

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

The equine asthma model of airway remodeling: from a veterinary to a human perspective

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1722234> since 2020-01-10T13:40:00Z

Published version:

DOI:10.1007/s00441-019-03117-4

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

The equine asthma model of airway remodeling: from a veterinary to a human perspective

Michela Bullone¹ and Jean-Pierre Lavoie²

¹ Department of Veterinary Sciences, Università degli Studi di Torino, Grugliasco, Italy.

² Faculty of Veterinary Sciences, University of Montreal, St-Hyacinthe, Quebec, Canada.

Correspondence to: Jean-Pierre Lavoie, Faculty of Veterinary Sciences, University of Montreal,
3200 rue Sicotte, Saint-Hyacinthe, Quebec, Canada. Email: jean-pierre.lavoie@umontreal.ca

Abstract

Human asthma is a complex and heterogeneous disorder characterized by chronic inflammation, bronchospasm and airway remodeling. The latter is a major determinant of the structure-function relationship of the respiratory system and is likely contributing to the progressive and accelerated decline in lung function observed in patients over time. Anti-inflammatory drugs such as corticosteroids are the cornerstone of asthma treatment. While their action on inflammation and lung function is well characterized, their effect on remodeling remains largely unknown. An important hindrance to airway remodeling as a major focus in asthma research is that the physiologic and clinical consequences of airway wall thickening and altered composition are not well understood. In this perspective, equine asthma provides a unique and ethical (non-terminal) preclinical model for hypothesis testing and generation. Severe equine asthma is a spontaneous disease affecting adult horses characterized by recurrent and reversible episodes of disease exacerbations. It is associated with bronchoalveolar neutrophilic inflammation, bronchospasm, excessive mucus secretion. Severe equine asthma is also characterized by bronchial remodeling, which is only partially improved by prolonged period of disease remission induced by therapy or antigen avoidance strategies. This review will focus on the similarities and differences of airway remodeling in equine and human asthma, on the strengths and limitations of the equine model, and on the challenges the model has to face to keep up with human asthma research.

Keywords: horse, severe equine asthma, lung, airway smooth muscle, animal models.

Human asthma is a complex and heterogeneous disorder that affects over 334 million people worldwide; its prevalence is estimated to increase in the coming years, especially in low-income countries. It is a chronic inflammatory condition whose main clinical trait is a variable and (at least partially) reversible respiratory obstruction (Papi, et al., 2018). Another important feature of human asthma, first described in the early 20's (Huber and Koessler, 1922), is airway remodeling. This terminology describes a well-characterized set of changes in structural cells and tissues of the airways (Hirota and Martin, 2013), but whose impact on asthma clinical presentation is largely unknown (Prakash, et al., 2017). In this review we discuss the potential contribution of the equine model of asthma to this unsolved issue.

Airway remodeling

Morphological and/or phenotypic alterations are reported for all the structural cell types in the bronchi of asthmatic patients. Overall, the remodeled airways are thickened, mainly as a consequence of the increased deposition of submucosal extracellular matrix and of the increased airway smooth muscle mass. Also, the airway lumen is often reduced as a result of the increased secretion of mucus from the epithelium and submucosal glands. Asthma-associated traits of airway remodeling are typically described as subepithelial fibrosis, increased smooth muscle mass, gland enlargement, neovascularization and epithelial alterations (Bergeron, et al., 2010). The same traits are recognized in the airways of asthmatic horses (**Figure 1**).

Remodeling is generally considered as the consequence of chronic tissue inflammation and dysregulated repairs. However, the similar degree of remodeling observed in recent compared to long-standing mild asthmatic patients (Boulet, et al., 2000), the demonstration of bronchial remodeling in wheezing children (O'Reilly, et al., 2013, Saglani, et al., 2007), and the rapid remodeling response observed after antigen challenges in asthmatics (Kariyawasam, et al., 2007)

argue against a chronic insult being necessary for the development of all alterations found in human asthmatic airways. The results of a recent study showing increased collagen deposition below the basement membrane occurring rapidly in response to methacholine challenges in asthma patients also questions the need of (eosinophilic) inflammation in this process, at least once the disease has already developed/is already present (Grainge, et al., 2011). Collectively, these data support the theory that, albeit intimately linked, inflammation and remodeling maintain a certain degree of independence, most likely due to the fact that they can be regulated by separate pathways, and affecting different cell-types. Current knowledge on this matter is limited as most of the research has focused only on the inflammatory side, with a few glimpses on remodeling. Studying airway inflammation and remodeling in parallel and using a systematic approach might provide the much-needed information on the overlap and interdependence of asthma pathogenetic pathways.

Airway remodeling is a major determinant of the structure-function relationship of the respiratory system (West, et al., 2013). It significantly contributes to airflow obstruction in asthma, particularly during episodes of acute bronchoconstriction (asthma exacerbations). However, the relationship between airway structure and its function (meaning, how well/smoothly the air can pass/flow through the tube) is not a linear one (Jarjour, et al., 2012). In fact, airway remodeling has qualitative aspects as well (i.e. altered stiffness of the extracellular matrix, heterogeneous airway smooth muscle contraction), whose quantification is hard to achieve, and which perturb the system in a less predictable way. There is also evidence of a variable effect of asthma (and of diverse phenotypes of asthma) at different levels of the bronchial tree. To complicate matters further, the structure-function relationship of the respiratory system physiologically changes along the bronchial tree and with aging, even in healthy people (James and Carroll, 2000, Thannickal, et al., 2015).

As a result, asthma alters the physiologic structure-function relationship of the aging human lung, leading to an accelerated decline of airway function (Lange, et al., 1998, Lange, et al., 2006). The major determinant of this accelerated decline in asthmatic patients remains undetermined, however.

Indeed, although it is tempting to speculate that airway remodeling is the most plausible cause for the loss of lung function observed in asthma, recent reports suggest that only inflammation and acute exacerbations are likely to play a role in this process (Bai, et al., 2007, Coumou, et al., 2018, Newby, et al., 2014, Ortega, et al., 2018), disregarding the effect they are likely to have on remodeling.

Airway remodeling as a major focus in human asthma research

Asthma-associated airway remodeling has gained importance only in the last decades (Boulet and Sterk, 2007). Up to now and to the best of our knowledge, however, it has never been listed among the primary outcomes of randomized clinical trials for testing the efficacy of asthma treatment. Due to technical and economic constraints, most of our understanding of the putative determinants and impact of airway remodeling on the clinically relevant outcomes of human asthma derives from small-to-medium size observational cross-sectional studies. Nevertheless, this approach has significantly increased our understanding of “what remodeling is” and, albeit only partly, of “how remodeling changes in different asthma phenotypes”; but still leaves unanswered the questions on “what causes remodeling” and on “what remodeling does”. An official research statement by the American Thoracic Society details the challenges hindering research and therapeutic advances concerning our understanding on airway remodeling (Prakash, Halayko, Gosens, Panettieri, Camoretti-Mercado, Penn, Structure and Function, 2017). It underlines that the limited understanding of the physiologic and clinical consequences of airway wall thickening in asthma has prevented the study of airway remodeling as a major focus in human asthma research. Understanding airway remodeling mechanisms and their impact on disease is the key toward the development of anti-remodeling therapies. To date, airway remodeling is thought to be minimally affected by current treatments (Prakash, Halayko, Gosens, Panettieri, Camoretti-Mercado, Penn, Structure and Function, 2017), but the data available is too scarce and heterogeneous to draw

reliable conclusions on this matter. While there is no doubt that human tissues are the gold standard to study the implications of airway remodeling in human asthma, it is ethically and economically unconceivable to obtain (repeated) airway biopsies, especially from the peripheral airways, from such a high number of patients as it would be necessary in order to study all the possible factors implicated in these processes. Also, while imaging modalities such as multidetector computed tomography (CT) and magnetic resonance imaging with hyperpolarized helium (He^3) might have a role in quantifying airway thickness, they do not provide sufficient detail to study remodeling, especially when studying the small peripheral airways. In this perspective, animal models are unavoidable resources, but a mindful approach is necessary, given the limited understanding of the triggering factors responsible for the development of airway remodeling in human asthma.

Measures of efficacy of human asthma treatment

Asthma may be transient in some patients, especially when developing during childhood. However, due to the chronic nature of the disorder and to the unavailability of curative therapies, most asthma patients are doomed to lifelong pharmacologic treatment in order to maintain airway patency and adequate respiratory airflow. Besides lung function, other clinical outcomes of asthma that have gained importance in the last years to assess the efficacy of new therapeutic approaches are blood/bronchial inflammation, exacerbation rate, asthma control, and the corticosteroid maintenance daily dose (only when assessing add-on or biological treatments, and non-pharmacological interventions). Inflammation in trials for asthma therapies refers mostly to eosinophilic inflammation, either in the blood or in the airways. This outcome appears to work well for those treatments directed towards the T-2/Th-2 pathways (Bhakta and Woodruff, 2011, Fahy, 2015, Fajt and Wenzel, 2015, Ingram and Kraft, 2012), while it might reveal inadequate for neutrophilic patients. The exacerbation rate defines how frequently the patient sustains acute deterioration of its clinical status (due to an obstructive event) requiring the attention of the

attending clinician and/or intensive care unit access/hospitalization/intubation within an interval of time (Fuhlbrigge, et al., 2012, Reddel, et al., 2009). Asthma control is a questionnaire-based measure that aims to quantify how much the daily activities of a patient are affected by asthma-associated symptoms (it can be viewed as a proxy of the quality of life). Of note, unlike other measures of asthma control, neutrophilic inflammation and asthma exacerbation rate appear to be less responsive to corticosteroid treatment, the pillar of asthma therapeutic approach (Harrison, et al., 2004, Jatakanon, et al., 1999, Macedo, et al., 2009, Moore, et al., 2014, Osborne, et al., 2009, Roy and Milgrom, 2010). It is tempting to speculate that thickened airways are more prone to more frequent acute obstruction, as partly supported by studies finding a positive association between increased wall thickness and disease severity (Bai, 2010, Montaudon, et al., 2009, Niimi, et al., 2004), but this relationship remains speculative.

Given its high prevalence and the chronicity of its treatment, human asthma is associated with high healthcare costs. Moreover, the majority of asthma-related costs address the needs of a small subset of patients with the severe form of the disease (O'Neill, et al., 2015), which is associated with a pronounced airway remodeling (Aikawa, et al., 1992, Benayoun, et al., 2003, James, et al., 2009, James, et al., 2012). In this perspective, understanding whether and how airway remodeling influences the clinical outcome employed to assess the efficacy of asthma treatments is paramount.

An oversimplification of asthma

Clinically, asthma shows as a combination of the clinical outcomes listed in the previous paragraph. Any of these outcomes of asthma, or even their sum, can be seen simplistically as:

$$\textit{Asthma} = k + \textit{inflammation} + \textit{remodeling} + \textit{ASM activation}$$

Where k represents the reference population data (and its genetic variability) and asthma stands for the sum of the clinical outcomes associated with this disorder. Obviously, this model does not take into account time, the complex interactions between these parameters, or the latency of effect of any of the factors included. Also, genetic factors that might affect inflammation, remodeling, or ASM activation are not explicitly reported; and the effects of smoke exposure, aging, and comorbidities are disregarded. However, it is deliberately written in these terms to focus on the single component causes of the basic causal mechanism of asthma. It is worth noting that, in this view, the treatment is included in the term “asthma”, but the equation can be rewritten as:

$$Asthma = (k + inflammation + remodeling + ASM\ activation) - treatment$$

Given that most asthma treatments act on several pathways, it makes sense to break up the effect of treatment into several terms:

$$Asthma = (k - treatment) + (inflammation - treatment) + (remodeling - treatment) + (ASM\ activation - treatment)$$

Thus, as $(k - treatment)$ is the effect of treatment on healthy people and it is likely to be equal to k :

$$Asthma = k + (inflammation - treatment) + (remodeling - treatment) + (ASM\ activation - treatment)$$

Where $(inflammation - treatment)$ is the effect of the treatment on asthma-related inflammation; $(remodeling - treatment)$ is the effect of the treatment on asthma-related airway remodeling; and $(ASM\ activation - treatment)$ is the effect of treatment on asthma-related bronchoconstriction.

Ideally, in order to study the specific effect of airway remodeling on the clinical outcomes of asthma, a model (or a treatment) where inflammation and ASM activation could be modulated and, possibly, nullified, would be required. While this cannot be achieved *in toto* in human patients or in animal models of asthma, the latter offer much more margin for maneuver.

Animal models of asthma

Animal models of asthma can be classified as models of induced disease, genetically manipulated models, and spontaneous models (Rosenberg and Druey, 2018). The animal model used is selected based on the research question to be addressed. Mouse models (either challenge-induced disease or genetically manipulated) are commonly studied in asthma research due to their convenience and contained costs. They are well suited for genetic manipulations when the aim of the study is biomechanistical. However, their appropriateness for translational research is more limited. This is due mainly to anatomical and physiological differences between mice and men, likely affecting at least some of the outcomes most frequently studied. Indeed, mice bronchial tree is less developed than in humans, and most of the lung is represented by lung parenchyma (at the expenses of small airways). The size of the largest intrapulmonary airway in mice is approximately that of a small humans airway (<2 mm in diameter), with important implications for data interpretation. Murine airways also lack submucosal glands, as well as a normal intra-pulmonary systemic (bronchial) circulation, and have limited airway smooth muscle mass compared to humans (Shin, et al., 2009); thus, the study of these features for human asthma research has important limitations. Mice size, breathing frequency and respiratory volumes cause their structural cells to undergo very different loads and frequency of loads compared to the human ones (Fehrenbach, et al., 2017, Rosenberg and Druey, 2018). Given the importance of these factors in human lung physiology, lung function results derived from mouse model studies should be interpreted with caution. Second, mice are not naturally prone to develop clinical pulmonary conditions associated with inflammation or allergic disorders. Thus, the ability to induce asthma-like airway inflammation by allergen challenge in this model does not prove our ability to reproduce the complexity of the mechanisms underlying spontaneous asthma. As stated by Stephen T. Holgate: «While asthma is an inflammatory disorder [...], inflammation itself does not explain the origin(s) of this disease nor why the airways are so

susceptible to a range of different environmental factors» (Holgate, 2011). Indeed, even after sensitization and allergen-challenge, allergic mice – whose airways are inflamed – do not show increased airway resistance unless spasmogens are administered (Rosenberg and Druey, 2018). Lastly, it has to be noticed that most remodeling data obtained from asthmatic mice are obtained from (boosted) allergic or Th2-‘high’ models, which we now know reflect the status of only a portion of asthmatic patients (Douwes, et al., 2002, Woodruff, et al., 2009). Rats show anatomical advantages over mice when studying airway remodeling: first, the submucosal and the smooth muscle layers are more pronounced in the proximal large airways of rats than mice; second, rats possess a bronchial circulation and their mucosa and submucosa are provided with capillaries and veins while in mice only the trachea and the first bronchial ramifications have an arterial blood supply (Tschernig, et al., 2008). Rats also show an earlier response to an allergen challenge compared to mice in terms of airway remodeling (Fehrenbach, Wagner and Wegmann, 2017). Different challenges can induce both airway smooth muscle and extracellular matrix remodeling in rat airways, making this species adapt to study several aspects of airway remodeling (Martin and Tamaoka, 2006).

In nature, cats, monkeys and horses suffer from spontaneous forms of asthma-like conditions. Feline asthma affects approximately 1 to 5% of adult cats and is characterized clinically by recurrent episodes of cough, mucus hypersecretion and bronchoconstriction in the presence of airway hyperresponsiveness and eosinophilic inflammation (Trzil and Reinero, 2014). A disadvantage of the feline model of asthma is the unawareness of the triggers inducing spontaneous disease exacerbations. House dust mite and Bermuda grass allergens have been successfully used for sensitization and to induce exacerbations and asthma-associated bronchial remodeling both in central and peripheral airways in normal cats. However, chronic sensitization appears to blunt the eosinophilic response associated with feline asthma (Norris Reinero, et al., 2004), unlike what is observed in the spontaneous disease. When used as a model for asthma, monkeys need to be

sensitized with these allergens, although spontaneous sensitization to certain allergens such as those from *Ascaris spp.*, dust mite and cedar pollen occurs in monkeys. They are a model for human allergic asthma, and when challenged for prolonged period of time they also develop remodeling features similar to those observed in humans. Their use is however limited by costs and ethical issues (Coffman and Hessel, 2005).

Equine asthma, a proxy for human airway remodeling research

History and definitions

Equine asthma is a term that encompasses a broad set of terminologies previously employed by veterinarians for non-infectious, inflammatory, recurrent (chronic) and reversible disorders of adult horses characterized clinically by airflow obstruction associated with a cough, mucus hypersecretion and airway hyperreactivity (**Figure 2**). Equine asthma is now broadly classified as mild to severe based on the severity of airway obstruction (and recurrence of the condition). Severe asthma (heaves, RAO...) describes a well characterized and clinically recognizable phenotype of the disease. Horses with severe asthma are typically 7 years or older (adult), afebrile, with repeated episodes of increased respiratory effort at rest, and respond generally to corticosteroid or bronchodilator treatment (Leclere, et al., 2011b). Of note, bronchodilator treatments such as beta-2 adrenergic agonists or anticholinergic drugs, the most commonly used in horses, induce a clinical improvement (Calzetta, et al., 2017, Derksen, et al., 1999) but does mask the underlying inflammation and might be detrimental in terms of hyperreactivity when chronically administered (Bullone, et al., 2017c). Typically, horses with severe asthma have a predominant neutrophilic inflammation in their lower airways, which makes of severe asthma a neutrophilic disorder. However, some studies have also reported an increase in mast cells and one of their products (tryptase) in bronchoalveolar lavage fluid (BALF) of severely asthmatic horses (Dacre, et al., 2007,

Leclere, et al., 2011a). Lastly, it has to be emphasized that severe equine asthma should be seen as a clinical phenotype or a syndrome, rather than a disorder with a unique etiology. Indeed, at least 2 groups of etiological agents are able to cause (separately or in conjunction) clinically overlapping conditions commonly referred to as severe asthma exacerbations in horses. They are the antigens related to hay and straw exposure – typically dusts, mites and LPS (Pirie, et al., 2002, Pirie, et al., 2003a, Pirie, et al., 2001, Pirie, et al., 2003b) – and those related to seasonal pollens (Bullone, et al., 2016b, Costa, et al., 2006, Seahorn and Beadle, 1993).

Mild (to moderate) equine asthma represents a wider phenotype, where all non-infectious respiratory conditions causing chronic (>4 weeks) cough, poor performance, and/or hyperresponsiveness are gathered together (Pirie, et al., 2016), with blurred boundaries available to define pathophysiologically different events (Bond, et al., 2018, Pirie, Couetil, Robinson and Lavoie, 2016). Mild to moderate asthma affect horses of all ages, concurrently with increases in neutrophil, mast cell, and/or eosinophil counts in BALF. Differently from its human counterpart, mild to moderate asthma in adult horses is considered a condition that may be transient (a curable condition). Only a small subset of horses with mild asthma will progress to the more severe form of the condition, but no studies to date have documented the natural history of equine asthma over extended period of time.

Airway remodeling has been studied systematically only in severe equine asthma, while one recent study reported remodeling of the central airways in horses with mild to moderate disease (Bessonnat, 2018). Indirect evidence suggests that bronchial remodeling might occur in mild cases of equine asthma as well (Ter Woort, et al., 2018).

Defining K in horses

Based on the over-simplistic model of asthma reported above, asthma results from the sum of inflammation, remodeling and ASM activation over what we expect to be the normal condition of a healthy subject with the same characteristics of age, sex, weight... but without asthma. Indeed, it has to be acknowledged that, at least for some of the variables used to define human asthma (i.e. inflammation or lung function), there is a certain degree of physiological variation due to aging and its associated processes, such as oxidative stress, increased rates/prevalence of comorbidities and altered structure composition. While this is well described in humans, little or no data are available for horses. An important step required for a thoughtful scientific use of this animal model should be to characterize what is “normal”. While a few data are available concerning variations in BALF cell counts associated with aging (Flaminio, et al., 2000, Hostetter, et al., 2017, Pacheco, et al., 2014), there are still conflictual results (Gerber, et al., 2003). Moreover, little is known on the anatomic and physiologic changes induced by aging in the equine lung (Bullone, et al., 2017b, Mauderly and Hahn, 1982, van Brantegem, et al., 2007). Overall, this indicates that more efforts should be spent in this direction in order to maximize the information we might gather from this spontaneous animal model of asthma.

Airway remodeling in severe equine asthma

Descriptive changes in the anatomy and structure of the lungs of asthmatic horses date some decades ago and have been confirmed over time (Kaup, et al., 1990a, Kaup, et al., 1990b, Thurlbeck and Lowell, 1964, Viel, 1983, Winder, et al., 1989). However, it is in more recent times that these alterations have been quantified. This has corroborated the initial observations that the magnitude of airway remodeling varies along the bronchial tree, and it is most marked peripherally (airways < 2 mm in diameter).

Most remodeling data collected in asthmatic horses concerns ASM, which is reasonable given its importance in disease pathophysiology. The available evidence suggests that ASM mass is increased both in central/intermediate and peripheral airways, but this process is more accentuated peripherally. Indeed, while an average 50% increase in ASM mass has been reported in central airways of asthmatic vs. control horses (Bullone, et al., 2015), up to a 300% increase has been reported in small peripheral airways (Herszberg, et al., 2006, Leclere, Lavoie-Lamoureux, Gelin-Lymburner, David, Martin and Lavoie, 2011a). Both hyperplasia and hypertrophy of ASM cells are likely to contribute to this process (Herszberg, Ramos-Barbon, Tamaoka, Martin and Lavoie, 2006, Leclere, Lavoie-Lamoureux, Gelin-Lymburner, David, Martin and Lavoie, 2011a), but their dynamics of occurrence are still not elucidated. Neutrophil-mediated ASM cell proliferation might represent one of the mechanisms of increased smooth muscle mass in asthmatic horses (Vargas, et al., 2016). A different distribution of extracellular matrix (ECM) components has also been described in the ASM layer of large and small airways of horses with severe asthma (Bullone, Vargas, Elce, Martin and Lavoie, 2017c), resembling the findings in healthy humans but not in asthmatic patients (Araujo, et al., 2008, Yick, et al., 2013). However, no data are available on ASM composition of healthy horses to draw any conclusion on the implication of such findings in equine asthma pathology.

Compared to humans, equine asthma is characterized by a less marked ASM remodeling of the large/intermediate airways when this is measured in whole airway sections using similar approaches in both species (Bullone, Beauchamp, Godbout, Martin and Lavoie, 2015, Elliot, et al., 2015, James, Bai, Mauad, Abramson, Dolhnikoff, McKay, Maxwell, Elliot and Green, 2009, James, Elliot, Jones, Carroll, Mauad, Bai, Abramson, McKay and Green, 2012). Given the substantial contribution of the large airways to total airway resistance (West, 2008), this might explain why fatal asthma attacks in horses are not reported. A morphological phenotype of severe asthma has been described in a limited number of human patients with ASM remodeling observed only in the

small airways and with a reduced use of ICS (Elliot, Jones, Abramson, Green, Mauad, McKay, Bai and James, 2015).

Due to obvious physical and ethical limitations, morphometric assessment of remodeling of whole airways requires surgical biopsies, which it is almost impossible for large airways. Instead, the most easily applicable tool to assess ASM remodeling in the large airways of living human patients is endobronchial biopsy, whose results in terms of ASM remodeling have never been directly compared to those measured in post-mortem samples with more accurate methods to the best of our knowledge. Although endobronchial biopsy is considered the “gold standard” for the study of central airway remodeling, a standardization of the methods to be employed for analysis still lacks (Jeffery, et al., 2003) and biological variability is high (James and Carroll, 2000). Moreover, data obtained from humans and in horses using different analytical methods do not correlate (Bullone, et al., 2014, Labonte, et al., 2008), which should discourage direct and deliberate comparison of studies employing different measuring units.

The extracellular matrix lying within the lamina propria is also altered in severe equine asthma, both in the central and peripheral airways (Bullone, Chevigny, Allano, Martin and Lavoie, 2014, Setlakwe, et al., 2014). Collagen I and III are abundant in the airways and total collagen content is increased the peripheral airways of asthmatic horses (Setlakwe, Lemos, Lavoie-Lamoureux, Duguay and Lavoie, 2014). Collagen III is related to tissue distensibility while collagen I is the major matrix element that resists tensile stresses. Interestingly, airway collagen was positively correlated with pulmonary resistance in asthmatic horses in remission of the disease (Setlakwe, Lemos, Lavoie-Lamoureux, Duguay and Lavoie, 2014). Only when combined, collagen I and III were increased in peripheral airways of asthmatic horses (Furness, et al., 2010, Setlakwe, Lemos, Lavoie-Lamoureux, Duguay and Lavoie, 2014), while no information is available concerning their distribution in large airways. Collagen I is increased while collagen III was shown to be decreased in human asthmatic peripheral airways, which is indicative of a profibrotic process leading to stiffer

and less distensible airways (Brown, et al., 2007, Dolhnikoff, et al., 2009, Wilson, et al., 1993). In large human airways, proteoglycans as well as the two forms of collagen are increased in severe asthmatic vs. healthy individuals, while differences are less marked or even undetectable between mild asthmatics and control subjects (Benayoun, Druilhe, Dombret, Aubier and Pretolani, 2003, Chakir, et al., 2003, Chu, et al., 1998, de Kluijver, et al., 2005, Huang, et al., 1999, Minshall, et al., 1997, Pini, et al., 2007). The increased lamina propria thickness observed in asthmatic horses compared to controls is in contradiction with what is described in human asthma, despite the increased deposition of ECM elements in humans. Indeed, two studies investigating this parameter in asthmatic patients have found a decreased epithelium-smooth muscle distance in occupational asthma compared to controls (Sumi, et al., 2007), and in severe compared to moderate asthmatics (Pepe, et al., 2005). In the first study, asthmatic patients were in remission from occupational asthma (not symptomatic and not taking any treatment) for more than 14 years on average. In the second study, severe asthmatics were on higher doses of corticosteroid treatment compared to moderate asthmatics. The hypothesis that this could have caused a greater decrease of the lamina propria thickness in that group is not supported by in vitro data, however (Jacques, et al., 2010). There are no obvious reasons explaining this discrepancy between asthmatic men and horses. A selective inward vs. outward growth of the ASM cells might be involved. Alternatively, collagen fibers could be more densely packed in asthma (Roche, et al., 1989). Lastly, and as discussed below, both remission state and the use of corticosteroids have been shown to nearly normalize the collagen content in asthmatic horses (Leclere, et al., 2012b), suggesting that discrepancies between the results from equine and human subjects may be due to therapy.

Differently from human asthma, the basement membrane (or lamina reticularis) is not thickened in asthmatic horses, possibly due to the fact that severe equine asthma is not an eosinophilic disease (Dubuc and Lavoie, 2014). Basal membrane thickness, indeed, has been repeatedly associated with

eosinophilia in human asthma (Grainge, Lau, Ward, Dulay, Lahiff, Wilson, Holgate, Davies and Howarth, 2011).

Elastic fiber deposition is increased almost 4-fold in the peripheral airways of asthmatic horses vs. controls, but might be dysfunctional (Setlakwe, Lemos, Lavoie-Lamoureux, Duguay and Lavoie, 2014). Contrarily, no difference was observed between healthy people and non-fatal asthmatic concerning the quantity of elastic fibers in their peripheral bronchial wall. They were increased in fatal asthma vs. non-fatal asthma instead (Araujo, Dolhnikoff, Silva, Elliot, Lindeman, Ferreira, Mulder, Gomes, Fernezlian, James and Mauad, 2008). Elastic fibers of human asthmatic central bronchi were initially described as enlarged (or hypertrophic) and fragmented, but overall not increased in quantity compared to healthy subjects (Bousquet, et al., 1996, Gabbrielli, et al., 1994, Godfrey, et al., 1995). Also, the alterations observed in the large airways of asthmatics were not linked to the severity or duration of asthma (Bousquet, Lacoste et al. 1996). Since then, few studies have addressed this question using quantitative approaches, showing a reduced or unchanged quantity of elastin in human asthmatic airways most of the time (Reddel, et al., 2012). A recent work support the hypothesis that IL-13, a pleiotropic cytokine strongly involved in asthma pathogenesis, might be one of the causes of the reduced elastin synthesis in asthmatics (Ingram, et al., 2015).

The bronchial epithelium of asthmatic horses is characterized by an increased expression of a genetic variant of secretoglobin 1A1 (SCGB 1A1, Clara cell secretory protein), which also increases neutrophil oxidative burst and phagocytosis compared to the classical SCGB 1A1 (Cote, et al., 2014, Cote, et al., 2012). The increased expression of this genetic variant could be the reason why previous work have reported depleted granules in absence of concurrent mucus accumulation in these cells in the bronchioles of asthmatic horses (Katavolos, et al., 2009). Also, a reduced quantity of ciliated epithelial cells is reported (Kaup, Drommer and Deegen, 1990b). Semi-quantitative analyses have provided conflicting results on the number of mucous cells in equine airways

(Bullone, et al., 2016a, Lugo, et al., 2006). However, a linear relationship exists between bronchial epithelial metaplasia, stored muco-substances and inflammation in this species (Lugo, Harkema, deFeijter-Rupp, Bartner, Boruta and Robinson, 2006).

Reversibility of airway remodeling

Whether airway remodeling is reversible, and, if so, to which extent, remains a matter of debate. Data available in human asthma suggest that at least ASM remodeling is not, or not completely, reversible with the current pharmacological treatments (namely, corticosteroids and bronchodilators) (Girodet, et al., 2016, James, Elliot, Jones, Carroll, Mauad, Bai, Abramson, McKay and Green, 2012). However, cohorts of asthmatic have not been prospectively evaluated before and after treatment in order to definitely answer this question. Studies investigating the effect of biologicals on airway remodeling are lacking. Myocyte hyperplasia, at least in the in large airways, has also been consistently identified in asthma in studies in which stereology-based approaches were employed (Ebina, et al., 1993, James, Elliot, Jones, Carroll, Mauad, Bai, Abramson, McKay and Green, 2012, Woodruff, et al., 2004), suggesting this is a less reversible trait compared to hypertrophy, which is less commonly observed in patients with stable disease. This is consistent to what is observed in equine severe asthma. Indeed, asthmatic horses, even in remission of the disease, have twice as much ASM as that observed in healthy horses in their peripheral airways (Leclere, Lavoie-Lamoureux, Gelinus-Lymburner, David, Martin and Lavoie, 2011a). Even if repeated studies have proven that ASM mass in horses in remission of the disease is decreased (about 30%) compared to what observed during disease exacerbations, it appears that there is a portion of ASM remodeling which cannot be reversed by corticosteroid treatment, either alone or in association with a bronchodilator (Bullone, Vargas, Elce, Martin and Lavoie, 2017c, Leclere, et al., 2012a). ASM cell number or proliferation markers did not significantly improve with treatment in these same studies, while a rapid (4 weeks) response was observed in terms of reduced cell size,

especially in large airways. Of note, however, ASM cell size was greater in large than in small airways during severe equine asthma exacerbation, which might have magnified the effect of treatment in the large airways. Altogether, these findings suggest that, both in human and equine asthma, increased ASM mass cannot be completely abolished by current first line asthma treatments. Also, the available evidence suggests that the increase in ASM occurring during exacerbations and due to hypertrophy of ASM cells might be more prominent in large central than in small peripheral airways and can be reversed with treatment, at least partly. However, to which extent any available any pharmacological or biological treatment effectively reduces the number of ASM cells in the airways of asthmatic patients remains largely unknown.

Most studies investigating the effect of corticosteroid treatment on airway remodeling in human patients have focused on changes of the bronchial extracellular matrix in endobronchial biopsy samples. It is now recognized that long-term high-dose corticosteroids treatment can decrease the basal membrane thickness in central asthmatic airways (Laitinen, et al., 1997, Olivieri, et al., 1997, Trigg, et al., 1994). However, this reversal is not observed in patients receiving low-dose treatment for the same length of time and showing a similar clinical improvement (Chetta, et al., 2003).

Whether corticosteroids can also decrease the amount of ECM deposition within the lamina propria and to which extent in central and peripheral airways is less obvious. Endothelin, a protein involved in the process of lung repair and fibrosis, is increased in corticosteroid-naïve asthmatics compared to ICS-treated asthmatics and controls (Redington, et al., 1997). It has been reported that 2 weeks of oral corticosteroid did not reverse collagen deposition (Chakir, Shannon, Molet, Fukakusa, Elias, Laviolette, Boulet and Hamid, 2003), while a significant decrease was observed after 6 months of inhaled budesonide given at high dosage, modulated by tissue MMPs and TIMPs (Hoshino, et al., 1999). On the other hand, a 2-year treatment with low-dose corticosteroids did was not successful at decreasing collagen deposition within the lamina propria in human asthma (Chakir, et al., 2010).

Another study reported that neither short-term fixed dose nor long-term (< 6 months) treatment with

variable doses of inhaled steroids significantly altered the collagen content of the tissue (Godfrey, Lorimer, Majumdar, Adelroth, Johnston, Rogers, Johansson and Jeffery, 1995). The synthesis of ECM proteins is stimulated by TGF- β , which is increased in asthmatic airways and blood, and respiratory secretions (Halwani, et al., 2011). Corticosteroid treatment can partly decrease serum and sputum TGF- β expression in the long but not in the short-term (Chakir, Shannon, Molet, Fukakusa, Elias, Laviolette, Boulet and Hamid, 2003, Kai, et al., 2007, Kawayama, et al., 2008, Manuyakorn, et al., 2008); nevertheless, TGF- β levels remain higher in asthmatic patients compared to healthy subjects (Manuyakorn, Kamchaisatian, Atamasirikul, Sasisakulporn, Direkwattanachai and Benjaponpitak, 2008, Yamaguchi, et al., 2008). TGF- β expression within the bronchial wall is not affected by corticosteroid treatment at all (Tomkowicz, et al., 2008). Elastin is another ECM component possibly undergoing remodeling in asthma. However, corticosteroid treatment does not affect elastin content of the tissue in large airways (Bousquet, Lacoste, Chanez, Vic, Godard and Michel, 1996, Godfrey, Lorimer, Majumdar, Adelroth, Johnston, Rogers, Johansson and Jeffery, 1995). Overall, these findings suggest that airway fibrosis and ECM deposition is not directly targeted or effectively treated by corticosteroids alone in asthmatic patients. Although restricted to few studies, there is evidence that β_2 -agonists could modulate remodeling of the bronchial ECM in asthma when administered for an extended period of time. Twelve weeks of treatment with salbutamol reduced the tenascin but not the collagen content of the bronchial wall in biopsies from mild asthmatics (Altraja, et al., 1999), while only six weeks of salmeterol treatment but had no effect on the underlying remodeling processes despite improving the clinical indices of the disease (Roberts, et al., 1999). The recent and somehow surprising finding that bronchoconstriction itself can induce remodeling in asthmatic patients (Grainge, Lau, Ward, Dulay, Lahiff, Wilson, Holgate, Davies and Howarth, 2011) rises legitimate questions on whether the opposite occurs as well, that is, whether bronchodilation *per se* could reverse airway remodeling.

In this context, asthmatic horses might be employed to investigate hypotheses that would be unethical in human subjects. We have recently shown that long term (3 months) treatment with salmeterol, a β_2 -adrenergic agonist, effectively reduced ECM remodeling in the equine model of asthma, albeit not controlling airway hyperresponsiveness or inflammation (Bullone, Vargas, Elce, Martin and Lavoie, 2017c). Further studies have shown a more marked effect in central than in peripheral airways concerning the inhibition of the deposition of ECM elements in the lamina propria of asthmatic horses (Bullone, Vargas, Elce, Martin and Lavoie, 2017c, Leclere, Lavoie-Lamoureux, Joubert, Relave, Lanctot Setlakwe, Beauchamp, Couture, Martin and Lavoie, 2012a). Whether this was due to impaired deposition of the inhaled drugs at the peripheral levels of the bronchial tree or to different pathophysiological mechanisms sustaining remodeling will have to be elucidated. Studies in asthmatic patients and horses suggest that asthma-associated ECM remodeling, at least quantitatively, might be completely reversible by current pharmacological treatment if correctly addressed. It remains to be clarified whether the mechanical properties of the asthmatic airways in which remodeling has been reversed and of the healthy airways are the same. Few studies have investigated the effect of corticosteroid treatment on human bronchial epithelial structure or function *in vivo*, reporting an increased proliferation and a restitution of the epithelial thickness to normal values (Vignola, et al., 2001), joined to a reduction of epithelial cells in BALF (Ward, et al., 2002). Also, a normalization of the ciliated to goblet cell ratio has been reported following inhaled corticosteroid treatment (Laitinen and Laitinen, 1995). In horses, unpublished data suggest that ICS treatment can reduce epithelial thickness, epithelial proliferation as well as goblet cell density in asthmatic horses (Bullone, et al., 2017a). However, these differences were not noticed when asthmatic horses in exacerbation and remission of the diseases were studied cross-sectionally using semi-quantitative methods (Bullone, Helie, Joubert and Lavoie, 2016a).

Determinants of airway remodeling

In order to study the effect that any intervention might induce on airway remodeling, additive effects due to inflammation and ASM contraction should ideally be separated or nullified. While preventing ASM contraction can be achieved by means of bronchodilators or spasmogens, inflammation can hardly be kept stable or nullified in human asthmatics.

In this perspective, severe equine asthma offers a unique model where researchers are able to differently modulate inflammation and remodeling. Different studies have shown that BALF neutrophilia can be reversed only when the horse is removed from the offending environment (that is, only when exposure to the antigen mixture causing the exacerbation is stopped) for a prolonged interval of time (Bullone, Vargas, Elce, Martin and Lavoie, 2017c, Leclere, Lavoie-Lamoureux, Joubert, Relave, Lanctot Setlakwe, Beauchamp, Couture, Martin and Lavoie, 2012a). If this is not achieved, BALF neutrophilia is maintained while lung function and remodeling of the airways is improved (Bullone, Vargas, Elce, Martin and Lavoie, 2017c, Leclere, Lavoie-Lamoureux, Joubert, Relave, Lanctot Setlakwe, Beauchamp, Couture, Martin and Lavoie, 2012a), providing a rare opportunity to study remodeling in the presence vs. absence of BALF neutrophilic inflammation. The same reasoning can be repeated using selected bronchodilators (with more or less pronounced anti-inflammatory properties) and other anti-inflammatory drugs. The limitation of this approach is that it is restricted to the neutrophilic inflammatory processes detected in equine BALF, and it is not known to what extent it reflects what happens in the airway wall. The available information using this model suggests that bronchial wall inflammation is modulated differently by treatment at the central and peripheral levels of the bronchial tree. Indeed, while even a short-term treatment with corticosteroids reduces inflammatory cell counts in the submucosa of the large airways, no such effect is detected in peripheral airways (Bullone, 2016, Leclere, Lavoie-Lamoureux, Joubert, Relave, Lanctot Setlakwe, Beauchamp, Couture, Martin and Lavoie, 2012a).

Strengths and limitations of the model

Due to the spontaneous occurrence of disease, their long lifespan and large dimensions, horses are well-suited models for human asthma. Main physiopathological differences between species are the lack of a predominant eosinophilic signature in the severe form of equine asthma, the increased thickness of the lamina propria – rather than a thinning as reported in severe human asthma (Pepe, Foley, Shannon, Lemiere, Olivenstein, Ernst, Ludwig, Martin and Hamid, 2005), and the major involvement of small (<2 mm in diameter) rather than large airways in equine asthma. In this perspective, horses might, however, represent a well-suited model for studying non-eosinophilic human asthma. Also, horses might be a more suitable model for studying peripheral rather than central airway remodeling. Indeed, even the largest bronchi in men reach dimensions of approximately 1-1.5 cm (Montaudon, et al., 2007, Zahedi-Nejad, et al., 2011), which correspond to the size of intermediate airways of the 7th-9th generation in a 500 kg adult horse, considering its monopodial branching scheme. This anatomic difference could lead to different structure-function relationship in the two species when considering large airways. Furthermore, the central airways are easily accessible in humans, decreasing the need of an animal model to study changes occurring at this level of the airways.

Equine asthma model is not free from limitations. First of all, there are practical limitations such as the need of large dedicated facilities, personnel and equipment; the fact that asthmatic horses must be looked for and recruited from animal owners, and cannot be “produced on demand”. Moreover, working with the equine model of asthma requires employing many products, devices and instruments that are not developed for the use in horses. This requires their adaptation to this species and additional efforts. The availability of horse-adapted devices will grow only proportionally to its use in asthma research. On the other hand, to maximize the translational yield of this model, increased commitment is required by equine asthma scientists towards the improvement of the phenotyping/endotyping process of equine asthma, based (at least initially) on human knowledge. Having this information available could foster the preclinical research on

biologics as well. To these aims, the question on whether large scale/multi-centric epidemiological studies need to be implemented in asthmatic horses remains open and deserves future attention.

Conclusions

In summary, equine asthma provides a spontaneous model of asthma-associated airway remodeling which closely resemble that occurring in human asthma especially at the level of the small peripheral airways. Given the paucity of information on the effect of many asthma therapies on airway remodeling, and the growing interest in this field by human asthma researchers, equine asthma might provide a good and ethical (non-terminal) preclinical model for hypothesis testing and generation.

Compliance with Ethical Statements

Conflict of interest statement:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Funding:

The author(s) received no specific funding for this work.

Informed consent:

Not required.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T (1992) Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 101:916-921
- Altraja A, Laitinen A, Meriste S, Marran S, Martson T, Sillastu H, Laitinen LA (1999) Regular albuterol or nedocromil sodium--effects on airway subepithelial tenascin in asthma. *Respir Med* 93:445-453
- Araujo BB, Dolhnikoff M, Silva LF, Elliot J, Lindeman JH, Ferreira DS, Mulder A, Gomes HA, Fernezlilian SM, James A, Mauad T (2008) Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 32:61-69
- Bai TR (2010) Evidence for airway remodeling in chronic asthma. *Curr Opin Allergy Clin Immunol* 10:82-86
- Bai TR, Vonk JM, Postma DS, Boezen HM (2007) Severe exacerbations predict excess lung function decline in asthma. *Eur Respir J* 30:452-456
- Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M (2003) Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 167:1360-1368
- Bergeron C, Tulic MK, Hamid Q (2010) Airway remodelling in asthma: from benchside to clinical practice. *Can Respir J* 17:e85-93
- Bessonnat A (2018) Évaluation du remodelage des voies respiratoires centrales de chevaux asthmatiques. Department of Clinical Sciences, vol M. Sc. University of Montreal, Montreal
- Bhakta NR, Woodruff PG (2011) Human asthma phenotypes: from the clinic, to cytokines, and back again. *Immunol Rev* 242:220-232
- Bond S, Leguillette R, Richard EA, Couetil L, Lavoie JP, Martin JG, Pirie RS (2018) Equine asthma: Integrative biologic relevance of a recently proposed nomenclature. *J Vet Intern Med* 32:2088-2098
- Boulet LP, Sterk PJ (2007) Airway remodelling: the future. *Eur Respir J* 30:831-834
- Boulet LP, Turcotte H, Laviolette M, Naud F, Bernier MC, Martel S, Chakir J (2000) Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Influence of inhaled corticosteroids. *Am J Respir Crit Care Med* 162:1308-1313
- Bousquet J, Lacoste JY, Chanez P, Vic P, Godard P, Michel FB (1996) Bronchial elastic fibers in normal subjects and asthmatic patients. *Am J Respir Crit Care Med* 153:1648-1654
- Brown NJ, Salome CM, Berend N, Thorpe CW, King GG (2007) Airway distensibility in adults with asthma and healthy adults, measured by forced oscillation technique. *Am J Respir Crit Care Med* 176:129-137
- Bullone M (2016) Reversibility of airway remodeling in equine asthma: contribution of anti-inflammatory and bronchodilator therapies. . Clinical Sciences, vol PhD Thesis. Université de Montréal, Montréal, pp 421

- Bullone M (2018) Severe equine asthma: a naturally-occurring model of neutrophilic asthma. *Official Journal of the Italian Association of Hospital Pneumologists* 1:8-14
- Bullone M, Beauchamp G, Godbout M, Martin JG, Lavoie JP (2015) Endobronchial Ultrasound Reliably Quantifies Airway Smooth Muscle Remodeling in an Equine Asthma Model. *PLoS One* 10:e0136284
- Bullone M, Boivin R, Vargas A, Lavoie JP (2017a) Il-13 Gene Expression In Endobronchial Biopsies: A Marker Of Airway Smooth Muscle Hypercontractility In Severe Equine Asthma? *Am J Respir Crit Care Med* 195:
- Bullone M, Chevigny M, Allano M, Martin JG, Lavoie JP (2014) Technical and physiological determinants of airway smooth muscle mass in endobronchial biopsy samples of asthmatic horses. *J Appl Physiol* (1985) 117:806-815
- Bullone M, Helie P, Joubert P, Lavoie JP (2016a) Development of a Semiquantitative Histological Score for the Diagnosis of Heaves Using Endobronchial Biopsy Specimens in Horses. *J Vet Intern Med* 30:1739-1746
- Bullone M, Murcia RY, Lavoie JP (2016b) Environmental heat and airborne pollen concentration are associated with increased asthma severity in horses. *Equine Vet J* 48:479-484
- Bullone M, Pouyet M, Lavoie JP (2017b) Age associated changes in peripheral airway smooth muscle mass of healthy horses. *Veterinary Journal* 226:62-64
- Bullone M, Vargas A, Elce Y, Martin JG, Lavoie JP (2017c) Fluticasone/salmeterol reduces remodelling and neutrophilic inflammation in severe equine asthma. *Sci Rep* 7:8843
- Calzetta L, Roncada P, di Cave D, Bonizzi L, Urbani A, Pistocchini E, Rogliani P, Matera MG (2017) Pharmacological treatments in asthma-affected horses: A pair-wise and network meta-analysis. *Equine Vet J* 49:710-717
- Chakir J, Loubaki L, Laviolette M, Milot J, Biardel S, Jayaram L, Pizzichini M, Pizzichini E, Hargreave FE, Nair P, Boulet LP (2010) Monitoring sputum eosinophils in mucosal inflammation and remodelling: a pilot study. *Eur Respir J* 35:48-53
- Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet LP, Hamid Q (2003) Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 111:1293-1298
- Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R, Baraldo S, Testi R, Saetta M, Olivieri D (2003) Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 167:751-757
- Chu HW, Halliday JL, Martin RJ, Leung DY, Szeffler SJ, Wenzel SE (1998) Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 158:1936-1944
- Coffman RL, Hessel EM (2005) Nonhuman primate models of asthma. *J Exp Med* 201:1875-1879

- Costa LR, Johnson JR, Baur ME, Beadle RE (2006) Temporal clinical exacerbation of summer pasture-associated recurrent airway obstruction and relationship with climate and aeroallergens in horses. *Am J Vet Res* 67:1635-1642
- Cote O, Clark ME, Viel L, Labbe G, Seah SY, Khan MA, Douda DN, Palaniyar N, Bienzle D (2014) Secretoglobin 1A1 and 1A1A differentially regulate neutrophil reactive oxygen species production, phagocytosis and extracellular trap formation. *PLoS One* 9:e96217
- Cote O, Lillie BN, Hayes MA, Clark ME, van den Bosch L, Katavolos P, Viel L, Bienzle D (2012) Multiple secretoglobin 1A1 genes are differentially expressed in horses. *BMC Genomics* 13:712
- Coumou H, Westerhof GA, de Nijs SB, Zwinderman AH, Bel EH (2018) Predictors of accelerated decline in lung function in adult-onset asthma. *Eur Respir J* 51:
- Dacre KJ, McGorum BC, Marlin DJ, Bartner LR, Brown JK, Shaw DJ, Robinson NE, Deaton C, Pemberton AD (2007) Organic dust exposure increases mast cell tryptase in bronchoalveolar lavage fluid and airway epithelium of heaves horses. *Clin Exp Allergy* 37:1809-1818
- de Kluijver J, Schrumpf JA, Evertse CE, Sont JK, Roughley PJ, Rabe KF, Hiemstra PS, Mauad T, Sterk PJ (2005) Bronchial matrix and inflammation respond to inhaled steroids despite ongoing allergen exposure in asthma. *Clin Exp Allergy* 35:1361-1369
- Derksen FJ, Olszewski MA, Robinson NE, Berney C, Hakala JE, Matson CJ, Ruth DT (1999) Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. *Am J Vet Res* 60:689-693
- Dolhnikoff M, da Silva LF, de Araujo BB, Gomes HA, Fernezlian S, Mulder A, Lindeman JH, Mauad T (2009) The outer wall of small airways is a major site of remodeling in fatal asthma. *J Allergy Clin Immunol* 123:1090-1097, 1097 e1091
- Douwes J, Gibson P, Pekkanen J, Pearce N (2002) Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 57:643-648
- Dubuc J, Lavoie JP (2014) Airway wall eosinophilia is not a feature of equine heaves. *Vet J* 202:387-389
- Ebina M, Takahashi T, Chiba T, Motomiya M (1993) Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am Rev Respir Dis* 148:720-726
- Elliot JG, Jones RL, Abramson MJ, Green FH, Mauad T, McKay KO, Bai TR, James AL (2015) Distribution of airway smooth muscle remodelling in asthma: relation to airway inflammation. *Respirology* 20:66-72
- Fahy JV (2015) Type 2 inflammation in asthma--present in most, absent in many. *Nat Rev Immunol* 15:57-65
- Fajt ML, Wenzel SE (2015) Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. *J Allergy Clin Immunol* 135:299-310; quiz 311

- Fehrenbach H, Wagner C, Wegmann M (2017) Airway remodeling in asthma: what really matters. *Cell Tissue Res* 367:551-569
- Flaminio MJ, Rush BR, Davis EG, Hennessy K, Shuman W, Wilkerson MJ (2000) Characterization of peripheral blood and pulmonary leukocyte function in healthy foals. *Vet Immunol Immunopathol* 73:267-285
- Fuhlbrigge A, Peden D, Apter AJ, Boushey HA, Camargo CA, Jr., Gern J, Heymann PW, Martinez FD, Mauger D, Teague WG, Blaisdell C (2012) Asthma outcomes: exacerbations. *J Allergy Clin Immunol* 129:S34-48
- Furness MC, Bienzle D, Caswell JL, Delay J, Viel L (2010) Immunohistochemical identification of collagen in the equine lung. *Vet Pathol* 47:982-990
- Gabrielli S, Di Lollo S, Stanflin N, Romagnoli P (1994) Myofibroblast and elastic and collagen fiber hyperplasia in the bronchial mucosa: a possible basis for the progressive irreversibility of airway obstruction in chronic asthma. *Pathologica* 86:157-160
- Gerber V, Robinson NE, Luethi S, Marti E, Wampfler B, Straub R (2003) Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. *Equine Vet J* 35:491-495
- Girodet PO, Allard B, Thumerel M, Begueret H, Dupin I, Ousova O, Lassalle R, Maurat E, Ozier A, Trian T, Marthan R, Berger P (2016) Bronchial Smooth Muscle Remodeling in Nonsevere Asthma. *Am J Respir Crit Care Med* 193:627-633
- Godfrey RW, Lorimer S, Majumdar S, Adelroth E, Johnston PW, Rogers AV, Johansson SA, Jeffery PK (1995) Airway and lung elastic fibre is not reduced in asthma nor in asthmatics following corticosteroid treatment. *Eur Respir J* 8:922-927
- Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, Holgate S, Davies DE, Howarth PH (2011) Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 364:2006-2015
- Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q (2011) Role of transforming growth factor-beta in airway remodeling in asthma. *Am J Respir Cell Mol Biol* 44:127-133
- Harrison TW, Osborne J, Newton S, Tattersfield AE (2004) Doubling the dose of inhaled corticosteroid to prevent asthma exacerbations: randomised controlled trial. *Lancet* 363:271-275
- Herszberg B, Ramos-Barbon D, Tamaoka M, Martin JG, Lavoie JP (2006) Heaves, an asthma-like equine disease, involves airway smooth muscle remodeling. *J Allergy Clin Immunol* 118:382-388
- Hirota N, Martin JG (2013) Mechanisms of airway remodeling. *Chest* 144:1026-1032
- Holgate ST (2011) The sentinel role of the airway epithelium in asthma pathogenesis. *Immunol Rev* 242:205-219
- Hoshino M, Takahashi M, Takai Y, Sim J (1999) Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and

tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 104:356-363

- Hostetter SJ, Clark SK, Gilbertie JM, Wiechert SA, Jones DE, Sponseller BA (2017) Age-related variation in the cellular composition of equine bronchoalveolar lavage fluid. *Vet Clin Pathol* 46:344-353
- Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M (1999) Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 160:725-729
- Huber HL, Koessler KK (1922) The pathology of bronchial asthma. *Arch Intern Med* 30:689-760
- Ingram JL, Kraft M (2012) IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. *J Allergy Clin Immunol* 130:829-842; quiz 843-824
- Ingram JL, Slade D, Church TD, Francisco D, Heck K, Sigmon RW, Ghio M, Murillo A, Firszt R, Lugogo NL, Que L, Sunday ME, Kraft M (2015) Matrix Metalloproteinases-1 and -2 Mediate IL-13-induced Suppression of Elastin in Airway Fibroblasts in Asthma. *Am J Respir Cell Mol Biol*
- Jacques E, Semlali A, Boulet LP, Chakir J (2010) AP-1 overexpression impairs corticosteroid inhibition of collagen production by fibroblasts isolated from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 299:L281-287
- James A, Carroll N (2000) Airway smooth muscle in health and disease; methods of measurement and relation to function. *Eur Respir J* 15:782-789
- James AL, Bai TR, Mauad T, Abramson MJ, Dolnikoff M, McKay KO, Maxwell PS, Elliot JG, Green FH (2009) Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 34:1040-1045
- James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, Bai TR, Abramson MJ, McKay KO, Green FH (2012) Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am J Respir Crit Care Med* 185:1058-1064
- Jarjour NN, Erzurum SC, Bleecker ER, Calhoun WJ, Castro M, Comhair SA, Chung KF, Curran-Everett D, Dweik RA, Fain SB, Fitzpatrick AM, Gaston BM, Israel E, Hastie A, Hoffman EA, Holguin F, Levy BD, Meyers DA, Moore WC, Peters SP, Sorkness RL, Teague WG, Wenzel SE, Busse WW, Program NSAR (2012) Severe asthma: lessons learned from the National Heart, Lung, and Blood Institute Severe Asthma Research Program. *Am J Respir Crit Care Med* 185:356-362
- Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ (1999) Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 160:1532-1539
- Jeffery P, Holgate S, Wenzel S, Endobronchial Biopsy W (2003) Methods for the assessment of endobronchial biopsies in clinical research: application to studies of pathogenesis and the effects of treatment. *American Journal of Respiratory and Critical Care Medicine* 168:S1-17
- Kai S, Nomura A, Morishima Y, Ishii Y, Sakamoto T, Kiwamoto T, Iizuka T, Sekizawa K (2007) Effect of inhaled steroids on increased collagen synthesis in asthma. *Respiration* 74:154-158

- Kariyawasam HH, Aizen M, Barkans J, Robinson DS, Kay AB (2007) Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. *Am J Respir Crit Care Med* 175:896-904
- Katavolos P, Ackerley CA, Viel L, Clark ME, Wen X, Bienzle D (2009) Clara cell secretory protein is reduced in equine recurrent airway obstruction. *Vet Pathol* 46:604-613
- Kaup FJ, Drommer W, Damsch S, Deegen E (1990a) Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). II: Pathomorphological changes of the terminal airways and the alveolar region. *Equine Vet J* 22:349-355
- Kaup FJ, Drommer W, Deegen E (1990b) Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). I: Alterations of the larger conducting airways. *Equine Vet J* 22:343-348
- Kawayama T, Kinoshita T, Imaoka H, Gauvreau GM, O'Byrne PM, Aizawa H (2008) Effects of inhaled fluticasone propionate on CTLA-4-positive CD4+CD25+ cells in induced sputum in mild asthmatics. *Respirology* 13:1000-1007
- Labonte I, Laviolette M, Olivenstein R, Chakir J, Boulet LP, Hamid Q (2008) Quality of bronchial biopsies for morphology study and cell sampling: a comparison of asthmatic and healthy subjects. *Can Respir J* 15:431-435
- Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA (1997) Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 156:951-958
- Laitinen LA, Laitinen A (1995) Inhaled corticosteroid treatment for asthma. *Allergy Proc* 16:63-66
- Lange P, Parner J, Vestbo J, Schnohr P, Jensen G (1998) A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 339:1194-1200
- Lange P, Scharling H, Ulrik CS, Vestbo J (2006) Inhaled corticosteroids and decline of lung function in community residents with asthma. *Thorax* 61:100-104
- Leclere M, Lavoie-Lamoureux A, Gelinas-Lymburner E, David F, Martin JG, Lavoie JP (2011a) Effect of antigenic exposure on airway smooth muscle remodeling in an equine model of chronic asthma. *Am J Respir Cell Mol Biol* 45:181-187
- Leclere M, Lavoie-Lamoureux A, Joubert P, Relave F, Lanctot Setlakwe E, Beauchamp G, Couture C, Martin JG, Lavoie JP (2012a) Corticosteroids and Antigen Avoidance Decrease Airway Smooth Muscle Mass in an Equine Asthma Model. *Am J Respir Cell Mol Biol* 589-596
- Leclere M, Lavoie-Lamoureux A, Joubert P, Relave F, Setlakwe EL, Beauchamp G, Couture C, Martin JG, Lavoie JP (2012b) Corticosteroids and antigen avoidance decrease airway smooth muscle mass in an equine asthma model. *American Journal of Respiratory Cell and Molecular Biology* 47:589-596
- Leclere M, Lavoie-Lamoureux A, Lavoie JP (2011b) Heaves, an asthma-like disease of horses. *Respirology* 16:1027-1046

- Lugo J, Harkema JR, deFeijter-Rupp H, Bartner L, Boruta D, Robinson NE (2006) Airway inflammation is associated with mucous cell metaplasia and increased intraepithelial stored mucosubstances in horses. *Vet J* 172:293-301
- Macedo P, Hew M, Torrego A, Jouneau S, Oates T, Durham A, Chung KF (2009) Inflammatory biomarkers in airways of patients with severe asthma compared with non-severe asthma. *Clin Exp Allergy* 39:1668-1676
- Manuyakorn W, Kamchaisatian W, Atamasirikul K, Sasisakulporn C, Direkwattanachai C, Benjaponpitak S (2008) Serum TGF-beta1 in atopic asthma. *Asian Pac J Allergy Immunol* 26:185-189
- Martin JG, Tamaoka M (2006) Rat models of asthma and chronic obstructive lung disease. *Pulm Pharmacol Ther* 19:377-385
- Mauderly JL, Hahn FF (1982) The effects of age on lung function and structure of adult animals. *Adv Vet Sci Comp Med* 26:35-77
- Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, Hamid Q (1997) Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 17:326-333
- Montaudon M, Desbarats P, Berger P, de Dietrich G, Marthan R, Laurent F (2007) Assessment of bronchial wall thickness and lumen diameter in human adults using multi-detector computed tomography: comparison with theoretical models. *J Anat* 211:579-588
- Montaudon M, Lederlin M, Reich S, Begueret H, Tunon-de-Lara JM, Marthan R, Berger P, Laurent F (2009) Bronchial measurements in patients with asthma: comparison of quantitative thin-section CT findings with those in healthy subjects and correlation with pathologic findings. *Radiology* 253:844-853
- Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, Wenzel SE, Peters SP, Meyers DA, Bleecker ER, National Heart L, Blood Institute's Severe Asthma Research P (2014) Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 133:1557-1563 e1555
- Newby C, Agbetile J, Hargadon B, Monteiro W, Green R, Pavord I, Brightling C, Siddiqui S (2014) Lung function decline and variable airway inflammatory pattern: longitudinal analysis of severe asthma. *J Allergy Clin Immunol* 134:287-294
- Niimi A, Matsumoto H, Takemura M, Ueda T, Nakano Y, Mishima M (2004) Clinical assessment of airway remodeling in asthma: utility of computed tomography. *Clin Rev Allergy Immunol* 27:45-58
- Norris Reinero CR, Decile KC, Berghaus RD, Williams KJ, Leutenegger CM, Walby WF, Schelegle ES, Hyde DM, Gershwin LJ (2004) An experimental model of allergic asthma in cats sensitized to house dust mite or bermuda grass allergen. *Int Arch Allergy Immunol* 135:117-131
- O'Neill S, Sweeney J, Patterson CC, Menzies-Gow A, Niven R, Mansur AH, Bucknall C, Chaudhuri R, Thomson NC, Brightling CE, O'Neill C, Heaney LG, British Thoracic Society Difficult Asthma N (2015) The cost of treating severe refractory asthma in the UK: an

economic analysis from the British Thoracic Society Difficult Asthma Registry. *Thorax* 70:376-378

- O'Reilly R, Ullmann N, Irving S, Bossley CJ, Sonnappa S, Zhu J, Oates T, Banya W, Jeffery PK, Bush A, Saglani S (2013) Increased airway smooth muscle in preschool wheezers who have asthma at school age. *J Allergy Clin Immunol* 131:1024-1032, 1032 e1021-1016
- Oborne J, Mortimer K, Hubbard RB, Tattersfield AE, Harrison TW (2009) Quadrupling the dose of inhaled corticosteroid to prevent asthma exacerbations: a randomized, double-blind, placebo-controlled, parallel-group clinical trial. *Am J Respir Crit Care Med* 180:598-602
- Olivieri D, Chetta A, Del Donno M, Bertorelli G, Casalini A, Pesci A, Testi R, Foresi A (1997) Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study. *Am J Respir Crit Care Med* 155:1864-1871
- Ortega H, Yancey SW, Keene ON, Gunsoy NB, Albers FC, Howarth PH (2018) Asthma Exacerbations Associated with Lung Function Decline in Patients with Severe Eosinophilic Asthma. *J Allergy Clin Immunol Pract* 6:980-986 e981
- Pacheco AP, Paradis MR, Hoffman AM, Hermida P, Sanchez A, Nadeau JA, Tufts M, Mazan MR (2014) Age effects on blood gas, spirometry, airway reactivity, and bronchoalveolar lavage fluid cytology in clinically healthy horses. *J Vet Intern Med* 28:603-608
- Papi A, Brightling C, Pedersen SE, Reddel HK (2018) Asthma. *Lancet* 391:783-800
- Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P, Ludwig MS, Martin JG, Hamid Q (2005) Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol* 116:544-549
- Pini L, Hamid Q, Shannon J, Lemelin L, Olivenstein R, Ernst P, Lemiere C, Martin JG, Ludwig MS (2007) Differences in proteoglycan deposition in the airways of moderate and severe asthmatics. *Eur Respir J* 29:71-77
- Pirie RS, Collie DD, Dixon PM, McGorum BC (2002) Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves. 2: Effects of inhaled HDS on control and heaves horses. *Equine Vet J* 34:337-342
- Pirie RS, Collie DD, Dixon PM, McGorum BC (2003a) Inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (organic dust-induced asthma). *Clin Exp Allergy* 33:676-683
- Pirie RS, Couetil LL, Robinson NE, Lavoie JP (2016) Equine asthma: An appropriate, translational and comprehensible terminology? *Equine Vet J* 48:403-405
- Pirie RS, Dixon PM, Collie DD, McGorum BC (2001) Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. *Equine Vet J* 33:311-318
- Pirie RS, Dixon PM, McGorum BC (2003b) Endotoxin contamination contributes to the pulmonary inflammatory and functional response to *Aspergillus fumigatus* extract inhalation in heaves horses. *Clin Exp Allergy* 33:1289-1296

- Prakash YS, Halayko AJ, Gosens R, Panettieri RA, Jr., Camoretti-Mercado B, Penn RB, Structure ATSAoR, Function (2017) An Official American Thoracic Society Research Statement: Current Challenges Facing Research and Therapeutic Advances in Airway Remodeling. *Am J Respir Crit Care Med* 195:e4-e19
- Reddel CJ, Weiss AS, Burgess JK (2012) Elastin in asthma. *Pulm Pharmacol Ther* 25:144-153
- Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szeffler SJ, Thomas MD, Wenzel SE, American Thoracic Society/European Respiratory Society Task Force on Asthma C, Exacerbations (2009) An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 180:59-99
- Redington AE, Springall DR, Meng QH, Tuck AB, Holgate ST, Polak JM, Howarth PH (1997) Immunoreactive endothelin in bronchial biopsy specimens: increased expression in asthma and modulation by corticosteroid therapy. *J Allergy Clin Immunol* 100:544-552
- Roberts JA, Bradding P, Britten KM, Walls AF, Wilson S, Gratziau C, Holgate ST, Howarth PH (1999) The long-acting beta2-agonist salmeterol xinafoate: effects on airway inflammation in asthma. *Eur Respir J* 14:275-282
- Roche WR, Beasley R, Williams JH, Holgate ST (1989) Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1:520-524
- Rosenberg HF, Druey KM (2018) Modeling asthma: Pitfalls, promises, and the road ahead. *J Leukoc Biol* 104:41-48
- Roy SR, Milgrom H (2010) Managing outpatient asthma exacerbations. *Curr Allergy Asthma Rep* 10:56-66
- Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK (2007) Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 176:858-864
- Seahorn TL, Beadle RE (1993) Summer pasture-associated obstructive pulmonary disease in horses: 21 cases (1983-1991). *J Am Vet Med Assoc* 202:779-782
- Setlakwe EL, Lemos KR, Lavoie-Lamoureux A, Duguay JD, Lavoie JP (2014) Airway collagen and elastic fiber content correlates with lung function in equine heaves. *Am J Physiol Lung Cell Mol Physiol* 307:L252-260
- Shin YS, Takeda K, Gelfand EW (2009) Understanding asthma using animal models. *Allergy Asthma Immunol Res* 1:10-18
- Sumi Y, Foley S, Daigle S, L'Archeveque J, Olivenstein R, Letuve S, Malo JL, Hamid Q (2007) Structural changes and airway remodelling in occupational asthma at a mean interval of 14 years after cessation of exposure. *Clin Exp Allergy* 37:1781-1787
- Ter Woort F, Caswell JL, Arroyo LG, Viel L (2018) Histologic investigation of airway inflammation in postmortem lung samples from racehorses. *Am J Vet Res* 79:342-347

- Thannickal VJ, Murthy M, Balch WE, Chandel NS, Meiners S, Eickelberg O, Selman M, Pardo A, White ES, Levy BD, Busse PJ, Tuder RM, Antony VB, Sznajder JI, Budinger GR (2015) Blue journal conference. Aging and susceptibility to lung disease. *Am J Respir Crit Care Med* 191:261-269
- Thurlbeck WM, Lowell FC (1964) Heaves in Horses. *Am Rev Respir Dis* 89:82-88
- Tomkowicz A, Kraus-Filarska M, Bar J, Rabczynski J, Jelen M, Piesiak P, Fal A, Panaszek B (2008) Bronchial hyper-responsiveness, subepithelial fibrosis, and transforming growth factor-beta(1) expression in patients with long-standing and recently diagnosed asthma. *Arch Immunol Ther Exp (Warsz)* 56:401-408
- Trigg CJ, Manolitsas ND, Wang J, Calderon MA, McAulay A, Jordan SE, Herdman MJ, Jhalli N, Duddle JM, Hamilton SA, et al. (1994) Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 150:17-22
- Trzil JE, Reinero CR (2014) Update on feline asthma. *Vet Clin North Am Small Anim Pract* 44:91-105
- Tschernig T, Neumann D, Pich A, Dorsch M, Pabst R (2008) Experimental bronchial asthma - the strength of the species rat. *Curr Drug Targets* 9:466-469
- van Brantegem L, de Cock HE, Affolter VK, Duchateau L, Govaere J, Ferraro GL, Ducatelle R (2007) Antibodies to elastin peptides in sera of Warmblood horses at different ages. *Equine Vet J* 39:414-416
- Vargas A, Roux-Dalvai F, Droit A, Lavoie JP (2016) Neutrophil-Derived Exosomes: A New Mechanism Contributing to Airway Smooth Muscle Remodeling. *Am J Respir Cell Mol Biol* 55:450-461
- Viel L (1983) Structural-functional correlations of the lung in horses with small airway disease. Faculty of Graduate Studies, vol PhD. University of Guelph, pp 178
- Vignola AM, Chiappara G, Siena L, Bruno A, Gagliardo R, Merendino AM, Polla BS, Arrigo AP, Bonsignore G, Bousquet J, Chanaz P (2001) Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. *J Allergy Clin Immunol* 108:738-746
- Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH (2002) Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 57:309-316
- West AR, Sytyong HT, Siddiqui S, Pascoe CD, Murphy TM, Maarsingh H, Deng L, Maksym GN, Bosse Y (2013) Airway contractility and remodeling: links to asthma symptoms. *Pulm Pharmacol Ther* 26:3-12
- West JB (2008) *Respiratory physiology: the essentials*. Lippincott Williams & Wilkins, Baltimore, MD, USA
- Wilson JW, Li X, Pain MC (1993) The lack of distensibility of asthmatic airways. *Am Rev Respir Dis* 148:806-809

- Winder NC, Gruenig G, Hermann M, Howald B, von Fellenberg R (1989) Comparison of respiratory secretion cytology and pulmonary histology in horses. *Zentralbl Veterinarmed A* 36:32-38
- Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, Solberg OD, Carter R, Wong HH, Cadbury PS, Fahy JV (2004) Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 169:1001-1006
- Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV (2009) T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 180:388-395
- Yamaguchi M, Niimi A, Matsumoto H, Ueda T, Takemura M, Matsuoka H, Jinnai M, Otsuka K, Oguma T, Takeda T, Ito I, Chin K, Mishima M (2008) Sputum levels of transforming growth factor-beta1 in asthma: relation to clinical and computed tomography findings. *J Investig Allergol Clin Immunol* 18:202-206
- Yick CY, Zwinderman AH, Kunst PW, Grunberg K, Mauad T, Fluiter K, Bel EH, Lutter R, Baas F, Sterk PJ (2013) Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. *Am J Respir Crit Care Med* 187:1076-1084
- Zahedi-Nejad N, Bakhshayesh-Karam M, Kahkoei S, Abbasi-Dezfouly A, Masjedi MR (2011) Normal dimensions of trachea and two main bronchi in the Iranian population. *Pol J Radiol* 76:28-31

Figure

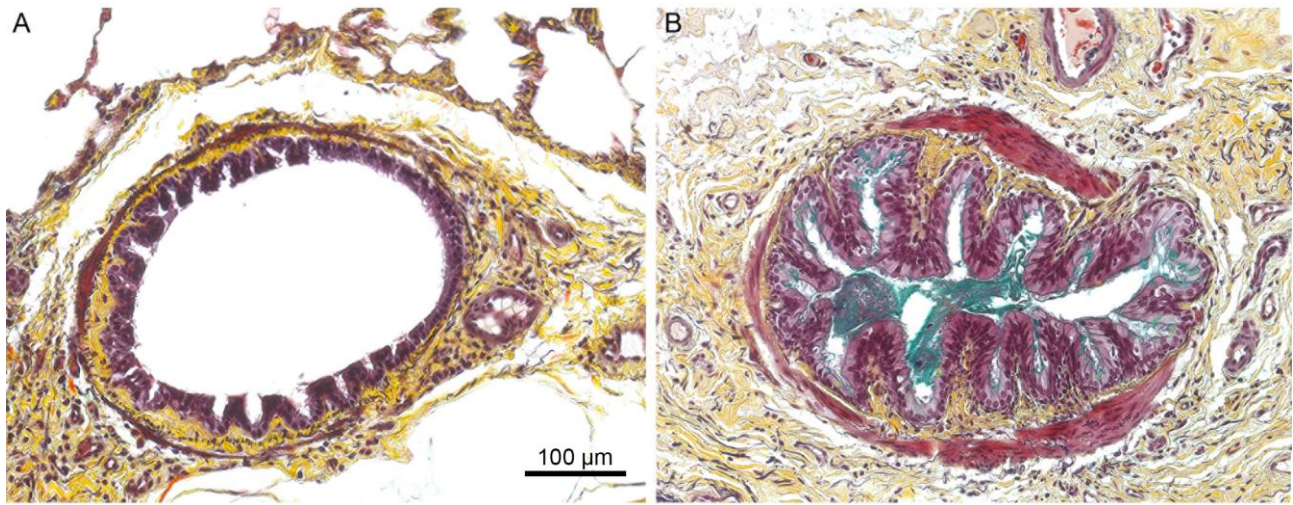


Figure 1. Bronchial section from a non-asthmatic (A) and from an asthmatic horse (B). Bronchial remodeling in equine asthma closely resembles what is observed in human asthmatics. Structural alterations are visible in the right panel affecting the smooth muscle layer as well as the adventitia and lamina propria, all thickened and markedly rearranged. In equine asthma, is also appreciable the increased presence of mucus (green) within the airway lumen, at this level of the bronchial tree mainly produced by metaplastic bronchial epithelial cells. Histologic sections were stained with modified Russel-Movat pentachrome. Scale bar is the same for both images.

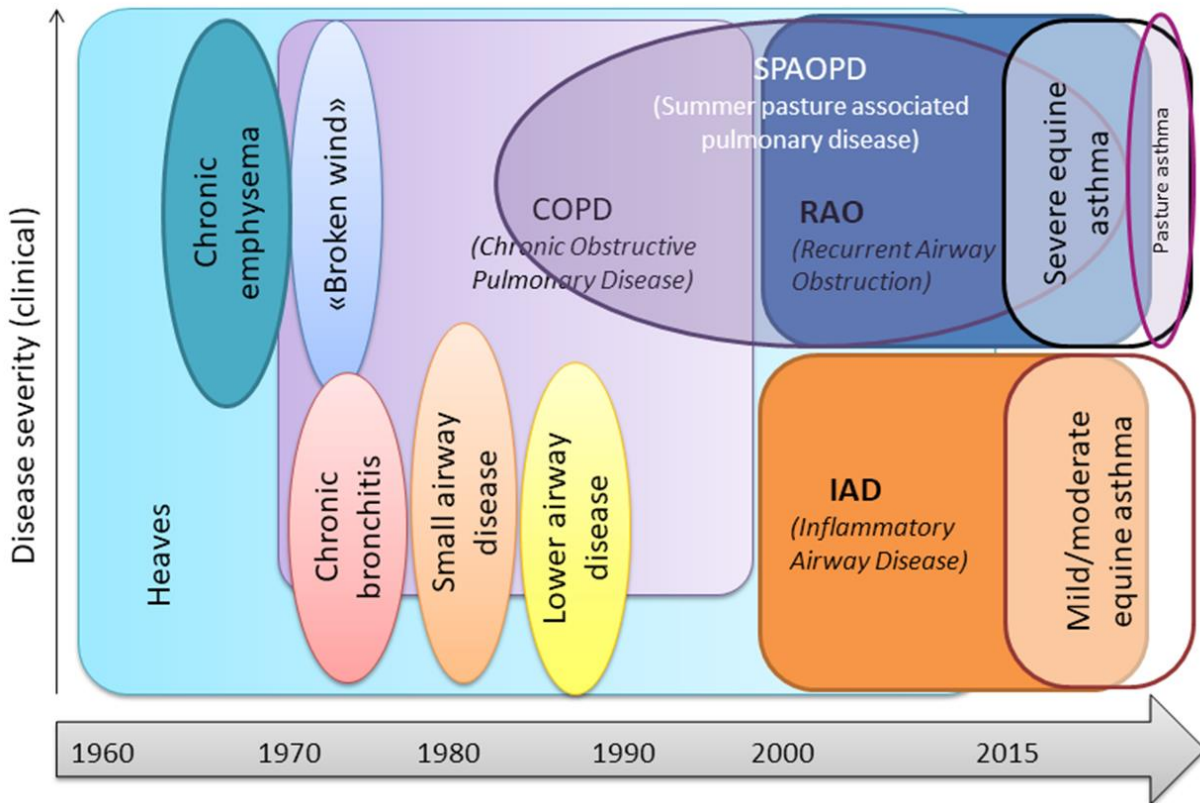


Figure 2. Terminology used in the scientific literature to define the asthma-like disease affecting adult horses (modified from (Bullone, 2018)).