Asthma "of horses and men"-How can equine heaves help us better understand human asthma immunopathology and its functional consequences?

Asthma "of horses and men" – how can equine heaves help us better understand human asthma immunopathology and its functional consequences?

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Highlights:

- Heaves is an asthma-like disease regulated by genetic and environmental factors.
- Innate immune activation is a feature of heaves and neutrophilic asthma.
- Airway neutrophilic inflammation is associated with a Th2 biased immune response.
- Bronchial wall remodeling features closely resemble those of human asthma.

Abstract

Animal models have been studied to unravel etiological, immunopathological, and genetic attributes leading to asthma. However, while experiments in which the disease is artificially induced have helped discovering biological and molecular pathways leading to allergic airway inflammation, their contribution to the understanding of the causality of the disease has been more limited. Horses naturally suffer from an asthma-like condition called "heaves" which presents sticking similarities with human asthma. It is characterized by reversible airway obstruction, airway neutrophilic inflammation, and a predominant Th2 immune response. This model allows one to investigate the role of neutrophils in asthma, which remains contentious, the regulation of chronic neutrophilic inflammation, and their possible implication in pulmonary allergic responses. Furthermore, the pulmonary remodeling features in heaves closely resemble those of human asthma, which makes this model unique to investigate the kinetics, reversibility, as well as the physiological consequences of tissue remodeling. In conclusion, heaves and asthma share common clinical presentation and also important immunological and tissue remodeling features. This makes heaves an ideal model for the discovery of novel pathways implicated in the asthmatic inflammation and associated tissue remodeling.

1. Introduction

Major discoveries related to human diseases have been gained through animal experiments. It is undoubted that mice models have helped uncovering novel immunological mechanisms responsible for the development of different disease processes. Nevertheless, therapeutic strategies derived from these studies have been for the most part disappointing when translated to human diseases, including asthma (Clienti et al., 2011; Giembycz and Newton, 2011; Nair et al., 2012). This may be in part due to different transcriptional responses to acute inflammatory insults in mice and men (Seok et al., 2013).

Development of animal models better mimicking human diseases not only in their clinical presentation, but also taking into account genetic diversity and the complexity of immunopathological mechanisms leading to disease ontogeny, is considered crucial for the discovery of novel therapeutic approaches (Hein and Griebel, 2003). Domestic animal species spontaneously develop diseases having striking similarities with human conditions. Lifespan and size of large animals are more similar to men than to rodents, as is their developmental, innate, and mucosal immunity. For instance, mice lack the gene encoding for the interleukin-8 (Hol et al., 2010), a cytokine implicated in severe neutrophilic asthma and in respiratory virus-induced asthma exacerbations (Nakagome et al., 2012; Rohde et al., 2014), and also essential for neutrophil recruitment in men, cattle, and horses (Caswell et al., 1999; Caswell et al., 2001; Cook et al., 2009; Douglass et al., 1996; Franchini et al., 1998; Kaur and Singh, 2013).

2. Equine heaves, as a naturally occurring model of asthma

Horses naturally develop an asthma-like condition currently known in the veterinary scientific community as "heaves" or RAO (recurrent airway obstruction) (Robinson, 2001). This condition has also been known in the past as chronic bronchiolitis, broken-wind, hay sickness, emphysema, small airway disease, allergic airway disease, and chronic obstructive pulmonary disease. As “heaves” was the term used to introduce the horse as an animal model for asthma, we will employ

this term in this review. The name "Inflammatory Airway Disease" (IAD) has been coined to describe a milder form of equine respiratory inflammatory disease in which no respiratory effort is observable at rest. It is characterized by mild clinical signs (nasal discharge, cough, decreased athletic performance) detected in presence of inflammatory abnormalities of the bronchoalveolar lavage fluid (BALF) cytology (Couetil et al., 2007). It has recently been proposed that heaves and IAD in all their clinical variants are grouped together under the definition of “Equine Asthma” (Lavoie, personal communication).

Both human asthma and equine heaves are heterogeneous diseases which might present in a variety of clinical forms depending upon the stage of the disease, the chronicity of the condition, and possibly upon different pathogenetic pathways leading to its development. We recognize that not all forms or stages of human asthma necessarily share the same attributes as equine heaves. Based on definition of the most recent GINA guidelines (http://www.ginasthma.org/), we believe that heaves represent an ideal animal model for the study of non-allergic asthma, late-onset asthma, and severe asthma.

2.1. “Heaves” and asthma

Heaves is a chronic obstructive respiratory condition naturally affecting 10-15% of adult horses living in temperate climates (Hotchkiss et al., 2007). It shares remarkable similarities with human asthma (**Table I**). In heaves, disease exacerbations, during which horses suffer from respiratory distress episodes comparable to those affecting severe asthma patients, are triggered by inhalation of environmental antigens (Pirie et al., 2003). It had been postulated that heaves was analogous to allergic pneumonitis in man (Farmer's lung disease), as moldy hay is an important triggering factor for both diseases. However, these 2 conditions are otherwise different in their clinical presentation, lung pathology, and underlying immunopathological mechanisms. For instance, bronchiolitis and alveolitis with granuloma formation and extensive fibrosis leads to a restrictive respiratory pattern in allergic pneumonitis (Costabel et al., 2012), while in heaves these changes are not present.

The exposure to hay and dusts leading to heaves is rather a consequence of the human influence on horses' natural environment. Molds and fungi are indeed common antigens in the stables, suggesting that heaves is a disease of "domestication". However, horses can develop a similar condition while at pasture, with grass pollen being then the likely triggering factor (Dixon and McGorum, 1990; Seahorn and Beadle, 1993). Therefore, the antigens towards which horses develop an asthma-type response vary according to environmental exposure.

During clinical exacerbation of heaves, horses develop a pulmonary neutrophilic inflammation (Jean et al., 2011) (**Figure 1**). While asthma is commonly described as an eosinophilic disease, it is now recognized that neutrophilic inflammation may be present in asthma of all severities, although it is more common in severe asthmatic patients and during acute disease exacerbations (Nakagome et al., 2012; Qiu et al., 2007; Wenzel, 2012). Eosinophils, metachromatic cells or neutrophils may infiltrate the airway lumen when horses develop the mild to moderate asthmatic-type response seen in IAD.

Both heaves and asthma are characterized by reversible airflow obstruction, as a consequence of bronchospasms, increased mucus production, airway hyperresponsiveness, and pulmonary remodeling (**Figure 2**). During periods of remission of the disease, when offending antigens are removed from the horses' environment, horses with heaves are clinically indistinguishable from healthy animals, and their airway function and bronchial cytology normalize (Leclere et al., 2011). However, as reported in neutrophilic human asthma (Wood et al., 2012), systemic inflammation (Lavoie-Lamoureux et al., 2012b; Lavoie-Lamoureux et al., 2012c) and subclinical airway obstruction (Leclere et al., 2012a; Van Erck et al., 2006) persist in these horses. The latter is explained, at least in part, by a persistent remodeling of the airways (Lanctot Setlakwe et al., 2014; Leclere et al., 2012a).

2.2. Advantages of the equine asthma model

Heaves is a naturally-occurring disease in which pathogenetical mechanisms are likely to be similar to those observed in human asthma, possibly triggered by immunological "defects" rather than from external manipulations as it happens in rodent models. Also, similar to the natural history of human asthma, horses with heaves experience repeated episodes of airway obstruction occurring over periods of years or sometimes even decades. This contrasts with rodent models in which chronicity can rarely be achieved for more than a 3 month period (Nials and Uddin, 2008; Yang et al., 2013). Furthermore, as the environmental triggering conditions are known, disease status may be modulated as required by specific research needs, avoiding the use of drugs or antigens irrelevant to disease development to induce bronchoconstriction. This represents a unique strength of equine heaves for the study of human asthma.

The horse is especially well suited for prospective studies requiring multiple analyses repeated overtime and on the same subjects. Indeed, additional advantages of horses are those linked to their size. Venipuncture and blood analysis can be performed recurrently allowing the non-invasive collection of large quantities of circulating cells without altering the animal immune response. Bronchoscopy is performed in standing sedated animals, allowing mucus collection, tracheal wash (TW) aspirates, bronchoalveolar lavage (BAL) and/or bronchial epithelial brushing. Furthermore, the equine tracheobronchial tree offers more than 40 reachable carinae for endobronchial biopsy collection (**Figure 3**). Although endobronchial biopsies have been shown to be inadequate samples for quantitative studies of airway smooth muscle (ASM) mass (Bullone et al., 2014a), they provide valuable information regarding epithelial, extracellular matrix components, and ASM cell phenotypes (Jeffery et al., 2003; Leclere et al., 2011; Leclere et al., 2012a; Leguillette et al., 2009; Pini et al., 2007). Horses' lungs also permit harvesting large peripheral lung biopsies by means of thoracoscopic surgery (Lugo et al., 2002; Relave et al., 2008; Relave et al., 2010), which makes heaves perhaps the only animal model allowing the study of small airways remodeling over time in the same subjects. Furthermore, techniques such as spirometry, impulse oscillometry, and

endobronchial ultrasound among others, allow studying lung function as well as structural remodeling in this species (Bullone et al., 2014b; Couetil et al., 2000; Van Erck et al., 2006).

The effect of aging on immunological variables is often disregarded when rodent models are employed in experimental settings, but evidences support important immunological functions to be dependent on or to change with age (Busse and Mathur, 2010; Lee et al., 2012). Horses' lifespan (≈ 30 years) is undoubtedly more similar to that of man than those of rodents, cats or dogs. Also, horses develop heaves in their adulthood, which is reminiscent of late-onset human asthma, with both conditions generally displaying neutrophilic pulmonary inflammation and a less pronounced allergic component (Brazil et al., 2005; Wenzel, 2012).

The treatments of choice for heaves are corticosteroids, either inhaled or systemically administered. Also, both adrenergic and anticholinergic bronchodilators have been proven effective at inhibiting the bronchospasm associated with heaves. many molecules targeting specific intracellular pathways or mediators shown to be involved in murine asthma models were effective when tested in mice, but not in horses or in man (**Table II**). For instance, p38 MAPK inhibitors (Bhavsar et al., 2010; Chopra et al., 2008; Lavoie et al., 2008), PDE4 inhibitors (Giembycz and Newton, 2011; Lavoie et al., 2006; Matera et al., 2014), as well as cysteinyl-leukotriene antagonists, were poorly effective as sole therapy in horses with heaves (Kolm et al., 2003; Lavoie et al., 2002) despite showing promising outcomes in rodent models (Bos et al., 2007; Pera et al., 2011). These drugs are now considered either ineffective or only as add-on therapies for uncontrolled asthma rather than as first-choice monotherapies in asthma international guidelines (Busse and Lemanske, 2007; Turner et al., 2011). Thus, equine heaves appears to be a valuable preclinical model for reliably testing the efficacy of new drugs for asthmatic patients.

2.3. Disadvantages of the equine model of asthma

Using horses as an asthma animal model is not free of drawbacks. A direct consequence of their large size is limited accessibility and higher cost for drugs, breeding facilities and equipment,

procedure materials, and ordinary care. An equine tissue bank has been developed for respiratory research (http://www.ertb.ca), which makes this model available to researchers lacking the facilities or the technical expertise required for handling these animals. Also, few antibodies have been validated for this species (Schnabel et al., 2013) and newly-discovered immune system cells may not yet be characterized in horses. However, the equine genome has been entirely sequenced (Wade et al., 2009), which facilitates the identification of homologous sequences among different species in order to improve cross-reactivity. Studying subjects of different breed, size, age and origin increases intra-group variability and further complicates data analysis and interpretation. However, it provides a heterogeneous population similar the human one.

3. Heaves and immunology

3.1. Neutrophils and the Th2 paradigm in asthma

Asthma is generally considered as an eosinophilic disease, especially in its allergic form, driven by a Th2-type inflammatory response. However, the kinetic of inflammatory cell recruitment into the airway lumen of antigen-challenged asthmatics identified eosinophil accumulation to occur after the early asthmatic response (Lommatzsch et al., 2006). Interestingly, neutrophil recruitment to the airway lumen is common in acute asthma exacerbations (Fahy et al., 1995; Lopuhaa et al., 2002; Norzila et al., 2000; Ordonez et al., 2000) and occurs as early as 4 hours after allergen challenge (Nocker et al., 1999). These findings support a role for neutrophils during uncontrolled phases of the disease (exacerbations or asthma attacks). However, while neutrophil recruitment in experimental settings is a clear consequence of antigenic challenge, it is not established whether during real-life asthma attacks, it precedes or follows the fall in lung function. Also, the immunologic mechanisms linking neutrophil activation/recruitment to the Th2-predominant immune response in asthma remain incompletely elucidated.

In horses with heaves, a predominant Th2-type immune response associated with an airway neutrophilia has been described both in the acute and chronic phases of the diseases (Beadle et al.,

2002; Cordeau et al., 2004; Klukowska-Rotzler et al., 2012a; Lavoie et al., 2001). Pulmonary neutrophilia develops as early as 5-6 hours after antigen exposure, preceding the development of airway obstruction (Brazil et al., 2005; Fairbairn et al., 1993; Franchini et al., 2000). Peripheral neutrophils are primed in heaves, also during periods of remission of the disease (Lavoie-Lamoureux et al., 2012a). An increased number of neutrophils express IL-5 and IL-9 receptors in heaves-affected horses compared to controls, which could link the observed Th2-type immune response to the neutrophilic chronic inflammatory phenotype (Dewachi et al., 2006). Also, recombinant equine IL-4 stimulation induced an increase in IL-8 and IL-4R expression in equine neutrophil *ex vivo*, suggesting that Th2 cytokines may contribute to the recruitment and activation of neutrophils during allergic inflammation (Lavoie-Lamoureux et al., 2010).

Disease chronicity has also been associated with induction/activation of Th17-mediate immunity in heaves (Ainsworth et al., 2006; Debrue et al., 2005). Th17 cytokines may therefore contribute to the sustained airway neutrophilic inflammation in this disease, as reported in human asthma (Linden and Dahlen, 2014). However, Th17 and Th2 responses may not be mutually exclusive but rather sequentially expressed in the airways (Lavoie-Lamoureux et al., 2010), hallmarks of consecutive phases of the inflammatory process. Also, and as reported in human asthma, multiple molecular phenotypes or endotypes possibly occur in heaves, as predominant Th1 and mixed Th1/Th2-type responses has been reported, suggesting complex immune processes contributing to the disease in some circumstances (Ainsworth et al., 2003; Ainsworth et al., 2006; Beadle et al., 2002; Giguere et al., 2002). Clearly, and similarly to human asthma, the pathways responsible for the asthmatic phenotype in heaves are likely to be complex and influenced by both genetic and environmental factors.

3.2. Unraveling the mechanisms linking asthma clinical signs, inflammation, and tissue remodeling

Asthma is a chronic obstructive disease characterized by airway hyperresponsiveness, inflammation, and remodeling. The identification of excessive and possibly inappropriate airway

inflammation as a phenomenon underlying asthma pathophysiology was recognized in the late 80s, and led to the proposal of the "inflammatory paradigm", by which local chronic inflammation would trigger tissue remodeling and consequently amplify the constrictive effect of airway hyperresponsiveness (Walter and Holtzman, 2005). Remodeling has been documented to occur at all levels of the bronchial wall, with ASM being the structure whose architectural and phenotypical alterations more profoundly impact asthma clinical manifestations (Lambert et al., 1993; Oliver et al., 2007). Nevertheless, the mechanisms regulating remodeling and inflammation in asthma as well as their effect on airway hyperresponsiveness are far from clear, and are subject of extensive research.

The study of remodeling features in asthmatic patients is complicated by both a difficulty in obtaining tissue samples (mainly small airways) and by technical impossibility of controlling for several variables linked to the environment in which the patient live (antigen exposure, alimentary habits, environmental temperature and humidity, medication adherence and compliance), which can affect asthma clinical presentations and thus remodeling features. The horse is a model that, despite the biological/genetic variability among different subjects, allows removal of most of the "environmental noise". Furthermore, lung samples can be sequentially obtained from both central and peripheral airways from the same horses.

While studying equine heaves as an asthma model, our group has shown that, following corticosteroid inhalation therapy, clinical manifestations of the disease and small ASM remodeling were dissociated from pulmonary neutrophilia (Leclere et al., 2012a). Conversely, when remission of the disease was achieved by long-term antigen avoidance strategies, normalization of airway neutrophilia preceded the decrease in ASM mass. Also, IL-8 and TNF-α mRNA expression in BAL cells remained elevated in horses receiving inhaled fluticasone when compared to horses treated with antigen avoidance strategies. These results indicate that a greater degree of activated neutrophils were indeed present within the bronchi of horses showing the better outcome in terms of ASM remodeling features. Alterations in the architecture of the airway wall are not limited to the

ASM cells in asthma. The extracellular matrix (ECM), a dynamic three-dimensional fibrous network essential to the mechanical properties of the airways, is also altered in quantity and composition in the asthmatic airways (Roche et al., 1989; Wilson and Li, 1997). We recently reported an increase in collagen and elastic fiber content in the peripheral airways of horses with heaves in remission, which was correlated with alterations in airway function (Lanctot Setlakwe et al., 2014). These findings indicate that increased collagen content contribute to the residual airway obstruction in asthmatic horses, while increased and disorganized elastic fiber content decreased the elastic properties (compliance) of the lung. Interestingly, while collagen remodeling is considered poorly responsive to intervention, we observed a reduction in airway collagen content after a 1-year treatment with either inhaled corticosteroids or antigen avoidance strategies (Leclere et al., 2012b). The reduced collagen content was not associated with decreased TGF-ß or inflammatory cytokine expression by BAL cells in these horses. Taken together, these findings suggest that BAL neutrophilic inflammation is not strictly associated with small airway remodeling or airway obstruction in chronic disease, but a direct link seems to exist between airway hyperreactivity and tissue remodeling.

3.3. Mechanical regulation of the bronchial immune response

Increasing evidences suggest that mechanical stimulation of the airways prompts activation of several resident cells, with consequent upregulation of inflammatory gene expression and changes in phenotype of structural components (Le Bellego et al., 2009; Ludwig et al., 2004; Mohamed et al., 2010; Park et al., 2010; Park and Tschumperlin, 2009). Interestingly, smooth muscle cells can switch from their normal contractile phenotype to a more proliferative/synthetic one when chronic mechanical loads are imposed (DiSanto et al., 2003; Hirota et al., 2009). The (+)insert smooth muscle myosin heavy chain (SMMHC) isoform is a marker of the smooth muscle proliferative/hypercontractile phenotype, and was found to be significantly increased in endobronchial biopsies from asthmatics compared to controls (Leguillette et al., 2009). To the same

extent, (+)insert SMMHC is overexpressed in central and peripheral airways of horses with heaves compared to controls, indicating phenotype switching of ASM along the bronchial tree (Boivin, 2014). The relative contribution of mechanical load and inflammation to (+)insert SMMHC regulation is unknown. However, as corticosteroid administration and long-term antigen avoidance led to a significant reduction of the (+)insert expression in the airways of heaves-affected horses (Boivin, 2014), inflammation likely contributes to this process.

3.4 Innate immune activation is a feature of neutrophilic asthma

Chronic innate immune activation is present in neutrophilic human asthma as in equine heaves, which persists also during remission of the disease (Fu et al., 2013; Lavoie-Lamoureux et al., 2012b; Wood et al., 2012). It has been speculated that the chronic inflammatory response of the asthmatic airways could be the result of a defective innate immune system. An inappropriately developed or altered innate immune response could lead to an exaggerated reaction to normally no-noxious stimuli. Alternatively, there may be an inability of such system to be "switch off" after being activated. Indeed, chronic activation itself could prevent adequate negative feedback systems to act properly.

Neutrophils are first-line defense cells of the innate immune system. They are considered the hallmark of acute inflammatory processes, as they quickly congregate at sites of damaged or infected tissues in response of several chemotactic agents liberated during the initial insult. They directly fight the noxious agent by liberating anti-microbial and protease-rich granules, by producing extracellular traps (NETs, **Figure 4**), and through phagocytosis. They also liberate several pro and anti-inflammatory cytokines, thus possibly modulating the inflammatory response. Peripheral blood neutrophils are activated in human asthma, and in equine heaves (Dewachi et al., 2006; Lavoie-Lamoureux et al., 2012a; Mann and Chung, 2006; Marr et al., 1997; Tremblay et al., 1993; Wood et al., 2012). Available data support an early neutrophilic wave to happen as early as 5 hours after antigen exposure in both asthma and heaves (Brazil et al., 2005; Nocker et al., 1999).

Interestingly, healthy human and equine subjects develop mild but significant neutrophilic pulmonary inflammation after allergen challenge, which spontaneously resolves within few days/hours despite protraction of the stimulus (Leclere et al., 2011; Nocker et al., 1999). Such natural clearance is not observed in asthma or in heaves. Furthermore, neutrophils recruited to the airways are activated in horses with heaves but not in control animals exposed to the same environment, as shown by the increased identification of NETs in the first group only (Cote et al., 2014). Whether systemic neutrophilic activation results from an intrinsic defect of the neutrophils, or neutrophils are activated secondary to the lung inflammation remains to be definitively determined. Basal peripheral neutrophil activation is not dissimilar between horses with heaves in remission and controls, but changes in response to specific stimuli, suggesting that alterations of the innate immune response to specific noxa may be associated with heaves pathology (Lavoie-Lamoureux et al., 2012b). These differences being found even in absence of lung inflammation provides some evidence of an intrinsic defect of neutrophil activation in heaves.

3.5 Role of the bronchial epithelium in heaves

The ASM-centric paradigm leading asthma research in the last decades is slowly evolving towards asthma pathogenesis being driven by both the ASM and the bronchial epithelium (Erle et al., 2014). Bronchial epithelial cells obtained before and after antigen challenge showed an increased expression of transcription factors able to regulate the immunologic response (NF-Kβ, AP-1 and CREB) in horses with heaves compared to controls (Bureau et al., 2000; Couetil et al., 2006). Protein expression of IL-6, IL-10 and TNF-α and gene expression of CXCL1 and TLR4 were similar in bronchial epithelial cells of heaves and healthy horses early after antigen challenge (Ainsworth et al., 2006; Riihimaki et al., 2008). Bronchial epithelial cell cultured from horses with heaves and controls showed a similar increase in TLSP gene expression 6h after hay dust suspension challenge (Klukowska-Rotzler et al., 2012a). However, epithelial cells from horses with heaves showed an increased expression of IL-8 and TLR-4 compared to controls later on in the

development of the disease (Ainsworth et al., 2006; Berndt et al., 2007), suggesting that these cells likely contribute to the persistent airway inflammation.

3.6. Heaves and immunity towards helminths

As suggested by the "hygiene hypothesis", regular use of modern antihelmintics and a decreased exposure to parasites could increase the risk for horses developing heaves and other allergic diseases (Strachan, 1989). Clearance of extracellular parasites, including helminths, is mediated by Th2-type immune response. Equine studies linking heaves to parasite immunity have been derived from data collected from 2 half-sibling families of Swiss horses affected with heaves (Ramseyer et al., 2007). Some of these horses (all belonging to the same family) have an increased resistance to intestinal parasite infestation when compared to the other family of heaves affected horses, or to control animals (Brundler et al., 2011; Neuhaus et al., 2010). These differences between the 2 heaves-affected families were associated with microsatellite markers near the gene of the IL-4 receptor alpha chain (IL4Rα), affecting its expression during disease exacerbations (Jost et al., 2007; Klukowska-Rotzler et al., 2012b; Racine et al., 2011). Also, hay dust and cyathostomin extract increased the expression of IL4Rα, IL-4, and IL-10 by isolated blood leukocytes only in the family showing increased parasite resistance (Lanz et al., 2013). Interestingly, IL4Rα is associated with defense against parasites in humans and animals, and a correlation between susceptibility to asthma and resistance to parasitic infections has been reported for asthmatics as well (Barnes et al., 2005; Hopkin, 2009). These findings suggest that a Th2-biased immune response is "genetically programmed" in some equine and human subjects, rather than being a consequence of an increased hygiene. While this genetic trait would be advantageous by providing increased parasite protection in the wild, it may be disadvantageous when the horse is moved into a domesticated milieu and exposed to high burdens of inhaled allergens, promoting an exaggerated and inappropriate pulmonary immune response.

3.7. Ageing

Asthma incidence in the elderly population is growing and these patients are more likely to be underdiagnosed and undertreated than young asthmatic subjects (Hanania et al., 2011). Ageing in healthy individuals is a physiological process associated with important changes in the immune function collectively known as immunosenescence or "inflamm-ageing". It results in a reduced capacity to cope with a variety of stressors and in a progressive pro-inflammatory status (Franceschi et al., 2000). Inflamm-ageing has been demonstrated to occur in horses (Fermaglich and Horohov, 2002; Horohov et al., 2010), supporting the use of these animals for age-related immunological studies. Specifically, T cells of aged horses (>20 years old) show a decreased proliferative response (Adams et al., 2008; Horohov et al., 2002) when compared to younger animals, and an increased production of IFNγ and TNFα by lymphocytes and monocytes, respectively, (Hansen et al., 2014; Hansen et al., 2013; Katepalli et al., 2008). Furthermore, specific immune-ageing processes appear to vary according to the anatomical locations. For instance, TNFα production increases with age in peripheral mononuclear cells but not in BALF cells. Also, while IL-1β mRNA expression increases with age in peripheral blood, they decrease in BALF cells (Adams et al., 2008; Hansen et al., 2013). Whether and how these findings correlate with the development of airway diseases, and whether they are affected by common anti-asthma medications remains to be elucidated.

5. Conclusions

In conclusion, heaves and asthma share common clinical presentation but also important immunological basis. While equine heaves does not necessarily share the same attributes of all forms or stages of human asthma, the natural history of the disease and the similarities in the airway remodeling processes make heaves an ideal model to study the cellular and molecular pathways associated with the asthmatic airway response and its reversibility, especially regarding late-onset and severe asthma. Significant similarities in the therapeutic responses between horses suffering from heaves and asthmatic patients further support the study of equine heaves as a model for human

asthma. Finally, the role of neutrophils in asthma (and in heaves) remains to be established. Progress in this area fostering the ability of regulating or re-programming the neutrophilic response in subjects with heaves could be important for elucidating the implication of such cells into asthma development.

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Figures

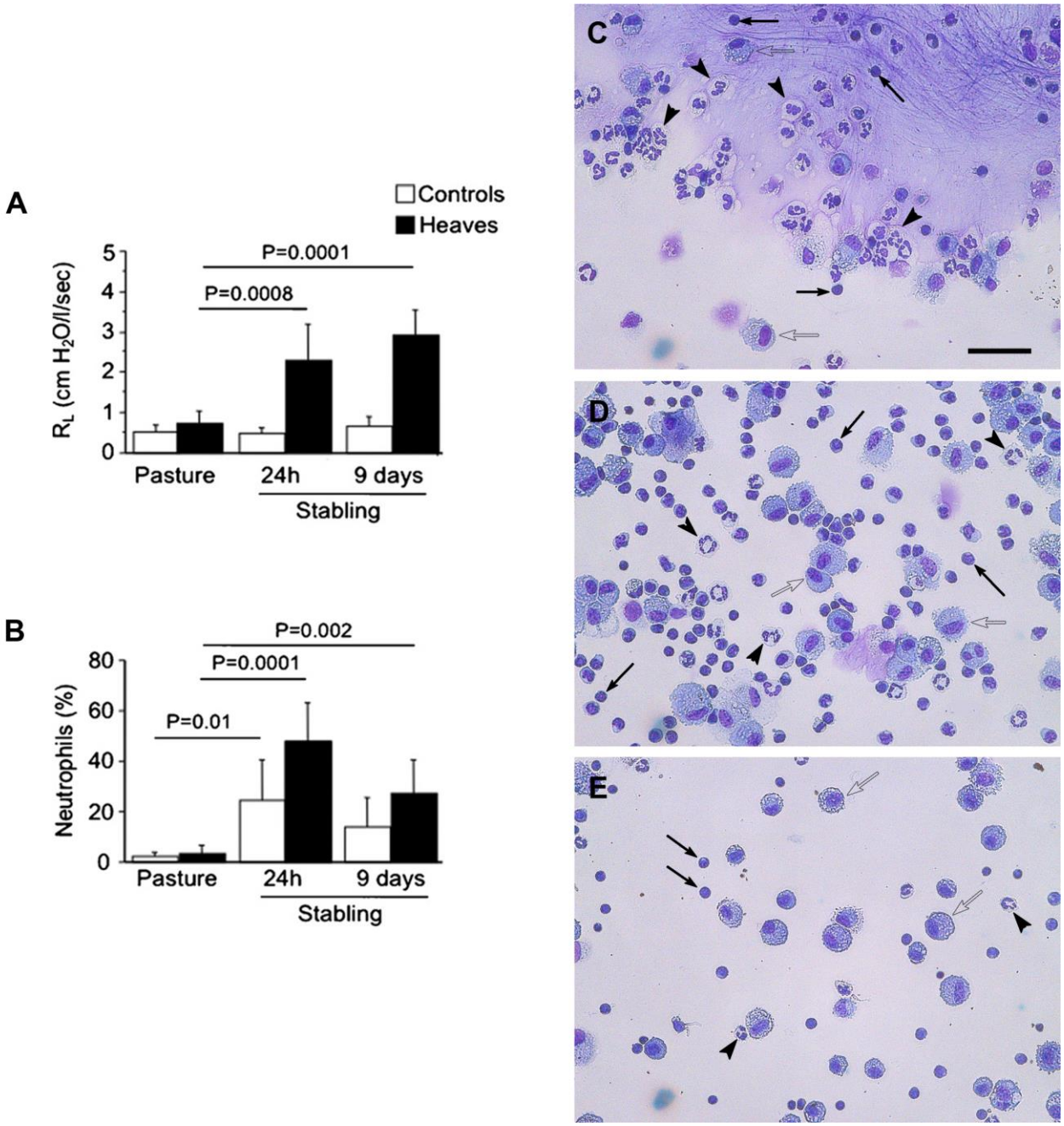

Figure 1. A, B) Horses with heaves during clinical remission of the disease have pulmonary resistance (R_L) values and neutrophil percentages in BALF similar to those of healthy controls. However, after antigen exposure, sustained increased in R_L and neutrophil percentages in BALF are present only in horses with heaves (reprinted from Joubert et al., 2011 with the permission of Elsevier, license number: 3423941179982). C, D, E) BAL fluid cytology of a horse with heaves during exacerbation (C) and remission (D) of the disease, and of a healthy horse (E). Note the

presence of mucus (upper right), the increased cellularity of BAL fluids and also the increase in neutrophil percentage in the horse suffering from heaves during exacerbation of the disease. Neutrophils are indicated by black arrowheads; lymphocytes by black arrows and macrophages by transparent arrows.

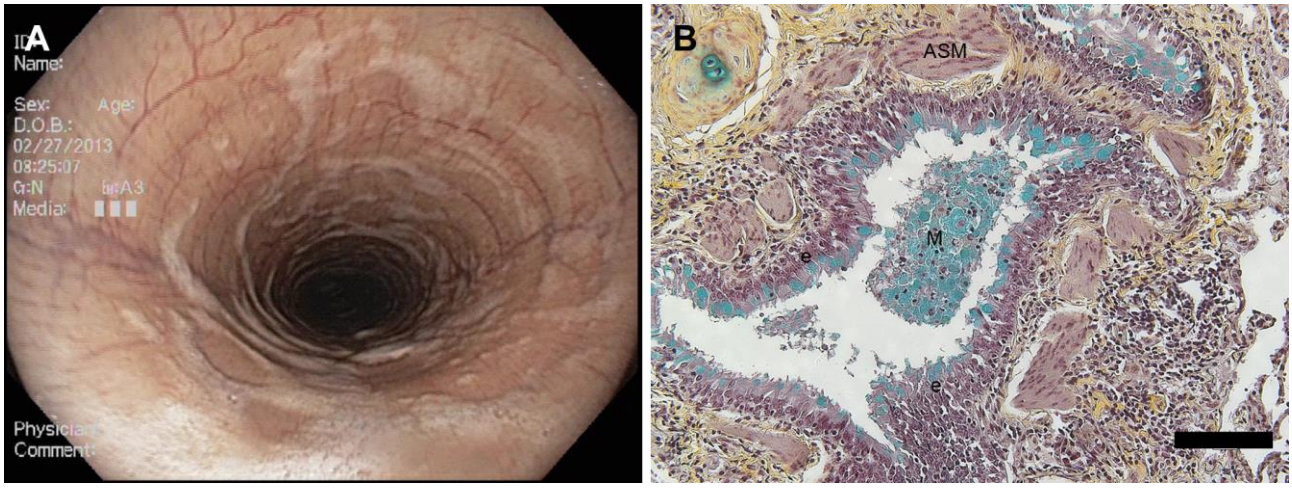

Figure 2. A) Endoscopic image of the trachea of a horse suffering from heaves during disease exacerbation. Note the mucus accumulation. B) Histologic section of the lung parenchyma of a horse with heaves. The bronchial lumens of the smaller airways are filled with mucus (green) containing inflammatory and epithelial cells. Epithelial mucus-producing cells are increased in number. Sub-epithelial inflammatory infiltrate is also evident. Airway smooth muscle mass is increased compared to normal horses (scale bar = 100 μm). ASM: airway smooth muscle; M: mucus; e: bronchial epithelium.

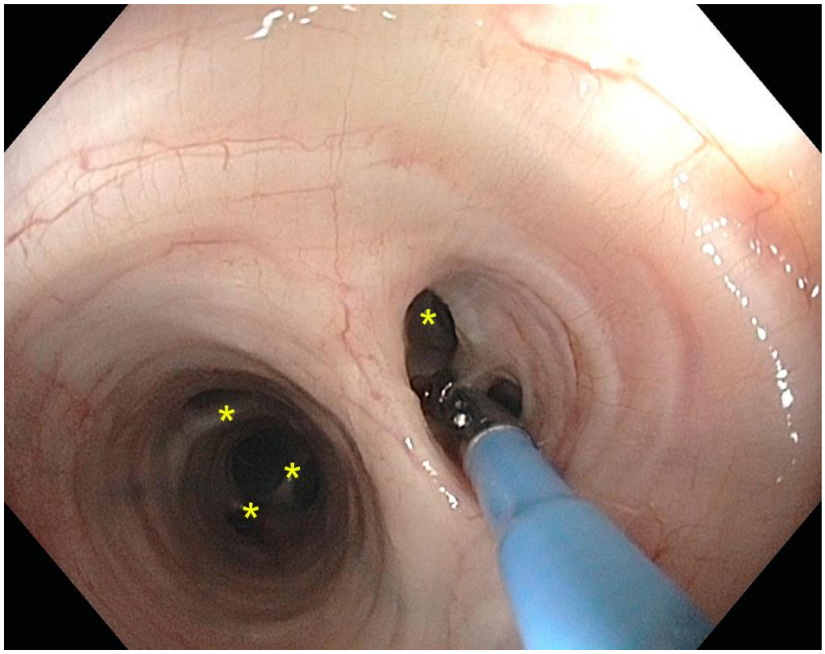

Figure 3. Endobronchial biopsy procedure in a horse. Carinae from which endobronchial biopsies can be withdrawn are numerous (marked with yellow stars). Biopsies may be withdrawn from carinae of 1st, 2nd, 3rd and 4th generation. This is due to the branching pattern of the equine bronchial tree (monopodial), in which several lateral ancillary bronchi stems from the main caudal bronchus before reaching the maximal caudal accessible sites where it is possible to lodge the endoscope.

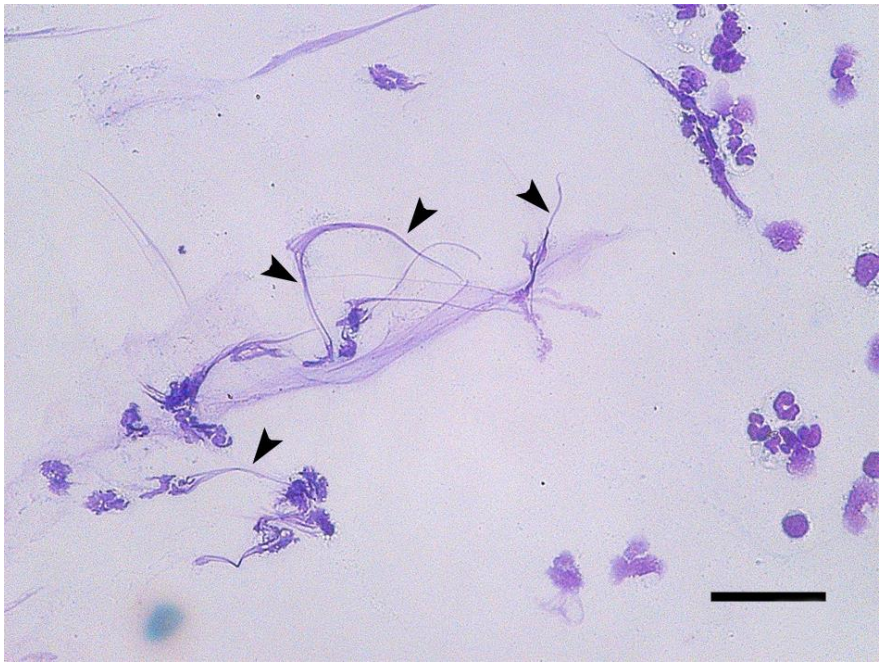

Figure 4. BALF cytology of a horse with heaves during disease exacerbation. Note the presence of NETs, as indicated by the black arrow heads (40x magnification, scale bar = 50 μm).