

REVIEW

Equine asthma: Integrative biologic relevance of a recently proposed nomenclature

Stephanie Bond¹  | Renaud Léguillette¹  | Eric A. Richard^{2,3}  | Laurent Couetil⁴  |
Jean-Pierre Lavoie⁵ | James G. Martin⁶ | R. Scott Pirie⁷

¹Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta

²Equine Immunity & Inflammation, LABÉO Frank Duncombe, Caen, France

³BIOTARGEN, Normandie Univ, UniCaen, Biotargen, France

⁴Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, Indiana

⁵Department of Clinical Sciences, Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, Quebec

⁶Meakins Christie Laboratories, McGill University Health Center Research Institute, McGill University, Montreal, Quebec

⁷The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, University of Edinburgh, Midlothian, Scotland, United Kingdom

Correspondence

Renaud Léguillette, University of Calgary, Faculty of Veterinary Medicine, HRIC-GAA10-3330 Hospital Drive, NW, Calgary, AB, T2N 4N1, Canada.
Email: rleguill@ucalgary.ca

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

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; IL4R α , IL-4 receptor α -chain; LT, leukotriene; NF- κ B, nuclear factor- κ B; RAO, recurrent airway obstruction; TNF, tumor necrosis factor

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

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 non-septic 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 effort¹; 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 phenotypes³¹; 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 categories¹¹ 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-naïve 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 human¹¹ 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 heterogeneity⁴¹ (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	<ul style="list-style-type: none"> Allergic trigger associated with respiratory symptoms/ expiratory airflow limitation Often commences in childhood Past/family history of allergic disease (eczema/ allergic rhinitis/food or drug allergy) Sputum often reveals eosinophilic airway inflammation Usually respond well to ICS treatment Th-2 CD4+ lymphocyte response—IL-5-mediated eosinophil recruitment IL4Rα gene associated with the development of asthma, skin allergies and parasite defense 	<p>IAD</p> <ul style="list-style-type: none"> Antigenic triggers central to development of lower airway inflammation 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 Antigenic triggers (eg, dust, mold spores) associated with increased neutrophil/mast cell% in BALF Antigenic triggers associated with clinical signs (eg, coughing, poor performance) Often occurs in young horses Eosinophilic phenotype associated with dust exposure in young horses Usually respond well to ICS treatment Th-2 response—Increase in IL-4 and IL-5 in BALF linked with mastocytic phenotype 	Yes	<ul style="list-style-type: none"> Eosinophil involvement in pathogenesis of IAD Effect of BALF phenotype on performance Role of IgE in IAD and RAO Longitudinal and cross-sectional studies investigating an “atopic march” in horses Comprehensive study investigating the effect of various allergenic triggers on both lower airway pathology and clinical signs (ie, investigate causality rather than association)
		<p>RAO</p> <ul style="list-style-type: none"> Allergic trigger (molds \pm LPS) associated with clinical signs and pathology (increased neutrophil % in BALF, increased respiratory effort at rest) Associated with multiple hypersensitivities in some families of horses (insect bite hypersensitivity, urticaria, increased parasite resistance) Good response to ICS Association between IL4Rα and RAO IL4Rα upregulates IL-4 expression during disease exacerbation, which promotes isotype switching from IgM to IgE Increased IgE in BALF in horses with RAO 	Yes	
Non-allergic asthma	<ul style="list-style-type: none"> Not associated with allergy Sputum can be neutrophilic eosinophilic or paucigranulocytic Often respond less well to ICS Chronically activated mast cells in bronchial mucosa (can be associated with non-allergic stimulus) Th-1 response—cell-mediated immunity and phagocyte-dependent inflammation 	<p>IAD</p> <ul style="list-style-type: none"> BALF can reveal neutrophilia and/or eosinophilia and/or mast cells accumulation Th-1 response—mRNA encoding TNF-α, IL-1β, and IFN-γ in BALF Th-17 response—Increase in IL-17 and IL-23 linked with increased neutrophil % in BALF Often respond less well to ICS 	Yes	<ul style="list-style-type: none"> Role of neutrophil/mast cell activation in the development of lower airway inflammation
		<p>RAO</p> <ul style="list-style-type: none"> BALF can be neutrophilic or paucigranulocytic (in severe cases where BALF return is low) Chronic innate immune activation - chronic activation of peripheral neutrophils Often respond less well to ICS 	Yes	
Late-onset asthma	<ul style="list-style-type: none"> Initial presentation as adult (particularly women) Less likely to be atopic Decreased baseline pulmonary function Often refractory to ICS/require higher doses for control 	<p>IAD</p> <ul style="list-style-type: none"> Insufficient evidence 	No	<ul style="list-style-type: none"> Disease progression from IAD to RAO over time Correlation between inflamm-aging and development of chronic inflammatory airway disease
		<p>RAO</p> <ul style="list-style-type: none"> Decreased baseline pulmonary function during disease exacerbation Mature/older animals Can require higher doses for control 	Yes	

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	<ul style="list-style-type: none"> Chronic asthma patients with fixed airflow limitation; thought to be because of airway wall remodeling Increased airway smooth muscle mass and extracellular matrix at all levels of bronchial tree Postbronchodilator FEV₁ < 70% (predicted) 	<p>IAD</p> <ul style="list-style-type: none"> Insufficient evidence <p>RAO</p> <ul style="list-style-type: none"> Tissue remodeling is reversible—long-term antigen avoidance strategies and corticosteroid therapy decrease airway smooth muscle mass and subepithelial collagen area 	<p>No</p> <p>Insufficient evidence</p>	<ul style="list-style-type: none"> Airway remodeling in IAD 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	<ul style="list-style-type: none"> Dyspnea on exertion Requires objective measurement of variable airflow limitation—Obesity-associated respiratory symptoms can mimic asthma Little eosinophilic airway inflammation 	<ul style="list-style-type: none"> Correlation between body condition score and body fat (%) and increased expression of IL-1 and TNF-α in plasma 	Insufficient evidence	<ul style="list-style-type: none"> Expression of inflammatory cytokines in BALF or increased pulmonary resistance in obese/equine metabolic syndrome horses

Abbreviations: BALF, bronchoalveolar lavage fluid; FEV₁, 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 inflammation³³; 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 particulates⁴⁴ 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 (IL4R α) gene on equine chromosome 13.⁴¹ Importantly, the IL4R α 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 hypersensitivity⁶¹ and urticaria,⁶² as well as increased parasite resistance⁶³; 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 IL4R α gene, resulting in an upregulation of IL-4 during RAO disease exacerbation.⁶⁴ However, the association between IL4R α 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 RAO⁸⁰ 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- κ B (NF- κ B) 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 asthma⁹³; 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-1 β 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- κ B 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:

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ORCID

Stephanie Bond  <https://orcid.org/0000-0002-6700-5127>

Renaud Léguillette  <https://orcid.org/0000-0003-0381-3640>

Eric A. Richard  <https://orcid.org/0000-0002-5890-8658>

Laurent Couetil  <https://orcid.org/0000-0002-1516-6666>

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