **151**: 1303-1310.

11. Yan Z, Stewart ZA, Sinn PL, *et al.* Ferret and pig models of cystic fibrosis: prospects and promise for gene therapy. *Hum Gene Ther Clin Dev* 2015; **26**: 38-49.
12. Sanchez-Sampedro L, Perdiguero B, Mejias-Perez E, *et al.* The evolution of poxvirus vaccines. *Viruses* 2015; **7**: 1726-1803.
13. Rubin H. The early history of tumor virology: Rous, RIF, and RAV. *Proc Natl Acad Sci U S A* 2011; **108**: 14389-14396.
14. Hunfeld KP, Hildebrandt A, Gray JS. Babesiosis: recent insights into an ancient disease. *Int J Parasitol* 2008; **38**: 1219-1237.
15. Safransky RJ. The Morrill Acts. In *Encyclopedia of Law and Higher Education*, Russo CJ (ed). SAGE, 2010; 317-319.
16. Enserink M. Medicine. Initiative aims to merge animal and human health science to benefit both. *Science* 2007; 1553; DOI 316/5831/1553a [pii] 10.1126/science.316.5831.1553a.
17. Cardiff RD, Ward JM, Barthold SW. 'One medicine---one pathology': are veterinary and human pathology prepared? *Lab Invest* 2008; **88**: 18-26.
18. Zinsstag J, Schelling E, Waltner-Toews D, *et al.* From "one medicine" to "one health" and systemic approaches to health and well-being. *Prev Vet Med* 2011; **101**: 148-156.
19. van Helden PD, van Helden LS, Hoal EG. One world, one health. Humans, animals and the environment are inextricably linked-a fact that needs to be remembered and exploited in our modern approach to health. *EMBO Rep* 2013; **14**: 497-501.
20. Gibbs EP. The evolution of One Health: a decade of progress and challenges for the future. *Vet Rec* 2014; **174**: 85-91.

21. Serre D, Paabo S. Evidence for gradients of human genetic diversity within and among continents. *Genome Res* 2004; **14**: 1679-1685.
22. Burri PH. Structural aspects of postnatal lung development - alveolar formation and growth. *Biol Neonate* 2006; **89**: 313-322.
23. Grubb BR, Boucher RC. Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiol Rev* 1999; **79**: S193-214.
24. KE PJaP. Gross and subgross anatomy of lungs, pleura, connective tissue septa, distal airways, and structural units. In *Comparative Biology of the Normal Lung*, (2nd Edition edn), RA P (ed). Academic Press: London, 2015.
25. Schwartz LW, Dungworth DL, Mustafa MG, *et al.* Pulmonary responses of rats to ambient levels of ozone: effects of 7-day intermittent or continuous exposure. *Lab Invest* 1976; **34**: 565-578.
26. Tyler WS, Tyler NK, Last JA, *et al.* Comparison of daily and seasonal exposures of young monkeys to ozone. *Toxicology* 1988; **50**: 131-144.
27. Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. *N Engl J Med* 1974; **291**: 755-758.
28. Norris Reinero CR, Decile KC, Berghaus RD, *et al.* An experimental model of allergic asthma in cats sensitized to house dust mite or bermuda grass allergen. *Int Arch Allergy Immunol* 2004; **135**: 117-131.
29. Williams KJ, Malarkey D, Cohn L, *et al.* Identification of spontaneous feline Idiopathic Pulmonary Fibrosis: morphology and ultrastructural evidence for a Type II Pneumocyte defect. *Chest* 2004; **125**: 2278-2288.
30. Rennard SI, Drummond MB. Early chronic obstructive pulmonary disease: definition, assessment, and prevention. *Lancet* 2015; **385**: 1778-1788.
31. Wright JL, Cosio M, Churg A. Animal models of chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**: L1-15.

32. Bartalesi B, Cavarra E, Fineschi S, *et al.* Different lung responses to cigarette smoke in two strains of mice sensitive to oxidants. *Eur Respir J* 2005; **25**: 15-22.
33. March TH, Wilder JA, Esparza DC, *et al.* Modulators of cigarette smoke-induced pulmonary emphysema in A/J mice. *Toxicol Sci* 2006; **92**: 545-559.
34. Janoff A, White R, Carp H, *et al.* Lung injury induced by leukocytic proteases. *Am J Pathol* 1979; **97**: 111-136.
35. Sahebji H. Effects of postnatal starvation and refeeding on rat lungs during adulthood. *Am Rev Respir Dis* 1986; **133**: 769-772.
36. Mizgerd JP, Skerrett SJ. Animal models of human pneumonia. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**: L387-398.
37. Brain JD, Knudson DE, Sorokin SP, *et al.* Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Environ Res* 1976; **11**: 13-33.
38. Hutchinson J, Fogarty A, Hubbard R, *et al.* Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur Respir J* 2015; **46**: 795-806.
39. Raghu G, Rochwerg B, Zhang Y, *et al.* An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med* 2011; **192**: e3-e19; DOI 10.1164/rccm.201506-1063ST.
40. Gharaee-Kermani M, Gyetko MR, Hu B, *et al.* New insights into the pathogenesis and treatment of idiopathic pulmonary fibrosis: a potential role for stem cells in the lung parenchyma and implications for therapy. *Pharm Res* 2007; **24**: 819-841.

41. Hambly N, Shimbori C, Kolb M. Molecular classification of idiopathic pulmonary fibrosis: Personalized medicine, genetics and biomarkers. *Respirology* 2015; DOI 10.1111/resp.12569.
42. Richeldi L, du Bois RM, Raghu G, *et al.* Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014; **370**: 2071-2082.
43. King TE, Jr., Bradford WZ, Castro-Bernardini S, *et al.* A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2014; **370**: 2083-2092.
44. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**: L152-160.
45. Moeller A, Ask K, Warburton D, *et al.* The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? *Int J Biochem Cell Biol* 2008; **40**: 362-382.
46. Bagnato G, Bitto A, Pizzino G, *et al.* Simvastatin attenuates the development of pulmonary and cutaneous fibrosis in a murine model of systemic sclerosis. *Rheumatology (Oxford)* 2013; **52**: 1377-1386.
47. Sisson TH, Mendez M, Choi K, *et al.* Targeted injury of type II alveolar epithelial cells induces pulmonary fibrosis. *Am J Respir Crit Care Med* 2010; **181**: 254-263.
48. Sime PJ, Xing Z, Graham FL, *et al.* Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 1997; **100**: 768-776.
49. Holmes AM, Solari R, Holgate ST. Animal models of asthma: value, limitations and opportunities for alternative approaches. *Drug Discov Today* 2011; **16**: 659-670.

50. Alderton WK, Angell AD, Craig C, *et al.* GW274150 and GW273629 are potent and highly selective inhibitors of inducible nitric oxide synthase in vitro and in vivo. *Br J Pharmacol* 2005; **145**: 301-312.
51. Liu Y, Zhang S, Li DW, *et al.* Efficacy of anti-interleukin-5 therapy with mepolizumab in patients with asthma: a meta-analysis of randomized placebo-controlled trials. *PlosOne* 2013; **8**: e59872; DOI 10.1371/journal.pone.0059872 PONE-D-12-37483 [pii].
52. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech* 2008; **1**: 213-220.
53. Riley JP, Fuchs B, Sjoberg L, *et al.* Mast cell mediators cause early allergic bronchoconstriction in guinea-pigs in vivo: a model of relevance to asthma. *Clin Sci (Lond)* 2013; **125**: 533-542.
54. Skoglund P, Ersmark E, Palkopoulou E, *et al.* Ancient Wolf Genome Reveals an Early Divergence of Domestic Dog Ancestors and Admixture into High-Latitude Breeds. *Curr Biol* 2015; **25**: 1515-1519.
55. Driscoll CA, Menotti-Raymond M, Roca AL, *et al.* The Near Eastern origin of cat domestication. *Science* 2007; **317**: 519-523.
56. Axelsson E, Ratnakumar A, Arendt ML, *et al.* The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 2013; **495**: 360-364.
57. Montague MJ, Li G, Gandolfi B, *et al.* Comparative analysis of the domestic cat genome reveals genetic signatures underlying feline biology and domestication. *Proc Natl Acad Sci USA* 2014; **111**: 17230-17235.
58. Heikkila-Laurila HP, Rajamaki MM. Idiopathic pulmonary fibrosis in West Highland white terriers. *Vet Clin North Am Small Anim Pract* 2014; **44**: 129-142.

59. Williams K, Malarkey D, Cohn L, *et al.* Identification of spontaneous feline idiopathic pulmonary fibrosis: morphology and ultrastructural evidence for a type II pneumocyte defect. *Chest* 2004; **125**: 2278-2288.
60. Cohn LA, Norris CR, Hawkins EC, *et al.* Identification and characterization of an idiopathic pulmonary fibrosis-like condition in cats. *J Vet Intern Med* 2004; **18**: 632-641.
61. Miele A, Dhaliwal K, Du Toit N, *et al.* Chronic pleuropulmonary fibrosis and elastosis of aged donkeys: similarities to human pleuroparenchymal fibroelastosis. *Chest* 2014; **145**: 1325-1332.
62. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med* 2000; **161**: 646-664.
63. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002; **165**: 277-304.
64. Corcoran BM, Cobb M, Martin MW, *et al.* Chronic pulmonary disease in West Highland white terriers. *Vet Rec* 1999; **144**: 611-616.
65. Norris AJ, Naydan DK, Wilson DW. Interstitial lung disease in West Highland White Terriers. *Vet Pathol* 2005; **42**: 35-41.
66. Webb JA, Armstrong J. Chronic idiopathic pulmonary fibrosis in a West Highland white terrier. *Can Vet J* 2002; **43**: 703-705.

67. Heikkila-Laurila HP, Rajamaki MM. Idiopathic pulmonary fibrosis in West Highland white terriers. *Vet Clin North Am Small Anim Pract* 2014; **44**: 129-142.
68. Heikkila HP, Lappalainen AK, Day MJ, *et al.* Clinical, bronchoscopic, histopathologic, diagnostic imaging, and arterial oxygenation findings in West Highland White Terriers with idiopathic pulmonary fibrosis. *J Vet Intern Med* 2011; **25**: 433-439.
69. Lilja-Maula LI, Laurila HP, Syrja P, *et al.* Long-term outcome and use of 6-minute walk test in West Highland White Terriers with idiopathic pulmonary fibrosis. *J Vet Intern Med* 2014; **28**: 379-385.
70. Heikkila HP, Lappalainen AK, Day MJ, *et al.* Clinical, bronchoscopic, histopathologic, diagnostic imaging, and arterial oxygenation findings in West Highland White Terriers with idiopathic pulmonary fibrosis. *J Vet Intern Med* 2011; **25**: 433-439.
71. Syrja P, Heikkila HP, Lilja-Maula L, *et al.* The histopathology of idiopathic pulmonary fibrosis in West Highland white terriers shares features of both non-specific interstitial pneumonia and usual interstitial pneumonia in man. *J Comp Pathol* 2013; **149**: 303-313.
72. Lilja-Maula LI, Palviainen MJ, Heikkila HP, *et al.* Proteomic analysis of bronchoalveolar lavage fluid samples obtained from West Highland White Terriers with idiopathic pulmonary fibrosis, dogs with chronic bronchitis, and healthy dogs. *Am J Vet Res* 2013; **74**: 148-154.
73. Lilja-Maula L, Syrja P, Laurila HP, *et al.* Comparative study of transforming growth factor-beta signalling and regulatory molecules in human and canine idiopathic pulmonary fibrosis. *J Comp Pathol* 2014; **150**: 399-407.

74. Krafft E, Lybaert P, Roels E, *et al.* Transforming growth factor Beta 1 activation, storage, and signaling pathways in idiopathic pulmonary fibrosis in dogs. *J Vet Intern Med* 2014; **28**: 1666-1675.
75. Khalil N, O'Connor RN, Flanders KC, *et al.* TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. *Am J Respir Cell Mol Biol* 1996; **14**: 131-138.
76. Khalil N, O'Connor RN, Unruh HW, *et al.* Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1991; **5**: 155-162.
77. Roels E, Krafft E, Antoine N, *et al.* Evaluation of chemokines CXCL8 and CCL2, serotonin, and vascular endothelial growth factor serum concentrations in healthy dogs from seven breeds with variable predisposition for canine idiopathic pulmonary fibrosis. *Res Vet Sci* 2015; **101**: 57-62.
78. Roels E, Krafft E, Farnir F, *et al.* Assessment of CCL2 and CXCL8 chemokines in serum, bronchoalveolar lavage fluid and lung tissue samples from dogs affected with canine idiopathic pulmonary fibrosis. *Vet J* 2015; **206**: 75-82.
79. Cohn LA NC, Hawkins EC, Dye JA, *et al.* Identification and Characterization of an Idiopathic Pulmonary Fibrosis- Like Condition in Cats. *J Vet Intern Med* 2004; **18**: 632-641.
80. Evola MG, Edmondson EF, Reichle JK, *et al.* Radiographic and histopathologic characteristics of pulmonary fibrosis in nine cats. *Vet Radiol Ultrasound* 2014; **55**: 133-140.
81. Williams KJ, Maes R, Del Piero F, *et al.* Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. *Vet Pathol* 2007; **44**: 849-862.

82. Williams KJ, Robinson NE, Lim A, *et al.* Experimental induction of pulmonary fibrosis in horses with the gammaherpesvirus equine herpesvirus 5. *PLoS One* 2013; **8**: e77754.
83. Lok SS, Egan JJ. Viruses and idiopathic pulmonary fibrosis. *Monaldi Arch Chest Dis* 2000; **55**: 146-150.
84. Vannella KM, Moore BB. Viruses as co-factors for the initiation or exacerbation of lung fibrosis. *Fibrogenesis Tissue Repair* 2008; **1**: 2; DOI 1755-1536-1-2 [pii] 10.1186/1755-1536-1-2.
85. Arase Y, Suzuki F, Suzuki Y, *et al.* Hepatitis C virus enhances incidence of idiopathic pulmonary fibrosis. *World J Gastroenterol* 2008; **14**: 5880-5886.
86. Ueda T, Ohta K, Suzuki N, *et al.* Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus. *Am Rev Respir Dis* 1992; **146**: 266-268.
87. Kuwano K, Nomoto Y, Kunitake R, *et al.* Detection of adenovirus E1A DNA in pulmonary fibrosis using nested polymerase chain reaction. *Eur Respir J* 1997; **10**: 1445-1449.
88. Dworniczak S, Ziora D, Kapral M, *et al.* Human cytomegalovirus DNA level in patients with idiopathic pulmonary fibrosis. *J Physiol Pharmacol* 2004; **55 Suppl 3**: 67-75.
89. Kelly BG, Lok SS, Hasleton PS, *et al.* A rearranged form of Epstein-Barr virus DNA is associated with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2002; **166**: 510-513.
90. Stewart JP, Egan JJ, Ross AJ, *et al.* The detection of Epstein-Barr virus DNA in lung tissue from patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1999; **159**: 1336-1341.

91. Tang YW, Johnson JE, Browning PJ, *et al.* Herpesvirus DNA is consistently detected in lungs of patients with idiopathic pulmonary fibrosis. *J Clin Microbiol* 2003; **41**: 2633-2640.
92. Pulkkinen V, Salmenkivi K, Kinnula VL, *et al.* A novel screening method detects herpesviral DNA in the idiopathic pulmonary fibrosis lung. *Ann Med* 2012; **44**: 178-186.
93. Wangoo A, Shaw RJ, Diss TC, *et al.* Cryptogenic fibrosing alveolitis: lack of association with Epstein-Barr virus infection. *Thorax* 1997; **52**: 888-891.
94. Bell SA, Balasuriya UB, Gardner IA, *et al.* Temporal detection of equine herpesvirus infections of a cohort of mares and their foals. *Vet Microbiol* 2006; **116**: 249-257.
95. Wang L, Raidal SL, Pizzirani A, *et al.* Detection of respiratory herpesviruses in foals and adult horses determined by nested multiplex PCR. *Vet Microbiol* 2007; **121**: 18-28.
96. Wong DM, Belgrave RL, Williams KJ, *et al.* Multinodular pulmonary fibrosis in five horses. *J Am Vet Med Assoc* 2008; **232**: 898-905.
97. Morrow LD, Smith KC, Piercy RJ, *et al.* Retrospective analysis of post-mortem findings in 1,444 aged donkeys. *J Comp Pathol* 2011; **144**: 145-156.
98. Miele A, Dhaliwal K, Du Toit N, *et al.* Chronic pleuropulmonary fibrosis and elastosis of aged donkeys: similarities to human pleuroparenchymal fibroelastosis. *Chest* 2014; **145**: 1325-1332.
99. Travis WD, Costabel U, Hansell DM, *et al.* An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013; **188**: 733-748.

100. Frankel SK, Cool CD, Lynch DA, *et al.* Idiopathic pleuroparenchymal fibroelastosis: description of a novel clinicopathologic entity. *Chest* 2004; **126**: 2007-2013.
101. Reddy TL, Tominaga M, Hansell DM, *et al.* Pleuroparenchymal fibroelastosis: a spectrum of histopathological and imaging phenotypes. *Eur Respir J* 2012; **40**: 377-385.
102. von der Thusen JH. Pleuroparenchymal Fibroelastosis: Its Pathological Characteristics. *Curr Respir Med Rev* 2013; **9**: 238-247.
103. Pirie RS. Recurrent airway obstruction: a review. *Equine Vet J* 2014; **46**: 276-288.
104. Bullone M, Lavoie JP. Asthma "of horses and men"--how can equine heaves help us better understand human asthma immunopathology and its functional consequences? *Mol Immunol* 2015; **66**: 97-105.
105. Reinero CR. Advances in the understanding of pathogenesis, and diagnostics and therapeutics for feline allergic asthma. *Vet J* 2011; **190**: 28-33.
106. Trzil JE, Reinero CR. Update on feline asthma. *Vet Clin North Am Small Anim Pract* 2014; **44**: 91-105.
107. Reinero CR, DeClue AE, Rabinowitz P. Asthma in humans and cats: is there a common sensitivity to aeroallergens in shared environments? *Environ Res* 2009; **109**: 634-640.
108. Hotchkiss JW, Reid SW, Christley RM. A survey of horse owners in Great Britain regarding horses in their care. Part 2: Risk factors for recurrent airway obstruction. *Equine Vet J* 2007; **39**: 301-308.
109. Tesarowski DB, Viel L, McDonell WN. Pulmonary function measurements during repeated environmental challenge of horses with recurrent airway obstruction (heaves). *Am J Vet Res* 1996; **57**: 1214-1219.

110. Clarke AF, Madelin T. Technique for assessing respiratory health hazards from hay and other source materials. *Equine Vet J* 1987; **19**: 442-447.
111. Jean D, Vrins A, Beauchamp G, *et al.* Evaluation of variations in bronchoalveolar lavage fluid in horses with recurrent airway obstruction. *Am J Vet Res* 2011; **72**: 838-842.
112. Gerber V, Robinson NE, Venta RJ, *et al.* Mucin genes in horse airways: MUC5AC, but not MUC2, may play a role in recurrent airway obstruction. *Equine Vet J* 2003; **35**: 252-257.
113. Jefcoat AM, Hotchkiss JA, Gerber V, *et al.* Persistent mucin glycoprotein alterations in equine recurrent airway obstruction. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L704-712.
114. Venema CM, Patterson CC. Feline asthma: what's new and where might clinical practice be heading? *J Feline Med Surg* 2010; **12**: 681-692.
115. Corcoran BM, Foster DJ, Fuentes VL. Feline asthma syndrome: a retrospective study of the clinical presentation in 29 cats. *J Small Anim Pract* 1995; **36**: 481-488.
116. Dye JA, McKiernan BC, Rozanski EA, *et al.* Bronchopulmonary disease in the cat: historical, physical, radiographic, clinicopathologic, and pulmonary functional evaluation of 24 affected and 15 healthy cats. *J Vet Intern Med* 1996; **10**: 385-400.
117. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004; **22**: 789-815.
118. Hunninghake GW, Schwarz MI. Does current knowledge explain the pathogenesis of idiopathic pulmonary fibrosis? A perspective. *Proc Am Thorac Soc* 2007; **4**: 449-452.

Figure Legends:

Figure 1. Comparative histological anatomy of the terminal acinus. (A) In rodents, ruminants, and equids the terminal bronchiole ends in an alveolar duct (AD) which consists of a duct lined by alveoli (arrow) without a continuation of bronchiolar structures. (B) In primates, including humans, and carnivores the terminal bronchiole transitions into a respiratory bronchiole (RB) which consists of islands of bronchiole (arrow) separated by alveolar outpocketings.

Figure 2. Gross and histological pathology of canine pulmonary fibrosis in the West Highland White Terrier (WHWT). (A) Sections of lung from a WHWT with pulmonary fibrosis. The fibrosis is present within the subpleura (arrows) as well as within the deeper lung parenchyma. (B) Histologically, abundant collagen expands the alveolar wall in pulmonary fibrosis in the WHWT.

Figure 3. Histological pathology of feline pulmonary fibrosis. (A) Fibrosis is present within the sub-pleura (arrow) as well as within the deeper lung parenchyma. Note the temporal heterogeneity, with fibrotic lung adjacent to relatively unaffected lung. (B) Higher magnification showing the markedly hypertrophied and hyperplastic bundles of smooth muscle (arrowheads) present in feline pulmonary fibrosis. Masson's Trichrome histochemistry.

Figure 4. Gross and histological pathology of Equine Multinodular Pulmonary Fibrosis (EMPF). (A) The foci of fibrosis develop as individual and coalescing nodules of dense fibrosis (arrow). (B) Histologically there is abundant interstitial collagen that separates enlarged airspaces that contain moderate amounts of neutrophilic and

macrophagic inflammation and which are lined by cuboidal epithelial cells. (C) Higher magnification illustrating the inflammation, cuboidal epithelium, and a large eosinophilic herpesviral inclusion body within an enlarged macrophage (arrow). Image (A) provided by Drs. Jon Patterson and Katryia Chankow, Michigan State University.

Figure 5. Histologic pathology of donkey pulmonary fibrosis. The pleura (arrow) is markedly thickened by dense collagen and elastin fibres. Collagen and elastin fibres are present within the subjacent alveolar spaces (arrowhead). Elastin Van Gieson histochemistry. Image kindly provided by Dr. Amy Miele, University of Edinburgh.

Figure 6. Histologic pathology of equine recurrent airway obstruction (RAO) and feline asthma. (A) Widespread filling of bronchioles with mucus and inflammation (arrows) is characteristic of RAO. (B) Mucus and inflammation are also present in feline asthma (arrow) along with widespread bronchiolar smooth muscle hypertrophy (arrowhead). Image (A) provided by Dr. Scott Fitzgerald, Michigan State University.

Table 1. Availability of models for respiratory conditions

Condition	Induced	Preferred Animal	Spontaneous	Animal
COPD	+	Rodents*	+	Dogs**
Asthma	+	Rodents*	+	Cat, Horse
Infection	+	Rodents*	+	Dog, Cat, Horse
Fibrosis	+	Rodents*	+	Dog, Cat, Horse, Donkey

* Mainly mice

** Bronchitis; emphysema not described

Table 2. Main advantages and disadvantages of animal models of respiratory disease

Induced model		Naturally-occurring model	
Advantages	Disadvantages	Advantages	Disadvantages
Availability	Interspecies variation (e.g., anatomy, genetics)	Spontaneous clinical presentation	Not readily available
Easy/rapid breeding	Intravascular physiological monitoring difficult	Similar environmental exposures as humans	Disorders not well characterized
Size, cost	Do not adequately mimic human disease	Mimic human disease	Difficulties in obtaining tissue
Controlled environment	Variations in providers	Cost	Reagents not always available
Reagents available (especially for mice)	Cost of equipment (e.g., aerosolization chambers)		Monitoring difficult
Suitable for genetic manipulation (especially for mice)	Costs related to length of study		Ethical considerations