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REVIEW

# Equine asthma: Integrative biologic relevance of a recently proposed nomenclature

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The term "equine asthma" has been proposed as a unifying descriptor of inflammatory airway disease (IAD), recurrent airway obstruction (RAO), and summer pasture-associated obstructive airway disease. Whilst the term will increase comprehensibility for both the lay and scientific communities, its biologic relevance must be compared and contrasted to asthma in human medicine, recognizing the limited availability of peer-reviewed equine-derived data, which are largely restricted to clinical signs, measures of airway obstruction and inflammation and response to therapy. Such limitations constrain meaningful comparisons with human asthma phenotypes. Suggested minimum inclusion criteria supporting the term asthma, as well as similarities and differences between IAD, RAO, and multiple human asthma phenotypes are discussed. Furthermore, differences between phenotype and severity are described, and typical features for equine asthma subcategories are proposed. Based on shared features, we conclude that mild/moderate (IAD) and severe (RAO) equine asthma are biologically appropriate models for both allergic and non-allergic human asthma, with RAO (severe equine asthma) also being an appropriate model for late-onset asthma. With the development of new biologic treatments in humans and the application of more targeted therapeutic approaches in the horse, it would appear appropriate to further investigate the allergic (Th-2) and non-allergic (non-Th-2) phenotypes of equine asthma. Further research is required to more fully determine the potential clinical utility of phenotype classification.

**KEYWORDS**

animal model, disease severity, horse, IAD, RAO

## 1 | INTRODUCTION

Numerous terms have been used to describe chronic inflammatory lower airway disease in horses, including heaves, recurrent airway obstruction (RAO), equine chronic obstructive pulmonary disease, inflammatory airway disease (IAD), tracheal IAD, bronchial IAD, small airway disease, chronic bronchitis, summer pasture-associated chronic obstructive pulmonary disease, summer pasture-associated obstructive pulmonary disease, summer pasture-associated obstructive airway disease, summer heaves, and summer RAO. Progressive awareness of various clinical and pathological features of equine inflammatory lower airway disease precipitated the evolution of the above nomenclature; however, this has become unsustainable, resulting in confusion within both the veterinary and lay communities. It has recently been proposed that chronic non-infectious inflammatory lower airway disease in horses be reassigned the designation "equine asthma."[1–3] As highlighted during the 6th World Equine Airway

**Abbreviations:** AP-1, activator protein-1; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CXCL2, chemokine (C-X-C motif ) ligand 2; ECM, extracellular matrix; IAD, inflammatory airway disease; IFN, interferon; IL, interleukin; IL4Rα, IL-4 receptor α-chain; LT, leukotriene; NF-κB, nuclear factor-κb; RAO, recurrent airway obstruction; TNF, tumor necrosis factor

Symposium (2017), the biological appropriateness of applying the term "equine asthma" must be considered in light of its current use in human medicine before its widespread adoption in the veterinary literature.[4] Increasing comprehensibility amongst the horse-owning public and the veterinary profession would constitute a clear benefit of the newly proposed terminology; however, the validity and limitations of the proposed change in nomenclature must first be considered and described. Before the proposed use of the term "equine asthma," RAO/Heaves, and IAD have been widely used and accepted because of their accurate descriptions of the disease processes to which they refer. While a distinction between these 2 phenotypes was initially proposed for research purposes to facilitate comparison between study results,[5,6] it was not the intent of the workshop participants to suggest that they were 2 separate conditions. However, different names lead clinicians to subsequently consider them to be distinct and both have individually been the subject of expert panels' workshops[5,7,8] and publications.[1,6,9] In contrast to, and distinct from IAD, horses with RAO exhibit increased respiratory effort at rest.[6] This distinguishing feature is attributable to the magnitude of bronchoconstriction, increased mucus production and bronchiolar inflammation associated with this disorder.[9,10] While IAD and RAO are considered as separate diseases, it is presently unclear whether this distinction reflects a dissimilar pathogenesis, or simply a difference in the clinical severity. There are many factors which potentially differ (ie, clinical signs, pathogenesis, recurrence) among the spectrum of diseases which fall within the proposed new "equine asthma" classification, including severity of clinical signs, pathogenetic pathways, and rates of recurrence. Therefore, further differentiation of the term into mild, moderate, and severe equine asthma has been advocated.[2] Although application of these qualifying terms is currently limited to clinical severity, with mild/moderate and severe equine asthma being analogous to IAD and RAO, respectively,[2] it is hoped that future subclassification efforts might consider additional criteria such as pathogenetic pathways and immunological characteristics. The aims of this review are to: (1) propose minimum inclusion criteria supporting utilization of the term "equine asthma," (2) compare and contrast features of equine asthma with the most common human asthma phenotypes, (3) propose typical features for subcategories of equine asthma, and (4) provide recommendations for future research directions.

## 2 | INCLUSION CRITERIA

The biological appropriateness of the term "equine asthma" must be considered relative to its current use in human medicine. It is important to consider both a minimum set of criteria shared by *all* human and equine asthma phenotypes, as well as additional criteria shared between *specific* human and equine asthma phenotypes.[4]

## 3 | MINIMUM INCLUSION CRITERIA FOR APPLICATION OF THE TERM "ASTHMA"

Asthma in humans is a heterogeneous disease characterized by nonseptic chronic airway inflammation.[11] Patients have a history of signs of respiratory disease (coughing, wheezing, shortness of breath and tightness of the chest) which vary in intensity and over time, combined with airway hyperresponsiveness and expiratory airflow limitation of fluctuating severity.[11] Bronchoconstriction, airway wall thickening, increased mucus secretion, and airway remodeling are accompanying this phenotype.[11] With the exception of shortness of breath and chest tightness, which, as subjective descriptors of a perceived sensation, are not feasibly applicable to the horse, this phenotype is largely shared by both IAD and RAO. Horses with RAO exhibit the same pathophysiologic features as human asthma; namely bronchoconstriction, airway wall thickening, increased mucus production and airway remodeling.[12,13] This pathophysiology is associated with the increased respiratory effort observed at rest in horses with RAO.[13] Horses with IAD have inflammation of the trachea and bronchi, with an excessive accumulation of mucus in the airways,[14,15] resulting in a mild increased resistance to airflow.[16–18] Mild equine asthma decreases racing performance in Thoroughbred racehorses.[19] The pathology exhibited by horses with IAD typically manifests in clinical signs that are subtle at rest, with horses exhibiting chronic (>3 weeks) occasional coughing and normal respiratory effort[1]; and coughing, increased nasal discharge, poor performance, or a combination of these during exercise.[1] Impaired pulmonary gas exchange limits performance, and intensely exercising horses with IAD have worsening of exercise-induced hypoxemia.[20–22] However, the bronchoconstriction in horses with IAD is sufficiently mild to evade clinical detection via the appreciation of increased respiratory effort at rest without bronchoprovocation. Whilst airway remodeling has not yet been studied in horses with IAD, peribronchiolar infiltration of inflammatory cells (82/95 horses) and bronchiolar smooth muscle hyperplasia (93/95 horses) are common in racehorses.[23] Although eosinophils or mast cells (or both) are present in the bronchiolar wall of some racehorses, it was not possible for the authors to determine if these findings correspond to a clinical diagnosis of IAD.[23] Notably absent from this list of minimum inclusion criteria is the predominant airway inflammatory cell; this notable omission is further discussed in Section 7.

## 4 | DIAGNOSIS

A diagnosis of asthma in human patients with signs of respiratory disease is initially based on a detailed clinical history, physical examination (which can be normal at the time of presentation), radiography, and screening questionnaires.[24,25] Despite the value of context-specific questionnaires in positively screening for high-risk chronic airway disease patients, international guidelines emphasize the diagnostic importance of spirometry.[11] This is especially pertinent considering the shared features common to both asthma and chronic obstructive pulmonary disease. Similarly, a presumptive diagnosis of IAD or RAO is generally based on the horses' history and clinical presentation, the latter of which has been incorporated into both the independently validated risk-screening questionnaire (RSQ) and horse owner assessed respiratory signs index (HOARSI).[26,27] Whilst these clinical-sign–based screening tools have both excellent sensitivity and negative predictive values for detecting severe lower airway inflammation (RAO), they fail

to differentiate between healthy horses and those with mild airway inflammation (IAD).[28–30] Furthermore, in light of the poor diagnostic sensitivity of coughing, mucoid nasal discharge and poor performance, reliance is placed on additional tests, such as tracheal endoscopy, bronchoalveolar lavage fluid (BALF) cytology and lung function evaluation,[1] in an attempt to maximize diagnostic accuracy of both RAO and IAD.

## 5 | ADDITIONAL INCLUSION CRITERIA BETWEEN SPECIFIC HUMAN ASTHMA AND IAD/RAO PHENOTYPES

Any efforts to advocate equine asthma as an appropriate disease model for the study of human asthma must take into consideration the fact that multiple human asthma phenotypes exist, not all of which will share attributes with RAO and IAD. Similar considerations also relate to the translational application of human asthma-derived scientific findings to the horse, and vice versa. Therefore, the appropriateness of any such cross-species comparisons necessitates the application of additional criteria which specifically distinguish certain human asthma and IAD/RAO phenotypes based on disease-specific key features. It has been proposed that RAO is an ideal equine model for the study of non-allergic, late-onset, and severe asthma phenotypes[31]; however, the biologic appropriateness of IAD for the study of specific human asthma phenotypes has not yet been investigated and is a focus of this review.

## 6 | PHENOTYPE VERSUS SEVERITY

An "asthma phenotype" is a recognizable cluster of demographic, clinical, pathophysiological, or any combination of these characteristics[32–34]; however, these do not always have a strong correlation with specific pathologic processes, or even treatment responses.[11] In humans, various asthma management guidelines have described methods to categorize asthma severity; however, there are substantial theoretical and practical differences between recommendations.[35] Asthma severity is differentiated into mild, moderate and severe categories[11] and is predominantly based on the level of treatment required to control symptoms and exacerbation; it is not a static feature of the disease and changes over time. In some instances, it is also used to describe the intensity of symptoms or the magnitude of airflow limitation. However, these approaches do not focus on quantifying markers of airway inflammation, which would assess the severity of the disease process itself. For practical reasons, asthma is only classified after institution of effective treatment and therefore assessment is always subject to treatment effect. To date, there are no treatment-naive predictors of disease severity.

It has been proposed that mild/moderate equine asthma replace IAD, and severe equine asthma replace RAO.[1,2] Certain criteria have recently been proposed for the subcategorization of equine asthma based on severity. Specific cutoff values or recommendations were proposed for the following methods: clinical presentation, airway endoscopy, airway cytology, and pulmonary function tests.[36] However, applicability of these criteria to RAO and IAD subcategorization remains arbitrary. A meta-analysis of published studies based on client-owned horses with IAD and RAO would likely offer valuable information on the relative contributions of each of the above criteria to the overall equine asthma subcategorization exercise. Moreover, a poor correlation exists between specific diagnostic results (ie, severe inflammatory bronchoalveolar lavage [BAL] profile) and clinical signs (ie, increased respiratory effort at rest). Although the inclusion of severity of clinical signs as a key criterion in the subcategorization of equine asthma is easy to comprehend (particularly among the horse-owning public), it should not be applied exclusively, particularly in light of the inconsistent correlation between severity of airway inflammation and clinical signs in both human[11] and equine asthma.[37–39] Despite the challenges facing any effort to further subcategorize equine asthma, such an exercise can clearly be justified by its potential to reveal more specific therapeutic and prophylactic targets.

## 7 | PHENOTYPES

There is a need to identify and apply criteria to further subcategorize equine asthma, and it has been suggested that a new classification based on immunological signature data could have greater relevance,[4] particularly in the context of novel, targeted biologic therapeutic approaches.[40] In humans, it is recognized that asthma is a heterogeneous disease, with the underlying pathogenesis differing among phenotypes.[11] There is evidence that RAO has a genetic background with possible locus heterogeneity[41] (discussed in Section 8). In comparison, while genetic susceptibility is suspected in IAD, it has not been investigated. In light of the biologic characteristics common to both equine and human asthma and the marked disease heterogeneity in both, endeavoring to apply currently defined human asthma phenotypes to the horse seems to represent a logical starting point in the process of equine asthma subcategorization. There are multiple human asthma phenotypes, the most common of which are allergic asthma, non-allergic asthma, late-onset asthma, asthma with fixed airflow limitation, and asthma in obese patients.[11] While RAO and IAD do not necessarily share attributes with all phenotypes, similarities and differences between these equine diseases and human asthma are discussed below, and summarized in Table 1. Furthermore, Table 1 also identifies the equine diseases which, at this time, the authors propose to be biologically appropriate models for each human asthma phenotype. The authors acknowledge the requirement for further research to better support these preliminary proposals. Our review aims to focus on the biologic relevance of the proposed nomenclature; however, for an extensive discussion of the advantages and disadvantages of the equine asthma model, the reader is referred to the excellent review article.[31]

## 8 | ALLERGIC ASTHMA

One of the most common human asthma phenotypes is "allergic asthma," a term which reflects the triggering role of allergens in this particular subgroup. Allergic asthma is generally associated with a

**TABLE 1** Features of asthma phenotypes in humans and IAD/RAO in horses, appropriateness of equine asthma model, and areas identified for future research

| Asthma phenotype | Features in humans | Features supporting phenotype model in horses | Equine model appropriate? | Areas identified for future equine research |
|---|---|---|---|---|
| Allergic asthma | • Allergenic trigger associated with respiratory symptoms/expiratory airflow limitation<br>• Often commences in childhood<br>• Past/family history of allergic disease (eczema/allergic rhinitis/food or drug allergy)<br>• Sputum often reveals eosinophilic airway inflammation<br>• Usually respond well to ICS treatment<br>• Th-2 CD4+ lymphocyte response—IL-5–mediated eosinophil recruitment<br>• IL4Rα gene associated with the development of asthma, skin allergies and parasite defense | IAD<br>• Antigenic triggers central to development of lower airway inflammation<br>• Stabling exposes horses to high levels of airborne particulates (eg, dust, endotoxin, fungi, molds, ultrafine particles, noxious gases), and is a risk factor for IAD<br>• Antigenic triggers (eg, dust, mold spores) associated with increased neutrophil/mast cell% in BALF<br>• Antigenic triggers associated with clinical signs (eg, coughing, poor performance)<br>• Often occurs in young horses<br>• Eosinophilic phenotype associated with dust exposure in young horses<br>• Usually respond well to ICS treatment<br>• Th-2 response—Increase in IL-4 and IL-5 in BALF linked with mastocytic phenotype | Yes | • Eosinophil involvement in pathogenesis of IAD<br>• Effect of BALF phenotype on performance<br>• Role of IgE in IAD and RAO<br>• Longitudinal and cross-sectional studies investigating an "atopic march" in horses<br>• Comprehensive study investigating the effect of various allergenic triggers on both lower airway pathology and clinical signs (ie, investigate causality rather than association) |
| | | RAO<br>• Allergenic trigger (molds ± LPS) associated with clinical signs and pathology (increased neutrophil % in BALF, increased respiratory effort at rest)<br>• Associated with multiple hypersensitivities in some families of horses (insect bite hypersensitivity, urticaria, increased parasite resistance)<br>• Good response to ICS<br>• Association between IL4Rα and RAO<br>• IL4Rα upregulates IL-4 expression during disease exacerbation, which promotes isotype switching from IgM to IgE<br>• Increased IgE in BALF in horses with RAO | Yes | |
| Non-allergic asthma | • Not associated with allergy<br>• Sputum can be neutrophilic eosinophilic or paucigranulocytic<br>• Often respond less well to ICS<br>• Chronically activated mast cells in bronchial mucosa (can be associated with non-allergenic stimulus)<br>• Th-1 response—cell-mediated immunity and phagocyte-dependent inflammation | IAD<br>• BALF can reveal neutrophilia and/or eosinophilia and/or mast cells accumulation<br>• Th-1 response—mRNA encoding TNF-α, IL-1β, and IFN-γ in BALF<br>• Th-17 response—Increase in IL-17 and IL-23 linked with increased neutrophil % in BALF<br>• Often respond less well to ICS | Yes | • Role of neutrophil/mast cell activation in the development of lower airway inflammation |
| | | RAO<br>• BALF can be neutrophilic or paucigranulocytic (in severe cases where BALF return is low)<br>• Chronic innate immune activation - chronic activation of peripheral neutrophils<br>• Often respond less well to ICS | Yes | |
| Late-onset asthma | • Initial presentation as adult (particularly women)<br>• Less likely to be atopic<br>• Decreased baseline pulmonary function<br>• Often refractory to ICS/require higher doses for control | IAD<br>• Insufficient evidence | No | • Disease progression from IAD to RAO over time<br>• Correlation between inflamm-aging and development of chronic inflammatory airway disease |
| | | RAO<br>• Decreased baseline pulmonary function during disease exacerbation<br>• Mature/older animals<br>• Can require higher doses for control | Yes | |

(*Continues*)

**TABLE 1** (Continued)

| Asthma phenotype | Features in humans | Features supporting phenotype model in horses | Equine model appropriate? | Areas identified for future equine research |
|---|---|---|---|---|
| Asthma with fixed airflow limitation | • Chronic asthma patients with fixed airflow limitation; thought to be because of airway wall remodeling<br>• Increased airway smooth muscle mass and extracellular matrix at all levels of bronchial tree<br>• Postbronchodilator $FEV_1$ < 70% (predicted) | IAD<br>• Insufficient evidence<br>RAO<br>• Tissue remodeling is reversible—long-term antigen avoidance strategies and corticosteroid therapy decrease airway smooth muscle mass and subepithelial collagen area | No<br>Insufficient evidence | • Airway remodeling in IAD<br>• Reversibility of airway remodeling in human asthmatics/horses with IAD/horses with RAO; there is limited data studying airway remodeling of the peripheral airways of human asthmatics and reversibility in response to therapy, and limited data available in horses with RAO |
| Asthma in obese patients | • Dyspnea on exertion<br>• Requires objective measurement of variable airflow limitation—Obesity-associated respiratory symptoms can mimic asthma<br>• Little eosinophilic airway inflammation | • Correlation between body condition score and body fat (%) and increased expression of IL-1 and TNF-α in plasma | Insufficient evidence | • Expression of inflammatory cytokines in BALF or increased pulmonary resistance in obese/equine metabolic syndrome horses |

Abbreviations: BALF, bronchoalveolar lavage fluid; $FEV_1$, forced expiratory volume in 1 s; IAD, inflammatory airway disease; ICS, inhaled corticosteroid; RAO, recurrent airway obstruction, TNF, tumor necrosis factor.

past/family history of allergic disease (eg, eczema, food allergy) and pretreatment induced sputum from affected patients often reveals eosinophilic airway inflammation[33]; the response to inhaled corticosteroid treatment is generally favorable. Currently, IAD in the horse can be further subcategorized based on the predominant inflammatory cell in BALF; namely, neutrophilic, eosinophilic, mastocytic, or mixed granulocytic. Whilst the pathogenesis of IAD is incompletely defined, it is widely understood to be a multifactorial disease with the relative contribution of etiological influences varying with environment, husbandry, location, season, and preventive medicine strategies.[42,43] Antigenic triggers are central to the development of lower airway inflammation. Horses kept in conventional stables with poor ventilation are exposed to high levels of airborne particulates[44] including dust, endotoxin, fungi, molds, ultrafine particles and noxious gases, and there is strong evidence that stabling of horses is a risk factor for IAD.[45–48] However, the level of respirable particulates in the overall stall air does not necessarily reflect the level of challenge a horse experiences, as the majority of dust exposure occurs in the breathing zone during feeding.[48] Exposure to hay and its accompanying mold spores, such as *Aspergillus fumigatus*, *Saccharopolyspora rectivirgula*, and *Thermoactinomyces vulgaris*, are a risk factor in the development of lower airway inflammation.[28,49,50] Furthermore, compared to feeding hay from the ground feeding hay in a net has a 4-fold increase in breathing zone respirable particle concentration.[48] There is little information regarding an association between antigenic triggers (ie, dust, mold spores) and specific IAD phenotypes. A prospective, cross-over study did reveal an association between stabling of young horses and an IAD phenotype characterized by increased airway neutrophils.[45] This phenotype has been associated with coughing and poor performance (discussed above in minimum inclusion criteria for application of the term "asthma"), both of which form the basis for the diagnosis of IAD. In contrast with the human allergic asthma phenotype, eosinophils are less commonly detected in equine BALF, with the exception of a subgroup of IAD mainly found in young horses yet with an overall prevalence lower than other IAD cytological subtypes.[28,51–53] In young horses, the recruitment of airway eosinophils appears to be associated with dust exposure[43,44] and increased BALF eosinophil ratios have been associated with pulmonary hyperresponsiveness.[54] Further studies are clearly warranted to more fully clarify the role of eosinophils in IAD pathogenesis and their effect on respiratory function.[1] Nevertheless, regardless of the BALF cytologic profile, it appears that antigenic triggers are associated with both the clinical signs and pathology of lower airway inflammation observed in horses with IAD. Similarly, yet more widely reported in the literature, antigenic triggers are strongly associated with both clinical exacerbations and pathologic changes (eg, airway remodeling) in horses with RAO.[31] Of note, however, eosinophils are absent from the airway wall of RAO-affected horses.[55]

In humans, an "atopic march" has been described, whereby the first clinical manifestation of allergic disease, atopic dermatitis, is followed by the subsequent development of food allergy, rhinitis, and asthma.[56] Evidence suggests that 75% of young children that experience severe atopic dermatitis will develop allergic rhinitis, and 50% will develop asthma.[57] In horses, while data supporting the existence of an "atopic march" are lacking, there is genetic, epidemiological and clinical evidence of multiple co-existing manifestations of allergic disease within a single individual. There is a genetic association between RAO and microsatellite markers syntenic with the IL-4 receptor α-chain (IL4Rα) gene on equine chromosome 13.[41] Importantly, the IL4Rα gene is associated with the development of asthma, skin allergies, and parasite defense in humans.[58–60] RAO is associated with multiple hypersensitivities, including insect bite hypersensitivity[61] and urticaria,[62] as well as increased parasite resistance[63]; specifically, members of a half-sibling family with a high-incidence of RAO shed fewer strongylid eggs compared to genetically unrelated RAO-unaffected pasture mates. Furthermore, RAO-affected offspring within the high-prevalence family had lower strongylid egg counts than RAO-unaffected descendants. In this instance, the RAO-

phenotype was associated with the expression of microsatellite markers near the IL4Rα gene, resulting in an upregulation of IL-4 during RAO disease exacerbation.[64] However, the association between IL4Rα and RAO is neither absolute nor universal. The fact that it is not observed in every high-prevalence RAO family supports the existence of genetic heterogeneity within the currently defined RAO phenotype. Although IL-4 promotes isotype switching from IgM to IgE,[65] there is inconclusive evidence within the veterinary literature regarding the role of IgE in RAO; one study reported an increase in mold-specific serum IgE in RAO horses compared with control horses,[66] whilst several studies failed to generate similar findings.[67,68] There is an increase in BALF IgE concentrations in horses with RAO.[67] Whilst there are presently no reports on the role of IgE in IAD, a Th-2 cytokine signature has been detected in BAL cells derived from mastocytic forms of IAD, characterized by increased expression of IL-4 and IL-5 mRNA.[69,70] Whilst further data, derived from longitudinal studies, are required to support the existence of an "atopic march" in the horse, an "allergic equine asthma" phenotype currently appears biologically appropriate.

## 9 | NON-ALLERGIC ASTHMA

A common asthma phenotype in human adults is "non-allergic asthma," where there is no apparent association with allergy. Analysis of pretreatment patient-derived sputum reveals neutrophilic, eosinophilic, or paucigranulocytic inflammation. Paucigranulocytic asthma is associated with normal or near-normal levels of eosinophils and neutrophils. Human asthma, particularly the allergic phenotype, displays an IL-5–mediated eosinophil recruitment predominantly driven by a Th-2 CD4+ lymphocyte response. However, the role of a Th-1 immune response and its ability to evoke cell-mediated immunity and phagocyte-dependent inflammation is exhibited both in chronic severe asthma and acute asthma exacerbations, the latter being associated with airway neutrophil recruitment as early as 4 hours after allergen exposure. Furthermore, in chronic asthma in humans, there are persistently activated mast cells in the bronchial mucosa, evident as elevated cytokine expression and synthesis.[71–73] Although mast cell activation is often assumed to be allergen induced, there are multiple non-allergenic stimuli which can cause this activation, including proteases,[74] cytokines,[75] and Toll-like receptor ligands.[75] These and other mechanistic pathways are described in detail in a review article.[76] In addition to the varied mechanisms (both allergenic and non-allergenic) which underpin mast cell degranulation, differences also exist with respect to the kinetics of degranulation. In contrast to the rapid mast cell degranulation observed after allergen challenge, the ultrastructural appearance of some asthmatic airway mast cells appears consistent with a slower degranulation process.[73] Whilst mast cells are well known for their role in allergic and anaphylactic reactions (where rapid degranulation is observed as part of a Th-2-biased response), increasing evidence supports an alternative role of mast cells in inflammation, whereby they exhibit "differential" or "selective" secretion of mediators without degranulation.[77] Similarly, there is evidence that both the Th-1 and Th-2 immune responses are involved in the pathogenesis of IAD and RAO. However, when interpreting the gene expression data derived from horses with IAD, it is important to consider whether the diagnosis was based on a generalized increase in airway inflammatory cells or an increase in a specific inflammatory cell (neutrophilic, mastocytic, eosinophilic). Evidence of a Th-1 response in the lower respiratory tract, characterized by upregulation of IFN-γ mRNA in BALF-derived cells, has repeatedly been reported in association with a *generalized* increase in BAL inflammatory cells, both in the presence and absence of clinical signs.[53,70,78] Additionally, a Th-17 response has been implicated in neutrophilic IAD, with an association between the BALF neutrophil ratio and increased IL-17 and IL-23 mRNA expression.[53,69] It is important to consider that these responses might reflect sequential phases of the chronic inflammatory process in the respiratory tract; consequently, it might not be appropriate to consider them as mutually exclusive.[79] Such considerations remain speculative, particularly in naturally occurring cases, and additional studies are required for clarification.

Chronic innate immune activation is a feature of both neutrophilic human asthma, as well as RAO, which persists during disease remission.[80,81] The chronic activation of peripheral blood neutrophils reported in RAO[80] could, in part, contribute to the greater disease severity compared with IAD, whereby exposure to an inhaled stimulus (eg, dust, mold spores) could result in an exaggerated and inappropriate inflammatory response. Although such exposures can induce mild neutrophilic pulmonary inflammation in both healthy horses and humans, the degree of cellular activation decreases in hours/days, even if the inciting stimulus is maintained.[82,83] In contrast, if exposure to an antigenic stimulus is maintained in horses with IAD, pulmonary inflammation persists for up to 3 months.[45] Whilst further research into the innate immune response in IAD and RAO is required to fully understand the role of neutrophil activation in the development of lower airway inflammation, given that a non–Th-2 immune response has also been associated with both IAD and RAO, the proposed existence of a "non-allergic equine asthma" phenotype currently appears biologically appropriate.

## 10 | LATE-ONSET ASTHMA

Some patients (particularly women) present with asthma for the first time as adults. These patients are less likely to be atopic, as "age of onset" is significantly lower in patients with allergic asthma, compared with those with non-allergic asthma.[84] They also have decreased baseline pulmonary function and are either refractory to inhaled corticosteroid therapy or require higher doses of inhaled corticosteroids to achieve asthma control.[33] Horses with RAO exhibit decreased baseline pulmonary function during disease exacerbation, and tend to be mature to older animals.[1] "Inflamm-aging" describes a reduction in the capacity of the aging body to cope with a variety of stressors together with a progressively increasing chronic low-grade inflammatory status, associated with aging and provoked by a continuous antigenic load.[85] There are age-related increases in pro-inflammatory cytokines in both humans and horses, with aged healthy horses having increased expression of IL-6, IL-8, IFN-γ, and peripheral blood mononuclear cell-derived TNF-α mRNA concentration in plasma.[86] Furthermore, T-cells of geriatric horses (>20 years) exhibit a lower proliferative response

than those of younger animals,[87] and peripheral blood lymphocytes and monocytes derived from this cohort exhibit an increased basal expression of IFN-γ and TNF-α mRNA, respectively.[88] However, age-related changes appear to be more tightly regulated in the lungs than in the systemic circulation. Inflammatory cell populations in the lung represent a balance between cellular recruitment, via airway epithelial cell and macrophage-derived chemotactic cytokines, and removal, via apoptosis and phagocyte-mediated clearance. Lung granulocytes (neutrophils and macrophages) in horses with RAO exhibit altered apoptosis,[89] which together with increased activity of transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1)[90] might contribute to the maintenance of neutrophilic inflammation in horses treated with glucocorticoids and maintained in an allergenic environment.[91] Whilst no age-related trends in BALF cytological profiles in horses with IAD or RAO have been reported, there is an age-associated increase in mRNA expression of IFN-γ producing lymphocytes in stimulated BAL cells.[88] Whilst there is a paucity of definitive data on the progression of IAD to RAO over time, there is anecdotal evidence suggesting the progression from IAD in younger age to RAO in some horses. Although potentially influenced by the high prevalence of IAD, such a phenomenon of disease progression does warrant further study. There is no current correlation between inflamm-aging and the development of chronic inflammatory airway diseases. However, based on the human phenotype, we believe it is biologically appropriate to use RAO as an equine model for late-onset asthma, as recently reviewed.[92]

## 11 | ASTHMA WITH FIXED AIRFLOW LIMITATION

Patients with fixed airflow obstruction are often grouped under the heading of chronic obstructive pulmonary disease (COPD), with distinct pathological and functional characteristics compared to those with a history of asthma[93]; for example, asthmatic patients do not exhibit a loss of airways as observed in COPD.[94] It is thought that fixed airflow limitation in asthmatic patients is because of airway wall remodeling, with both airway smooth muscle (ASM) mass and extracellular matrix (ECM) deposition being increased at all levels of the bronchial tree,[95] with the increased ASM mass being the functionally dominant alteration.[96] Consequently, in addition to the clinical similarities between RAO and human asthma, both diseases also share certain structural features. The structural alterations seen in human patients with fixed airflow limitation are currently thought to be irreversible; however, appropriate studies are lacking to verify if indeed this is correct. In contrast, tissue remodeling in RAO is partially reversible under certain circumstances.[13] In horses with RAO, long-term corticosteroid therapy (fluticasone) and antigen avoidance strategies have been shown to significantly decrease both smooth muscle mass (30% decrease over 3 months, but remained twice that of healthy controls) and subepithelial collagen area.[97,98] Corticosteroid administration increased the rate of decline in smooth muscle mass, although antigen avoidance was better at controlling airway inflammation.[97] Airway remodeling in horses with IAD has not yet been investigated. In light of the paucity of studies investigating peripheral airway remodeling and its reversibility in human asthma and the limited data derived from RAO horses, there is currently insufficient evidence to determine the suitability of equine asthma as a model for asthma with fixed airflow limitation.

## 12 | ASTHMA WITH OBESITY

In humans, obese patients with asthma can have moderate to severe respiratory symptoms, with little eosinophilic airway inflammation; there is no evidence for an increase in sputum inflammatory cells. Whilst it is unknown whether obesity per se contributes to asthma, there are marked alterations to respiratory physiology including an increased demand for ventilation and work of breathing. Breathing at low lung volumes enhances airway responsiveness which improves after bariatric surgery.[99,100] The altered mechanics of breathing that favor airway narrowing and airway hyperresponsiveness can result in a more severe clinical presentation than that predicted upon consideration of the underlying inflammatory cytologic profile. Whilst there is evidence that obesity increases the risk of developing asthma in people, some studies suggest that insulin resistance, systemic IL-6 inflammation and clinical features of metabolic dysfunction have a stronger association with more severe asthma than body mass index (BMI) or body mass.[101] Whilst there is a positive correlation between both body condition score and body fat (%) and IL-1 and TNF-α in equine plasma,[102] there is currently no report of increased expression of inflammatory cytokines in BAL fluid or increased pulmonary resistance in horses with obesity. Furthermore, to the best of authors' knowledge there are no reports of a link between equine metabolic syndrome and the presence of chronic lower airway inflammation in horses. Therefore, there is currently insufficient evidence to consider equine asthma a suitable model for human asthma associated with obesity.

## 13 | PHENOTYPE VERSUS ENDOTYPE

Our inability to identify consistent genetic and environmental correlations with IAD and RAO can potentially be attributed to our limited understanding of the various pathophysiologic mechanisms underlying these diseases. In human medicine, “asthma endotypes” are disease subtypes defined by their distinct, underlying pathophysiologies.[103] The broad syndrome of asthma can therefore be divided into distinct disease entities, or subtypes, on the basis of 7 variables; these include clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and response to treatment.[103] Recently, several groups have used transcriptomic data derived from stimulated peripheral blood mononuclear cells (ex vivo)[104] and bronchial epithelium (in vivo)[105] to identify differentially expressed genes and pathways between RAO and non-RAO horses. Stimulation with hay dust extract resulted in the greatest differential gene expression,[104] the most dominant among the upregulated genes being those involved in immune cell trafficking, neutrophil chemotaxis, immune and inflammatory responses, and cell cycle regulation and apoptosis.[104,105] The most upregulated hay dust extract-induced chemokine was

CXCL13,[104,106] a B cell chemoattractant predominantly produced by Th17, but not Th1 or Th2, cells.[107] Rather than indicating a primary gene dysregulation, this might represent an abnormal response to allergens in horses with RAO. Interestingly, levels of CXCL13 have been shown to be upregulated 8-fold in BALF from human asthmatics compared to controls.[108] Furthermore, treatment of a sensitized murine asthma model with an anti-CXCL13 antibody reduces inflammatory cell recruitment, bronchial-associated lymphoid tissue formation, and airway inflammation, potentially supporting CXCL13 as a novel treatment target.[108] Another potential mechanistic pathway which could underpin the inflammatory cascade in RAO is the activation of neutrophils by the bronchial epithelium, leading to epithelial injury and impaired repair and differentiation.[105] With the development of new biologic treatments in human asthma and the application of more targeted therapeutic approaches in the horse, it is appropriate to further investigate and clarify the clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and response to treatment to better elucidate the pathophysiologic mechanisms in RAO, thus enabling the description of the allergic (Th-2), non-allergic (non-Th-2) and late-onset endotypes of equine asthma.

## 14 | RESPONSE TO TREATMENT

Human asthma control is assessed in terms of both symptom control and risk of future adverse outcomes. The level of control is the extent to which symptoms are experienced by the patient, and is determined by interactions between the patient's genetics, underlying disease processes, treatment, environment, and psychosocial factors.[109] In comparison, there are multiple challenges associated with assessing the control of signs of respiratory disease in equine asthma; therefore, the majority of peer-reviewed studies are short-term therapeutic efficacy clinical trials. As maintaining appropriate air hygiene, through a reduction in antigen and airborne dust exposure, constitutes the most important therapeutic and prophylactic approach to both IAD and RAO, one of the greatest challenges in the design of clinical trials is maintaining a degree of control over environmental exposures. Currently, there is a need for a long-term longitudinal study assessing the relative and combined beneficial effects of both drug therapy and environmental management on the control of IAD clinical signs. Indeed, even clinical research on the efficacy of treatments on airway hypersensitivity and hyperreactivity in IAD cases is limited,[110] with treatment decisions typically based on clinical experience, data derived from horses with RAO, or both.[1,111] Initially, therapeutic trials in RAO focused primarily on the beneficial effects of bronchodilators, in light of the lower airway obstruction and increased respiratory effort at rest exhibited by these cases. Recently, however, the therapeutic research focus in equine asthma has partly shifted towards the control of airway inflammation.

Airway inflammation is due in part to the increased activity of transcription factors that in turn lead to an increased production of inflammatory mediators and recruitment of inflammatory cells. Therefore, the efficacy of anti-inflammatory drugs, such as corticosteroids, in RAO has partly been evaluated via their influence on the expression of selected inflammatory genes in both BALF-derived cells[98,112,113] and bronchial epithelium.[113] Additionally, airway cytology has been used as a marker of therapeutic success with a reduction in airway neutrophilia being achieved by transferring horses to a low dust feed, with a greater level of improvement achieved by the additional administration of oral dexamethasone.[113] However, in most studies, corticosteroids as sole therapy, whether administered systemically or by inhalation, failed to normalize the airway neutrophilia, even after up to 6 months of treatment, and this might also be true in IAD.[97,98,110,114,115] However, glucocorticoid therapy downregulates some of the neutrophil functions in the airways of horses with RAO.[116] Compared to the use of low dust feed alone, dexamethasone administration resulted in a decreased expression of IL-8, chemokine (C-X-C motif) ligand 2 (CXCL2), and IL-1β in BALF-derived cells; whereas, both treatments decreased expression of IL-8 and CXCL2 in airway epithelium, compared to baseline.[113] Similarly, low dust feed resulted in a greater decrease of IL-8 expression than that of inhaled fluticasone.[97] Furthermore, as the anti-inflammatory properties of glucocorticoids are thought to be mediated by suppression of inflammatory gene expression via inhibition of transcription factors NF-κB and AP-1, the effect of glucocorticoid administration on these factors in BALF-derived cells and bronchial epithelium in horses with RAO have also been investigated: no significant treatment effect was observed on the expression of either transcription factor.[91] There are currently no published studies assessing the effects of glucocorticoid administration on the activity of transcription factors beyond a treatment period of 2 weeks.

New immunomodulatory agents have been investigated in both human and equine allergic (Th-2) asthma. Recently, nonspecific CpG-GNP (nanoparticle-bound cytosine-phosphate-guanosine oligodeoxynucleotides) based immunotherapy was shown to provide an effective, allergen-independent approach to treatment of horses with RAO.[117] Briefly, CpG is recognized by Toll-like receptors (TLR9), that are expressed in equine pulmonary neutrophils, macrophages, and epithelial cells.[118] Ligand-binding results in the stimulation of Th-1 response, leading to the downregulation of any Th-2 bias associated with an allergenic trigger (as in seen during an RAO exacerbation). Furthermore, Treg lymphocytes are stimulated, helping to re-establish T-helper cell homeostasis.

## 15 | CONCLUSIONS

Upon consideration of the shared factors between human asthma, IAD and RAO, we conclude that adoption of the term equine asthma is appropriate, whilst acknowledging that important heterogeneity exists within this broad disease category. We therefore support the proposal that the term mild/moderate equine asthma replace IAD and severe equine asthma replace RAO in the literature from this point onwards, whilst recognizing the need to preserve the spectrum of diseases which fall within the proposed new “equine asthma” classification. Furthermore, in addition to the subcategorization of equine asthma based on severity, we propose that equine equivalents to specific human asthma phenotypes exist, based on shared clinical and pathophysiological characteristics. Finally, with the development of new biologic treatments in human asthma and the application of more

targeted therapeutic approaches in the horse, it might be appropriate to further investigate and clarify the allergic (Th-2), non-allergic (non–Th-2) and late-onset phenotypes of equine asthma; however, further research is required to more fully determine the potential clinical utility of such a phenotypic classification exercise. Currently, there is insufficient evidence to recommend an equine model for asthma with fixed airflow limitation, and asthma in obese patients.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION:

Authors declare no IACUC or other approval was needed.

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## REFERENCES

1. Couëtil L, Cardwell J, Gerber V, et al. Inflammatory airway disease of horses—revised consensus statement. *J Vet Intern Med*. 2016;30:503-515.
2. Lavoie J-P. Which is the most appropriate in 2017: "Mild to Severe Equine Asthma" or heaves, RAO, equine COPD, IAD, tracheal IAD, bronchial IAD, small airway disease, chronic bronchitis, SPACOPD, SPOPD, summer heaves or summer RAO? *World Equine Airway Symposium*. Vol 6. Copenhagen, Denmark; 2017.
3. Lavoie JP. Is the time primed for equine asthma? *Equine Vet Educ*. 2015;27:225-226.
4. Pirie RS. RAO/SPAOAD - severe equine asthma? *World Equine Airway Symposium*. Vol 8. Copenhagen, Denmark: University of Copenhagen; 2017.
5. Robinson N. Inflammatory airway disease: defining the syndrome. Conclusions of the Havemeyer workshop. *Equine Vet Educ*. 2003;15:61-63.
6. Robinson NE. International workshop on equine chronic airway disease. Michigan State University 16-18 June 2000. *Equine Vet J*. 2001;33:5-19.
7. Marti E, Gerber V, Wilson A, et al. Report of the 3rd Havemeyer workshop on allergic diseases of the horse, Hólar, Iceland, June 2007. *Vet Immunol Immunopathol*. 2008;126:351-361.
8. Richard E, Robinson N. Inflammatory airway disease congress: one syndrome, multiple pathways: a Dorothy Russell Havemeyer symposium. *Equine Vet Educ*. 2016;28:9-12.
9. Couëtil LL, Hoffman AM, Hodgson J, et al. Inflammatory airway disease of horses. *J Vet Intern Med*. 2007;21:356-361.
10. Robinson NE, Berney C, Eberhart S, et al. Coughing, mucus accumulation, airway obstruction, and airway inflammation in control horses and horses affected with recurrent airway obstruction. *Am J Vet Res*. 2003;64:550-557.
11. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: http://ginasthma.org.
12. Gerber V, Lindberg Å, Berney C, Robinson NE. Airway mucus in recurrent airway obstruction–short-term response to environmental challenge. *J Vet Intern Med*. 2004;18:92-97.
13. Bullone M. *Reversibility of Airway Remodeling in Equine Asthma: Contribution of Anti-Inflammatory and Bronchodilator Therapies*. Montréal, Québec, Canada: Université de Montréal; 2017.
14. Cardwell JM, Wood JLN, Smith KC, Newton JR. Descriptive results from a longitudinal study of airway inflammation in British National Hunt racehorses. *Equine Vet J*. 2011;43:750-755.
15. Koblinger K, Nicol J, McDonald K, et al. Endoscopic assessment of airway inflammation in horses. *J Vet Intern Med*. 2011;25:1118-1126.
16. Couetil LL, Rosenthal FS, DeNicola DB, Chilcoat CD. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am J Vet Res*. 2001;62:538-546.
17. Bedenice D, Mazan M, Hoffman A. Association between cough and cytology of bronchoalveolar lavage fluid and pulmonary function in horses diagnosed with inflammatory airway disease. *J Vet Intern Med*. 2008;22:1022-1028.
18. Richard E, Fortier G, Denoix JM, et al. Influence of subclinical inflammatory airway disease on equine respiratory function evaluated by impulse oscillometry. *Equine Vet J*. 2009;41:384-389.
19. Ivester KCL, Moore G. Role of particulate exposure and airway inflammation in racing performance. *World Equine Airway Symposium*. Vol 145. Copenhagen, Denmark: University of Copenhagen; 2017.
20. Couetil LL, Denicola DB. Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease. *Equine Vet J Suppl*. 1999;31:77-82.
21. Sanchez A, Couetil LL, Ward MP, et al. Effect of airway disease on blood gas exchange in racehorses. *J Vet Intern Med*. 2005;19:87-92.
22. Courouce-Malblanc A, Deniau V, Rossignol F, et al. Physiological measurements and prevalence of lower airway diseases in trotters with dorsal displacement of the soft palate. *Equine Vet J*. 2010;42:246-255.
23. ter Woort F, Caswell JL, Arroyo LG, Viel L. Histologic investigation of airway inflammation in postmortem lung samples from racehorses. *Am J Vet Res*. 2018;79:342-347.
24. Thiadens H, De Bock G, Dekker F, et al. Identifying asthma and chronic obstructive pulmonary disease in patients with persistent cough presenting to general practitioners: descriptive study. *BMJ*. 1998;316:1286-1290.
25. Tinkelman DG, Price DB, Nordyke RJ, et al. Symptom-based questionnaire for differentiating COPD and asthma. *Respiration*. 2006;73:296-305.
26. Hotchkiss JW, Reid SW, Christley R. Construction and validation of a risk-screening questionnaire for the investigation of recurrent airway obstruction in epidemiological studies of horse populations in Great Britain. *Prev Vet Med*. 2006;75:8-21.
27. Ramseyer A, Gaillard C, Burger D, et al. Effects of genetic and environmental factors on chronic lower airway disease in horses. *J Vet Intern Med*. 2007;21:149-156.
28. Wasko AJ, Barkema HW, Nicol J, et al. Evaluation of a risk-screening questionnaire to detect equine lung inflammation: results of a large field study. *Equine Vet J*. 2011;43:145-152.
29. Laumen E, Doherr M, Gerber V. Relationship of horse owner assessed respiratory signs index to characteristics of recurrent airway obstruction in two Warmblood families. *Equine Vet J*. 2010;42:142-148.
30. Rettmer H, Hoffman A, Lanz S, Oertly M, Gerber V. Owner-reported coughing and nasal discharge are associated with clinical findings, arterial oxygen tension, mucus score and bronchoprovocation in horses with recurrent airway obstruction in a field setting. *Equine Vet J*. 2015;47:291-295.

31. Bullone M, Lavoie J-P. Asthma "of horses and men"—how can equine heaves help us better understand human asthma immunopathology and its functional consequences? *Mol Immunol*. 2015;66:97-105.
32. Bel EH. Clinical phenotypes of asthma. *Curr Opin Pulm Med*. 2004;10:44-50.
33. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *Am J Respir Crit Care Med*. 2010;181:315-323.
34. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. 2012;18:716-725.
35. Colice GL. Categorizing asthma severity: an overview of national guidelines. *Clin Med Res*. 2004;2:155-163.
36. Gerber V. Sport horse IAD - moderate equine asthma? *World Equine Airway Symposium*. Vol 9. Copenhagen, Denmark: University of Copenhagen; 2017.
37. Couëtil LL, Chilcoat CD, DeNicola DB, et al. Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction. *Am J Vet Res*. 2005;66:1665-1674.
38. Leguillette R, Desevaux C, Lavoie JP. Effects of pentoxifylline on pulmonary function and results of cytologic examination of bronchoalveolar lavage fluid in horses with recurrent airway obstruction. *Am J Vet Res*. 2002;63:459-463.
39. Lapointe JM, Lavoie JP, Vrins AA. Effects of triamcinolone acetonide on pulmonary function and bronchoalveolar lavage cytologic features in horses with chronic obstructive pulmonary disease. *Am J Vet Res*. 1993;54:1310-1316.
40. Klier J, Lehmann B, Fuchs S, et al. Nanoparticulate CpG immunotherapy in RAO-affected horses: phase I and IIa study. *J Vet Intern Med*. 2015;29:286-293.
41. Jost U, Klukowska-Rötzler J, Dolf G, et al. A region on equine chromosome 13 is linked to recurrent airway obstruction in horses. *Equine Vet J*. 2007;39:236-241.
42. Rosenthal FS, Gruntman A, Couetil LL. A comparison of total, respirable, and real-time airborne particulate sampling in horse barns. *J Occup Environ Hyg*. 2006;3:599-605.
43. Riihimaki M, Raine A, Elfman L, et al. Markers of respiratory inflammation in horses in relation to seasonal changes in air quality in a conventional racing stable. *Can J Vet Res*. 2008;72:432.
44. Ivester K, Couetil L, Moore G, Zimmerman NJ, Raskin RE. Environmental exposures and airway inflammation in young thoroughbred horses. *J Vet Intern Med*. 2014;28:918-924.
45. Holcombe S, Jackson C, Gerber V, et al. Stabling is associated with airway inflammation in young Arabian horses. *Equine Vet J*. 2001;33:244-249.
46. Gerber V, Robinson N, Luethi S, Marti E, Wampfler B, Straub R. Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. *Equine Vet J*. 2003;35:491-495.
47. Millerick-May M, Karmaus W, Derksen F, et al. Local airborne particulate concentration is associated with visible tracheal mucus in thoroughbred racehorses. *Equine Vet J*. 2013;45:85-90.
48. Ivester K, Couetil L, Zimmerman N. Investigating the link between particulate exposure and airway inflammation in the horse. *J Vet Intern Med*. 2014;28:1653-1665.
49. Pirie RS. Recurrent airway obstruction: a review. *Equine Vet J*. 2014;46:276-288.
50. Robinson N, Karmaus W, Holcombe S, Carr EA, Derksen FJ. Airway inflammation in Michigan pleasure horses: prevalence and risk factors. *Equine Vet J*. 2006;38:293-299.
51. McGorum B, Dixon P. The analysis and interpretation of equine bronchoalveolar lavage fluid (BALF) cytology. *Equine Vet Educ*. 1994;6:203-209.
52. Hughes K, Malikides N, Hodgson D, et al. Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses 1. Evaluation of cytological stains and the percentage of mast cells and eosinophils. *Aust Vet J*. 2003;81:681-684.
53. Hughes KJ, Nicolson L, Da Costa N, et al. Evaluation of cytokine mRNA expression in bronchoalveolar lavage cells from horses with inflammatory airway disease. *Vet Immunol Immunopathol*. 2011;140:82-89.
54. Hare JE, Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. *J Vet Intern Med*. 1998;12:163-170.
55. Dubuc J, Lavoie J-P. Airway wall eosinophilia is not a feature of equine heaves. *Vet J*. 2014;202:387-389.
56. Barnetson RSC, Rogers M. Childhood atopic eczema. *BMJ*. 2002;324:1376-1379.
57. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol*. 1999;103:1173-1179.
58. Hershey GKK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. *N Engl J Med*. 1997;337:1720-1725.
59. Ober C, Leavitt SA, Tsalenko A, et al. Variation in the interleukin 4–receptor α gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet*. 2000;66:517-526.
60. Scales H, Ierna M, Lawrence C. The role of IL-4, IL-13 and IL-4Rα in the development of protective and pathological responses to Trichinella spiralis. *Parasite Immunol*. 2007;29:81-91.
61. Lanz S, Brunner A, Graubner C, Marti E, Gerber V. Insect bite hypersensitivity in horses is associated with airway Hyperreactivity. *J Vet Intern Med*. 2017;31:1877-1883.
62. Kehrli D, Jandova V, Fey K, Jahn P, Gerber V. Multiple hypersensitivities including recurrent airway obstruction, insect bite hypersensitivity, and Urticaria in 2 Warmblood horse populations. *J Vet Intern Med*. 2015;29:320-326.
63. Neuhaus S, Bründler P, Frey C, et al. Increased parasite resistance and recurrent airway obstruction in horses of a high-prevalence family. *J Vet Intern Med*. 2010;24:407-413.
64. Lavoie JP, Maghni K, Desnoyers M, et al. Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. *Am J Respir Crit Care Med*. 2001;164:1410-1413.
65. Grünig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*. 1998;282:2261-2263.
66. Derksen FJ, Scott JS, Miller DC, Slocombe RF, Robinson NE. Bronchoalveolar lavage in ponies with recurrent airway obstruction (heaves) 1–3. *Am Rev Respir Dis*. 1985;132:1066-1070.
67. Schmallenbach K, Rahman I, Sasse H, et al. Studies on pulmonary and systemic Aspergillus fumigatus-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Vet Immunol Immunopathol*. 1998;66:245-256.
68. Tahon L, Baselgia S, Gerber V, et al. In vitro allergy tests compared to intradermal testing in horses with recurrent airway obstruction. *Vet Immunol Immunopathol*. 2009;127:85-93.
69. Beekman L, Tohver T, Leguillette R. Comparison of cytokine mRNA expression in the bronchoalveolar lavage fluid of horses with inflammatory airway disease and bronchoalveolar lavage mastocytosis or neutrophilia using REST software analysis. *J Vet Intern Med*. 2012;26:153-161.
70. Lavoie J, Cesarini C, Lavoie-Lamoureux A, et al. Bronchoalveolar lavage fluid cytology and cytokine messenger ribonucleic acid expression of racehorses with exercise intolerance and lower airway inflammation. *J Vet Intern Med*. 2011;25:322-329.
71. Ying S, Humbert M, Barkans J, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol*. 1997;158:3539-3544.
72. Broide DH, Gleich GJ, Cuomo AJ, et al. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J Allergy Clin Immunol*. 1991;88:637-648.
73. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis*. 1989;139:806-817.
74. Machado DC, Horton D, Harrop R, Peachell PT, Helm BA. Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol*. 1996;26:2972-2980.
75. Okumura S, Kashiwakura J-i, Tomita H, et al. Identification of specific gene expression profiles in human mast cells mediated by toll-like receptor 4 and FcεRI. *Blood*. 2003;102:2547-2554.
76. Bradding P. Asthma: eosinophil disease, mast cell disease, or both? *Allergy Asthma Clin Immunol*. 2008;4:84-90.

77. Theoharides TC, Kempuraj D, Tagen M, Conti P, Kalogeromitros D. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev*. 2007;217:65-78.
78. Richard E, Depecker M, Defontis M, et al. Cytokine concentrations in bronchoalveolar lavage fluid from horses with neutrophilic inflammatory airway disease. *J Vet Intern Med*. 2014;28:1838-1844.
79. Lavoie-Lamoureux A, Moran K, Beauchamp G, et al. IL-4 activates equine neutrophils and induces a mixed inflammatory cytokine expression profile with enhanced neutrophil chemotactic mediator release ex vivo. *Am J Physiol Lung Cell Mol Physiol*. 2010;299:L472-L482.
80. Lavoie-Lamoureux A, Beauchamp G, Quessy S, Martin JG, Lavoie JP. Systemic inflammation and priming of peripheral blood leukocytes persist during clinical remission in horses with heaves. *Vet Immunol Immunopathol*. 2012;146:35-45.
81. Wood LG, Baines KJ, Fu J, Scott HA, Gibson PG. The neutrophilic inflammatory phenotype is associated with systemic inflammation in asthma. *Chest J*. 2012;142:86-93.
82. Leclere M, Lavoie-Lamoureux A, Gélinas-Lymburner É, David F, Martin JG, Lavoie JP. Effect of antigenic exposure on airway smooth muscle remodeling in an equine model of chronic asthma. *Am J Respir Cell Mol Biol*. 2011;45:181-187.
83. Nocker RE, Out TA, Weller FR, et al. Influx of neutrophils into the airway lumen at 4 h after segmental allergen challenge in asthma. *Int Arch Allergy Immunol*. 1999;119:45-53.
84. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D, and the EGEA Cooperative Group. Allergic vs nonallergic asthma: what makes the difference? *Allergy*. 2002;57:607-613.
85. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000; 908:244-254.
86. McFarlane D, Holbrook T. Cytokine dysregulation in aged horses and horses with pituitary pars intermedia dysfunction. *J Vet Intern Med*. 2008;22:436-442.
87. Adams A, Breathnach C, Katepalli M, Kohler K, Horohov DW. Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. *Mech Ageing Dev*. 2008;129: 656-664.
88. Hansen S, Sun L, Baptiste KE, Fjeldborg J, Horohov DW. Age-related changes in intracellular expression of IFN-γ and TNF-α in equine lymphocytes measured in bronchoalveolar lavage and peripheral blood. *Develop Comp Immunol*. 2013;39:228-233.
89. Niedzwiedz A, Jaworski Z, Tykalowski B, Smialek M. Neutrophil and macrophage apoptosis in bronchoalveolar lavage fluid from healthy horses and horses with recurrent airway obstruction (RAO). *BMC Vet Res*. 2014;10:29.
90. Barnes PJ, Adcock IM. Transcription factors and asthma. *Eur Respir J*. 1998;12:221-234.
91. Couëtil LL, Art T, Moffarts B, et al. Effect of beclomethasone dipropionate and dexamethasone isonicotinate on lung function, bronchoalveolar lavage fluid cytology, and transcription factor expression in airways of horses with recurrent airway obstruction. *J Vet Intern Med*. 2006;20:399-406.
92. Bullone M, Lavoie J-P. The contribution of oxidative stress and Inflamm-aging in human and equine asthma. *Int J Mol Sci*. 2017;18:2612.
93. Fabbri LM, Romagnoli M, Corbetta L, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;167:418-424.
94. McDonough JE, Yuan R, Suzuki M, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med*. 2011;365:1567-1575.
95. Lambert R, Wiggs B, Kuwano K, Hogg JC, Pare PD. Functional significance of increased airway smooth muscle in asthma and COPD. *J Appl Physiol*. 1993;74:2771-2781.
96. Oliver MN, Fabry B, Marinkovic A, Mijailovich SM, Butler JP, Fredberg JJ. Airway hyperresponsiveness, remodeling, and smooth muscle mass: right answer, wrong reason? *Am J Respir Cell Mol Biol*. 2007;37:264-272.
97. Leclere M, Lavoie-Lamoureux A, Joubert P, et al. Corticosteroids and antigen avoidance decrease airway smooth muscle mass in an equine asthma model. *Am J Respir Cell Mol Biol*. 2012;47:589-596.
98. Bullone M, Vargas A, Elce Y, Martin JG, Lavoie JP. Fluticasone/salmeterol reduces remodelling and neutrophilic inflammation in severe equine asthma. *Sci Rep*. 2017;7:8843.
99. Boulet L-P, Turcotte H, Martin J, Poirier P. Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir Med*. 2012;106:651-660.
100. Dixon AE, Pratley RE, Forgione PM, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. *J Allergy Clin Immunol*. 2011;128:508-515. e502.
101. Peters MC, McGrath KW, Hawkins GA, et al. Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. *Lancet Respir Med*. 2016;4:574-584.
102. Vick M, Adams A, Murphy B, et al. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *J Anim Sci*. 2007;85:1144-1155.
103. Lötvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol*. 2011;127:355-360.
104. Pacholewska A, Jagannathan V, Drögemüller M, et al. Impaired cell cycle regulation in a natural equine model of asthma. *PLoS One*. 2015;10:e0136103.
105. Tessier L, Côté O, Clark ME, et al. Impaired response of the bronchial epithelium to inflammation characterizes severe equine asthma. *BMC Genomics*. 2017;18:708.
106. Pacholewska A, Kraft MF, Gerber V, Jagannathan V. Differential expression of serum MicroRNAs supports CD4+ T cell differentiation into Th2/Th17 cells in severe equine asthma. *Genes*. 2017;8:383.
107. Takagi R, Higashi T, Hashimoto K, et al. B cell chemoattractant CXCL13 is preferentially expressed by human Th17 cell clones. *J Immunol*. 2008;181:186-189.
108. Baay-Guzman GJ, Huerta-Yepez S, Vega MI, et al. Role of CXCL13 in asthma: novel therapeutic target. *Chest*. 2012;141:886-894.
109. Taylor D, Bateman E, Boulet L, et al. A new perspective on concepts of asthma severity and control. *Eur Respir J*. 2008;32:545-554.
110. Leguillette R, Tohver T, Bond S, et al. Effect of dexamethasone and fluticasone on airway Hyperresponsiveness in horses with inflammatory airway disease. *J Vet Intern Med*. 2017;31:1193-1201.
111. Mazan MR. Update on noninfectious inflammatory diseases of the lower airway. *Vet Clin North Am*. 2015;31:159-185.
112. Giguere S, Viel L, Lee E, et al. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. *Vet Immunol Immunopathol*. 2002;85:147-158.
113. DeLuca L, Erb H, Young J, Nicol JA, McDonald KJ. The effect of adding oral dexamethasone to feed alterations on the airway cell inflammatory gene expression in stabled horses affected with recurrent airway obstruction. *J Vet Intern Med*. 2008;22:427-435.
114. Lavoie JP, Leguillette R, Pasloske K, et al. Comparison of effects of dexamethasone and the leukotriene D4 receptor antagonist L-708,738 on lung function and airway cytologic findings in horses with recurrent airway obstruction. *Am J Vet Res*. 2002;63:579-585.
115. Lavoie JP, Pasloske K, Joubert P, et al. Lack of clinical efficacy of a Phosphodiesterase-4 inhibitor for treatment of heaves in horses. *J Vet Intern Med*. 2006;20:175-181.
116. Vargas A, Boivin R, Cano P, Murcia Y, Bazin I, Lavoie JP. Neutrophil extracellular traps are downregulated by glucocorticosteroids in lungs in an equine model of asthma. *Respir Res*. 2017;18:207.
117. Klier J, Geis S, Steuer J, et al. A comparison of nanoparticullate CpG immunotherapy with and without allergens in spontaneously equine asthma-affected horses, an animal model. *Immun Inflamm Dis*. 2018; 6:81-96.
118. Schneberger D, Caldwell S, Suri SS, Singh B. Expression of toll-like receptor 9 in horse lungs. *Anat Rec*. 2009;292:1068-1077.


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