

**International Workshop on Equine Chronic Airway Disease
Michigan State University
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Chairperson's introduction

In recent years, there has been growing concern about the terminology used for equine chronic airway disease. This has arisen because it is now clear that 1) human and equine chronic obstructive pulmonary disease (COPD) are quite different conditions and 2) the term equine COPD is used widely and often inappropriately to describe horses with airway inflammation. The term 'equine COPD' was introduced by Sasse (1971) to describe the syndrome of airway inflammation and obstruction that affects the mature horse. In its most severe form, this syndrome is known to horse owners as 'heaves' or 'broken wind'. The term COPD was selected because 1) it is descriptive, i.e. the disease is chronic and causes obstruction and 2) the characteristic mucus accumulation and neutrophilic inflammation also occur in the human disease of the same name. Since the introduction of the term equine COPD, there has been a huge increase in understanding of both human and equine inflammatory obstructive airway diseases. It is now clear that equine 'heaves' is characterised by reversible airway narrowing that is due to bronchospasm. In this regard, it more closely resembles human asthma than human COPD, which is a progressive disease with little reversibility that is usually related to smoking. Use of the term equine COPD therefore leads to misunderstanding and hinders discussion among pulmonary scientists.

Since the term equine COPD originated, its use among equine veterinarians has widened to include horses with excess mucopurulent secretions in their airways and a history of poor exercise performance. Therefore, a young horse that has recently won a major competition but whose performance is below expectation may be said to have COPD. Never would such a diagnosis be made in a human college athlete who was coughing and not performing up to par. That individual might be said to have bronchitis. Use of the term equine COPD for such a young horse suggests that its condition is permanent and progressive. There is no evidence for such conclusions. Inclusion of such horses in research populations of animals with COPD results in a mixed bag of animals with a wide variety of conditions, because neutrophils and increased mucus are normal nonspecific responses of the airways to many agents.

These then were the concerns that initiated the idea of a workshop on equine chronic airway disease. In setting objectives for the workshop, it was decided to focus on what is known about airway disease and not to struggle with the unknowns. In this regard, 'heaves' has been extensively studied because affected animals have a measurable functional abnormality and are easy to define. The first objective was, therefore, to summarise the state of knowledge about 'heaves' and define the phenotype by clinical, physiological and clinicopathological means. With this definition in place, it should be possible to identify other phenotypes and compare those to 'heaves'. Identification of similarities and differences between different airway syndromes increases knowledge about pathogenesis and eventually leads to improved diagnosis, treatment and prevention. Another advantage to phenotype definition is facilitation of research collaboration. As different laboratories develop specialised molecular techniques of investigation, sharing samples from clearly defined populations increases the sample size of studies and gives more confidence to the results.

In planning the workshop, the chairperson was assisted by Dorothy Ainsworth (Cornell University), Jean-Pierre Lavoie (University of Montreal), and Laurent Viel (University of Guelph). Their assistance in defining objectives, developing the format of the workshop, chairing sessions and leading discussion was second to none. The Matilda Wilson

Fund and Boehringer Ingelheim Animal Health graciously provided the funding that allowed us to invite the leaders in equine airway disease research from around the world. Thanks are due to all of these individuals and to my assistant Victoria Hoelzer-Maddox.

The format of the report follows the format of the workshop. Summaries of current knowledge are followed by consensus statements about the current understanding of equine chronic airway disease, criteria for definition of the 'heaves' phenotype, recommendations to standardise the technique for bronchoalveolar lavage, and recommendations for research. Because it represents the views of an international group of leaders in equine airway disease research, it is our hope that the report will be a watershed in equine airway disease research and clinical management.

Human asthma and chronic obstructive pulmonary disease (COPD)

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Asthma and COPD are diseases triggered by environmental factors and which affect susceptible hosts; both are characterised by airflow limitation and airway inflammation. There are overlapping clinical features, such as the development of acute episodes of reversible airway narrowing (exacerbations), usually following viral upper respiratory tract infections (URTI). The 2 conditions are, however, usually distinguishable readily on clinical grounds. There is a school of thought that adheres to the hypothesis that asthma and COPD are fundamentally one and the same disease. The generic nature of the term chronic obstructive pulmonary disease should not be construed as including all pulmonary conditions associated with chronic airflow limitation, but is employed because of uncertainty related to the relative components of the airway dysfunction attributable to emphysema and airway disease.

Asthma has 3 defining features, airway hyperresponsiveness (AHR; defined as excessive airway narrowing on exposure to bronchoconstrictive stimuli), eosinophilic airway inflammation and reversible airway narrowing. Clinical signs are triggered by environmental factors, such as aeroallergen (dust mites, animal proteins), pollutants (ozone, sulphur dioxide, oxides of nitrogen) and viral URTI. The inflammatory process has been studied extensively on bronchial biopsies and has been shown to be driven by activated T cells, present in increased numbers in the airway walls. The effects of the T cells, predominantly CD4+ in type, on other cells are mediated by the secretion of soluble mediators, cytokines. The profile of cytokines (IL-4, IL-5 and IL-13) that is characteristic of allergic inflammation has led to these T cells being called Th2 in type. IL-4 and IL-13 favour IgE synthesis by B cells but also have effects on vascular endothelial adhesion molecules and airway smooth muscle (ASM) and IL-5 is required for eosinophilia. The hyperresponsiveness of the airways in asthma probably involves altered ASM function, but the mechanisms are as yet poorly understood. Both mechanical and biochemical explanations have been advanced to account for AHR. An increase in ASM mass, an important component of the airway remodelling associated with asthma, is the probable structural change accounting for AHR. Changes in ASM contractile properties have been observed with IgE binding to the ASM cell surface through low affinity receptors as well as exposure to certain cytokines, notably IL-1, IL-5 and IL-13. Airway narrowing is the result of the action of inflammatory mediators, in particular the cysteinyl-leukotrienes (LTC₄, LTD₄ and LTE₄). Other

mediators are probably important also, but a complete characterisation of the other bronchoconstrictive agents is awaited.

COPD is a gradually progressive disease, usually smoking-related, which evolves slowly over time. The symptoms that bring the disease to attention are usually a reflection of advanced airflow limitation, presumably because the slow development of disease leads to adjustments of lifestyle. The progression of COPD is similar to an accelerated ageing process. Ageing is associated with a normal decline in lung function, but smoking and other noxious stimuli cause an accelerated decline in lung function. Although the rapid decline in lung function can be slowed, the reversibility of lost function in COPD is minimal, which is a major distinguishing feature from asthma. Once sufficient lung function has been lost as a result of smoking, eventual symptomatic disease is inevitable from lung ageing, unless death occurs prematurely from other causes.

The inflammation of COPD has a major neutrophilic component, which has led to the theory that neutrophil-derived proteolytic enzymes cause lung destruction. Alpha-1 antitrypsin deficiency is associated with a pattern of emphysema termed panlobular at a younger age than usual COPD, consistent with a role for proteolytic/antiproteolytic imbalance. Macrophage elastase has also been implicated through studies of knockout mice. T cell involvement has been proposed also and CD8+ T cells have been found to be the dominant subset. The CD4+ cells appear to be of the Th1 phenotype (IFN- γ expressing). Whether cytotoxic properties of the CD8+ cells are involved in the process of airway damage and parenchymal destruction is not as yet known. Detailed description of the inflammatory process in the airway wall in COPD is lacking. Epithelial-derived cytokines appear to be implicated. For example, IL-8, a neutrophil chemoattractant, has been found to be expressed in COPD. Other cytokines that are associated with the disease are TNF- α and IFN- γ . The neutrophil lipid chemoattractant LTB₄ is also synthesised in increased amounts. The eosinophil appears to be recruited in increased numbers during acute exacerbations of the disease but is not a feature of the expectorated sputum.

Asthma and COPD are usually functionally distinct diseases whose basis appears to be in the inflammatory process engendered by allergen/viruses/pollutants in asthma and by smoking/viruses/pollutants in COPD. Distinct patterns of inflammation appear to be present. Human asthma appears to have more relevance for 'heaves' than COPD, based principally on reversibility of lung function. Further research is required more fully to characterise the inflammation of COPD and to understand the overlap with pathogenetic mechanisms in asthma.

Clinician's perspective of equine airway disease ('heaves')

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Equine chronic airway disease (also known as recurrent obstructive airway disease or 'heaves') causes a variety of clinical signs. According to Bracher *et al.* (1991), 60–80% of Swiss horses older than 8 years suffer from some degree of the disease. Problems may be seasonal and more intense while horses are housed indoors. On pasture, improvement is seen in most patients. However, summer-pasture-associated obstructive pulmonary disease is clinically indistinguishable from 'heaves' (Seahorn and Beadle 1993; Seahorn *et al.* 1996). Clinical signs often wax and wane, depending on the environmental conditions. Obstruction may be worsened by exposure to dust, poor quality hay, ammonia fumes, fungal spores and other nonspecific stimuli (Thomson and McPherson 1984; Dixon *et al.* 1995d). However, even severely affected horses may experience long periods of relief while staying in the same environment.

Exercise intolerance is one of the main complaints and it usually worsens slowly. The most striking clinical signs are cough, respiratory distress, mucopurulent nasal discharge, abnormal lung sounds and an enlarged lung percussion field. However, lack of or very mild clinical signs, such as occasional cough or a minimal abdominal effort, may render the diagnosis difficult. Rebreathing increases lung sounds, but ancillary diagnostic techniques often are required (Dixon *et al.* 1995c).

Determination of arterial blood gases at rest, immediately and 5 min after exercise gives reliable information about the degree of gas exchange impairment, especially if Pao₂ values at rest are low (normal range at sea level 100 ± 5 mmHg or 13.3 ± 0.7 kPa; Sasse 1971). More discriminating is the alveolar-arterial O₂ gradient (A-aDO₂), which should not exceed 7 mmHg (1 kPa) at rest. After exercise, high values of A-aDO₂ persist significantly longer in diseased horses (Deegen 1999). Lung function tests demonstrate an increased airway resistance in most of these horses (Janssen 1996). But, in a minority of cases, even this technique fails to demonstrate functional problems in horses with histologically confirmed lower airway obstruction (Sasse 1971). Possibly, obstructions restricted to the smallest bronchioli cannot be detected. Horses with clinically confirmed COPD, subjected to euthanasia in a period when they are free of clinical signs, still show histological alterations (van den Ingh 1985). The clinical signs are recurrent, the disease is permanent.

From the clinician's perspective, tracheo-bronchoscopy, cytology of tracheal aspirate or bronchoalveolar lavage (BAL) fluid are very helpful (Winder *et al.* 1990; Dixon 1997). Histamine bronchoprovocation (Klein and Deegen 1986) and lung biopsies (Naylor *et al.* 1992) are even more discriminating. These techniques not only provide a means to confirm alterations in the small airways, but also may detect aetiological differences of clinically indistinguishable lower airway diseases.

Treatment is aimed at reducing hyperresponsiveness and its sequela, bronchoconstriction, and at increasing mucociliary clearance (Turgut and Sasse 1989; Dixon 1992). Corticosteroids (Andrews and Schmeitzel 1999) and bronchospasmolytics, such as albuterol (Derksen *et al.* 1999), and clenbuterol (Petro 1999) are used widely. In countries where they are available, bronchodilators, mucolytics, and bronchomotoric drugs or hyperinflation of saline (Schusser *et al.* 1987; Bosler 1986; Sasse 1995) can be used to assist clearance.

Pathology of the airways

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In horses with airway disease, there remains a frustrating incongruity between the severity of clinical disability and the extent of structural abnormality. There are many excellent reviews of the lesions associated with equine chronic airway disease (Gerber 1973; McPherson and Lawson 1974; Cook 1976; Breeze 1979; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990a,b; Beech 1991; Naylor *et al.* 1992; Nyman *et al.* 1991; Robinson *et al.* 1996), but relationships between causal factors, structural changes, airway hyperreactivity and airway inflammation remain incompletely understood. Recent renewed interest in airway structure has seen a shift in emphasis from epithelial to submucosal compartments, because of the central role that remodelling has in the pathogenesis of asthma.

The macroscopic changes in horses with 'heaves' include a light pink, fluffy soft hyperinflated lung generally without emphysematous bullae (Breeze 1979; McPherson and Thomson 1983; Beech 1991). Hypertrophy of secondary respiratory muscles and the right ventricle have also been described (Beech 1991). The earliest structural changes develop in the most peripheral airways but, unlike the situation in human asthma, lesions do not progress centrally with chronicity or worsening disease (McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990a,b; Nyman *et al.* 1991; Naylor *et al.* 1992).

Acute exposure of horses to a challenge environment may lead to destruction of surface cilia and loss of cells from the epithelium associated with submucosal and intercellular oedema (Kaup *et al.* 1990a,b). In general, these changes are not reliably identified by routine light microscopy. Acute bronchiolar disease may lead to degeneration, necrosis and exfoliation of Clara cells (Winder and von Fellenberg 1987; Kaup *et al.* 1990b). Typically, chronic 'heaves' is associated with irregular hyperplastic foci of proliferating progenitor cells (McPherson and Lawson 1974; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Nyman *et al.* 1991; Naylor *et al.* 1992). Mucosal changes in COPD are irregularly distributed along

airways although dorso-caudal regions of lung are typically more frequently involved (McPherson and Lawson 1974; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Beech 1991; Nyman *et al.* 1991; Costa *et al.* 2000). The reason for this lack of uniformity is unknown.

Mucous cell metaplasia has been described multifocally in the small airways of horses with 'heaves' (McPherson and Lawson 1974; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Nyman *et al.* 1991; Naylor *et al.* 1992), and abnormal, viscous-appearing mucin often occludes small airways (Gerber 1973; Kaup *et al.* 1990a,b; McPherson and Lawson 1974; McPherson and Thomson 1983; Naylor *et al.* 1992). Occasionally, mucus rather than mucus is present (McPherson and Lawson 1974; Breeze 1979; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Beech 1991; Naylor *et al.* 1992). In cases of severe 'heaves', mucus may accumulate in large lakes within peribronchiolar alveoli, and this may mineralise. Whether this represents retrograde mucociliary transport, simple aspiration or some other mechanical effect is unclear, but such bronchiolar and alveolar plugs are common in advanced disease and probably contribute to irreversible, progressive declines in respiratory function. Enlargement and hypersecretion of submucosal glands in more central airways is an inconsistent finding (Breeze 1979; Kaup *et al.* 1990a,b; Naylor *et al.* 1992; McPherson and Lawson 1974; McPherson and Thomson 1983).

In the submucosa of horses with 'heaves', fibroplasia and fibrosis is a fairly common, albeit nonuniform finding (Gerber 1973; McPherson and Lawson 1974; Cook 1976; Breeze 1979; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990a,b; Beech 1991; Nyman *et al.* 1991; Naylor *et al.* 1992; Viel 1997). In severely affected cases there is also smooth muscle hyperplasia which appears accentuated when bronchioles are constricted. Although basement membrane thickening has been described, it is almost certainly not present, since ultrastructural studies on asthmatic airways demonstrated that basement membranes are unaltered but the submucosal matrix is expanded (Huang *et al.* 1999). Given the great importance now placed on airway remodelling in the genesis of airway hyperreactivity, and the involvement of constituent mesenchymal cells in this process (Busse *et al.* 1999; Elias *et al.* 1999, 2000; Kips and Pauwels 1999; Laprise *et al.* 1999; Fredberg 2000; Solway 2000), addressing these issues for horses with 'heaves' is overdue. The responses of the submucosal bronchiolar compartment to injury in horses may be substantially different to that of man, because fibroelastosis and elastin fragmentation described in asthmatics (Mauad *et al.* 1999; Carroll *et al.* 2000) does not occur to the same degree, if at all, in horses.

While the lumen of the airways contains large numbers of neutrophils, the submucosa of bronchioles of 'heaves'-affected horses contains variable numbers of lymphocytes, mast cells, plasma cells and sometimes eosinophils (Gerber 1973; McPherson and Lawson 1974; Cook 1976; Breeze 1979; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990a,b; Beech 1991; Nyman *et al.* 1991; Naylor *et al.* 1992). In extreme cases, lymphoid follicular structures may develop. Immunoglobulin producing cells are present at these sites (Winder and von Fellenberg 1987) and a dominance of CD4+ cells has been described. Dominance of a CD4+/TH₂ lymphocyte population has been implicated in the development of asthma (Finkbeiner 1999; Fredberg 2000; Holgate *et al.* 2000) and, although a similar pattern is suspected in horses, it is yet to be proven. Interestingly, the percentage of lymphocytes considered normal in lavage fluids varies widely between horses in Australia, UK and North America (Kydd *et al.* 1996; Balsan *et al.* 1997), but whether this influences the severity and progression of COPD in the different countries is unclear.

Normally, tethering of alveoli to bronchioles means that the state of bronchiolar dilation is in synchrony with that of alveolar expansion. Asynchrony is taken as an indicator of bronchiolar constriction and in horses is usually associated with alveolar hyperinflation without emphysema (McPherson and Lawson 1974; Breeze 1979; McPherson and Thomson 1983; Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Nyman *et al.* 1991; Naylor *et al.* 1992). Alveoli adjacent to airways may have interstitial fibrosis, chronic inflammation and obstruction with exudates or mucus, particularly when bronchioles are

similarly affected (Winder and von Fellenberg 1987; Kaup *et al.* 1990b; Costa *et al.* 2000). These changes are probably brought about both by physical extension from the airway and also via release of pro-inflammatory and remodelling factors released into the bronchiolar vasculature, which then percolate into adjacent lung tissue.

Lung function: limitations of current methods

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Rather than being diagnostic, results of equine pulmonary function testing (PFT) offer indications of the severity of clinical lung dysfunction and allow objective monitoring of a patient's progress. Equine PFT has traditionally involved horses with clinical signs of recurrent airway obstruction (RAO; for reviews see Willoughby and McDonnell 1979; Robinson *et al.* 1996) and has centred on the evaluation of breathing mechanics via simultaneous measurement of intrapleural pressure, airflow and tidal volume in the spontaneously ventilating, conscious animal. A number of variables derived from these measurements help provide quantitative assessment of changes in the elasticity of the lung and/or the resistance of the nonelastic portion of the respiratory tract: maximal changes in transpulmonary or pleural pressure ($\Delta P_{pl,max}$), dynamic lung compliance (C_{dyn}), pulmonary resistance (R_L), and work of breathing (W). In horses with classical RAO, $\Delta P_{pl,max}$, R_L and W are increased and C_{dyn} is decreased. Other PFT that can be assessed include the PaO_2 (low-normal to decreased, depending on disease severity), peak inspiratory and expiratory airflows (increased), ratio of inspiratory time to total breath time (reduced) and the shape of the tidal breathing flow-volume loop (Petsche *et al.* 1994).

Despite their established use as indicators of airway obstruction, PaO_2 , C_{dyn} and R_L are relatively insensitive measurements, as they often stray outside the normal range of values only when obstruction is so severe that it is already clinically apparent. This is particularly so when assessing small (peripheral) airway function. While comprising the majority of the lung's airways, small airways are normally responsible for <20% of R_L (Macklem and Mead 1967). Therefore, with diseases like RAO in which all or most pathological change is found in noncartilaginous airways, obstructive lesions are often extensive before significant changes in R_L occur. Because C_{dyn} is a measure of lung elastic recoil and is altered by uneven distribution of ventilation, it should theoretically be a better indicator of the status of the small airways. However, it is influenced by body size, lung volume, and breathing frequency (Otis *et al.* 1956) and is, therefore, subject to considerable inter- and intra-individual variation. Hence, C_{dyn} is a relatively insensitive test when assessing the peripheral airways and elastic properties of the lung.

A wide range of normal values for measurements of breathing mechanics has been reported for horses, and this makes interpretation of results from individual tests difficult. While real differences exist, other sources of variation include temporal, daily and seasonal variations (Stadler and Deegen 1986; Jean *et al.* 1999) and methodological differences, e.g. positioning of the oesophageal balloon tip, the volume of dead space in the mask worn by the horse and the use of sedatives (Broadstone *et al.* 1992), may each affect results. Failure adequately to phase test equipment may also limit the validity of tests. Calculation of C_{dyn} presumes that the contribution of inertial forces to breathing mechanics is negligible, although it may not be so in horses breathing rapidly, as may occur with RAO (Dosman *et al.* 1975; Art *et al.* 1989).

Finally, while RAO is usually characterised as being due to a generalised condition of small airways, the possibility that some inhomogeneity of pulmonary pathology exists cannot be discounted, particularly if it is early in the development of this condition. Conventional pulmonary function tests are currently not capable of detecting such inhomogeneities if they exist. Measurements of ventilation distribution offer a way to detect inhomogeneity (Votien *et al.* 1999).

Does measurement of airway reactivity assist in population definition?

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Airway hyperresponsiveness (AHR), as an integral feature of 'heaves', was first demonstrated by Obel and Schmitterlow (1948). They observed a profound increase in bronchoconstriction to i.v. histamine in these horses. Similar findings of AHR were reported by Derksen *et al.* (1985a) in ponies after exposure to mouldy hay. Soon after, Klein and Deegen (1986) described groups of horses with varying degrees of naturally occurring airway obstruction, that, without further provocation with mouldy hay, exhibited AHR. Doucet *et al.* (1991) correlated airway inflammation with AHR, and Fairbairn *et al.* (1993a) showed that a short exposure of 'heaves'-susceptible horses to hay induced AHR that persisted for days. What is apparent from these studies is that AHR can be provoked by an allergic stimulus in selected pony 'responders' or found in the natural population of stabled horses. Elegant *in vitro* studies have shown that the basis for AHR in horses with 'heaves' involves several mechanisms, including altered acetylcholine release, defective inhibitory nonadrenergic noncholinergic responses and decreased inhibitory function of prostanoids (Broadstone *et al.* 1991; LeBlanc *et al.* 1991; Yu *et al.* 1994a). Without a doubt, these horses are fundamentally different from controls in their response to agonists.

However, AHR is not an exclusive trait of 'heaves'. The degree of AHR is similar in horses with 'heaves' (i.e. in remission from a previous or recurrent episode of airway obstruction plus BAL neutrophilia, >15%, n = 30), and those classified as having small airway inflammatory disease (i.e. horses without a history of 'heaves', exhibiting cough and/or exercise intolerance and abnormal BAL cytology: neutrophils >5%, mast cells >2%, eosinophils >1%; n = 51). Both affected groups were significantly different from controls. Therefore, AHR was a satisfactory criterion only for segregation of controls (Hoffman *et al.* 1998; Hoffman 1999; Hoffmann and Mazan 1999).

The validity of AHR for defining horse populations is related to the risk of misclassification (i.e. false positives). We found that 5 out of 30 horses presenting in remission for 'heaves' did not show AHR. Of these, 4 had abnormal BAL neutrophil counts (>15%), and 3 had an elevated baseline respiratory resistance (R_{RS}). Hence 29/30 horses could be classified as 'heaves', based on AHR and BAL findings, and all 30 horses were identified as 'heaves' if baseline lung mechanics were included. In controls, we found that 6 out of 22 horses had AHR. Four of these also had elevated (>0.6 cm H₂O/l/s) baseline R_{RS} . These horses may have had small airway lesions despite the lack of BAL abnormalities. Obviously, the use of BAL alone for the gold standard is problematic based on these data, in that horses without detectable inflammation by BAL may still exhibit signs of airway dysfunction, defying further classification. In a similar vein, 11 out of 51 horses defined as small airway inflammatory disease on the basis of airway inflammation did not exhibit AHR. Only 2 of these 11 horses had 'normal' baseline lung mechanics (R_{RS} <0.6 cm H₂O/l/s and lack of frequency dependence). Hence, it is clear that a combination of AHR, baseline lung mechanics and BAL findings would improve the classification of horses into 'heaves', small airway inflammatory disease and controls. 'Heaves', of course, could be distinguished by markedly elevated BAL neutrophils, in addition to clinical history of recurrent episodes of expiratory distress.

Endoscopic scores, bronchoalveolar lavage and tracheobronchial cytology

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Endoscopy and bronchoalveolar lavage have been important milestones in the development of new concepts and understanding regarding horses affected with 'heaves'. Visualisation of the upper and lower airways is useful to identify mucopurulent secretions, mucosal oedema, mucosal hyperaemia and bronchospasm in horses with 'heaves' (Hare and Viel

1998). Endoscopic scores have been described and used to qualitatively and quantitatively assess the amount of oedema and exudate present in the airways of affected horses (Dixon *et al.* 1995c; Hare and Viel 1998).

Because the BAL procedure is safe, repeatable and has few complications (Viel 1980), it is a routine clinical procedure to assist in the evaluation of diffuse pulmonary disorders. A single lavage sample is representative of the entire lung in horses with 'heaves' (McGorum *et al.* 1993a). Consequently, our understanding of the role of inflammatory cells in the pathophysiology of many equine respiratory disease processes has been greatly increased. Analysis of BAL fluid has facilitated evaluation of the microbial inhabitants of the lower airways (Fogarty *et al.* 1983; Hoffman *et al.* 1992), noncellular constituents such as histamine (Hare *et al.* 1994) and various cytokines (Franchini *et al.* 1998).

Generally, the fluid infused (200–500 ml) consists of prewarmed (37°C) 0.9% physiological saline or phosphate-buffered saline in 1–3 boluses. This fluid is instilled into and re-aspirated from the lung via an endoscope or specialised tube. The volume of lavage fluid retrieved usually ranges from 40 to 60 percent but may decrease with 'heaves'. The appearance (colour, turbidity, presence of flocculent debris) of the fluid collected contributes to the clinical pathologist's overall interpretation.

In healthy animals, the major cell populations consist of alveolar macrophages (60%) and lymphocytes (35%). The other cell types - neutrophils, eosinophils, mast cells and epithelial cells - are usually in negligible numbers. During exacerbation of 'heaves', the cytology is characterised by a significant increase in neutrophils, as well as a lesser increase in eosinophils and mast cells (Vrins *et al.* 1991; Winder *et al.* 1991). These values return to baseline when the horse is in remission. Cellular morphology and noncellular components should also be described when evaluating BAL.

Transtracheal aspiration (TA) as a method for collecting respiratory tract samples for bacterial and cytological analyses has been extensively used in veterinary medicine since the 1970s (Mansmann and Knight 1972). The principle disadvantage of TA is the wide variability in results obtained in control horses and lack of correlation between TA cytology and BAL cytology. In studies by Derksen *et al.* (1989) and Larson and Busch (1985), the 95% confidence interval for neutrophil differential counts is 0–88% and 4–68%, respectively, limiting the procedure's clinical utility to screen horses for chronic respiratory disease. The study by Derksen *et al.* (1989) was unable to correlate any cellular differential counts for neutrophils, lymphocytes and macrophages between samples collected via BAL and TA.

Environmental factors as aetiological agents

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Equine 'heaves' resembles those human occupational lung diseases caused by inhaled organic dusts. Dust in horse stables contains over 50 species of moulds, large numbers of forage mites, endotoxins and inorganic components (Halls and Gudmundsson 1985; Clarke and Madelin 1987; Woods *et al.* 1993). Therefore, stable dust is a variable mixture of agents, many of which can induce or exacerbate pulmonary inflammation. The relative importance of each of these agents in the aetiology of 'heaves' is unknown, and it is probable that the pathogenicity of some agents (e.g. moulds) is potentiated by the presence of others (e.g. bacterial endotoxins).

Moulds

Stables may contain a wide range of airborne fungi and thermophilic actinomycetes (Clarke and Madelin 1987). While horses with 'heaves' have exaggerated pulmonary antibody responses to moulds (Halliwell *et al.* 1993; Schmallenbach *et al.* 1998), it is unclear whether this implies a causal role for antibody-mediated hypersensitivity in disease pathogenesis, or merely reflects sensitisation following exposure. McGorum *et al.* (1993e) showed that inhalation of aqueous extracts of *Aspergillus fumigatus* or *Faenia rectivirgula* induced a neutrophilic pulmonary inflammatory response and pulmonary dysfunction in

asymptomatic 'heaves'-affected horses, but not in controls. While this supports a causal role for these moulds, as the response to these agents was less than that to hay/straw exposure (McGorum *et al.* 1993d), other agents in stable dust probably contribute to 'heaves'. In contrast, Derksen *et al.* (1988) found that inhalation of *F. rectivirgula* induced airway neutrophilia in control and 'heaves' horses, but only pulmonary dysfunction in 'heaves'-affected horses. As control horses do not develop a significant pulmonary neutrophilia on exposure to hay/straw, the experimental *F. rectivirgula* challenge used in this study differed from that encountered in a stable.

Endotoxins

While the role of inhaled endotoxin in 'heaves' is unclear, it probably contributes to disease pathogenesis because (a) there are striking similarities between endotoxin-mediated lung diseases in other species and 'heaves', (b) concentrations of airborne endotoxin in stables may exceed the threshold that induces inflammation and hyperresponsiveness in man (McGorum *et al.* 1998) and (c) inhaled endotoxin induces airway neutrophilia in horses (Pirie 1998).

Forage mites

Hay and straw may contain large numbers of forage mites (Halls and Gudmundsson 1985; Clarke and Madelin 1987); and airborne stable dust contains high concentrations of *Lepidoglyphus destructor* antigens (Woods *et al.* 1993). While forage mites may contribute to occupational asthma in man (Cutthbert *et al.* 1979), their role in 'heaves' is unclear. Although several studies have assessed dermal reactivity to forage mite antigens in the horse (Hockenjos *et al.* 1981; Eriksen 1985; Sasse *et al.* 1985), no meaningful conclusions were obtained due to poor experimental design and technical problems.

β -D-glucans

The role of airborne β -D-glucans, which are components of the cell walls of fungi, plants and bacteria, in 'heaves' is unknown. β -D-glucans contribute to the development of organic-dust induced airway inflammation in other species, primarily by potentiating the response to other inhaled agents such as endotoxin (Williams 1997).

Other environmental factors

Because horses with symptomatic 'heaves' have airway hyperreactivity (Derksen *et al.* 1985a), other nonspecific environmental factors, such as airborne inorganic dusts, cold air and dry air may be important determinants of the day-to-day severity of pulmonary dysfunction.

Is 'heaves' a septic process?

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In evolutionary terms, the neutrophil has developed for host defence against bacterial pathogens, in particular by release of highly toxic proteases and oxygen-free radicals. In addition to their role as effector cells at the site of inflammation, neutrophils can also play a significant local regulatory role, including the recruitment of additional neutrophils, by cytokine synthesis and release. However, the range of diseases in which the neutrophil plays a major effector role is much wider than bacterial infections. The neutrophil is recognised as a prime effector cell in a wide range of noninfectious inflammatory disorders. In some cases, this can be to the detriment of the host due to release of their histotoxic contents (e.g. *Type 3* hypersensitivity vasculitis, psoriasis and rheumatoid arthritis in man, equine intestinal mucosal reperfusion injury) (Haslett 1997; Brazil 1999). In the human lung, the neutrophil is now recognised to play a pivotal role in the pathogenesis of a wide range of noninfectious disorders including neonatal hypoxic pulmonary damage, acute asthma, many interstitial pulmonary diseases and acute respiratory distress

syndrome (Fahy *et al.* 1995; Haslett 1997; Mecklenburgh *et al.* 1999; Ordonez *et al.* 2000). A further group of human neutrophil-mediated pulmonary disorders with parallels to equine 'heaves' are the endotoxin-mediated, occupational pulmonary diseases (e.g. cotton worker's lung) (Rylander and Bergstrom 1993; Jacobs 1997).

Neutrophilic bronchiolitis is the major pathological feature of equine 'heaves' (Nicholl 1978; Kaup *et al.* 1990a,b). In association with this, greatly elevated neutrophil ratios have been recorded consistently in the tracheal secretions and bronchoalveolar lavage fluid (BALF) of 'heaves'-affected horses, both clinically (Mair 1987; Dixon *et al.* 1995c) and experimentally (Derksen *et al.* 1985b; McGorum *et al.* 1993c; Brazil 1999) where they are recruited to the lungs within 6 h of suitable inhalation challenge (Fairbairn *et al.* 1993b).

The normal morphology of neutrophils (i.e. the absence of the so-called 'toxic neutrophils' or of significant neutrophil bacterial phagocytosis) in the respiratory secretions of 'heaves'-affected horses differs from the cytological findings in pulmonary bacterial infections (Dixon *et al.* 1995c). It is recognised that 'heaves'-affected horses often have increased numbers of a wide variety of organisms within their tracheal secretions (e.g. *Pseudomonas* or *Acinetobacter*). However, on cessation of the inhalation challenge, these bacteria disappear together with the associated pulmonary inflammation and excessive tracheal secretions. This indicates that these bacteria do not play a primary aetiological role in 'heaves' (Dixon 1992; Dixon *et al.* 1995b). In addition, the epidemiological pattern of 'heaves', i.e. of individual older (>7 years) horses being affected, contrasts with the pattern in infectious respiratory disease, where large numbers of in-contact horses (especially younger animals) are affected.

Many veterinarians and indeed many veterinary clinical laboratories are saddled with the simplistic concept that neutrophils equal infection and eosinophils equal allergy. Consequently, if tracheal secretions or BALF contain increased numbers of neutrophils, a diagnosis of bacterial infection is automatically made. An erroneously cited reason for this naïve viewpoint is that the primary inflammatory cells in the respiratory secretions of human asthmatics are eosinophils, with the implication that allergic pulmonary diseases are mediated only by eosinophils. While eosinophils are major inflammatory cells in the airways of chronic stable asthmatics, many recent studies (Fahy *et al.* 1995; Ordonez *et al.* 2000) have shown that, in fact, neutrophils are the predominant cells in acute severe asthma. The role of the neutrophil in a wide range of noninfectious pulmonary diseases needs to be recognised by veterinarians and by veterinary diagnostic laboratories.

Genetics of equine chronic airway disease

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In early reports, horse families with a high prevalence for 'heaves' were identified which suggested that a genetic predisposition for the disease may exist (Schäpfer 1939; Gerber 1989). However, no direct comparisons with control families without the disease were made, such that the genetic association was not solidly based. Marti *et al.* (1991) investigated if a genetic predisposition could be demonstrated for chronic airway disease, also called chronic bronchitis (CB). For that purpose, the occurrence of chronic airway disease was investigated in families at 2 studfarms and also among a group of half-siblings sired by 3 affected and 3 nonaffected stallions.

The family members at the 2 studfarms were born and raised under the same conditions. Diagnosis was based on long-term observations and multiple clinical examinations. In the first studfarm, 90 German Warmbloods (53 with CB and 37 nonaffected), whose parents were of known disease status, were examined. The offspring were grouped according to the diagnosis of their parents, i.e. both parents healthy, 1 or 2 parents affected with CB. When both parents were unaffected, 5/29 (17%) offspring were affected by CB. Twenty-three of 48 (48%) offspring with one CB-affected parent were suffering from CB and the number of affected offspring increased to 9/13 (67%) when both parents had CB ($P < 0.005$). In the second studfarm, similar results were obtained with 42 Lipizzan horses: mating of unaffected parents resulted

in 1/16 (6%) CB-affected offspring, and the percentage of affected offspring increased to 6/17 (35%) and 4/9 (44%) when one or both parents were affected ($P = 0.062$). Overall morbidity was lower in the second studfarm (26%) than in the first (41%), but the difference was not significant. Dam and sire influenced the pulmonary status of their offspring to the same extent: affected stallions bred to healthy mares produced 11 healthy and 9 affected offspring; 5 healthy and 5 affected offspring were born from mares suffering from CB mated to healthy stallions. In both cases, 50% of the offspring suffered from CB, regardless of the sex of the affected parent.

In the half-sibling group, the diagnosis was based on individual history and on a clinical examination. It was known in all these horses that clinical signs of CB were provoked by exposure to hay. The 153 Swiss Warmblood half-siblings were grouped according to their age (4 age groups: 5–7, 8–10, 11–13 and >14) and according to the pulmonary status of their sire. The quality of stabling (air ventilation, quality of hay) was also recorded. The overall results show that stallions with CB produced significantly more affected offspring (31/79, 39%) than healthy stallions (9/74, 12%). In each half-sibling group, the frequency of affected horses increased with increasing age. An analysis of variance showed that sire, age and environment all exerted a significant effect on the occurrence of CB. These results show that, similarly to asthma in man (Marsh *et al.* 1993), clinical manifestation of CB is the result of the interaction of genetic and environmental factors.

Inflammatory mediators, including leukotrienes

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Airway inflammation is a characteristic feature of equine 'heaves'. Neutrophils accumulate in large numbers in the lungs of affected horses following antigen challenge and T lymphocytes are also recruited to the airways (Robinson *et al.* 1996). These cells, together with those resident in the lung, such as mast cells, macrophages and endothelial and epithelial cells, are potential sources of inflammatory mediators which may contribute to the pathogenesis of 'heaves'.

A number of mediators have been shown to be present in increased amounts in the airways or circulation of horses with 'heaves' following antigen challenge, which is suggestive of a role in the disease process. Studying the effects of selective receptor antagonists or synthesis inhibitors for individual mediators on the response to antigen challenge has shed some light on their relative importance. Therefore, although histamine concentrations increase in bronchoalveolar lavage fluid (BALF) after antigen challenge of horses with 'heaves', histamine 1-receptor antagonists are not noted for producing significant beneficial effects (McGorum *et al.* 1993d). Similarly, plasma thromboxane B₂ and BALF prostaglandin (PG) levels increase, although local production of PGE₂ in the airways decreases, but the cyclo-oxygenase inhibitor flunixin did not affect airway obstruction or hyperreactivity (Gray *et al.* 1989, 1992; Watson *et al.* 1992). The phospholipid mediator, platelet-activating factor (PAF), has properties that are consistent with a role in COPD, yet a PAF receptor antagonist was without effect when given to COPD horses prior to antigen challenge (Marr *et al.* 1996).

In contrast to the lack of improvement in clinical signs after inhibition of the formation or action of other lipid mediators, some evidence has been obtained to suggest that 5-lipoxygenase (5-LO) inhibitors, which inhibit leukotriene (LT) formation, may reduce bronchoconstriction in some horses with 'heaves' (Marr *et al.* 1998a,b). However, others have reported no significant effects on airway function or BALF neutrophilia using an LTD₄ receptor antagonist or a 5-LO activating protein (FLAP)-antagonist (Robinson *et al.* 1998; Lavoie *et al.* 1999b). The significance of elevated circulating 15-hydroxyeicosatetraenoic acid, isoprostane and endothelin-1 levels in the airways has yet to be determined (Gray *et al.* 1992; Benamou *et al.* 1998; Kirschvink *et al.* 1999).

The mRNA for the chemokines, interleukin (IL)-8 and macrophage inflammatory protein (MIP)-2, both of which are potent neutrophil chemoattractants, is increased in stimulated equine alveolar macrophages

(Franchini *et al.* 1998). These findings suggest that inhibitors of chemokine formation or action warrant study in this condition.

Elevated levels of matrix metalloproteinases (MMPs), which are likely to be produced by activated neutrophils or macrophages, have been detected in respiratory tract secretions from horses with clinical signs of COPD (Koivunen *et al.* 1997a,b). A reduction in leucocyte-derived proteases, as well as free radicals and mediators, can be achieved by inhibition of cell activation through effects on intracellular signaling molecules. The actions of compounds such as isoenzyme selective phosphodiesterase inhibitors are worthy of investigation in equine 'heaves'.

Antibody and cytokine profiles

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The finding that exacerbation of clinical signs could be provoked by the inhalation of dusty hay led researchers to postulate that 'heaves' is an allergic reaction to inhaled moulds and fungi. The positive passive cutaneous anaphylaxis test observed using sera from horses with 'heaves' (Eyre 1972) and the elevated levels of IgE in BAL (Halliwell *et al.* 1993; Schmallenbach *et al.* 1998) of affected horses are supportive of the involvement of a *type I* hypersensitivity reaction. Skin testing, however, is not discriminating of health status, as most horses have a positive skin reaction to common barn allergens, although horses with 'heaves' tend to have a greater number of positive skin reactions (Eyre 1972; McPherson *et al.* 1979; Beech and Gunson 1981; Evans *et al.* 1992; McGorum *et al.* 1993b). A *type III* hypersensitivity reaction could also cause the neutrophilic inflammation in airway secretions of horses with 'heaves'. However, serum precipitin antibody levels correlate with poor barn environment rather than with the presence of 'heaves' (Lawson *et al.* 1979; Madelin *et al.* 1991; Chabchoub *et al.* 1993). A test for the detection of antigen specific IgE in the serum of horses is commercially available, but it has not been independently investigated and it is still unclear whether serum IgE is elevated in 'heaves' (Halliwell *et al.* 1993; Schmallenbach *et al.* 1998).

T cell-mediated immunity may be involved in the pathogenesis of inflammatory lung disease, via the secretion of various cytokines. Helper T cells (CD4+) and suppressor T cells (CD8+) may contribute to the modulation of inflammation. Various attempts have been made to define the predominant T-cell population in blood and BAL of horses with 'heaves'. While studies comparing horses with 'heaves' and control horses have identified differences in the total number and ratio of CD4 and CD8+ T-cells, there had been inconsistencies in the findings of the various reports, preventing definitive conclusions from being reached (McGorum *et al.* 1993c; Bendali-Ahcene *et al.* 1995; Watson *et al.* 1997; Kleiber *et al.* 1999).

The discovery of a divergence in CD4+ T helper cells (Th) into predominantly Th1 and Th2 subsets has improved our understanding of chronic inflammatory diseases. Cytokines produced by Th2 cells, such as interleukin (IL)-4 and IL-5, have been implicated in allergic inflammation (Romagnani 1994). Th1 cells are important for cell-mediated immunity by their production of INF- γ and other cytokines (O'Garra 1998). Using *in situ* hybridisation, we found that BAL cells from horses with 'heaves' have increased expression of IL-4 and IL-5 mRNA and decreased expression of INF- γ , which is consistent with a Th2 type cytokine response (Lavoie *et al.* 2000). However, when using RT-PCR on BAL cells, no consistent findings on cytokine mRNA expressions were found between studies (Lavoie *et al.* 1999a; Ainsworth *et al.* 2000; Giguere *et al.* 2000). A number of cytokines are probably implicated in the modulation of airway inflammation in 'heaves' and are currently being investigated.

Taken together, the studies cited above are suggestive, but not conclusive, that 'heaves' is an allergic condition. The variable results between studies are probably due to methodological factors, disease definition and sampling time. Clearly more studies are needed before the immunological events leading to pulmonary inflammation in 'heaves' are elucidated.

Oxidative stress

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The role of oxidative stress has been investigated recently in 'heaves'. Acute exacerbations are associated with a significant increase in markers of oxidative stress (i.e. oxidised glutathione and glutathione redox ratio) in pulmonary epithelial lining fluid (PELF). These markers are correlated significantly with neutrophilic count in bronchoalveolar lavage (Art *et al.* 1999a,b). This, together with the observation that 'heaves' is associated with an increase of metalloproteinase activity in PELF (Raulo and Maisi 1998), reinforces the importance of PELF neutrophils in the pathophysiology of 'heaves'.

Isoprostanes, i.e. prostanoids produced mainly by autoperoxidation of membrane phospholipids by reactive oxygen species, are also significantly increased in PELF of 'heaves'-affected horses in a way that is proportional to their pulmonary dysfunction (Kirschvink *et al.* 1999). In addition to being a reliable marker of oxidative stress, isoprostanes could also be one of the mediators of the 'heaves'-induced pulmonary dysfunction. Indeed, isoprostanes induce bronchoconstriction both *in vitro* and *in vivo* in equine airways, and 'heaves'-affected horses are hyperresponsive when compared to healthy ones. These prostanoids could act through the thromboxane TP-receptor (Kirschvink *et al.* 2000).

Transcription factors

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Equine 'heaves' is an inflammatory condition of the airways, in which a hypersensitivity response to inhaled antigens may be involved. Because inflammation associated with hypersensitivity results from an exaggerated expression of inflammatory genes, recent studies have aimed at identifying the mechanisms implicated in this inappropriate inflammatory gene induction in 'heaves'.

Transcription factor nuclear factor- κ B (NF- κ B) is highly activated in bronchial brushing samples (BBSs) and bronchoalveolar lavage (BAL) cells obtained from 'heaves'-affected horses during crisis, as compared with healthy horses. Three weeks after antigen challenge, NF- κ B activity in BBSs and BAL cells from diseased horses is generally maintained at moderate or high levels, and is highly correlated ($r = 0.88$) to the degree of residual lung dysfunction (Bureau *et al.* 2000a). Consequently, NF- κ B could be a putative target in 'heaves' therapy.

Active NF- κ B complexes found in BBSs and BAL cells from 'heaves'-affected horses are mainly p65 homodimers, rather than the classical p65-p50 heterodimers (Bureau *et al.* 2000a). Transcription of some genes, such as ICAM-1 and IL-8, depends on an atypical DNA site, which preferentially binds to p65 homodimers (Ledebur and Parks 1995). This is supported by the finding that expression of ICAM-1 parallels p65 homodimers activity in BBSs from healthy and 'heaves'-affected horses. Moreover, because p65 homodimers induce IL-8 (i.e. the most potent neutrophilic chemo-attractant), but not the other chemo-attractants, such as eotaxin (i.e. an eosinophilic chemo-attractant that is under p65-p50 control) (Matsukura *et al.* 1999), the presence of these particular NF- κ B complexes in horses' lungs could explain why 'heaves'-associated inflammation is neutrophilic.

Bronchial NF- κ B activity is strongly correlated to the percentage of neutrophils present in the bronchi, and is only abrogated after granulocytic death (Bureau *et al.* 2000b). This suggests that the sustained NF- κ B activity that occurs in the airways of 'heaves'-affected horses is driven mainly by the inflammatory cells that remain or appear in the bronchi after antigen challenge, which emphasises the importance of neutrophils in 'heaves'.

Activated neutrophils generate high amounts of IL-1 β and TNF- α , which activate NF- κ B, which in turn induces the expression of these pro-inflammatory cytokines, thereby initiating autoregulatory feedback loops. Accordingly, addition of both neutralising anti-IL-1 β and anti-TNF- α antibodies to cultured BBSs from 'heaves'-affected horses suppress the

NF- κ B activity (Bureau *et al.* 2000b).

In most cells, transactivating NF- κ B induces many inflammatory proteins, as well as its most abundant inhibitor, I κ B- α , thereby ensuring a transient response upon stimulation. Surprisingly, I κ B- β , whose expression is not upregulated by NF- κ B, unlike I κ B- α , is the most prominent NF- κ B inhibitor found in BBSs from horses. The amounts of I κ B- β are low in BBSs obtained from diseased as compared with healthy horses, but drastically increase after addition of the neutralising anti-IL-1 β and TNF- α antibodies, indicating that an imbalance between high levels of IL-1 β and TNF- α -mediated I κ B- β degradation and low levels of I κ B- β synthesis is probably the mechanism preventing NF- κ B deactivation in the airways of 'heaves'-affected horses before granulocytic death (Bureau *et al.* 2000b).

Apoptosis is significantly delayed in BAL neutrophils from 'heaves'-affected horses. This delay in neutrophilic apoptosis can be reversed by the use of anti-GM-CSF receptor antibodies, indicating that GM-CSF is the cytokine responsible for the resistance of neutrophils from 'heaves'-affected horses to apoptosis. Furthermore, Signal Transducer and Activator of Transcription-5 (STAT-5), the transcriptional activity of which is induced by GM-CSF, is drastically increased in BAL granulocytes from diseased horses (Bureau *et al.* 2000b). The delay in neutrophilic apoptosis found in 'heaves'-affected horses could therefore be involved in the persistence of inflammation observed in some 'heaves'-affected horses in remission.

What do we know about mucus?

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The presence of large quantities of thick sticky-appearing mucus in large and small pulmonary airways is a common bronchoscopic and *postmortem* observation in 'heaves'-affected horses (Schatzmann *et al.* 1973; Dixon 1992; Dixon *et al.* 1995a; Robinson *et al.* 1996). This clinical finding is similar to that observed in man with chronic inflammatory airway diseases, such as chronic bronchitis/bronchiolitis/rhinitis, asthma and cystic fibrosis. In these chronic airway diseases, copious amounts of luminal airway mucus are correlated with the histological appearance of numerous mucous (goblet) cells in the surface epithelium and subepithelial glands in airways that normally contain some mucous cells (e.g. mucous cell hyperplasia) and in regions that normally contain few or no mucous cells (i.e. mucous cell metaplasia) (Reid 1954; Dunnill 1960; Dunnill *et al.* 1969; Cutz *et al.* 1978; Aikawa *et al.* 1992). As in the above-mentioned human pathologies, mucus accumulation in horses is a characteristic, but nonspecific, airway response associated with various infectious and environmental inflammatory airway diseases (Dixon *et al.* 1995c).

The amount of mucus present in the lumen of airways is the result of a dynamic process that involves specific (mucin) gene expression, the production, storage and secretion of mucins into the airway lumen, and its clearance via cilia, lining the conducting airways. These are simple concepts and yet our understanding of the fundamental cellular and molecular mechanisms regulating these basic physiological processes, in normal and diseased airways, is not yet fully understood.

Mucus is a complex biofilm composed of water, electrolytes, enzymes, epithelial and inflammatory cells, and high molecular weight O-linked glycoproteins referred to as mucins. The major gel-forming mucins expressed in human and rodent airways (MUC2, MUC5AC and MUC5B) are encoded by members of a large family of mucin genes (Rose 1992; Gendler and Spicer 1995; Jeffery and Li 1997). There are currently no published reports of equine homologues of these gel-forming mucin genes, although this is an area of active research. These mucin gene products are produced and secreted by mucous (goblet) secretory cells in the surface epithelium and subepithelial glands. It is believed that, in horses, the bulk of secreted mucins is derived from the surface epithelial secretory cells (Kaup *et al.* 1990b; Dixon 1992), whereas in man the subepithelial glands contribute a much larger fraction of total secreted mucins present in airway lumens. Increased amounts of airway mucin glycoprotein in 'heaves'-affected horses (Jefcoat *et al.* 1999) might be due to increased numbers of airway

epithelial mucous cells compared to normal horses (i.e. no change in production or secretion per cell), or it might be due to elevated production and secretion of mucins by the same number of mucous cells found in normal horses (i.e. increased production and secretion per cell). Although there are numerous studies reporting mucous cell hyperplasia, metaplasia and hypertrophy in the pulmonary airways of 'heaves'-affected horses (Kaup *et al.* 1990b; reviewed in Dixon 1992) there are no published reports in which image analysis and rigorous morphometric techniques have been used to quantify both the amounts of stored intraepithelial mucosubstances in the surface epithelium (i.e. volume density; V_s) and the numeric density (i.e. mucous cells/mm basal lamina) of mucous secretory cells in the same tissue in normal and 'heaves'-affected horses.

Accumulation of mucus in 'heaves'-affected horses might also result from a decrease in mucociliary clearance. Decreased clearance in 'heaves'-affected horses may, in turn, result from alterations in the ciliary apparatus or in the physical properties of the mucus gel (Dixon 1992; King and Rubin 1994). The effectiveness of mucociliary clearance in 'heaves'-affected horses is, however, controversial. While some investigators have reported decreased clearance in 'heaves'-affected animals (Coombs and Webbon 1987; Turgut and Sasse 1989), Willoughby *et al.* (1991) found no difference compared to healthy horses. Kaup *et al.* (1990a) reported a loss of ciliated cells and abnormal ciliary structure in large conducting airways of 'heaves'-affected horses which might affect mucus transport. Studies on excised pieces of trachea from healthy horses have shown that variations of ciliary beat frequency at physiological temperatures have only a minor effect on mucociliary clearance rate. In contrast, the physical properties of mucus are very important (Gerber *et al.* 1997).

Alterations in viscoelastic properties of mucus result in changes in its clearability by ciliary and cough mechanisms (King and Rubin 1994). A critical factor affecting the viscoelasticity of mucus is hydration, i.e. the relative content of solids and water (Shih *et al.* 1977; Tomkiewicz *et al.* 1993). Another important factor is the presence of inflammatory cells (i.e. neutrophils). High molecular weight DNA (Armstrong and White 1950) and filamentous actin (Vasconcellos *et al.* 1994) released by necrotic neutrophils increase mucus viscoelasticity in cystic fibrosis patients and possibly also in patients with chronic bronchitis and asthma. In these human inflammatory airway diseases, the altered rheologic properties of airway mucus are thought to be associated with the severity of disease (reviewed by Kim 1997). Gerber *et al.* (2000) have recently reported that, although there is no difference in the rheologic properties of tracheal mucus obtained from normal horses and 'heaves'-affected horses in clinical remission, tracheal mucus obtained from 'heaves'-affected horses during acute stable-induced exacerbation of their disease have significantly increased mucus viscoelasticity. The water content of the mucus was not associated with these unfavourable changes. Other causes such as the changes in oligosaccharide side chains of mucus that occur in 'heaves'-affected horses (Jefcoat *et al.* 2000) remain to be investigated.

Smooth muscle and bronchodilators

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Bronchodilators are useful drugs for the relief of the respiratory distress associated with acute exacerbations of 'heaves' (Murphy *et al.* 1980; Pearson and Riebold, 1989; Erichsen *et al.* 1994). During acute airway obstruction induced by stabling and feeding hay, bronchodilator drugs decrease pulmonary resistance (R_L), increase dynamic compliance (C_{dyn}) and decrease the maximal change in pleural pressure during tidal breathing ($\Delta P_{pl_{max}}$). Both anticholinergic drugs (Broadstone *et al.* 1988; Robinson *et al.* 1993; Duvivier *et al.* 1997) and β_2 -adrenergic agonists (Sasse and Hajer 1977; Erichsen *et al.* 1994; Tesarowski *et al.* 1994; Derksen *et al.* 1996, 1999) are effective, but methylxanthines (McKiernan *et al.* 1990) are less consistent. When aerosol β_2 -adrenergic agonists are administered to maximal effect, there is a 50–70% improvement in R_L and $\Delta P_{pl_{max}}$, but the effect on C_{dyn} is more variable (Derksen *et al.* 1996). Therefore, in horses with 'heaves', bronchospasm is responsible for most of the airway obstruction, particularly in the larger airways. Even when

much of the airway obstruction has been relieved by stabling horses in a low-dust environment, there is still some bronchospasm that can be treated by bronchodilator drugs (Jackson *et al.* 2000).

The bronchospasm of 'heaves' may have multiple causes. Serotonin (5-HT) (Doucet *et al.* 1990; Olszewski *et al.* 1999a,b), histamine (H) (Derksen *et al.* 1985a; Klein and Deegen, 1986; Doucet *et al.* 1990; Guthrie *et al.* 1992; Olszewski *et al.* 1997, 1999a,b) and leukotriene D_4 (LTD₄) (Doucet *et al.* 1990; Marr *et al.* 1998b; Olszewski *et al.* 1999a,b), mediators associated with acute exacerbations of 'heaves', can all increase tension in equine airway smooth muscle via activation of their specific muscle receptors. Their actual role in 'heaves' remains unknown because there are no studies of the effects of specific antagonists on airway obstruction. The similar bronchodilator efficacy of anticholinergic agents and β_2 -adrenergic agonists suggests that most of the bronchospasm is mediated through pathways that involve the muscarinic receptor. In this regard, histamine, 5-HT and LTD₄ increase the smooth muscle response to endogenous acetylcholine (ACh) released by activation of parasympathetic nerves (Olszewski *et al.* 1999b). Histamine and 5-HT also augment the release of ACh from such nerves (Olszewski *et al.* 1999b). There is no evidence of an increased response of airway smooth muscle to ACh (Broadstone *et al.* 1991; LeBlanc *et al.* 1991; Yu *et al.* 1994a) and no evidence that neutrophil products cause smooth muscle contraction either directly or by action on parasympathetic nerves (Olszewski *et al.* 1995, 1999b). There is evidence for and against a defect in the neuronal muscarinic autoreceptor that provides negative feedback control of ACh release (Wang *et al.* 1995; Zhang *et al.* 1999). Such a defect could lead to increased ACh release and bronchospasm.

Defective inhibitory control of airway smooth muscle could also participate in the aetiology of bronchospasm. Inhibition of equine airway smooth muscle contraction occurs via activation of β_2 -adrenoceptors (Broadstone *et al.* 1991; LeBlanc *et al.* 1991; Torneke *et al.* 1997, 1998), release of nitric oxide (NO) from inhibitory nonadrenergic noncholinergic (iNANC) nerves (Yu *et al.* 1994b) and release of inhibitory prostanoids such as PGE₂ from the airway mucosa (Yu *et al.* 1993). During acute 'heaves', iNANC-mediated relaxation is absent in bronchi (Broadstone *et al.* 1991; Yu *et al.* 1994a) and there is a shift in mucosal prostanoid production from inhibitory prostanoids such as PGE₂ to excitatory prostanoids (Yu *et al.* 1994a). There is no evidence for altered β_2 -adrenoceptor function in 'heaves' (Broadstone *et al.* 1991; LeBlanc *et al.* 1991).

The magnitude of airway narrowing that occurs in response to smooth muscle contraction is determined by the thickness of the smooth muscle layer and the width of the mucosal layer between the muscle and the airway lumen (Moreno *et al.* 1986). Thickening of the airway amplifies the airway lumen narrowing effect of bronchospasm. The remodelling of the airway wall (mucus cell hyperplasia and metaplasia) that occurs in 'heaves' could have such an effect so that a small degree of smooth muscle contraction resulting from the factors described above could result in major narrowing of the lumen.

Corticosteroid therapy

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The therapeutic emphasis for asthma in human patients has shifted from symptomatic bronchodilator therapy to daily anti-inflammatory therapy to prevent episodes of airway obstruction (Varner and Busse 1996). Inflammation is now recognised as the underlying pathophysiological process in all cases of bronchoconstriction, and daily anti-inflammatory therapy can eliminate the need for bronchodilator therapy. Corticosteroids are the only drug class to demonstrate sustained modification of disease in human asthmatic patients. Nonsteroidal anti-inflammatory drugs and antihistamines are ineffective for treatment of allergic inflammation of the lower respiratory tract and regular administration of β_2 agonists produces deterioration in pulmonary function, increased airway responsiveness and more frequent exacerbations of bronchoconstriction (Sears 1995).

Systemic administration of potent corticosteroids is effective for treatment of equine recurrent airway obstruction. Triamcinolone acetonide (0.09 mg/kg bwt i.m., single dose) relieves airway obstruction for up to 4 weeks; however, marked adrenal suppression is evident for 4 weeks following administration (LaPointe *et al.* 1993). Dexamethasone (0.1mg/kg bwt i.v. s.i.d) reduces airway obstruction and inflammation within 3 to 7 days and improvement persists for approximately 7 days (Rush *et al.* 1998a,b). Administration of dexamethasone produces marked suppression of endogenous cortisol production, which persists approximately 3 days after discontinuation of drug (Rush *et al.* 1998c). Oral prednisone (400 mg/day s.i.d) does not improve clinical signs of airway obstruction or reduce pulmonary inflammation in horses (Traub-Dargatz *et al.* 1992). Additionally, oral prednisone (2.2 mg/kg bwt) administered in conjunction with environmental management provides no additional benefit over environmental management alone (Jackson *et al.* 2000).

Aerosolised corticosteroids are also effective for horses with recurrent airway obstruction. Beclomethasone (500 to 1500 µg b.i.d., Equine Aerosol Drug Delivery System) reduces pulmonary inflammation (Rush *et al.* 1998a, 2001), improves pulmonary function (Rush *et al.* 1998b) and improves ventilation imaging of horses with recurrent airway obstruction (Rush *et al.* 1999a). There is no immediate drug effect; however, clinical signs and pulmonary function improve within 24 h of administration (Rush *et al.* 2001). Administration of beclomethasone (3,200 µg q. 12h) using the AeroMask, improves parameters of pulmonary function and arterial oxygen tension approximately 3 or 4 days after initiation of therapy (Ammann *et al.* 1998). Of the commercially available aerosolised corticosteroid preparations, fluticasone is the most potent, has the longest pulmonary residence time and the least potential for adrenal suppression (Varner and Busse 1996). Fluticasone (2000 µg q. 12 h, AeroMask, reduces pulmonary neutrophilia, improves pulmonary function and reduces airway hyperresponsiveness in 'heaves'-affected horses (Viel *et al.* 1999). Although aerosolised corticosteroids are effective for treatment of 'heaves', clinical signs return after drug withdrawal if the horse remains in an allergen-challenged environment.

Horses are more sensitive to the adenosuppressive effects of aerosolised corticosteroids than humans (Rush *et al.* 1998c, 1999b). Endogenous cortisol production is suppressed within days of beclomethasone (>1000 µg q. 12h) and fluticasone (2000 µg q. 12h) administration in horses, but recovers approximately 2 days after discontinuation of drug. The threshold for adrenal suppression in horses is approximately 500µg beclomethasone administered b.i.d. (Rush *et al.* 1999b). The therapeutic efficacy of 500 µg beclomethasone is equivalent to the efficacy of doses in excess of 1000 µg b.i.d. (Rush *et al.* 2001).

Combination therapy with bronchodilators and corticosteroids may be the most appropriate approach in horses with moderate to severe recurrent airway obstruction. The role of corticosteroid therapy is to reverse underlying inflammation and alleviate bronchoconstriction and excess mucus formation. Corticosteroids do not provide immediate relief of airway obstruction (Rush *et al.* 2001) therefore, β_2 -adrenergic bronchodilators remain first-line therapy for emergency relief of severe obstruction. β_2 -adrenergic agents do not address the underlying inflammatory process and tolerance develops rapidly. Corticosteroids prevent down-regulation of receptors and induce formation of new β -adrenergic receptors. Deterioration in pulmonary function with frequent β_2 -adrenergic use is less likely to occur in patients receiving concurrent corticosteroid therapy.

Future therapies for the treatment and/or prevention of 'heaves'

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Environmental management that decreases inhaled foreign particulate loads will always remain one of the most effective strategies for preventing or reducing the pulmonary inflammatory response of 'heaves' (Jackson *et al.* 1999). Nevertheless, effective environmental

control can be impossible to achieve for the performance animal, necessitating the use of medications.

For the immediate future, inhaled corticosteroids will probably remain the treatment of choice for the management of symptomatic horses or asymptomatic horses exposed to untoward environments. These agents, coupled with bronchodilators that improve medicament delivery to the pulmonary airways, will remain the most effective means of decreasing the pulmonary inflammatory response (Ammann *et al.* 1998; Rush *et al.* 1999b). However, successful management of affected horses will be dependent upon the universal availability of an easy-to-use delivery device (Tesarowski *et al.* 1994; Derksen *et al.* 1996) and a long-acting topical corticosteroid exhibiting a rapid onset of action and lacking systemic effects (Rush *et al.* 1999b; Viel *et al.* 1999). For usage in the performance animal, the absence of a demonstrable performance-enhancing effect will be required. Undoubtedly, continued research efforts will define the optimal treatment protocols required for both symptomatic and asymptomatic horses. Specific questions that need to be addressed in future longitudinal trials (Pedersen 2000) include: (1) does the time of initiation of therapy (relative to the onset of disease) have an impact on how successfully horses are managed in their later years?; (2) for what length of time should inhaled medications be administered after the remission of clinical signs? and (3) how should remission of disease be assessed - by clinical signs or bronchial hyperreactivity?

Other therapies, including the use of antileukotriene agents (receptor antagonists or 5-lipoxygenase inhibitors) may prove to be useful alternatives to corticosteroids. Experimental data regarding their efficacy in 'heaves'-affected horses are conflicting (Marr *et al.* 1998b; Lavoie *et al.* 2000) but may, in part, reflect phenotypic differences in the study populations as occurs in human asthmatics (Drazen and Israel 1998).

Finally, in the not too distant future, it is to be hoped that technological advances in human airway epithelium gene therapies will be applied to the therapeutic management of 'heaves'. It is probable that as the pivotal role of nuclear factor-kappa B (NF- κ B) transcription factor in the propagation of the pulmonary inflammatory response of 'heaves' becomes more completely understood (Bureau *et al.* 2000a,b), gene therapy trials targeting this transcription factor in horses will be initiated. Nuclear factor- κ B plays a crucial role in the activation of genes encoding for inflammatory cytokines, chemokines, adhesion factors and inflammatory enzymes. Down regulation of NF- κ B activity (and attenuation of the inflammatory process) could be achieved by (1) gene transfer of naturally occurring intracellular inhibitors, such as I κ B-alpha, or (2) gene transfer of oligonucleotide decoys that competitively bind NF- κ B (Makarov *et al.* 1997; Griesenback *et al.* 2000). Such a physiological approach would eliminate problems associated with medication usage in the performance animal.

Workshop conclusions

1. Equine inflammatory airway disease can have various causes and is observed in various degrees of severity. It is inappropriate to use one term, such as COPD, for all these syndromes.
2. New information in human and equine medicine has revealed major differences between human COPD and equine 'heaves' so that, at present, it is no longer appropriate to use the term COPD in equine medicine.
3. The term 'heaves' or recurrent airway obstruction (RAO) should be used for the mature horse with airway obstruction that is reversed by a change in environment or use of bronchodilators.
4. The workshop participants recognise the syndrome of nonseptic lower airway disease, particularly in young horses, and recommend the term inflammatory airway disease (IAD) be used until the aetiology and principal anatomical site of lower respiratory disease is better defined.
5. The relationship between IAD in young horses and 'heaves' is unknown. The former may not necessarily progress to the latter.
6. At present, there is no way to identify a young horse that may develop 'heaves' later in life.
7. In horses with 'heaves', morphological changes are primarily located in the small airways, but functional changes may occur throughout the tracheobronchial tree.

8. During exacerbations of 'heaves', the major cause of airway obstruction is bronchospasm.
9. In addition to bronchospasm, airway obstruction is also a result of accumulation of mucus and inflammatory exudates in the airway lumen and thickening of the airway wall.
10. During clinical remission in 'heaves'-affected horses, residual airway inflammation, obstruction or hyperresponsiveness may persist.
11. During exacerbations of 'heaves', the predominant inflammatory cell in the lumen of the airway is the neutrophil. Eosinophilic inflammation is not typical of 'heaves'. The presence of neutrophils does not imply a septic process.
12. Bacterial infection is not a primary component in the aetiopathogenesis of 'heaves'.
13. 'Heaves' exacerbations are caused by exposure to respirable organic dusts which may contain moulds, endotoxins and other proinflammatory agents. Organic dusts may be found indoors and outdoors.
14. There is accumulating evidence to support an allergic component in the aetiology of 'heaves'.
15. Other factors, such as inhaled endotoxin, may contribute to the inflammation of 'heaves'.
16. There is evidence that genetic factors play a role in the expression of 'heaves' as a clinical disease.
17. Because no relationship has been demonstrated between airway and dermal reactivity, the use of skin testing to identify culprit aeroallergens is not justified.
18. There is no scientific evidence demonstrating the efficacy of hyposensitisation in the treatment of 'heaves'.
19. There is currently no scientific evidence to support the use of serum antibody testing for the diagnosis and treatment of 'heaves'.
20. Environmental control is paramount for the management of 'heaves'.
21. In the majority of horses with 'heaves', corticosteroids are effective in reducing inflammation and in improving airway function and clinical signs. Corticosteroids do not provide immediate relief of bronchospasm.
22. During acute exacerbations of 'heaves', bronchodilators rapidly improve airway function and reduce the signs of respiratory distress. Bronchodilator therapy does not alleviate the underlying inflammatory process.
23. There was a lack of consensus in the assembled body regarding the use of drugs that affect the production and clearance of mucus and airway secretions for the treatment of 'heaves'.

Criteria for defining the 'heaves' phenotype

1. Demonstrated reversible lower airway obstruction induced by an environmental challenge, as documented by either lung function or a defensible clinical score (Ohnesorge *et al.* 1998a,b; Robinson *et al.* 2000).
2. Airway obstruction is reversible by use of a bronchodilator or by return to a nonchallenge environment.
3. An increase in BALF neutrophils during environmental challenge.
4. During environmental challenge, there is evidence of airway obstruction determined either by a ΔPpl_{max} greater than 15 cm H₂O (2 kPa) or by use of a validated clinical score. Airway obstruction must be accompanied by more than 25% neutrophils in BALF.
5. Before environmental challenge, control horses must have ΔPpl_{max} greater than 10 cm H₂O, or an equivalent validated clinical score, and less than 10% neutrophils in BALF. After environmental challenge, the control horses should have ΔPpl_{max} less than 10 cm H₂O.
6. Because the neutrophil count is sensitive to the collection method, BALF analysis is recommended for detecting pulmonary neutrophilia in horses with 'heaves'. These guidelines should be followed: use a sampling tube 10–13 mm in diameter; if sampling frequently, use an endoscope to sample different sites of the lung; if blind sampling by a BAL tube, allow 7 days between sample collection; the volume of lavage fluid should range from 250 to 500 ml. Dilutional effects may be problematic, but no standardised means of adjusting BALF constituents has been determined.

Recommendations for research

Diagnosis

1. Develop a standardised, internationally accepted clinical scoring system for equine lower airway disease.
2. Develop diagnostic techniques that are more sensitive for detection of the early stages of airway disease.
3. Develop field tests that aid in the diagnosis of lung disease in equine clinical practice.
4. Establish the predictive value of airway reactivity for the development of 'heaves'.
5. Improve usefulness of bronchoalveolar lavage by establishing criteria to distinguish a 'heaves'-susceptible horse in remission from age-matched control.

Therapy

1. Compare the efficiency of drug delivery devices currently available for aerosol therapy.
2. Compare the safety and efficacy of currently available corticosteroids.
3. Compare the safety and efficacy of currently available bronchodilators.
4. Develop a long-acting bronchodilator for aerosol administration.
5. Determine the safety and efficacy of long-term low-dose corticosteroid administration to prevent episodes of airway obstruction and improve athletic performance.
6. Determine the ideal timing of corticosteroid administration to maximise efficacy and minimise adrenal suppression.
7. Define the interactions between corticosteroids and β_2 -adrenergic agents during treatment of 'heaves'.
8. Investigate systemic and pulmonary immune function after administration of aerosolised corticosteroids.
9. Determine the most effective protocol(s) by which 'heaves'-susceptible horses can be managed to prevent the development of clinical signs when environmental management is not feasible.
10. Clarify the importance of leukotrienes in 'heaves'.
11. Examine the effects of inhibitors of chemokine formation or action, including anti-inflammatory cytokines such as IL-10, in 'heaves'-affected horses.
12. Examine the effects of drugs which decrease leucocyte activation and, thereby, mediator formation, in 'heaves'-affected horses.
13. Use selective inhibitors of the formation or action of inflammatory mediators implicated in 'heaves' to establish their significance in disease pathogenesis.
14. Determine to what extent inhibition of NF- κ B activity can prevent the pulmonary inflammatory response in 'heaves'.
15. Investigate local therapy to induce granulocyte apoptosis, to inhibit IL-1 β and TNF- α and NF- κ B activation and to induce anti-inflammatory cytokines.
16. Investigate antioxidant treatment by both local and systemic routes.
17. Investigate pharmacological targeting of the neutrophil to modify (a) recruitment to the lungs, (b) priming, (c) activation, (d) apoptosis or (e) clearance.

Pathogenesis

1. Determine the role of inhaled endotoxin in 'heaves' pathogenesis.
2. Quantify β -D-glucan in airborne stable dust and determine the response to inhalation of this agent.
3. Quantify and characterise proteases present in stable dust.
4. Investigate causes of differing prevalence of 'heaves' in different countries despite apparently similar poor stable hygiene.
5. Develop monoclonal anti-equine IgE antibodies to better characterise the role of allergy in 'heaves'.
6. Determine if pathophysiological/immunological parameters (bronchial hyperreactivity, histamine release, immunoglobulin E levels, cytokine profiles etc) are influenced by genetic factors in the horse.
7. Search for genetic markers for chronic airway disease by collecting

- DNA from well-characterised horses descending from clinically well-characterised parents with and without chronic airway disease.
8. Identify the key cytokines and chemokine involved in the modulation of airway inflammation and bronchoconstriction in 'heaves'.
 9. Determine if cytokine profiles in 'heaves'-affected horses correspond to the TH₂ dominant immunological state described in human asthmatics.
 10. Determine the cell populations within the lower airways that play a key role in the modulation of lower airway inflammation via cytokine production.
 11. Clarify the importance of leukotrienes in 'heaves'.
 12. Examine the effects of the modulation of selected cytokines and chemokines in 'heaves'-affected horses.
 13. Examine the effects of drugs that decrease leucocyte activation, and thereby mediator formation, in 'heaves'-affected horses.
 14. Use selective inhibitors of the formation or action of inflammatory mediators implicated in 'heaves' to establish their significance in disease pathogenesis.
 15. Determine in horses with 'heaves' if airway hyperreactivity is an acquired phenotype, and if it is reversible.
 16. Determine if the basis for airway hyperreactivity in horses with 'heaves' differs from that of horses without 'heaves'.
 17. Determine the role of bronchospasm in less severe forms of airway inflammatory disease.
 18. Investigate interactions between β_2 -adrenoceptors and cholinergically mediated smooth muscle contraction in 'heaves'-affected animals.
 19. Determine effects of airway wall remodelling on mechanics of airway narrowing and in airway hyperreactivity.
 20. Investigate the important mesenchymal remodelling events that occur in the small airways of 'heaves'-affected horses.
 21. Determine the agents in the stable environment that induce airway remodelling resulting in overproduction/hypersecretion of mucus in 'heaves'-susceptible horses.
 22. Determine the clinical and functional significance of excessive mucus accumulation in 'heaves'-affected horses and by what mechanisms it occurs.
 23. Investigate differential mucin gene expression and altered patterns of glycosylation of specific mucin gene products in 'heaves'-affected horses.
 24. Determine the role of inflammatory cells in the pathogenesis, persistence and exacerbation of mucus overproduction/hypersecretion in 'heaves'-affected horses.
 25. Investigate the cell signalling pathways involved in upregulation of mucin production and secretion in 'heaves'-affected horses.

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