



In: **Equine Respiratory Diseases**, P. Lekeux (Ed.)

Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Inflammatory Airway Disease (16-Aug-2002)

J. L. Hodgson and D. R. Hodgson

University Veterinary Centre, Faculty of Veterinary Science, University of Sydney, Werombi Rd, Camden, NSW, Australia

Glossary

BAL – Bronchoalveolar Lavage

BALF – Bronchoalveolar Lavage Fluid

cfu – colony forming unit

EIPH – Exercise-Induced Pulmonary Hemorrhage

IAD – Inflammatory Airway Disease

LRT – Lower Respiratory Tract

LRTI – Lower Respiratory Tract Inflammation

NIAD – Non-infectious Inflammatory Airway Disease

PAM – Pulmonary Alveolar Macrophage

RAO – Recurrent Airway Obstruction

SAD – Small Airway Disease

SAID – Small Airway Inflammatory Disease

TA – Tracheal Aspirate

Introduction

Respiratory disease continues to be a major problem for horse industries worldwide [1,2]. However, to date the focus of respiratory disease research has centred on diseases of older horses, particularly those suffering from recurrent airway obstruction (RAO) or heaves. In recent years it has become increasingly apparent that airway inflammation is also a common disorder of young racehorses [3-8] and that this inflammatory process is likely to impact upon the ability of these horses to achieve peak performance [9]. This has lead researchers in diverse geographical regions to investigate this syndrome and apply the broad term inflammatory airway disease (IAD) to young racehorses found to have lower airway inflammation [4,10].

A universally accepted definition of IAD does not exist. This partially stems from the fact that our present understanding of the etiology, pathogenesis, clinical signs, diagnosis and effect on performance of this syndrome remain controversial. As a consequence a number of terms have been used, at times interchangeably, to describe the presence of airway inflammation in young performance horses. These include small airway inflammatory disease (SAID) [11-13], lower respiratory tract inflammation (LRTI) [14], small airway disease (SAD) [15], non-infectious inflammatory airway disease (NIAD) [16], bronchiolitis, and allergic airway disease [17]. However, in the current context, the authors believe the term IAD is most appropriate. In this respect, the term has no implications with regard to the specific etiology (infectious versus non-infectious), the site of inflammation (large versus small airway) or the type of inflammatory cell (neutrophil, eosinophil or mast cell) involved. Recent research demonstrates that inflammation involving all of these alternatives occur in young racehorses and are currently being encompassed under the one term, inflammatory airway disease. Until we better define the syndrome of IAD it is likely that a variety of clinical entities that occur in young racehorses will be included within this one syndrome.

This chapter will review the literature on lower airway inflammation in young to middle aged racehorses. It will include data derived from studies investigating airway inflammation associated with both infectious and non-infectious causes. Therefore, we will not be adopting the definition of IAD proposed by the International Workshop on Equine Chronic Airway Disease in which IAD was defined as a **non-septic** airway disease in younger, athletic horses that does not have a clearly defined etiology [18]. As the role of infectious and non-infectious agents in the pathogenesis of IAD has not been

definitively determined, we suggest discussion of both causes should be included until this matter is resolved. In some cases, the term IAD has been applied *post hoc* to studies of inflammatory disorders of the lower respiratory tract in young racehorses, but which the study may have referred to as RAO or chronic obstructive pulmonary disease (COPD - the older term applied to RAO). This review will not contain data on the syndrome of mild airway inflammation reported commonly in middle-aged or older horses from the Northern Hemisphere that is referred to as mild or sub-clinical COPD, bronchitis or chronic bronchiolitis. However, the possible relationships between IAD and these syndromes will be discussed.

Prevalence

Until a strict definition of IAD is determined and adhered to, the true prevalence of this syndrome will vary according to the individual researcher's case definition [17]. However, it is apparent that regardless of the definition, or whether it causes overt signs of respiratory disease, airway inflammation is common in young, performance horses [3-7,9]. Furthermore, respiratory disease has been found to be second only to musculoskeletal disease as the most common cause of poor performance, interruption of training, days lost to training, and premature retirement among racehorses in the UK and Australia [1,2]. The resultant economic losses attributable to IAD are likely to be considerable, and originate from the costs attributable to poor performance, days lost to training, as well as the direct cost of veterinary fees, treatment and control measures.

To date, studies on the prevalence of airway inflammation in young performance horses have defined IAD as either the presence of increased amounts of mucopus within the trachea (as observed via endoscopy), or the presence of increased neutrophils within tracheal washes (as identified by cytological evaluation). On the basis of these definitions airway inflammation occurs in 11.3 to 50% of Thoroughbred and Standardbred racehorses [3-7,9].

Although these values are often cited in discussions of the prevalence of IAD, care must be taken in their interpretation due to the definition of airway inflammation adopted in some studies. For example, MacNamara and co-workers [9] examined 965 Standardbred racehorses in race training using endoscopy and found that 22% had evidence of increased mucopurulent exudate within the trachea or tracheal bifurcation. Similarly, Burrell [3] found that 50% of the 118 Thoroughbred horses examined had evidence of increased amounts of tracheal mucus. In both of these studies it was concluded that these horses had airway inflammation. However, cytological evaluation for the presence of inflammatory cells was not performed on samples in either study. Since it may be difficult to distinguish between excess mucus (with no inflammatory cells) and mucopurulent exudates based on gross observation [19], these studies may have included horses with increased amounts of mucus, but not increased numbers of inflammatory cells as has been reported to occur [19]. Therefore the prevalence of IAD may have been overestimated in these studies.

Studies on prevalence of IAD using cytological evidence of inflammation have focused on samples obtained by tracheal aspiration (TA). Therefore, the location of the inflammation (large or small airway) cannot be defined. Sweeney and co-workers found that 27% of young Thoroughbred racehorses with no overt signs of respiratory tract disease could be characterized as having IAD as there were >20% neutrophils in their TA [4]. Similarly, Burrell and co-workers [5] showed that lower respiratory tract disease, as evidenced by increased amounts of mucopus visible by endoscopic examination of the distal trachea and by a neutrophilic response evident in TA, was more common than other more clinically apparent forms of respiratory disease such as nasal discharge, pyrexia and coughing. These investigators concluded that this group of horses spent as much as 33% of their time in training experiencing a degree of lower airway disease that could have impaired their performance.

In a longitudinal epidemiological study in the UK, the prevalence of IAD was 13.8%, and the incidence was 8.9 cases per 100 horses per month [7]. In this study the mean duration of disease was 7.8 weeks, which is similar to the 7 weeks estimated by Burrell [5], but shorter than the 3 months in Standardbred racehorses reported by Rush [10]. The prevalence and incidence of disease reported in the UK study, however, varied widely between training yards. In another study in the UK, a total of 1,235 TAs were taken from 724, two to ten year-old Thoroughbreds in race training [6]. Of the samples evaluated, 11.3% were classified as inflammatory, but this was as high as 18.8% if individual age groups were examined (2 year olds). Finally, in a recent study in Australia, the prevalence of IAD was determined after horses entered racing stables (Malikides, personal communication, 2002). Thoroughbred racehorses showing no evidence of airway inflammation (<20% neutrophils in a TA) upon entry to a racing stable were subsequently evaluated at two and four weeks post entry. Of the 165 horses evaluated, 41% developed evidence of airway inflammation as defined by >20% neutrophils within two weeks of stabling and half of these horses still had evidence of airway inflammation by day 28. It is not known whether the inflammation in these horses was continually present during the four weeks, or resolved and then recurred before the second sampling.

Risk Factors

A number of management and training practices are purported to predispose racehorses to the development of lower airway inflammation. For example, high-intensity exercise, transportation, co-mingling with other horses, exercise induced pulmonary hemorrhage (EIPH) [20], housing in stables, and age [21] are thought to influence the development of lower airway infection and/or inflammation. However, only a few epidemiological studies have been performed to analyse the influence of each of these factors in a population of racehorses [5-7,21,22].

Racing, training or intense exercise may increase the risk of developing lower airway inflammation [21]. Strenuous exercise impairs pulmonary macrophage function, alters peripheral lymphocyte function [20], increases the concentration of cortisol in serum for up to 24 hours [23] and promotes inhalation of dust particles and infectious agents deep into the lower respiratory tract. Alternatively, intense exercise in cold to temperate climates may allow unconditioned air to gain access to the lower airways [24]. Thus exercise allows the introduction of potentially inflammatory particles into the lungs whilst decreasing the host's ability to eliminate these agents. In a study examining the risk factors associated with coughing, horses that raced recently were found to be at increased risk [21]. This study also demonstrated that coughing was a specific indicator of lower airway inflammation, and therefore recent racing is also likely to be a risk factor for IAD.

Long distance transport depresses pulmonary macrophage function and promotes deep inhalation of dust [20]. In addition, if horses are confined for long periods with their heads elevated, as may occur during long distance transportation, a resultant accumulation of significant numbers of bacteria, inflammatory cells and mucus can occur within 6 to 12 hours [25,26]. Although these large numbers of bacteria and inflammatory cells are cleared within 8 to 12 hours after horses are released from confinement [26], they could pose a risk factor if superimposed on concomitant airway pathology or followed by strenuous exercise.

Co-mingling of horses allows frequent exposure to bacterial or viral respiratory pathogens [3,5,8,22,27,28]. Viral pathogens can damage mucociliary clearance mechanisms, destroy bronchial-associated lymphoid tissue, and impair pulmonary macrophage function [20]. This impairment of pulmonary clearance may predispose to bacterial colonisation, or alternatively decrease clearance of other pro-inflammatory agents deposited in the lower airways. Viral infections have been identified as potential risk factors for IAD [11].

The presence of blood in the pulmonary parenchyma, as occurs with EIPH, can initiate an inflammatory response as it is being cleared [29]. The ensuing airway inflammation may result in more fragile airways and may also lead to fibrosis [30]. Both of these effects may potentially predispose the horse to further pulmonary hemorrhage with exercise. Therefore, repeated episodes of EIPH could create a self-perpetuating cycle of lower respiratory tract inflammation and hemorrhage [11,30]. This may be reflected in the fact that EIPH often worsens with repeated exercise and age [30]. However, in a large study of racehorses in the UK, EIPH did not appear to be a risk factor for airway inflammation [6]. This result may reflect the method of diagnosis, TA, as compared to bronchoalveolar lavage (BAL), which is a more sensitive technique for detection of horses with EIPH [31].

There is conflicting information regarding the influence of age on the risk of development of IAD. Several studies have found that younger horses are more at risk of respiratory tract inflammation [5,6,21,22]. This may be influenced by the fact that younger racehorses are more at risk of acquiring bacterial [7,32] and viral [33] infections and the decreased risk with increasing age is a result of developing immunity. Alternatively, horses may develop tolerance to the etiological agents causing IAD [21]. In a study that examined the presence of increased mucus and neutrophils in TAs, the risk of lower airway disease was greatest in young horses (two-year olds and four-year olds), with a decreasing risk with age [6]. Similarly, studies in Australia and the UK found that the risk of coughing or airway inflammation decreased with age [7,21] and that two-year-old horses were about 7 times more likely to be affected than were three-year olds [5]. In contrast, a study in the US found an increasing risk of IAD in horses with increased age, where horses with normal BAL cytology had a mean average age of 5.3 years and the mean average age of horses with IAD was 8.5 years [34]. This difference in the effect of age may be explained by the different methods for determining the presence of IAD. In the studies in which younger horses were found to be more likely to develop IAD, samples were collected using TA, whereas in studies where older horses were more likely to develop IAD samples were collected using BAL. This may reflect the influence of age on the type of inflammation present (large versus small airway) and/or the etiological agents involved in each of these conditions.

The stable environment has been implicated as a risk factor for development of respiratory disease in young horses [4,11,21,35]. The cleanliness of the air in a stable may affect the incidence and severity of respiratory disease by affecting

the degree of challenge by micro-organisms, allergens or airway irritants, by affecting the rate at which pathogens are cleared from the respiratory tract, or by influencing a horse's local or systemic resistance. Although many studies have demonstrated that the stable environment plays a significant role in the development of chronic respiratory disease (e.g., RAO) in older horses, far fewer studies have examined the influence of this environment in younger racehorses. Studies in the UK found a positive influence of poor ventilation and bedding type on the development of IAD in racehorses [5,36]. In contrast, a study in Australia found that the risk of development of IAD decreased with the length of time in training, and therefore the length of time in a stable environment [21]. This finding may be explained by development of tolerance to airborne irritants such as endotoxin as has been demonstrated in humans [37].

Etiology

There has been considerable debate in recent literature regarding the pathogenesis of IAD and the exact etiological agents contributing to its development. Although there have been many proposed etiological causes, few of these have been substantiated by experimental or epidemiological studies. Furthermore, a wide variety of clinical entities are currently encompassed within the term IAD and include inflammation from different sites within the lower respiratory tract (large and small airways), inflammation characterized by different cell populations (neutrophil, eosinophil, mast cell, lymphocyte) and inflammation reported in horses of various ages undertaking diverse athletic pursuits. It is plausible that a variety of etiological agents are causing these different forms of airway inflammation. It is also possible that several agents may be required to act in concert for the development of IAD, and the temporal relationship between causes must be taken into consideration when discussing the pathogenesis of airway inflammation. Finally, it is unlikely that a single etiological factor is responsible for all cases of IAD [14]. Therefore, it is important to recognise the relative contribution that each etiological agent makes toward the overall prevalence of IAD and that this contribution is likely to vary considerably from country to country and even region to region.

In general, the proposed etiological agents fall into two categories, infectious and non-infectious. There has been much deliberation regarding the relative role of each of the two categories and whether inflammation associated with either of these categories should be included within the syndrome of IAD. However, as stated previously, the roles of infectious and non-infectious agents in the pathogenesis of IAD have not been definitively determined, and until they are a discussion of both causes should be included. Furthermore, a temporal relationship may exist between these two categories, and both may be necessary for development of clinically significant disease. Effective prevention and treatment of this syndrome will be impossible until the underlying causes and their interrelationships are elucidated. The putative etiological agents that have been proposed are summarised in Fig 1.

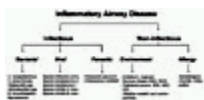


Figure 1. Proposed etiological agents of lower airway inflammation in young racehorses (IAD). - To view this image in full size go to the IVIS website at www.ivis.org . -

Respiratory Viruses - Respiratory viral infections are well-established causes of airway inflammation in horses, including young performance horses. The viruses most commonly associated with airway inflammation include equine influenza virus [38], equine herpesvirus type 4 and less commonly type 1 [39], and equine rhinitis A and B viruses [40] (formerly equine rhinovirus types 1 and 2).

Infections with equine herpesviruses types 4 and 1 (EHV-4, EHV-1) are primarily reported in foals or weanlings, but outbreaks in older horses in race training may occur [39]. These viruses are predominantly associated with pathology of the upper airways rather than the lower respiratory tract [39], but extension into the more distal pulmonary tree to cause tracheobronchitis, bronchiolitis and/or pneumonitis has been recorded [41-43]. In addition, outbreaks of EHV in horses in training yards have been associated with increased neutrophils in TAs in the absence of mucopus [44]. Herpesvirus infections may also predispose horses to opportunistic bacterial infections of the upper and lower respiratory tracts through either direct herpesvirus-inflicted damage to the protective respiratory mucosal barrier [39], decreased mucociliary clearance rates [45], or due to immunosuppressive effects [46]. These non-specific immunosuppressive effects can be detected for long periods after EHV-1 infection [46] and may explain why EHV infection preceded the occurrence of lower airway inflammation by 4 - 6 weeks in racehorses in one study [5]. However, these authors also warn that these events may be unrelated and this apparent sequence of events may be coincidental.

In contrast to EHV-4 infection, equine influenza virus commonly induces severe lower as well as upper respiratory tract disease, and is often accompanied by serious secondary bacterial infection of the lower airways [44]. This difference

between the effects of EHV and equine influenza virus may be related to the significant and prolonged impairment of tracheal mucociliary clearance that occurs after influenza but not EHV infection [5,45]. Influenza virus also can cause airway hyper-reactivity in horses, which may persist for at least 4 weeks following initial signs of clinical infection [47].

For many years most respiratory disease, and much loss of performance, in racehorses was attributed by owners, trainers and veterinary surgeons alike to viral infections, or "the virus" [48]. However, recent studies have questioned the role of viral infections in IAD and have revealed that most cases of lower airway inflammation occurring in young performance horses cannot be attributed to known equine respiratory viruses [5,7,8,22,44,49]. The viruses investigated in these studies included equine influenza virus, EHV-1, EHV-4, equine rhinitis A and B viruses, and equine adenovirus. No clear association was found with seroconversion to any of these viruses and signs of respiratory tract disease (coughing, nasal discharge or pyrexia) or cytological evidence of airway inflammation. The exception was equine influenza virus, which was strongly associated with acute disease in one study [22], but infection with this virus was also found to be uncommon. Furthermore, the prevalence of IAD in Australian racehorses (which are free of equine influenza virus infection) is similar to that reported in the UK and USA [8] and lends credence to the hypothesis that influenza is not a major contributor to IAD in young racehorses. These findings have important implications in the therapeutic and management procedures that should be recommended for control of IAD.

Although there is currently little evidence to suggest that a direct relationship between viral infections and IAD is common, the longitudinal relationship between viral infection and development of persistent IAD requires further investigation. For example, respiratory viruses cause alterations in the normal respiratory defense mechanisms of horses through decreased mucociliary clearance, inhibition of alveolar macrophage function [20] and may cause an increase in concentrations of IgA and IgE to mould allergens [50]. These alterations may in turn predispose to the development of bacterial infections or persistent, low-grade airway inflammation, which in turn increases the sensitivity of the airways to dust, moulds, pollens, and other irritants [14,27,28,51]. This may explain reports of prolonged airway hyper-reactivity following viral infection. Longitudinal epidemiological studies are required to better define these relationships.

Bacteria - In contrast to respiratory viral infections, a number of recent studies have shown that clinical or sub-clinical bacterial infections may be important contributors to IAD in young racehorses [3,5-8,22,32,44]. In these studies the presence of airway inflammation was evaluated by endoscopic evaluation of tracheal mucus and/or collection of TAs using methods to by-pass upper respiratory tract bacterial flora. In order to determine the significance of the bacteria isolated, the studies investigated a statistical association between the number of bacterial colony forming units (cfu's), clinical signs of respiratory tract disease and the presence of lower airway inflammation. Lower airway inflammation was defined as either increased amounts of mucus or the presence of a relative increase in the number of neutrophils. A number of studies further defined inflammation on the basis of a combined inflammation score [52] that was determined on the basis of numbers of nucleated cells, the relative number of neutrophils, and the amount of visible mucus on endoscopy [5,6].

The bacteria found to have a statistically significant association with lower airway inflammation in racehorses include *Streptococcus zooepidemicus*, *S. pneumoniae*, members of the Pasteurellaceae (including *Actinobacillus* spp), and *Bordetella bronchiseptica* [5,6,22,32,44,53]. Furthermore, the likelihood of culturing bacteria does not differ between age groups of racehorses [6], indicating that bacteria may play a role in airway inflammation observed in horses of a wide age range (two to ten years). Although these data may be interpreted to suggest that bacteria are involved in the pathogenesis of lower airway inflammation in young horses, the role of bacteria as primary pathogens remains controversial. Both *S. pneumoniae* [54] and *S. zooepidemicus* [55] have been shown experimentally to be primary pathogens, but definitive proof of their primary role in IAD does not exist. Furthermore, the role of other causes of airway inflammation, such as environmental agents and respiratory viruses, require better definition as they may predispose horses to bacterial infections, and by themselves also play an important contributory role to IAD [51].

The role of anaerobic bacteria in IAD is less clear. Although it is well documented that this group of bacteria play an important role in pleuropneumonia [56-58], their isolation from horses with IAD is rare. It is purported that anaerobic bacteria, particularly *Fusobacterium* spp, may play a role in IAD [5], but need damage produced by other causes of lower respiratory tract disease before they can colonise the lower respiratory tract. In addition, anaerobic bacteria were only isolated from cases involving older horses in one study [6] and were not isolated from any horses with IAD in another study [8].

Recent studies have demonstrated a role for Mycoplasma infections in IAD, particularly *M. felis* and *M. equirhinis* [7,22,44,59,60]. Analyses have shown that although they are relatively uncommon infections, when they do occur they are strongly associated with acute disease [22]. Furthermore, if large numbers of conventional bacteria are present in the equine lung, it is likely that mycoplasma infections will be present concurrently [59], and may reflect a synergistic relationship between infections with mycoplasma and conventional bacteria [5]. However, a study in Australia failed to isolate Mycoplasma in any case of IAD and there was no association between isolation of these bacteria and the presence of coughing [8]. This may reflect regional differences in the prevalence of different agents as causes of IAD.

The role of bacteria in IAD is, however, contentious. Studies investigating bacteria in the development of IAD have focused on the association between clinical signs of respiratory disease such as coughing and/or nasal discharge, the presence of airway inflammation, and isolation of significant numbers of bacteria from TAs. The authors of these studies have interpreted a strong association between these events as evidence for a role of bacteria in the pathogenesis of IAD. However, it is important to note that there is not a direct relationship between association and causation and that the increased numbers of bacteria observed in horses with airway inflammation may merely reflect impaired airway clearance rather than a causative role for the bacteria [17]. In addition, there are a number of studies that suggest bacterial infections are not common in young horses [15,61], and that although bacteria may be observed on cytological smears or isolated in low numbers in cultures, these isolates represent normal flora of the upper airways or temporary contaminants of the lower airways. However, results will have been influenced by the method of sampling used. In these studies samples were collected by BAL, which samples the distal, smaller airways and most commonly the caudo-dorsal regions of the lung [13]. Furthermore this technique is less sensitive for diagnosis of bacterial pneumonia [62]. The airway inflammation reported in these studies largely involved small airways with a diameter less than 0.05 cm, rather than the larger bronchi. Thus it is likely that bacteria do not feature in airway inflammation in which smaller airways or caudo-dorsal lung regions are predominantly involved, and as such this form of IAD is likely to have another inciting cause.

The epidemiological studies that incriminate bacteria in the pathogenesis of IAD have focused on samples obtained by TA, and therefore likely represent inflammation of the larger airways or with a more cranio-ventral distribution. There are a number of details reported in these studies to suggest that a causal relationship between this form of lower airway inflammation and the isolation of significant numbers of bacteria ($>10^3$ cfu/ml). First, there is a correlation between the risk of airway disease and the number of bacteria isolated (cfu/ml)[5, 8]. In addition, there is a large increase in the likelihood (risk) of airway disease when comparing isolation of $10^2 - 10^3$ cfu/ml and $10^3 - 10^4$ cfu/ml, where isolation of $10^3 - 10^4$ cfu/ml was between 10 to 20 times more likely to be associated with disease [8]. This is compatible with a biological effect of $>10^3$ cfu/ml bacteria. Second, the bacterial species associated with IAD in these studies are known to cause bacterial pneumonia in horses [25,54,56,63-65]. In contrast, organisms such as *Staphylococcus* spp and *Corynebacterium* spp, which are common isolates from the pharynx [66] yet infrequent causes of respiratory infections [56], were not associated with airway inflammation in any of the studies on IAD. Finally, disease was associated with identification of intracellular bacteria, but not extracellular bacteria or the absence of bacteria on cytology in one study [8], and represents active phagocytosis of bacteria as part of an inflammatory response to these organisms. Together these findings suggest that bacteria have a role in the pathogenesis of some forms of IAD in young racehorses.

The relative contribution of bacterial infections to the prevalence of IAD has been investigated in several studies in the UK and Australia. One large study in which a total of 1,235 tracheal aspirates were obtained from 724 Thoroughbred horses in race training, demonstrated that about 60% of samples with cytological evidence of lower airway inflammation contained significant numbers of bacteria [6]. Furthermore, a case control study in Australia determined that 42% of cases of coughing in young racehorses had significant numbers of bacteria in the lower airway [67]. Finally, a longitudinal epidemiological study in the UK concluded that bacteria were responsible for most of the airway inflammation detected in young Thoroughbreds in race training [7].

Parasites - Given the routine management procedures carried out in racing establishments, parasites are unlikely to be a common cause of bronchitis and pneumonitis in young racehorses. However, moderate to marked numbers of eosinophils may be found in TAs and BAL fluid (BALF) of racehorses [15,68-70] and may be due to either pulmonary parasites or a hypersensitivity reaction [52,68,71-73]. The most likely parasite involved in pulmonary disease of young racehorses is *Parascaris equorum*, with *Dictyocaulus arnfieldi* being a possible, but much less likely cause [74]. Often it is difficult to arrive at a definitive diagnosis in cases of suspected parasitic infestation of the lower airways, but a history of a poor anthelmintic regimen, or close association with donkeys may provide clues for the involvement of parasites. In addition, care must be taken not to rule out involvement of *P. equorum* due to a negative result for fecal floatation as the prepatent

period for this parasite is 71 to 110 days, but pulmonary migration occurs 7 to 14 days after ingestion of infective larvae [74].

Environmental Agents - The environment in which racehorses are housed and the management procedures to which they are exposed is frequently at odds with the requirement of optimal lung function during maximal performance. Inhalation of a variety of different non-infectious agents has been proposed to cause IAD and many of these agents are found in the stable and training environment (Fig. 2).



Figure 2. Dust in the environment of racehorses during training (Image courtesy of Prof. W.M. Bayly). - To view this image in full size go to the IVIS website at www.ivis.org . -

The airborne dust in equine stables contains a range of organic and inorganic compounds which include bacteria, viruses, moulds, mite debris and their feces, plant material, bacterial endotoxins, β -glucans and inorganic dusts [75,76]. Furthermore, the dust found in stables has a variable composition depending on the source and conditions under which the bedding and feed materials were grown, harvested, and stored [51]. These airborne compounds are potentially able to induce airway inflammation either by initiating infection, by inducing allergy, by direct toxicity, or indirectly by overwhelming pulmonary defense mechanisms [75]. The association between chronic respiratory tract disease in older horses (heaves) and dusty and mouldy hay or bedding is well established [77], and inhalation of respirable dusts is also thought to induce airway inflammation in younger horses [3-5,11,15,51,70,78,79].

When horses are housed in stables, they are likely to be exposed continuously to high concentrations of dust [51]. The burden of dust to which the horse is exposed can be divided into total and respirable dust fractions. The respirable fraction is the portion that will enter the airways within the thorax during inhalation [75] and is considered to be a reliable index of the respiratory hazard associated with dust inhalation. Dust concentrations measured in the corridors of horse barns are frequently below $3\text{mg}/\text{m}^3$ [51], but which approach recommended air hygiene standards for short-term dust exposure in cotton mill workers ($<2.5\text{mg}/\text{m}^3$)[51]. However, the dust concentrations measured in a corridor do not necessarily reflect the airborne dust concentrations measured in the stable, particularly when mucking out, or in the vicinity of the breathing zone of the horse (i.e., close to the nostril). As horses spend considerable periods with their muzzles in close contact with the feed and bedding, dust concentrations in the breathing zone more accurately represents the respiratory challenge encountered by the horse [75] and may be 20-fold higher than in the stall or corridor [80]. Total dust concentrations during mucking out may reach $10 - 15\text{mg}/\text{m}^3$, of which 20 - 60% may be respirable particles [75]. Total dust concentrations in the breathing zone vary with the type of feed and bedding and range from less than 1 to as high as $17.5\text{mg}/\text{m}^3$ of air, whilst respirable dust varies from less than 1 up to $9\text{mg}/\text{m}^3$ [80-82]. Dust concentrations of $10\text{mg}/\text{m}^3$ are known to be associated with a high prevalence of bronchitis in humans [83].

It is important to note that different sources of feed and bedding can have a major impact on the concentrations of total and respirable dust that are present in a stable. Therefore, feed and bedding quality and airflow through stables are important considerations when approaching the control of lower airway inflammation in stabled horses.

A number of investigations have examined the effects of environmental conditions on the development of lower airway disease in young horses [3,5,35,36]. One study found that while the incidence of lower respiratory tract disease did not increase in racehorses housed in poor environmental conditions in comparison with those housed in better conditions [3], the mean duration of respiratory disease in affected horses was longer when in poor stable environments. In contrast, a second study found that racehorses housed on straw in loose boxes were twice as likely to suffer from lower airway disease than those housed on shredded paper in American barns, with the former group also recovering more slowly from respiratory disease [5]. It was suggested that healthy horses may be able to tolerate considerable variations in environmental conditions, but the presence of dust and other environmental contaminants can substantially extend the convalescent period once horses are affected with respiratory disease [5]. Poor stable conditions with little natural ventilation also caused an increase incidence and severity of mucopus within tracheas of Thoroughbred horses as compared to horses stabled in boxes that were well ventilated [36]. Furthermore, this study found heavy fungal and actinomycete contamination of wood shavings in the heavily insulated, poorly ventilated housing, but not in the shavings of better ventilated boxes. These agents are well-documented causes of heaves in older horses [84] and may also play a role in the pathogenesis of IAD [51]. Finally, a study of young Arabian horses in either a stable or pasture environment demonstrated that stabling on straw bedding for three

months induced lower airway inflammation [35] as evidenced by significant increases in the total number of cells, the number of neutrophils, as well as the percent neutrophils and lymphocytes in BALF [35]. These studies indicate that a stable environment alone is capable of inducing lower airway inflammation in young horses.

No studies have linked specific agents within respirable dust to the pathogenesis of IAD. However, a number of different agents have been implicated, either because of their role in the development of chronic lower airway inflammation in older horses (RAO and chronic bronchiolitis) or their ability to induce airway inflammation in other species. For example, concentrations of endotoxin have been recorded in stable environments that are known to be associated with airway inflammation and hyper-reactivity in humans and horses [81,85] and could play a role in the development of IAD either directly or in association with respiratory allergens [86]. In addition, short duration experimental inhalation challenges with endotoxin induced a dose-dependent neutrophilic inflammation in horses that did not have heaves or other detectable pre-existing pulmonary disease [86]. Ozone alone can induce chronic bronchiolitis in humans with increases in BALF neutrophil numbers [11] and it has been suggested ozone may play a role in development of airway inflammation in racehorses housed in major capital cities [11,17,87]. However, horses are apparently less susceptible to the acute effects of ozone compared to humans or laboratory animals [24] and it is unlikely that ozone is a significant risk factor for the development of IAD in otherwise normal horses [24]. The ability of ozone to act either synergistically with other agents or with other pre-existing disease processes cannot be excluded [24]. Other agents implicated in the possible development of IAD in young racehorses include noxious gases such as ammonia and H₂S, other environmental pollutants (SO₂, NO₂, CO) or deep inhalation of small and ultrafine metallic and carbon particles [11,24,88] which can deposit on airway epithelium and initiate the production of cytokines contributing to an inflammatory response.

In addition to a direct role of dust and its various components in the induction of IAD, dust may also act in combination with other etiological agents. For example, acute respiratory diseases such as viral infections may result in airway hyper-reactivity, which is characterized by bronchoconstriction in response to otherwise innocuous concentrations of airway irritants such as inert dusts [51]. Thus, while the concentration of respirable dust found in stables may be tolerated by normal horses, in horses with airway hyper-reactivity caused by viral infections or other causes of inflammation, these dust concentrations may result in clinical signs of exercise intolerance, coughing and nasal discharge [51].

The relative contribution of non-infectious causes to IAD is controversial. Some authors maintain that all IAD are non-infectious, and that IAD is vanguard for the development of heaves, which is initiated by inhalation of organic dusts [15]. Other studies in the UK and Australia have also found a significant proportion of cases of IAD to be associated with cultivation of no bacteria from TAs and no evidence of concurrent seroconversion to known respiratory viruses. The percentage of cases of IAD suggested to be from non-infectious causes has varied from 35% in one study [6] to 58% in another [8]. Furthermore, in a longitudinal study in which development of IAD in stabled racehorses was investigated over 4 weeks, less than 10% of horses that developed cytological evidence of airway inflammation had significant numbers of bacteria cultured from TAs (Malikides, personal communication, 2002). However, it is known from other studies that bacteria are commonly associated with airway inflammation [3,5-8,22,32,44]. These findings indicate that non-infectious causes may be important initiating factors in the development of IAD, allowing subsequent bacterial colonisation of the more distal airways. The mechanism of this facilitation has not been elucidated.

Exercise and Unconditioned Air - Exercise alone can result in non-specific airway inflammation. One study found horses undergoing 10 weeks of training had more airway inflammation than non-exercised stable mates [31]. The cause of this inflammation was not documented, but exercise may cause increased deposition of irritant particles in the distal airways associated with the 20-fold or greater increase in minute ventilation that occurs during strenuous exercise. Strenuous exercise also impairs pulmonary macrophage function, alters peripheral lymphocyte function [14], and increases the concentration of cortisol in serum for up to 24 hours [23] all of which may help predispose to infection or inflammation.

Alternatively, exposure of the lower airways to unconditioned air, as may occur in cold climates, may cause airway inflammation as has been demonstrated in humans and laboratory animals [24]. It has been suggested that even in relatively moderate conditions (4°C and exercise at only 6 - 8 m/sec) unconditioned air may penetrate into the periphery of the equine lung during exercise [24]. Inhalation of unconditioned air causes reflex bronchoconstriction and mucosal sloughing in horses [24] that may induce airway inflammation. Therefore, hyperventilation, particularly with very cold air, may be a potential cause of inflammation of the lower airways [24].

Allergens - Other than for heaves, there is no direct evidence for a role of allergens in the pathogenesis of equine lower airway diseases [89] including IAD. However, there is much speculation that IAD is a precursor for the development of heaves and the underlying pathogenesis may be similar [15]. Heaves is probably initiated by several factors acting in concert and includes aeroallergens, endotoxin, and small particles and fibers [77]. Elevated concentrations of specific IgE in BALF [50] favour the hypothesis that heaves is initiated by an allergic response to thermophilic moulds and actinomycetes such as *Aspergillus fumigatus* and *Faenia rectivirgula*. In addition, horses with heaves manifest a Th2-type cytokine response [90] and environmental challenge can produce consistent exacerbation of disease [84]. Similar responses have not been demonstrated in horses with IAD. However, the presence of elevated numbers of eosinophils [15,68,70] or mast cells [15,70,91] in BALF of horses with poor performance, and the association of BALF mastocytosis with airway hyper-reactivity is indicative of a possible role of an allergic response in the pathogenesis of IAD [92,93]. This is supported by immunohistochemistry studies showing more IgA-containing cells and occasionally increased numbers of IgM and IgG-containing cells in the airways of horses with IAD [17].

Clinical Signs

The clinical signs of IAD in young performance horses are likely to represent the manifestation of several pathophysiological processes [14] and may differ depending on the site of inflammation, the etiological agent involved and the degree and type of inflammation present. Few studies have determined the specific clinical signs associated with the different presentations of IAD.

Clinically, IAD may be similar to many other respiratory conditions. Furthermore, clinical signs are often subtle and may be easily missed or overlooked by the trainer, horse owner and veterinarian [94]. Affected horses are typically afebrile, bright and responsive and have a normal appetite [14]. Auscultable pulmonary abnormalities are rarely present [20], though abnormal breath sounds (e.g., wheezes) and mildly increased respiratory efforts may be present on rare occasions [94]. When a re-breathing bag is used to facilitate auscultation of lung sounds, a cough may be elicited [16]. This hyper-reactivity of the airways may also be detected upon palpation of the trachea [94]. A slight abdominal lift on expiration may be observed intermittently [16] and a mild elevation in respiratory rate may be present [16]. However, the absence of bronchospasm and airway obstruction resulting in an overt and prominent increase in breathing effort distinguishes this syndrome from RAO or heaves [51,89]. Furthermore, clinical signs that can be reliably detected using scoring systems are characteristic of the horse with RAO, and not IAD [95].

Coughing may be acute or chronic and may occur intermittently [94]. It is often observed while eating or early during exercise [16]. Coughing during exercise is considered the most useful indicator of lower airway disease [5]. Although coughing is the most common clinical sign of respiratory disease reported by racehorse trainers [96], it remains a relatively insensitive indicator of lower airway inflammation. Studies report that horses with demonstrable IAD only coughed 38% [5] or 50% [10] of the time. However, one study also showed that although coughing is a relatively insensitive indicator of IAD, it is a specific indicator, as 84% of horses that coughed had IAD [5]. Furthermore, horses were more likely to cough if they had IAD for two successive months, and if both coughing and nasal discharge were present in the same month, IAD was extremely likely to be present [5]. Similar results for specificity were found in a study of coughing horses in Australia, where 80% of coughing horses had a relative increase in numbers of neutrophils in their lower airways [8].

Mild serous to mucoid to mucopurulent nasal discharge may be observed in horses with IAD (Fig. 3) [3,16,27,28,94] and when observed together with coughing, is a sensitive indicator of disease [5]. However, nasal discharge and/or pyrexia in the absence of coughing are not particularly useful predictors of the presence of IAD [5].



Figure 3. Mucoid nasal discharge observed in a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

Occasionally horses with IAD may have more severe clinical signs and include fever, signs of depression, and anorexia. In these cases bacteria, and possibly viruses, are more likely to be involved [20,49]. This is consistent with reports that show young racehorses with pyrexia are considerably more likely to have significant lower airway bacterial infections [49].

Sub-clinical IAD - Many horses with IAD do not exhibit overt clinical signs, and reduced exercise tolerance or poor performance may be the only sign reported [16,17,20,91,92,97]. For example, in one study, 30 of the 46 (65%) racehorses

referred for poor performance but with no clinical signs or history of respiratory disease, had evidence of airway inflammation in a TA, a BAL or both [98]. Similarly, another study found 76% of the 25 horses presented for either routine physical examination or poor performance, had evidence of airway inflammation based on endoscopy and TA findings [99]. Alternatively, horses may have no history of poor performance or signs referable to the respiratory tract yet have cytological evidence of IAD. This finding was reported to occur in 20 to 27% of racehorses [4,8]. These findings indicate that sub-clinical disease may be a common manifestation of IAD. Consequently, use of endoscopy and cytological evaluation of samples collected from the lower airways are necessary in order to fully investigate respiratory health of horses, particularly those with a history of poor performance.

Diagnosis

Many diagnostic techniques have been used to detect IAD. However, interpretation of results from the various techniques is currently one of the most contentious areas regarding this syndrome. Different techniques sample different areas of the lung, which influences the results obtained. In addition, these techniques are not interchangeable, and airway inflammation as determined by one method may not equate to a diagnosis of inflammation with other techniques. The various diagnostic techniques have different sensitivities and specificities for IAD. Thus, the method of diagnosis will influence the reported prevalence of airway inflammation. Finally, strong debate exists regarding the definition of normality in terms of physical properties and cytological values for the different techniques. Evidently, the relationship between "abnormal" values and respiratory health and performance requires further clarification.

History and Clinical Examination - A detailed history and thorough physical examination must be performed on all suspected cases of IAD. A thorough history is an essential part of the diagnostic evaluation due to the subtlety of clinical signs often present in horses with IAD. Questions regarding performance, management procedures and clinical history are required. Careful auscultation of the lung fields, including use of a re-breathing bag, is recommended. This examination may reveal the clinical signs discussed in the previous section. However, as sub-clinical IAD is relatively common, clinical signs are often sufficiently subtle to be missed on physical examination. In addition, other causes of pulmonary pathology found in young racehorses (e.g., EIPH) may present with similar history and clinical signs. Therefore IAD cannot be diagnosed from the history and clinical signs alone. As an early diagnosis of IAD is recommended to avoid further progression of the airway inflammation [16], if horses are presented with a history or clinical signs consistent with IAD, further confirmatory diagnostic tests should be recommended.

Thoracic Auscultation - Auscultation of the thorax using the re-breathing procedure has been advocated for racehorses with poor performance or exercise intolerance [20]. A good technique for undertaking this procedure is to place a medium sized plastic bag (about 25 L) over the distal aspect of the muzzle until the horse breathes deeply. This technique should at least double the tidal volume. The horse should be allowed access to fresh air around the side of the muzzle or the bag removed if it begins to show signs of mild anxiety. An additional advantage of this procedure is that it may induce coughing, a sign which may assist in a diagnosis of IAD [70]. When a re-breathing bag is utilised, the increased depth of respiration accentuates abnormal lung sounds, and may allow the clinician to subjectively evaluate the time period of induction of, and recovery from, dyspnea [20]. However, in the authors' experience, horses with IAD rarely have auscultable abnormalities even with re-breathing due to the insensitive nature of this procedure. In addition, rapid induction of, or prolonged recovery from, dyspnea is unlikely to be a consistent feature in horses with uncomplicated IAD.

Hematology and Biochemistry - Horses with IAD rarely have systemic manifestations of disease with the results of hematology and serum biochemical examinations usually being normal [51,100].

Endoscopy - The use of endoscopy is a standard diagnostic procedure in horses with poor performance and suspected IAD. Endoscopy allows not only direct visualisation of the mucosal surfaces of the upper and lower airways and determination of the presence of inflammatory exudates or blood, but also allows guided collection of samples from the lower trachea and large bronchi. These samples may be used for cytological and bacteriological investigations [16,20].

The mucociliary clearance mechanism in normal horses is efficient, such that mucus elimination keeps pace with production and secretion [52,101]. When pulmonary irritation occurs, mucus production increases [3,5,102,103] with resultant accumulation of mucus within the airways. Therefore excess mucus in the trachea and mainstem bronchi has been used as evidence for the presence of airway inflammation [3,9]. Excess mucus is a common endoscopic finding in racehorses [3,9,104]. For example, excess mucus has been reported in the trachea of 22 - 50% of racehorses examined at rest via endoscopy [3,9,51]. In addition, horses that cough frequently have excess mucus in their lower airways. One study found

that 62% of racehorses reported to cough had copious mucopus in their trachea whereas only 2% of those that coughed did not have any visible mucopus [52]. Another study found that 39% of racehorses with a cough had mild amounts of mucus, whereas 55% had moderate to marked quantities [8].

However, the quantity of mucus observed within the trachea constituting an abnormality has not been generally agreed upon [103]. Most reports concur that no mucus, or a few single mucus droplets in the trachea is normal (Fig. 4) [3,52,105], and may reflect proximal movement of mucus during exercise [3]. In addition, some racehorses living in stables, without overt signs of airway disease, occasionally have small ventrally confluent accumulations of mucus (Grade 1)[103]. However, when racehorses exhibit pooling of mucus, especially when extending for a considerable distance in the trachea (Grade 2 and 3), this finding has been regarded as abnormal and indicative of airway inflammation (Fig. 5 and Fig. 6) [3,100,103]. Care must be taken in this interpretation as mucus scores observed in healthy horses may considerably overlap those with IAD [8,103] and challenges the validity of the criteria whereby mucus accumulation alone is used for a diagnosis of this syndrome [103]. Additional care must be made when interpreting excess mucus in the lower airways based solely on gross observations. It can be difficult to distinguish between excess mucus with no polymorphonuclear cells and mucopurulent exudates [19]. In the case of increased amounts of mucus, cytology frequently demonstrates large numbers of active macrophages in a copious amount of mucus. The significance of these cytological findings is unresolved and thus it is not known if this result should also be classified as IAD.



Figure 4. Scant mucus in trachea of a healthy horse. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 5. Grade 2 mucus or mucopurulent secretions in the trachea of a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

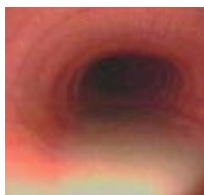


Figure 6. Grade 3 mucus or mucopurulent secretions in the trachea of a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

The effect of excess mucus on performance is also controversial, where mild mucus accumulation is a common finding in stabled horses [89]. However, studies have also demonstrated that horses with a lot of mucus in their tracheas may perform below expectation [9,106]. Clearly further studies are required to better define the significance of mucus accumulation in relation to performance.

Thoracic Radiographic Examination - Thoracic radiographic examination may be performed on horses with suspected IAD, but frequently is unrewarding. Horses with IAD may reveal mild to moderate bronchial, bronchointerstitial, or interstitial pulmonary patterns [107]. However, these findings are common in a wide variety of apparently normal horses and as such their significance in relation to IAD is questionable. Furthermore, no differences in the degree of bronchial or interstitial patterns were observed in the caudodorsal, caudoventral and craniodorsal lung regions between horses with either mild (IAD) or severe (RAO) airway inflammation [108]. These workers also found no correlation between radiographic findings and cytological or lung function scores [108]. They concluded that thoracic radiographs are of minimal use for grading the degree of lung dysfunction and airway inflammation in horses with suspected IAD or RAO.

Cytological Evaluation of Lower Airways Secretions - Cytological evaluation of secretions harvested from the lower airways is the most specific method for diagnosis of IAD. However, there is currently much debate regarding the technique of choice for collection of samples and also the interpretation of cytological findings.

It is important to recognise that inflammatory lesions in the lung may not be homogeneously distributed [51]. Instead, inflammation may be regionally localised [51,98,109] and may be restricted to the trachea and bronchi, extend into smaller airways such as bronchioles, or involve the pulmonary parenchyma [51]. The two most frequently used techniques for collection of samples from the lower airways of horses are TA and BAL. However, each of these techniques samples disparate areas of the lung and there is no correlation between the cytological findings of these samples when collected sequentially from an individual animal (Fig. 7) [98,110]. Therefore, a cytological diagnosis of IAD will be influenced by the method of sample collection and frequently will not correlate with a diagnosis made by the alternate technique (Table 1) [98,110]. Consequently, one should not assume that a "normal" sample obtained by either of these techniques indicates absence of disease in the entire lung.

Table 1. Contingency table showing the relationship between interpretation of TA and BAL specimens obtained from 46 racehorses after high-speed treadmill exercise. See section on Diagnosis for explanation of cut-off values used. (From [98]).

Technique	Tracheal Aspirate			
		>20% PMNs	≤20% PMNs	Total
Bronchoalveolar lavage	> 5% PMNs	12	5	17
	≤5% PMNs	13	16	29
Total		25	21	46

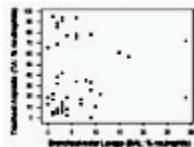


Figure 7. Scatter-plot showing relationship between relative percent neutrophils in TA and BAL specimens (From Malikides et al., [98]). - To view this image in full size go to the IVIS website at www.ivis.org . -

The choice of diagnostic techniques (TA versus BAL) for evaluation of the respiratory tract will be influenced by a number of factors including history and presenting signs, likely differential diagnoses, ease of technique, and stage of training. However, as these two techniques sample different areas of the lung and their results do not correlate, collection of both samples concurrently is indicated in order to assess the overall health of the lower airways. This is particularly the case in situations where a diagnosis is not readily evident, for example in cases of poor performance or coughing during exercise.

Tracheal Aspirates

Tracheal aspirates, also called tracheobronchial aspirations or tracheal washes, collect secretions, cells and debris that accumulate in the distal trachea and bronchi but which may also be derived from the more distal airways and alveoli [52,110]. As such they provide a non-homogenous sample and are not representative of any one segment of the lung [111]. The mucociliary apparatus moves secretions from all parts of the lung to the pharynx via the trachea, therefore TAs are more likely to reveal inflammation in disparate lung regions [51]. However, the relative contribution of cells and secretions from different areas of the lung cannot be determined for an individual TA. For example, a small lesion that is actively exudating or exfoliating cells may contribute a highly disproportionate quantity of cells and debris to the total pool [112]. Consequently, if inflammatory cells are detected in a TA these cells may have arisen from diverse regions in the lower respiratory tract including the large or small airways or alveoli, and from variably sized lesions within these regions.

Technique - Several methods for obtaining a TA have been developed with each technique having advantages and disadvantages. In general, aspirates may be obtained endoscopically, with or without a guarded catheter, or via a transtracheal (percutaneous) aspiration technique. The choice of sample technique, and the way in which the sample is obtained, can significantly affect numbers of bacteria, numbers of cells, and even the types of cells obtained [19,113]. For this reason it is recommended that standardisation of technique with regard to type of technique, time of sampling, volume and type of fluid instilled, and sample handling is performed to allow meaningful interpretation and comparison of results.

The most important consideration when choosing a technique is whether microbiological culture of the tracheobronchial secretions is indicated, such as when infectious causes of IAD are suspected. Samples obtained using an unguarded catheter within the biopsy channel of an endoscope are unsuitable for bacterial cultivation as they invariably become contaminated by upper airway flora in the nasopharynx [5,111]. However, guarded catheters have been shown to prevent this contamination from occurring and samples obtained with this technique are suitable for microbiological cultivation [114,115]. Alternatively, the transtracheal (percutaneous) aspiration technique may be used [116], but this is rarely indicated in racehorses in training given the invasive nature of the technique.

We recommend that routine collection of TAs in horses in race training be performed using guarded catheters via endoscopy (Fig. 8). The guarded catheters are passed through the biopsy channel of an endoscope, and samples aspirated whilst directly visualising the collection site [114]. A number of multi-lumen telescoping, plugged catheters have been assessed as to their suitability for aseptic collection of tracheal secretions via an endoscope, but their commercial availability is variable. Examples of guarded microbiology aspiration catheters currently available include Catheter EMAC800 (Mila International, Phoenix, AZ, USA) and Catheter V-EBAL-8.0-190 (Cook Veterinary Products (USA), Bloomington, IN, USA). Disinfection of the endoscope prior to each collection should be performed using an appropriate disinfectant (e.g., Cidex®), ensuring that the endoscope has 10 - 15 minutes of contact time at room temperature in order to adequately disinfect the biopsy channel. The endoscope including the biopsy channel should then be flushed with sterile saline before collection of samples.

Technical prowess definitely influences the quality of sample obtained using guarded catheters, especially if bacteriological cultivation of these samples is required. Factors that help prevent contamination from occurring include rapid collection of the sample, small volume of infused sterile isotonic saline (10 to 15 ml), and advancement of only the inner catheter into the tracheal "puddle" rather than the catheter as a whole (Fig. 9) [114]. In addition, if the horse has coughed frequently during the procedure, an increased risk of contamination with oro-pharyngeal organisms is likely, and these samples are rarely appropriate for bacteriological cultivation [8,114].



Figure 8. Collection of a TA using a guarded catheter via an endoscope. Samples collected using this technique are suitable for bacteriological cultivation. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 9. Visualisation of the inner guarded catheter inserted into the tracheal puddle during collection of a TA. Instillation of only 10 - 15 ml of sterile saline during collection will help minimise contamination with squamous epithelial cells and normal flora of the oropharynx during collection. - To view this image in full size go to the IVIS website at www.ivis.org . -

Factors influencing results of TAs - A number of other factors may influence the results of samples obtained by TA. These include long distance transportation prior to sample collection and the effect of exercise. Large increases in the numbers of bacteria and inflammatory cells in the LRT can occur within 6 to 12 hours of head confinement (cross tying or transportation) [25,26]. These bacteria and cells are a transient response to postural confinement, and are generally cleared within 12 hours of horses being released from confinement [26]. However, clearance may be prolonged if horses are dehydrated [117]. These findings have implications for interpretation of TAs from horses transported long distances prior to collection of samples and a careful history should be obtained to allow correct evaluation of results.

Collection of samples approximately one hour after moderate to intense exercise may yield specimens of greater diagnostic value. Samples obtained at this time more adequately represent different areas of the respiratory tract, are likely to contain extra secretions (more grossly opaque with additional mucus), and are more likely to reveal the presence of airway disease [3,52,118]. However, the effect of exercise on cytological variables of TA requires further clarification, as exercise can induce inflammation in lower airways of humans [119], and an increase in the relative numbers of neutrophils was demonstrated in TAs [118] but not BAL [120] samples collected shortly after intense exercise. Exercise increases lung movement and expiratory airflow, which may mechanically force secretions from lower regions to the proximal airway.

Alternatively, the effect of exercise may be to increase exposure of the trachea and proximal airways to environmental particles and infectious or toxic agents, too large to be deposited in the distal lower airways. This may result in an influx of neutrophils to this more proximal region and explain the elevations in neutrophil percentages in TA rather than in BAL samples. Exercise also increases the degree of oro-pharyngeal contamination of the lower airways, and thus influences the results of bacterial cultivation [121]. However, in the normal horse, this upper airway contamination is rapidly cleared by the mucociliary clearance mechanism [52,122] where in a healthy horse tracheal mucus transport velocity is about 2 cm/min [101]. Therefore, samples obtained approximately one hour after exercise rarely contain significant numbers of these contaminants [8].

Interpretation of Tracheal Aspirates

Although collection of TA is now considered a routine procedure in many practices, there remain a number of controversial issues associated with their interpretation. Most notable are the differing definitions of normal and abnormal cytological findings among investigators, the significance of increased numbers of inflammatory cells and mucus, particularly in performance horses, and the interpretation of bacterial cultivation. In addition, the significance of changes in cytological variables of TAs in relation to performance is not known, and further studies are required to correlate these alterations with variables that measure performance.

Mucus - Interpretation of the amount of mucus within a TA is best performed in conjunction with endoscopic evaluations of the lower airways and cytological evaluation of the cell types present. This will facilitate accurate estimation of the amount of mucus present in the airways as opposed to the amount of mucus collected with TA [3,5], and will allow determination of relative increases in the numbers and type of inflammatory cells. The amount of mucus in TAs increases when pulmonary irritation occurs, such as in cases of IAD (Fig. 14) [3,5,101,103]. However, the amount of mucus that constitutes an abnormality is controversial. This is particularly the case in horses where 1) there are only mild increases in the amount of mucus; 2) there is increased amounts of mucus but no, or mild, increases in the number of neutrophils; or 3) there is increased mucus with many activated macrophages. Furthermore, these findings may be present in horses not exhibiting overt signs of respiratory disease. It is currently contentious at which point mucus accumulation and/or airway inflammation become clinically significant, especially for different levels of performance [89]. However, cytological evidence of increased mucus, especially in conjunction with cytological evidence of airway inflammation, most likely represents a significant finding in racehorses.

Epithelial Cells - Tracheal aspirates from normal horses contain low to moderate numbers of epithelial cells, although increased numbers may be obtained when using endoscopic methods of collection [123]. They may be present as single cells or within clumps or sheets of cells (Fig. 10). The epithelial cells present in TA's are predominantly ciliated epithelial cells from the trachea and bronchi, but cuboidal cells from the smaller airways (distal bronchi and bronchioles) may also be observed [52,123]. Squamous epithelial cells should not be present in TA's from normal horses [114]. However, they are observed commonly and represent oropharyngeal contamination at the time of sampling or may be due to upper airway dysfunction [4,52]. Squamous cells are frequently covered by bacteria, and therefore provide a significant source of microbial contamination (Fig. 11) [52,114]. Identification of the presence of these cells is important in order to be able to accurately interpret results of microbial cultivation.



Figure 10. Clumps of ciliated columnar epithelial cells in a TA from a horse without signs of respiratory disease. Note variable degree of preservation of epithelial cells. - To view this image in full size go to the IVIS website at www.ivis.org . -

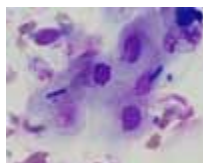


Figure 11. Squamous epithelial cells in TA from a horse. Note presence of *Simonsiella* spp on the surface of these cells, which is consistent with oropharyngeal contamination. - To view this image in full size go to the IVIS website at www.ivis.org . -

Mild changes to epithelial cells may be observed in normal horses and include nuclear changes and the loss of some cilia [52,123]. These changes probably represent normal wear and tear or turnover of cells [52], and care must be taken in the interpretation of these findings. Pathological changes to epithelial cells (epithelial atypia) result from inflammation and are

caused by inflammatory mediators liberated from leucocytes [52]. In addition, in cases of infectious respiratory tract disease there may be direct damage to the epithelium.

It is noteworthy that there are many causes of airway inflammation, and therefore the presence of epithelial atypia is not specific for a particular diagnosis or etiology. In addition, many of the changes described may be seen (albeit in lower numbers) in TA's from normal horses. Ciliocytophthoria is a specific form of cellular degeneration associated with viral infection in humans, and has been observed in horses with suspected viral upper respiratory tract infections [52,105]. Ciliocytophthoria results in the presence of two cellular elements; a spherical cell with pyknotic fragmented nuclear material and pink inclusions and small round ciliated cytoplasmic fragments or tufts. Both of these structures must be present for the term to be applied [124]. Claims that increased numbers of non-ciliated cells and ciliated tufts are observed in TAs from horses with poor performance, and are therefore indicative of this condition, remain unsubstantiated. Furthermore, care must be taken in the interpretation of isolated ciliated tufts (Fig. 12) as these fragments may be seen in any specimen where there is fragmentation of ciliated cells, for example acute bronchitis [124], and alone are not indicative of viral infections.

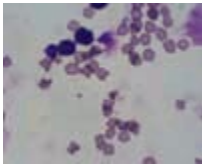


Figure 12. Isolated ciliated tuft together with many RBCs in TA from a horse. - To view this image in full size go to the IVIS website at www.ivis.org . -

Macrophages - Pulmonary alveolar macrophages (PAM) are the most abundant type of inflammatory cells present in TAs from normal horses (Fig. 13 and Table 2) [52,105]. Their presence, together with ciliated epithelial cells, is a pre-requisite for interpretation of TA cytology, as they originate from the terminal area of the respiratory tract [124] and therefore indicate that all levels of the pulmonary tree have been sampled [19,105,124]. Although PAMs are the most common inflammatory cell type in normal horses, increased numbers are rare in horses with IAD [6,19,52] and their significance is unknown [52]. In the 1,235 TAs examined in the study by Chapman et al. [6], the relative number of PAMs in TAs with a low inflammation score was 40.9% [± 0.44 (sem)] whereas horses with a high inflammation score (excess cells, mucus and/or neutrophils) had 45.7% [± 3.21 (sem)] [6]. It should be noted that the relative shift in neutrophils in these same samples was much greater (4.2% to 40.1%) between low and high inflammation scores.

Table 2. Relative proportion (%) of cells in TA obtained from horses without clinical signs of respiratory disease. (Note all values are mean % of cells \pm sd.)								
	No	Age (years)	Neut (%)	Macro (%)	Lymph (%)	Eosino (%)	Mast (%)	Epith (%)
Derksen [110]	10	6.9 (± 2.1)	32 ± 28.1	24 ± 12.7	8.2 ± 6.0	<1.0	0	34 ± 20.9
Larson [112]	92	Not given	39 ± 21.0	34 ± 18.0	4.9 ± 3.1	3.5 ± 2.0	1.6 ± 1.9	13.0 ± 5.8
Sweeney [4]	66	3	17.8 ± 21.8	44.1 ± 23.2	5.4 ± 4.1	0.7 ± 2.2	0.1 ± 0.2	30.4 ± 24.4
Mair [123] (endoscopic)	42	1 - 18	4.6 ± 4.9	43.0 ± 10.7	2.2 ± 2.4	0.7 ± 0.4	0.1 ± 0.2	49.1 ± 11.5
Mair [123] (transtracheal)	15	1 - 18	6.4 ± 5.5	65.0 ± 13.7	7.4 ± 3.8	1.2 ± 1.4	0.2 ± 0.4	19.8 ± 6.1
Christley [114]	9	3 - 6	10.0 ± 10.8	74.2 ± 11.1	13.0 ± 3.6	1.9 ± 1.2	0	NE

No = number of horses; Neut = neutrophils; Macro = macrophages; Lymph = lymphocyte; Eosino = eosinophils; Mast = mast cell; Epith = epithelial cell; NE = not evaluated.

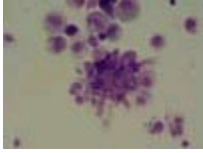


Figure 13. Multiple macrophages together with a clump of epithelial cells in a TA from a horse not showing signs of respiratory disease. - To view this image in full size go to the IVIS website at www.ivis.org . -

The activity of macrophages within a TA may vary considerably [52]. Their cytoplasmic inclusions reflect the amount and type of endogenous and exogenous material that the lower airways have been exposed to [52]. Macrophages with finely vacuolated cytoplasm are not considered abnormal, but marked increases in cytoplasmic vacuolation or large vacuoles that distort the cell and displace the nucleus are usually present only when there is evidence of pulmonary disease [52]. This increase in cytoplasmic reactivity occurs in response to infective, irritant and small airway allergic disease or obstruction. In these cases, the presence of ingested cellular debris or whole cells is common and reflects increased cellular turnover [19]. However, as stated previously, the clinical relevance of increased numbers of activated macrophages, especially in conjunction with increased mucus, is not known.

Lymphocytes - Lymphocytes are present in low numbers in normal TAs (Table 2) and they may be difficult to accurately differentiate from other cell types present such as small macrophages, and epithelial cells [52]. The numbers of lymphocytes may increase in cases of respiratory tract disease, but this is variable and no correlation has been made between cytological observations of this cell population in TA and specific disease processes.

Neutrophils - Although a population of moderately preserved neutrophils reside in horses' airways, the relative percentage of these cells in normal horses is reported to be low [4,105]. However, these cells respond to a large variety of stimuli, and their numbers may fluctuate rapidly (Fig. 14). In addition, in normal horses neutrophils are generally found in higher proportions in TAs than in BALs (compare Table 2 with Table 1 in the chapter by Hewson and Viel [125]). This possibly reflects the greater exposure to noxious influences, including bacteria, which may be present in the larger airway. Alternatively, tracheal secretions can derive from many areas of the lung, and increased numbers may therefore reflect this increased area of sampling.

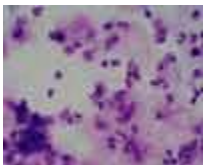


Figure 14. Many neutrophils and excess mucus in a TA of a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

The information derived from samples obtained by lavage of the lower airways that is most frequently used clinically is the differential cell count of recovered fluid [123]. The dilemma with interpretation of the relative numbers (%) of neutrophils found in TAs is to determine what value (if any) represents a significant change. Large variations in the relative percentage of neutrophils in TAs obtained from apparently healthy horses have been observed within [110] and between studies (Table 2). In addition, poor correlation between the relative number of cells in TA (including neutrophils) and pulmonary histopathology has been described [112]. As a result of this variation the clinical usefulness of cytological evaluation of TA samples, especially using a relative percentage of cells, in the diagnosis of lower airway inflammation has been questioned. However, studies in younger, more homogeneous populations of horses have found smaller variations in neutrophil ratios in normal horses [4,114,123]. For example, studies investigating airway inflammation in young racehorses demonstrated that in > 80% [8] and > 73 % [4] of clinically normal racehorses, neutrophils do not exceed 20% of the inflammatory cell types found in TA samples. Furthermore, Chapman et al., [6] reviewed 1235 TA samples obtained from 724 horses in race training, and found that in almost 90% of horses the relative percent neutrophil values were <10%. A strong statistical association between presence of > 20% neutrophils in TA specimens and signs of respiratory disease (i.e., coughing) in young racehorses [8] and the likelihood of isolating significant numbers of bacteria [6,8,22] has been reported. Therefore it is likely that > 20% neutrophils in TAs from young racehorses is abnormal, representing a significant inflammatory process and a strong risk for clinical respiratory disease [4,8,71,98,102,105,118,123].

The absolute numbers of cells present (TNCC) should also be taken into account when interpreting the significance of the relative proportions of neutrophils (and all inflammatory cells) in TA. Unfortunately, the absolute number of cells is frequently not determined for TAs in clinical practice, and in many reported studies, although they more accurately indicate

a shift in cell populations than determination of relative ratios. This reflects the difficulties that may be encountered during enumeration of cells in TAs due to the entrapment of cells within mucus, the presence of clumps of epithelial cells, the variable dilution of secretions by saline during collection and the variability of cellularity in smears [19,123]. Consequently, absolute counts are thought to be frequently inaccurate in TAs [19]. However, an estimate of cellularity in samples is useful, and may be incorporated in an inflammation score as has been advocated in a number of studies [5,6,52]. In this case, if the relative proportion of neutrophils is high but the overall cellularity is low, this will be reflected in a lower inflammation score, and will assist interpretation of the significance of the presence of neutrophils in TAs.

The presence of toxic or degenerative changes in neutrophils may also help interpretation in cases where overall cell numbers are low. In many cases of IAD, the majority of neutrophils are mature with only mild degenerate or toxic changes. The most common degenerative change observed in neutrophils is karyolysis, which involves nuclear swelling, loss of segmentation, decrease in nuclear staining intensity from purple to pink, and eventual lysis of the cell. The degree of nuclear disruption is roughly proportional to the degree of degeneration. Neutrophils with moderate to marked degenerative changes are most commonly observed in cases of IAD involving bacteria, and in these cases careful examination for intracellular bacteria is often diagnostically rewarding (Fig. 15). However, not all cases of IAD where significant numbers of bacteria can be cultured will have toxic neutrophils [114] and therefore this observation cannot be used for diagnosis of bacterial IAD.

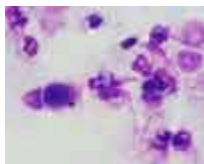


Figure 15. Intracellular bacteria in a neutrophil of a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

Eosinophils - Studies of clinically normal adult horses indicate that eosinophils are usually present in very low numbers in tracheobronchial secretions, though the numbers observed vary between studies and may reflect the horse's local environments (Table 2).

The relative proportion of eosinophils that is considered to be abnormal in TAs has not been determined. Increased numbers of eosinophils in TA (Fig. 16) are observed in cases of ascarid (*Parascaris equorum*) migration and lung worm (*Dictyocaulus arnfeldii*) infestation [74]. In these cases the relative percentage of eosinophils may be as high as 85% of cells in the TA. Due to management procedures practiced for racehorses, infestation with *D. arnfeldii* is unlikely in the majority of cases. However, in the experience of the authors, identification of adult ascarids in the small intestine of racehorses at postmortem is not an uncommon finding. Smaller elevations in the number of eosinophils in TA occur in the absence of parasitic infections, and are interpreted as evidence of a type I hypersensitivity response to inhaled allergens [15,68,70].

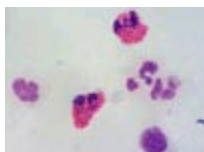


Figure 16. Eosinophils in the TA of a horse with IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -

Mast Cells - Mast cell may be identified by their characteristic staining granules, which are most easily observed in preparations where metachromatic stains are used (Toluidine blue or Leishmann's stain) [69]. In normal horses, mast cells in TA are rare or present in low numbers (Table 2). This is in contrast to samples obtained by BAL, in which higher numbers of mast cells may be noted. This difference may be explained by the predominant distribution of equine mast cells within secretions of the smaller airways and alveoli.

There is little information on normal or abnormal percentages of mast cells in TA from horses, or the significance of alterations in mast cell numbers. Furthermore, a study investigating the relative numbers of mast cells in TAs and BALs obtained sequentially from racehorses presented with poor performance found that BALs had significantly higher proportions of mast cells than TAs [69]. These authors suggested that TA may be less sensitive than BAL for detection of alterations in relative numbers of mast cells.

Bacterial Cultivation - Results of microbial cultivation warrant individual attention as misinterpretation is common in cases of IAD. The isolation of bacteria from TAs may represent infection, a transient lower airway population or contamination of

the TA at the time of sampling. It is essential for appropriate management of these cases to differentiate between these scenarios. Clinical signs consistent with bacterial involvement may help in this differentiation. These signs include fever, anorexia, coughing, and nasal discharge [20,49]. However, absence of these signs does not preclude bacterial infection, especially in milder cases of IAD with a bacterial component. If bacterial respiratory tract disease is suspected, a TA should be obtained using a guarded catheter in order to confirm the diagnosis.

Cytological evaluation of the TA should precede microbial cultivation. Tracheal aspirates from horses with bacterial lower respiratory tract infections will have increased mucus, increased total cell counts, and increased relative and absolute neutrophil counts with possibly degenerative neutrophils and intracellular bacteria [5,6,8]. There is no indication for cultivation of a sample without this cytological evidence of inflammation. In addition, it is preferable not to culture samples with large numbers of squamous epithelial cells, even when there are many neutrophils present, as this is evidence of oropharyngeal contamination [114]. If these samples are cultivated, and large numbers of bacteria are isolated, it is not possible to ascribe any significance to these isolates. Re-collection of the sample is recommended.

Aerobic cultivation should be routinely performed on samples with cytological evidence of airway inflammation. Anaerobic cultivation is rarely warranted, unless the horse is demonstrating clinical evidence of severe pulmonary disease. Quantitative cultures, which determine the number of colony forming units for each bacterial species, provide additional information. Aspirates collected in an appropriate fashion from healthy horses, or from horses with airway inflammation without a bacterial etiology, usually cultivate $<10^3$ bacteria (cfu)/ml and frequently no bacteria at all [5,6,8,114]. If $>10^3$ cfu/ml are cultivated, it is likely that these bacteria are contributing to the disease process [5,6,8], and identification of each species present in high numbers will assist in interpretation of their significance.

Identification of isolated bacteria allows differentiation of likely pathogens from likely contaminants. Bacteria that are most commonly isolated from uncomplicated IAD include *Streptococcus* spp, *Pasteurella* spp, *Actinobacillus* spp and occasionally *Bordetella bronchiseptica* and *Mycoplasma* spp [5,6,8,22,32,44,53]. Bacteria in the Enterobacteriaceae family (e.g., *E. coli*, *Klebsiella*) and anaerobic bacteria (*Bacteroides* spp, *Fusobacterium* spp) are rarely isolated from uncomplicated cases of IAD, but may be isolated if antimicrobial therapy has been initiated (Enterobacteriaceae) or pleuropneumonia or lung abscessation has evolved (anaerobes) [58]. Pathogenic bacteria that rarely cause disease in the lower airways, but are common contaminants during sampling include coagulase positive *Staphylococcus* spp, *Pseudomonas* spp, and *Proteus* spp. Care with interpretation of these isolates must be made and they are rarely significant. Isolation of non-pathogenic bacteria (coagulase negative *Staphylococcus* spp, *Corynebacterium* spp, *Bacillus* spp) indicates contamination at the time of sampling.

Bronchoalveolar Lavage

As stated earlier, inflammation of the lower airways is surprisingly compartmentalised [51], therefore BAL gives new information that cannot be obtained by TA. In addition, different cytological pictures of inflammation have been recognised in studies of BALF from young racehorse [15,70], leading to the speculation that different etiological agents or environmental exposures may be important to inflammatory phenotypes diagnosed with this technique [17].

Bronchoalveolar lavage yields cellular constituents and extracellular proteins from the epithelial surface of the pulmonary alveoli and terminal airways (segmental bronchi and bronchioles) and as such reflects the changes occurring in small airways in the region of the lung that is lavaged [20,61]. In studies of horses with diffuse pulmonary disease, cytological examination of BAL fluid accurately reflects histological abnormalities [61,126] and it is a more reliable technique than TA for investigation of peripheral or chronic pulmonary inflammation [110,123,125]. Furthermore, a single BAL sample has been shown to reflect the composition of BAL fluid throughout the lungs in horses with diffuse pulmonary disease such as heaves [127], but the equivalent study has not been performed in younger horses with IAD.

A number of different techniques have been described for collection of BALs (Fig. 17). These techniques, the methods for processing samples, and the cytological findings in BALF from healthy horses and horses with IAD are discussed in the chapter by Hewson and Viel [125].



Figure 17. Collection of a BAL using a Bivona® tube. - To view this image in full size go to the IVIS website at www.ivis.org . -

In young racehorses without signs of lower respiratory tract disease the majority of cells observed in BALF are macrophages and lymphocytes with differential cell counts of macrophages (59 - 68%) and lymphocytes (28 - 32%) reported [31,68,91,128]. Macrophages should appear uniform with minimal cytoplasmic vacuolation. The majority of lymphocytes in equine BAL fluid are T lymphocytes and approximately half are helper T cells (CD4+) lymphocytes, and half are suppressor T (CD8+) lymphocytes [10]. Non degenerate neutrophils constitute less than 5% of the total cells [10,68,91], and occasional eosinophils and mast cells are observed [123], where values of <2% for mast cells and <1% for eosinophils have been advocated as normal in young racehorses [12,7]. However, higher values for the relative numbers of mast cells have been reported by others in normal horses [73,129] but may reflect an older population of horse and the environment in which these horses were maintained. Relatively few epithelial cells are observed in BALF from normal racehorses and these are predominantly cuboidal or non-ciliated columnar epithelial cells. Identification of squamous epithelial cells or feed material in cytological preparations may indicate pharyngeal contamination of the BAL sample during catheter passage.

In comparison with healthy horses, cytological evaluation of BALF has demonstrated a number of different profiles in horses with IAD. Increased absolute numbers of neutrophils [35,97], lymphocytes [129], or neutrophils, lymphocytes and macrophages [10] have been reported. Furthermore, shifts in the relative numbers of cells have also been recorded with increased percentages of neutrophils, eosinophils, mast cells and lymphocytes and decreased macrophages either alone, or in various combinations reported [10,31,35,68,91,128]. The underlying pathogenesis of these cytological changes are not well documented in horses with IAD, but increased populations of mast cells and eosinophils are thought to indicate the presence of a type I hypersensitivity response [16,68], or possible parasitic infestation [74], increased neutrophils may be responding to pro-inflammatory agents lodged in the small airways [79] or alternatively be part of a type III hypersensitivity reaction [16], and increased lymphocytes an acute form of hypersensitivity pneumonitis resulting from chronic exposure to organic antigens [16]. Although a relationship between IAD and heaves has been suggested, the cytological profile of horses with IAD differs from those observed in BALF of horses with heaves. In IAD there is a relatively low elevation in the percentage of neutrophils (usually between 10 - 15%) [17] and there may be increases in mast cells, eosinophils and lymphocytes [16]. By comparison, in BALF of horses with heaves neutrophils usually predominate and may comprise almost the entire cell population [17]. Increased numbers of ciliated epithelial cells and ciliary tufts have been observed in BALF of horses with experimental EHV and equine influenza virus infection [130].

The significance of airway inflammation observed in BALF in young performance horses is not well defined. However, there is an increasing body of evidence to suggest that changes in cell populations in samples obtained by BAL may reflect physiologically significant processes. For example, horses presented for poor performance and endoscopic evidence of airway inflammation have elevated neutrophils, lymphocytes and macrophages in BALF [10]. In addition, airway hyper-reactivity occurs in young performance horses with exercise intolerance and is correlated with elevations in either eosinophils, mast cells or lymphocytes in BALF [68,92]. Finally, horses with elevated total nucleated cell counts or absolute neutrophil counts in BALF have more severe exercise-induced arterial hypoxemia as compared to control horses [97,131].

Lung Function Testing - Lung function tests such as measurement of maximal change in pleural pressure during tidal breathing, pulmonary resistance and dynamic lung compliance have been used to document the severity of airway obstruction in horses with heaves [132], and to monitor response of these horses to therapy [95]. However, the variability in these function tests makes them of little value for confirmation of diagnosis unless the animal is so severely affected that affirmation is unnecessary [77]. It is not therefore surprising that these tests are of no value for the diagnosis of IAD in which the pulmonary pathology and structural changes are less severe than in heaves.

Since the early 1990s several groups around the world have attempted to determine the usefulness of alternate techniques for diagnosis of airway dysfunction in horses with IAD. Airway hyper-reactivity, in response to provocation with histamine or metacholine, has been demonstrated in groups of young performance horses with exercise intolerance [68,92,133]. In the differential cell count of BALF obtained from these horses either eosinophils, mast cells or lymphocytes predominated. It is postulated that release of pro-inflammatory cytokines and chemokines by these cells induce airway hyper-reactivity through airway inflammation, airway wall thickening and autonomic nervous system dysfunction [68,92]. The airway wall thickening and remodelling may be reflected by changes in airway resistance as detected by forced oscillatory mechanics [34,134] and forced expiratory manoeuvres [129]. However, similar to the data for airway hyper-reactivity, these techniques revealed differences between groups of horses (heavy versus IAD versus control) but lacked appropriate sensitivity for demonstration of significant differences between individuals. Further refinement of these tests, or examination of alternate techniques such as capnography or scintigraphy, may help resolve this issue. Thus, although the volume of information

relating to respiratory function testing in horses continues to grow, in particular with reference to IAD, at this time the sensitivity of reported techniques limits their efficacy as routine clinical tools for assessing horses with this syndrome.

Pathology

Although no studies have directly examined the histological changes that occur in young racehorses with IAD, a number of studies have examined the pulmonary histology of young athletic horses. Findings from these studies may indicate the pathology present in IAD. Histological studies of horses with EIPH demonstrate multi-focal, small airway-centred changes as evidenced by thickened airways walls due to increased quantities of mucosal and peribronchiolar connective tissue, mononuclear bronchiolar cuffs, and extension of non-ciliated bronchiolar epithelial cells into alveolar ducts [135]. These findings may be in response to the presence of blood in the airways, but may also result from other causes of airway irritation as found in IAD. Similarly, clinically normal young Thoroughbred horses in training had histological evidence of increased collagen, disruption of epithelial basement membrane, and duplication of epithelial basement membranes [136] suggesting previous airway inflammation and epithelial injury. These authors speculated that these changes might be associated with excess deposition and retention of inhaled particulate matter during racing resulting in inflammation and injury to the epithelium and basement membrane. There is also evidence of an association between an increase in the numbers of inflammatory cells in BALF and bronchial and bronchiolar inflammatory changes in horses [61,126] including young performance horses [137]. These histological changes include inflammatory cell infiltrates of the mucosa, luminal exudates, peribronchiolar cuffing or fibrosis, bronchiolar epithelial hyperplasia and goblet cell metaplasia. However, one study showed that about 80% of horses without signs referable to the respiratory tract had low-grade peribronchiolitis [137], and thus the significance of these histological changes may be questioned. Further studies to better define the association between histological findings in the lungs of young racehorses and characteristics that currently define IAD, such as the presence of mucus in the lower airways or cytological evidence of airway inflammation, are required.

Treatment

Treatment strategies for IAD will be predicated to a large degree by the inciting cause of the inflammation, the horse's clinical signs, type of inflammation demonstrated in the lower respiratory tract (LRT), owner/trainer/manger compliance, cost of medication and prior experiences of the attending clinician. There is a growing body of evidence to suggest that most cases of IAD in young performance horses are not the result of viral infection. Environmental factors are implicated as being key in the pathogenesis of this disease, with bacteria being variably implicated. As a result the major aims of treatment should revolve, where possible, around identification of etiological agents, removal of the inciting cause, and management of airway inflammation. In addition, the presence of low-grade airway obstruction due to bronchoconstriction is postulated to occur in some cases of IAD as a result of airway inflammation [79], and may be one of the major causes of the clinical effects observed. Although this effect is by no means proven in all cases of IAD, the use of bronchodilators are indicated as part of the therapeutic plan for this syndrome. Thus, given our current understanding of IAD, therapy for this syndrome should be designed to achieve 3 main goals: 1) environmental management, 2) treatment of airway inflammation, and 2) bronchodilation [94].

Environmental management of IAD - Ideally medical management should be performed in conjunction with procedures to decrease exposure to pro-inflammatory agents present within the horse's environment. This may involve a change in the horse's environment, for example in more temperate climates and where space is readily available it is common practice for horses with IAD to be kept outdoors for much of the time (Fig. 18) and to be housed, when necessary, in stables with an open configuration thereby allowing optimal air quality (Fig. 19). Naturally, these management strategies may not be possible in all horse establishments throughout the world and as such treatment plans should focus on improving the immediate environment in which the horse is kept together with medical management. The former situation may involve ensuring the horse is kept on bedding known to have lower concentrations of respirable dust or other airway irritants (e.g., paper or cardboard, large wood shavings, peat moss) versus those likely to be contaminated with airway irritants (e.g., poor quality or old straw bedding, deep litter systems using shavings) (Fig. 20). Appropriate ventilation of the stable environment is essential also, with at least 8 air changes per hour required. Additionally, use of low-dust forages is to be recommended as traditional feedstuffs such as hay are likely to increase the concentration of pollutants in the horse's breathing zone by 3-6-fold when compared to low dust alternatives. Alternate methods for reducing dust challenge to horses via alterations in feed include use of complete pelleted feeds or silage and are preferable to wetting/soaking hay (Fig. 21) or use of artificially (i.e., kiln dried) cured hay [75]. Further discussion on environmental modification for horses with airway inflammation is found in the IVIS chapters on Recurrent Airway Obstruction (heaves) and Environmental Control of Respiratory Disease [75,77] and the principles discussed apply to horses with IAD.



Figure 18. Horse being managed outdoors for treatment of IAD. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 19. Stables with open plan to improve ventilation and decrease exposure to airway irritants. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 20. Straw used for bedding may be a source of dust and other airway irritants in a horse's environment. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 21. Soaking hay in water to decrease dust exposure is less effective than feeding a complete pelleted feed. - To view this image in full size go to the IVIS website at www.ivis.org . -

Medical management of IAD - Judicious use of drugs selected for their anti-inflammatory and bronchodilator effects have become central to the management of asthma in humans and heaves in horses. Not surprisingly as the understanding of IAD advances, in particular the proposed association between airway inflammation and bronchoconstriction, these therapeutic agents have gained popularity for treatment of this syndrome. This is based on the fact that the primary goals of IAD therapy are to control airway inflammation and relieve airflow obstruction. Medications for IAD may be administered parenterally or directly into the airways in the form of aerosols. Administration of therapeutic substances via inhalation has the advantage of delivering high concentrations of the drug directly into the airways while minimising the amount absorbed systemically. This latter effect should optimise the therapeutic effect whilst reducing the risk of adverse side effects. However, it should be noted that most of the drugs and dosages recommended for medical management of IAD are based on studies performed in horses with heaves. Despite this shortcoming, "good" clinical responses in horses with IAD have been reported when these treatment guidelines are adopted (Table 3). However, these findings need to be analysed in the light that management of the horse may have involved a variety of strategies all imposed at once. This may therefore have disguised the true efficacy of some management practices recommended for IAD. However, sufficient evidence currently exists to suggest that non-steroidal anti-inflammatory and "anti-histamine" drugs are ineffective for the treatment of IAD [94]. In contrast, systemic and aerosolised corticosteroids appear to be efficacious, with the risk of systemic effects and longer elimination half-times being greater when glucocorticoids are administered parenterally [20,94]. Therapeutic aerosols may be produced by nebulising a solution or using pre-packaged solutions delivered via metered-dose inhalers (MDI). An alternative delivery system utilises dry powder inhalers (DPI) where the drug is inhaled as powder form. A variety of devices have been developed to improve delivery of aerosol to the lower airways of the horse. These include face masks with spacers and holding chambers (e.g., Aeromask[®]) and nose pieces (EquineHaler[®], 3M Equine Inhaler[®]) (Fig. 22 and Fig. 23). Spacers and holding chambers are designed to alter the size distribution of particles originating from the MDI or nebulizer resulting in a reduction in upper airway deposition and an increase in the mass of drug contained in respirable particles [138]. A valve is usually present between the spacer and the horse's nostril, therefore precise synchronisation between MDI actuation and onset of inhalation is not required. The fraction of drug deposited into the lungs averages between 0.3 - 7.4% for nebulizers and between 6.1 - 23.3% for MDI delivery devices (6.1% for Aeromask[®]; 8.2% EquineHaler[®]; 23.3% for 3M Equine Inhaler[®]) [94].



Figure 22. Use of an Aeromask[®] to administer drugs via MDI. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 23. Use of an EquineHaler® to administer drugs via MDI. - To view this image in full size go to the IVIS website at www.ivis.org . -

Corticosteroids - Given the current state of knowledge relating to IAD, glucocorticoids should be considered one of the core therapies for this syndrome. Much information relating to use of corticosteroids in respiratory disease comes from studies in horses with heaves [139]. However, as mentioned previously, it remains unproven as to whether horses with IAD suffer the same pathophysiological responses to airway inflammation as those with heaves. Despite this, there is some limited controlled data and much empirical information is available to suggest the efficacy of these agents in IAD.

Corticosteroids may be administered systemically (usually oral, intravenous or intramuscular routes) or via inhalation. In general, use of the shorter acting forms of these agents should be considered as it is anticipated the IAD will be reversible, unlike heaves. Three systemically administered corticosteroids are indicated in the treatment of IAD. These are prednisolone, given orally, dexamethasone and dexamethasone-21-isoniconitate. Dexamethasone may be given orally, via intravenous or intramuscular routes whereas the dexamethasone-21-isoniconitate is given intramuscularly (see Table 4). If dexamethasone is administered per os an appropriate increase in the dose should be prescribed given that the drug is about 50% bio-available when given by this route [140]. Prednisone, an agent popular in the USA for many years for management of a variety of inflammatory disorders, has recently been shown to be ineffective in reducing airway inflammation [141] and as such has no indications for use in horses with IAD.

Table 3: Drugs given by inhalation suggested for use in horses for the management of IAD.

Drug	Trade Name (Laboratory)	Dose Delivered per Actuation	No of Doses per Canister	Device	Dose	Duration of Action
Bronchodilators						
Albuterol + Ipratropium	Combivent (Boehringer Ingelheim)	120 ug (+ 21 ug ipratropium)	200	inhaler	1-2 ug/kg	1-3 hr
Ipratropium bromide	Atrovent (Boehringer Ingelheim)	18 ug	200	Inhaler	0.5-1 ug/kg	4-6 hrs
	Atrovent (Boehringer) Combivent	0.02% solution for nebulisation	2.5 ml vial	Nebuliser	2-3 ug/kg	
Salmeterol	Serevent (Glaxo Wellcome)	25 ug	120 (13g canister)	Inhaler	0.2-1 g/kg	6-8 hrs
Corticosteroids						
Beclomethasone	Beclovent (GlaxoWellcome)	42 ug	200 (16.8 g canister)	Inhaler	1-3 ug/kg	8-12 hrs
Fluticasone	Flovent (GlaxoWellcome)	220 ug	120 (13 g canister)	Inhaler	2-4 ug/kg	8-12 hrs
Other						
Sodium cromoglycate	Intal (Rhone-Poulenc Rorer)	0.02% solution for nebulisation	2 ml vials	Nebuliser	200 mg	12-24 hrs
	Generic				80 mg	

Generally when systemic administration of corticosteroids is prescribed for IAD, horses are treated for 5 - 7 days in the first instance and then if possible, the degree of airway inflammation is re-evaluated. However, this may not be feasible in many

situations and duration of therapy is based frequently on apparent clinical improvement. Horses may also be provided local (inhalation) therapy during this period. Traditionally horses have been "weaned" off exogenous systemic corticosteroids using a tapering dosage schedule. Debate remains as to the necessity for this practice following short-term systemic administration of corticosteroids.

Table 4. Drugs given parenterally suggested for use in horses for the management of IAD.

Drug	Trade Name (Laboratory)	Mechanism of Action	Dose	Route	Remarks
Prednisolone	Various. Generic forms available	Corticosteroid	1-2 mg/kg q 12-24h	Oral	Cheap; well tolerated in feed or as paste; well absorbed from GIT
Dexamethasone	Azium (Schering Plough)	Corticosteroid	0.05-0.1 mg/kg q 24h	Oral, IV, IM	Effective; probably no more than 2-3 doses required
Dexamethasone-21-isonicotinate	Voren (Bio-Ceutic)	Corticosteroid	0.02-0.04 mg/kg q 3d	IM	Should not require more than one dose. Effect may not be evident for 2-3 days
Clenbuterol		Bronchodilator	0.8-3.2 ug/kg q 12h (oral); 0.8 ug/kg (IV) q 24h	Oral, IV	Also has mucolytic effects; enhances mucociliary escalator function; some side effects - sweating, tremors with IV and high oral doses
Trimethoprim Sulfonamide	Various	Antibiotic	15-30 mg/kg q 12h	Oral, IV	Active against many of the bacteria likely to be associated with IAD. Easy to administer per os, therefore popular. Diarrhea reported.
Penicillin G procaine	Various	Antibiotic	22 mg/kg q 12h for procaine salt	IM – procaine	Active against many bacteria associated with IAD. Procaine has long elimination time, restricting use close to competition
Penicillin G benzathine; Penicillin G sodium	Various	Antibiotic	22 mg/kg q 6h for aqueous salts	IV – aqueous salts	Active against many bacteria associated with IAD.
Ceftiofur sodium	Naxcel or Excenel (Upjohn)	Antibiotic	2.2-4.4 mg/kg q 12-24h	IV or IM	Active against many bacteria associated with IAD. Not registered for IV use. IM injections may cause muscle soreness. Diarrhea reported.
Gentamicin	Various	Antibiotic	6.6 mg/kg q 24h	IV	Active against most gram negative organisms associated with IAD. Often combined with penicillin to broaden spectrum.
Oxytetracycline	Various	Antibiotic	6.6 mg/kg q 12-24h	IV	Popular with many racetrack veterinarians for low grade respiratory infections, due to rapid withdrawal times, IV administration and apparent efficacy. Diarrhea reported.

Currently there are a variety of corticosteroids available for inhalation therapy. Availability and cost will vary according to country. In the United States there are five corticosteroids suggested for inhalation therapy in the horse. These include: beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, and triamcinolone acetonide. At present, beclomethasone dipropionate and fluticasone propionate are in common use in horses with respiratory inflammation (Table 3).

Again, extrapolation from data for horses with heaves is required when attempting to design treatment protocols for horses with IAD, and dosages effective for heaves are often recommended. In heavy horses beclomethasone dipropionate at doses ranging from 500 - 1500 ug twice a day (3M Equine Inhaler ®) to 3750 ug twice a day (Aeromask ®) result in significant improvement in clinical signs and lung function as well as reduction in pulmonary inflammation [142,143]. These therapeutic benefits are measurable within 24 hours of administration. One worker suggests doses of 500 - 1500 ug of beclomethasone dipropionate twice a day to treat IAD using commercially available MDI-delivery devices [94]. It should be noted that these doses result in significant systemic absorption manifest by adrenal suppression. Administration of the lower dose (500 ug) to horses with heaves results in similar efficacy as the higher dose but with less adrenal suppression [140]. The half-life of beclomethasone metabolites after inhalation in horses is not known but in humans varies from 1.5 to 15 hours [144].

Fluticasone propionate is reported to relieve signs of heaves when administered at 2000 ug twice daily using an Aeromask®. This dosage schedule resulted in improved clinical signs, decreased airway hyper-reactivity, and reduced pulmonary inflammation. Furthermore, these doses do not result in adrenal suppression. Half-lives for elimination of metabolites of fluticasone in the horse have not been reported.

Listings of common anti-inflammatory drugs suggested for therapy of IAD are included in Table 3 and Table 4.

Sodium Cromoglycate - This agent is a mast cell stabiliser that has been recommended for use in horses with IAD. This is particularly the case where there is an increase in the relative percentage of mast cells (>2%) in samples collected from the lower airway, particularly in BALF [70]. In these cases administration of sodium cromoglycate (200 mg twice daily) has been shown to improve clinical manifestations of respiratory disease and bronchial hyper-reactivity [70,91]. Sodium cromoglycate can be administered via a nebuliser or metered dose inhaler (MDI) using a face-mask (e.g., Aeromask®) [70]. As sodium cromoglycate is a mast cell stabiliser, clinical effects are dependent on the prevention of mast cell degranulation and as such onset of action may be delayed for several days following onset of treatment. Again, it needs to be stressed that this agent will only be effective when airway inflammation is the result of histamine release from degranulated mast cells.

Bronchodilators - Bronchodilators are indicated to relax airway smooth muscle and relieve airflow obstruction, the latter being a likely consequence of airway inflammation. At present two main classes of bronchodilators have been used in the horse: β_2 -agonists and anticholinergics. In general, these products appear to give best results when given via inhalation. However, there are products available for systemic use. It should be stressed that bronchodilators should not be used as the sole therapy for IAD because they do not suppress airway inflammation and do not reduce airway hyper-reactivity [145]. In addition, prolonged use of β_2 -agonists without corticosteroids has been found to induce receptor down regulation, which inevitably will render the drug(s) ineffective. In horses with significant airway obstruction, it is logical to administer bronchodilators prior to corticosteroids in order to optimise lung deposition of the latter product [94].

β_2 -agonists induce smooth muscle relaxation regardless of whether bronchoconstrictive mechanisms have been invoked. In addition, these agents inhibit mast cell degranulation to some degree. This latter effect may mitigate the need for sodium cromoglycate in horses with increased percentages of mast cells in BALF [145]. Albuterol, pirbuterol, and fenoterol are short acting bronchodilators (about 1 hour) with rapid onset of action (about 5 minutes) [146,147]. Salmeterol and formoterol are longer acting β_2 -agonists suitable for twice daily dosing. However, these drugs do have a slower onset of action. Given the nature of IAD, use of salmeterol and formoterol is most logical in horses with this syndrome, as longer-term bronchodilation should be considered one of the main objectives of therapy.

Ipratropium bromide is an anticholinergic drug chemically derived from atropine but devoid of apparent side effects when administered by inhalation. Administration at 2 ug/kg via nebulisation causes bronchodilation for approximately 6 hours [139]. Another advantage of the anticholinergic drugs on airway smooth muscle is that their effects are additive to those of the β_2 -agonists [145]. Ipratropium bromide therefore is suggested to be one of the bronchodilators of choice in horses with IAD.

The most widely used systemically administered bronchodilator in the horse is clenbuterol. This agent is usually administered at 0.8 ug/kg q12h *per os*, although a form suitable for intravenous administration is available. Generally if there is an apparent need for increases in dose rate this is recommended to occur in 0.8 ug/kg increments every 2 - 3 days. This is recommended to minimise adverse side effects such as tachycardia, muscle fasciculations, sweating and excitement. Doses of up to 3.2 ug/kg q 12h have been reported in horses with heaves. An added advantage of clenbuterol, apart from being administered orally, is the improvement in mucociliary clearance occurring in response to administration of this drug. The bronchodilators suggested for use in horses with IAD are included in table 3 and table 4.

Antibiotics - Bacteria may be isolated from TAs collected aseptically in up to 50% of cases of IAD. As a result there is a logical place for use of antimicrobial agents in selected cases of this syndrome. Bacteria involved most commonly include *Streptococcus* spp, *Pasteurella* spp, and *Actinobacillus* spp. These organisms probably colonise the LRT from their normal site of residence in the oropharynx. More rarely, there have been reports of *Bordetella bronchiseptica* and *Mycoplasma* spp being isolated from horses with IAD. Given the bacterial species involved, the antimicrobial agent of choice is penicillin G administered either as a procaine salt (22 mg/kg q 12h IM) or as aqueous (sodium or potassium) salts (22 mg/kg q 6h IV). However, a number of practitioners, particularly those dealing with race or competition horses are reluctant to use these agents particularly the procaine form. This form causes muscle soreness in some horses, is reported to make some horses

lethargic, and has a prolonged withholding period prior to competition. The requirement for six hourly intravenous administrations of the aqueous forms will limit the practical application of these agents, especially in horses not hospitalised for therapy. Several alternatives exist with one of the most popular choices being ceftiofur sodium. This antibiotic is a third generation cephalosporin with a spectrum of activity suitable for bacteria routinely isolated from horses with IAD. Ceftiofur sodium is recommended in these situations at a dose rate of 2.2 - 4.4 mg/kg q 12 - 24h IM or IV, with evidence available to suggest that the lower dose rate may be suitable when given IV q 12h. Similar to procaine penicillin G intramuscular injection of ceftiofur has been associated with apparent muscle soreness in some horses. The drug is not registered for intravenous use in the horse.

Combinations of trimethoprim and sulfonamides are utilised widely in horses with respiratory infections including cases of IAD. Much of the popularity of these combinations lies in the fact that they can be given per os and therefore can be readily administered by owners, trainers, grooms etc. Administration of trimethoprim-sulfonamides at doses of 15 mg/kg q 12h PO of trimethoprim are recommended by the manufacturer, although some specialists recommend dosing at 30-mg/kg q 12h PO. Absorption of these agents is optimised if they are given at least 30 minutes prior to feeding. In some countries an intravenous form of these agents is available. Although trimethoprim-sulfonamide combinations have a broader spectrum of activity than penicillin G, and are usually effective against the bacteria commonly associated with IAD, evidence exists that up to 30% of *Streptococcus* spp isolated from horses in the US show variable resistance to trimethoprim-sulfonamide combinations. As such this may limit their efficacy in some cases of LRT bacterial infections.

Over the past 20 years many practitioners, particularly those dealing with race or competition horses, have administered oxytetracycline to horses with LRT bacterial infections. These agents are popular as they are given intravenously and therefore do not cause muscle soreness. In addition, oxytetracycline, like the trimethoprim-sulfonamide combinations have a short withdrawal time prior to competition. However, the bacteria routinely isolated from cases of IAD have variable sensitivities to this antibiotic.

There are reports recommending the use of enrofloxacin in respiratory infections in the horse. However, in the case of IAD there are few indications for the use of this antimicrobial as; 1) a variety of antibiotics are available with a superior spectrum of activity against the common causative agents, particular *Streptococcus* spp for which enrofloxacin has little efficacy, 2) the high cost of this antibiotic compared to others, and 3) the risk of development of resistance against this important group of antimicrobials in human and veterinary medicine should limit its use to serious infections in which isolates have been identified and sensitivity testing performed.

Regardless of the antimicrobial agents selected for use in horses there is always the risk of adverse side effects. One of the most common and potentially devastating side effects is diarrhea. Although the etiopathogenesis of antibiotic induced diarrhea has not been elucidated fully, the most popular hypothesis is that the antimicrobial agents alter the gastrointestinal microbial flora such that colitis may ensue. In the majority of cases diarrhea is readily reversible when the drug is withdrawn. However, in some cases the colitis may be progressive and life threatening. Other rare yet reported side effects related to antibiotic administration include "procaine" reactions in horses following administration of procaine penicillin G. This may cause central nervous system stimulation following inadvertent intravascular injection during intramuscular administration. Anaphylactoid and type 1 hypersensitivity reactions have been reported rarely in horses associated with antibiotic administration.

Interferon Alpha - Although viral infections are reported to be an uncommon cause of IAD, respiratory viruses, and in particular equine influenza virus, are a potential cause of acute inflammation of the LRT in young racehorses. Furthermore, viral infections such as equine influenza virus and EHV can damage the mucociliary apparatus, which may take up to three weeks to recover [20]. As a result, resting the horse from the rigours of athletic activities should be considered one of the principal methods of management in cases where respiratory viruses are suspected or are positively diagnosed. However, given the costs involved in training horses and the pressures often imposed on trainer, such periods of inactivity may not be readily achievable.

Interferon-alpha (IFN- α) has been recommended for use in horses with viral respiratory tract infections [148]. In mammals, IFN- α is predominantly produced by leucocytes and is a proximal mediator acting early in the inflammatory cascade resulting in immunomodulation and antiviral activity [148]. Administration of exogenous IFN- α is thought to induce an antiviral state in target host cells by stimulating production of enzymes that inhibit viral protein synthesis and degrade viral RNA [148]. Additionally, IFN- α augments non-specific immunity via enhanced phagocytic activity of macrophages and

cytotoxic activity of natural killer and lymphokine-activated killer cells. Therefore, this agent may reduce pulmonary inflammation via immunomodulation or elimination of a persistent viral infection.

The clinical efficacy of IFN- α in horses with IAD was investigated by Rush [104]. In this study, natural human IFN- α was given to actively training Standardbred racehorses with IAD for 5 days per os. These horses were characterized with poor exercise tolerance, exudates in upper and lower airways, and airway inflammation. A semi-quantitative endoscopic score and cytological examination of BALF was performed prior to, 8 and 15 days after commencement of therapy. In this study neutrophil, macrophage, lymphocyte and nucleated cell counts in BALF were significantly lower compared with a placebo treated control. However, the proportion of CD4-, CD5-, and CD8-positive lymphocytes in BALF was not affected. Although this therapy is promising, the current cost and availability of this drug means that the use of this immunomodulator is likely to be limited in horses for some time.

Control

Effective control of IAD is predicated on achieving a better understanding of the underlying pathogenesis of this syndrome. Until the specific etiological agents of IAD are determined, regimens for control and prevention of this disorder remain imprecise. Most of the current control recommendations are based on conservative management techniques such as decreasing the horse's exposure to pro-inflammatory agents in the environment, judicious travel regimens, and appropriate convalescence after overt respiratory tract disease [20]. The efficacy of vaccination for viral respiratory pathogens for prevention of IAD is controversial given the relatively low prevalence of IAD caused by viruses. However, vaccination against equine influenza is mandatory in racing Thoroughbreds in Europe and is recommended for performance horses in the US.

Effect on Performance

The importance of IAD as a cause of poor performance is undefined. However, much speculation has been offered as to the likely effect of this syndrome on racehorses in which peak lung function is required to produce maximal performance and small decrements in this performance may have a major impact on economic outcomes.

The majority of horses with IAD do not exhibit respiratory distress, nor do they have consistent alterations in lung function that can be measured with current techniques [102]. In addition, in one study, horses with evidence of lower airway inflammation did not have a history of exercise intolerance or poor performance [4]. However, in a number of other studies, horses presented with poor performance commonly had endoscopic or cytological evidence of lower airway inflammation [9,34,44,91,98,118,149]. Care must be taken with interpreting this association between poor performance and airway inflammation as association does not equate to causation, and there are potentially many other causes of poor performance or exercise intolerance in any individual animal.

It has been theorised that mucus and inflammatory exudates cause uneven ventilation distribution that impair gas exchange and which may accentuate exercise-induced hypoxemia [79,150]. In support of this hypothesis, identification of excess mucus in large airways has been associated with poor race performance [9], and horses with elevated neutrophil counts in BALF had more severe exercise-induced arterial hypoxemia as compared to controls [97,131]. In contrast, Standardbred horses with mild bronchiolitis had no difference in pulmonary gas exchange when compared to healthy horses whilst undergoing a submaximal graded treadmill exercise test [151]. However, these horses had a significantly increased red cell volume to body weight ratio, which was interpreted as a compensatory mechanism for hypoxemia during maximal work.

Outcomes

The outcomes of IAD remain unclear. Some authors suggest that IAD represents a milder or earlier phase of heaves [15] and that if left untreated or uncontrolled will inexorably progress to this more severe form of chronic small airway inflammation. Others contest that the etiopathogenesis of IAD differs fundamentally from that of heaves [10,67] and that these two syndromes are unrelated. However, as a diverse group of diseases is currently encompassed within the term IAD, it is not surprising that there is evidence that both confirm and refute these hypotheses. Until this disorder is better defined, and specific disease entities within the syndrome delineated, it remains impossible to determine the outcome of lower airway inflammation in young racehorses.

Conclusion

It is clear that many aspects of the syndrome called "inflammatory airway disease" remain unknown or are contentious. Until reproducible criteria and distinguishing characteristics for "lower airway disease syndromes" are defined further, it is

important that detailed descriptions of individual diagnostic findings be offered in published works [89]. Then, as knowledge advances, several issues should be clarified and include: 1) the specific etiological agents causing airway inflammation in young racehorses, 2) the relationship between specific etiological agents and results of different diagnostic techniques, 3) the clinical relevance of mucus accumulation and airway inflammation especially with regard to their effects on performance, 4) the chronological interplay between various agents, 5) the time airway inflammation persists, and 6) the relationship, if any, with chronic respiratory diseases observed in older horses. This knowledge will expand the understanding of this important syndrome of young, athletic horses allowing development of more appropriate treatment and control stratagems.

References

1. Rosedale PD, Hopes R, Wingfield Digby NJ, et al. Epidemiological study of wastage among racehorses 1982 and 1983. *Vet Rec* 1985; 116:66-69.
2. Bailey CJ, Reid SWJ, Hodgson DR, et al. Impact of injuries and disease on a cohort of two- and three-year-old Thoroughbreds in training. *Vet Rec* 1999; 145:487-493.
3. Burrell MH. Endoscopic and virological observations on respiratory disease in a group of young Thoroughbred horses in training. *Equine Vet J* 1985; 17:99-103.
4. Sweeney CR, Humber KA, Roby KA. Cytologic findings of tracheobronchial aspirates from 66 Thoroughbred racehorses. *Am J Vet Res* 1992; 53:1172-1175.
5. Burrell MH, Wood JLN, Whitwell KE, et al. Respiratory disease in thoroughbred horses in training: the relationships between disease viruses, bacteria and environment. *Vet Rec* 1996; 139:308-313.
6. Chapman PS, Green C, Main JPM, et al. Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Vet Rec* 2000; 146:91-95.
7. Wood JLN, Newton JR, Chanter N, et al. A longitudinal epidemiological study of respiratory disease in racehorses: disease definitions, prevalence and incidence. In: Wernery U, Wade JF, Mumford JA, et al. eds. *Equine Infectious Diseases VIII*. Newmarket: R&W Publications Ltd, 1999; 64-70.
8. Christley RM, Hodgson DR, Rose RJ, et al. A case-control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. *Equine Vet J* 2001; 33:256-264.
9. MacNamara B, Bauer S, Lafe J. Endoscopic evaluation of exercise-induced pulmonary hemorrhage and chronic obstructive pulmonary disease in association with poor performance in racing Standardbreds. *J Am Vet Med Assoc* 1990; 196:443-445.
10. Rush Moore B, Krakowka S, Robertson JT, et al. Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. *Am J Vet Res* 1995; 56:562-567.
11. Hoffman AM. Small airway inflammatory disease in equids. In: *Proceedings of the Am Col Vet Int Med* 1995; 13:754-757.
12. Hoffman AM. Bronchoalveolar lavage technique and cytological diagnosis of small airway inflammatory disease. *Equine Vet Edu* 1999; 11:208-214.
13. Hoffman AM, Viel L. Techniques for sampling the respiratory tract of horses. *Vet Clin North Am Equine Pract* 1997;13(3):463-475.
14. Rush BR. Inflammatory Airway Disease. In: *Proceedings of the Br Equine Vet Assoc Congress* 1999; 38:144-145.
15. Viel L. Small airway disease as a vanguard for chronic obstructive pulmonary disease. *Vet Clin North Am Equine Pract* 1997; 13:549-560.
16. Viel L, Hewson J. BAL cytology in horses with exercise tolerance: what does it tell us? In: *Proceedings of the 2nd World Equine Airways Symp and 19th Comp Respir Soc Meet, CD-ROM*, 2001; 1-13.
17. Mazan MR. Inflammatory airway disease - current knowledge. In: *Proceedings of the Am Col Vet Int Med* 2002; 10:707-709.
18. Robinson NE. International workshop on equine chronic airway disease: Michigan State University, 16-18 June 2000. *Equine Vet J* 2001; 33:5-19.
19. Beech J. Tracheobronchial aspirates. In: Beech J, ed. *Equine respiratory disorders*. Philadelphia: Lea and Febiger, 1991; 41-53.
20. Moore BR. Lower respiratory tract disease. *Vet Clin North Am Equine Pract* 1996; 12:457-472.
21. Christley RM, Hodgson DR, Rose RJ, Hodgson JL, Wood JLN, Reid SWJ. Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. *Vet Rec* 2001; 148:99-104.
22. Newton JR, Wood JLN. Summary of a case control study of acute respiratory disease in young Thoroughbred racehorses. In: *Proceedings of the Br Equine Vet Assoc Congress* 1999; 38:190-191.

23. McCarthy RN, Jeffcott LB, Funder JW, et al. Plasma beta-endorphin and adrenocorticotrophin in young horses in training. *Aust Vet J* 1991; 68:359-361.
24. Davis MS, Foster WP. Inhalation toxicology in the equine respiratory tract. In: Lekeux P, ed. *Equine Respiratory Diseases*. New York: International Veterinary Information Service, 2002.
25. Racklyeft DJ, Love DN. Influence of head posture on the respiratory tract of healthy horses. *Aust Vet J* 1990; 67:402-405.
26. Raidal SL, Love DN, Bailey GD. Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 hours of confinement with the head elevated. *Aust Vet J* 1995; 72:45-50.
27. Clarke AF. Chronic pulmonary disease - a multifaceted disease complex in the horse. *Irish Vet J* 1987; 41:258-264.
28. Derksen FJ. Chronic obstructive pulmonary disease. In: Beech J, ed. *Equine respiratory disorders*. Philadelphia: Lea and Febiger, 1991; 223-235.
29. McKane SA, Slocombe RF. Sequential changes in bronchoalveolar cytology after autologous blood inoculation. *Equine Vet J Suppl* 1999; 30:126-130.
30. Erickson HH, Poole DC. Exercise-induced pulmonary haemorrhage. In: Lekeux P, ed. *Equine Respiratory Diseases*. New York: International Veterinary Information Service, 2002.
31. McKane SA, Canfield PJ, Rose RJ. Equine bronchoalveolar lavage cytology: survey of Thoroughbred racehorses in training. *Aust Vet J* 1993; 70:401-404.
32. Wood JLN, Burrell MH, Roberts CA, et al. Streptococci and Pasteurella spp. associated with disease of the equine lower respiratory tract. *Equine Vet J* 1993; 25:314-318.
33. Powell DG, Burrows R, Goodridge D. Respiratory viral infections among Thoroughbred horses in training during 1972. *Equine Vet J* 1974; 6:19-24.
34. Hoffman AM, Chase S, Mazan MR. Association between airway inflammation, airway obstruction and airway reactivity in horses presenting for exercise intolerance and cough: 138 cases. In: Havemeyer Foundation Monograph Series No. 4: Proceedings Workshop Equine Aller Dis Horses 2001; 12.
35. Holcombe SJ, Jackson C, Gerber V, et al. Stabling is associated with airway inflammation in young Arabian horses. *Equine Vet J* 2001; 33:244-249.
36. Clarke AF, Madelin TM, Allpress RG. The relationship of air hygiene in stables to lower airway disease and pharyngeal lymphoid hyperplasia in two groups of Thoroughbred horses. *Equine Vet J* 1987; 19:524-530.
37. Schwartz DA, Thorne PS, Jagielo PJ, et al. Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. *Am J Physiol* 1994; 267:L609-L617.
38. Daly JM, Mumford JA. Influenza infections. In: Lekeux P, ed. *Equine Respiratory Diseases*. New York: International Veterinary Information Service, 2002.
39. Allen GP. Respiratory infections by equine herpesvirus types 1 and 4. In: Lekeux P, ed. *Equine Respiratory Diseases*. New York: International Veterinary Information Service, 2002.
40. Mumford JA, Thomson GR. Studies on picornaviruses isolated from the respiratory tract of horses. In: Bryans JT, Gerber H, eds. *Equine Infectious Diseases IV*. Princeton: Veterinary Publications, 1978; 419-429.
41. Allen GP, Bryans JT. Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infections. *Prog Vet Microbiol Immunol* 1986; 2:78-144.
42. Kydd JH, Smith KC, Hannant D, et al. Distribution of Equid herpesvirus-1 (EHV-1) in the respiratory tract of ponies: implications for vaccination strategies. *Equine Vet J* 1994; 26:466-469.
43. Bryans JT, Allen GP. Herpesviral diseases of the horse. In: Wittmann G, ed. *Herpesvirus diseases of cattle, horses and pigs*. Boston: Kluwer, 1989; 176-229.
44. Wood JLN, Chanter N, Sinclair R, et al. The epidemiology of outbreaks of respiratory disease and poor performance in racing Thoroughbred horses. In: Nakajima H, Plowright W, eds. *Equine Infectious Diseases VII*. Newmarket: R&W Publications Ltd, 1994; 358-359.
45. Willoughby RA, Ecker G, McKee S, et al. The effects of equine rhinovirus, influenza virus and herpesvirus infection on tracheal clearance rate in horses. *Can J Vet Res* 1992; 56:115-121.
46. Hannant D, O'Neil T, Jessett DM, et al. Evidence for non-specific immunosuppression during development of immune responses to equid herpesvirus-1. *Equine Vet J Suppl* 1991; 12:41-45.
47. Hoffman AM, Viel L, McDonnell WN, et al. Airway hyperresponsiveness in ponies following a naturally acquired influenza infection. *Am Rev Respir Dis* 1992; 145:A432-.
48. Mumford JA, Rosedale PD. Virus and its relationship to the "poor performance" syndrome. *Equine Vet J* 1980; 12:3-9.
49. Burrell MH, Whitwell KE, Wood JLN, et al. Pyrexia associated with respiratory disease in young thoroughbred horses. *Vet Rec* 1994; 134:219-220.
50. Halliwell REW, McGorum BC, Irving P, et al. Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Vet Immunol Immunopathol* 1993; 38:201-215.

51. Derksen FJ. Evaluation of airway inflammation. In: Hodgson D, ed. Proceedings of the AEVA (Bain-Fallon Memorial Lectures) 2001; 23:21-41.
52. Whitwell KE, Greet TRC. Collection and evaluation of tracheobronchial washes in the horse. *Equine Vet J* 1984; 16:499-508.
53. Ward CL, Wood JLN, Houghton SB, et al. Actinobacillus and Pasteurella species isolated from horses with lower airway disease. *Vet Rec* 1998; 143:277-279.
54. Blunden AS, Hannant D, Livesay GJ, et al. Susceptibility of ponies to infection with Streptococcus pneumoniae (capsular type 3). *Equine Vet J* 1994; 26:22-28.
55. Varma KJ, Powers TE, Powers JD, et al. Standardization of an experimental disease model of Streptococcus zooepidemicus in the equine. *J Vet Pharmacol Ther* 1984;7:183-188.
56. Sweeney CR, Holcombe SJ, Barningham SO, et al. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *J Am Vet Med Assoc* 1991; 198:839-842.
57. Sweeney CR, Divers TJ, Benson CE. Anaerobic bacteria in 21 horses with pleuropneumonia. *J Am Vet Med Assoc* 1985; 187:721-724.
58. Raidal SL. Equine pleuropneumonia. *Br Vet J* 1995; 151:233-261.
59. Wood JLN, Chanter N, Newton, et al. An outbreak of respiratory disease in horses associated with Mycoplasma felis infection. *Vet Rec* 1997; 140:388-391.
60. Hoffman AM, Baird JD, Kloeze HJ, Rosendal S, Bell M. Mycoplasma felis pleuritis in two show-jumper horses. *Cornell Vet* 1992; 82:155-162.
61. Viel L. Structural functional correlations of the lung in horses with small airway disease. PhD dissertation, University of Guelph, Guelph, Canada, 1983.
62. Rossier Y, Sweeney CR, Ziemer EL. Bronchoalveolar lavage fluid cytologic findings in horses with pneumonia or pleuropneumonia. *J Am Vet Med Assoc* 1991; 198:1001-1004.
63. Bailey GD, Love DN. Oral associated bacterial infection in horses: studies on the normal anaerobic flora from the pharyngeal tonsillar surface and its association with lower respiratory tract and paraoral infections. *Vet Microbiol* 1991; 26:367-379.
64. Kamada M, Akiyama Y. Studies on the distribution of Streptococcus zooepidemicus in equine respiratory tracts. *Exp Report Equine Hlth Lab* 1975; 12:53-63.
65. Vandevenne S, Caudron I, Serteyn D, et al. Bordatella bronchiseptica respiratory infections in horses. *Ann Med Vet* 1995; 139:349-352.
66. Hoquet F, Higgins R, Lessard P, et al. Comparison of the bacterial and fungal flora in the pharynx of normal horses and horses affected with pharyngitis. *Can Vet J* 1985; 26:342-346.
67. Christley RM. Inflammatory airway disease in young thoroughbreds and comparison with chronic obstructive pulmonary disease. In: Proceedings of the Br Equine Vet Assoc 2000; 39:100-101.
68. Hare JE, Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. *J Vet Intern Med* 1998; 12:163-170.
69. Hughes KJ, Malikides N, Hodgson DR, et al. Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses 1: evaluation of cytological stains and the relative percentage of mast cells and eosinophils. *Aust Vet J* 2002 (in press).
70. Viel L. Lower airway inflammation in young performance horses. In: Robinson NE, ed. Current Therapy in Equine Medicine 4. Philadelphia: WB Saunders Co; 1997; 426-431.
71. Mair TS. Value of tracheal aspirates in the diagnosis of chronic pulmonary diseases in the horse. *Equine Vet J* 1987; 19:463-465.
72. Mackay RJ, Urquhart KA. An outbreak of eosinophilic bronchitis in horses possibly associated with Dictyocaulus arnfieldi infection. *Equine Vet J* 1979; 11:110-112.
73. McGorum BC, Dixon P, M. The analysis and interpretation of equine bronchoalveolar lavage fluid (BALF) cytology. *Equine Vet Educ* 1994; 6:203-209.
74. Darien B, J. Eosinophilic pneumonitis in foals and horses. *Compend Contin Educ Pract Vet* 1994; 16:1210-1212.
75. Art T, McGorum BC, Lekeux P. Environmental control of respiratory disease. In: Lekeux P, ed. Equine Respiratory Diseases. New York: International Veterinary Information Service, 2002.
76. Rylander R. The role of endotoxin and (1-3)-b-D-glucan as synergistic agents in lung inflammation and allergy. In: Proceedings of the 2nd World Equine Airways Symp and 19th Comp Respir Soc Meet, CD-ROM, 2001; 1-8.
77. Robinson NE. Recurrent Airway Obstruction. In: Lekeux P, ed. Equine Respiratory Diseases. New York: International Veterinary Information Service, 2002.
78. Clarke AF. A review of environmental and host factors in relation to equine respiratory disease. *Equine Vet J* 1987;

19:435-441.

79. Robinson NE. Pathogenesis and management of airway disease. In: Proceedings of the Annu Conv Am Assoc Equine Pract 1997; 43:106-115.
80. Woods PSA, Robinson NE, Swanson MC, et al. Airborne dust and aeroallergen concentration in a horse stable under two different management systems. *Equine Vet J* 1993; 25:208-213.
81. McGorum BC, Ellison J, Cullen RT. Total and respirable airborne dust endotoxin concentrations in three equine management systems. *Equine Vet J* 1998; 30:430-434.
82. Vandemput S, Istasse L, Nicks B, et al. Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. *Vet Q* 1997; 19:154-158.
83. Morgan WK. Occupational bronchitis. *Eur J Respir Dis* 1982; 63, Suppl. 123:117-124.
84. McGorum BC, Dixon PM, Halliwell REW. Responses of horses affected with chronic obstructive pulmonary disease to inhalation challenges with mould antigens. *Equine Vet J* 1993; 25:261-267.
85. Malikides N, Pike A, Kane K, et al. Endotoxin concentrations in respirable dust over time in horses bedded on straw versus sawdust. In: Proceedings of the Vet Comp Resp Soc Symp 2000; 18:51.
86. Pirie RS, Dixon P, M, Collie DDS, et al. Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. *Equine Vet J* 2001; 33:311-318.
87. Tyler WS, Jones JH, Birks EK, et al. Effects of ozone on exercising horses: a preliminary report. *Equine Exer Physiol* 1991; 3:490-502.
88. Donaldson K, MacNee W. Ultrafine Particle, oxidative stress and lung inflammation. In: Proceedings of the 2nd World Equine Airways Symp and 19th Comp Respir Soc Meet, CD-ROM, 2001; 1-11.
89. Gerber V, Straub R, Schott HD, et al. Is mild airway inflammation and mucus accumulation really abnormal and clinically significant? In: Havemeyer Foundation Monograph Series No. 4: Proceedings Workshop Equine Allergic Diseases of Horses 2001; 10.
90. Lavoie JP, Maghni K, Desnoyers M, et al. Neutrophilic airway inflammation in horses with heaves is characterised by a Th2-type cytokine profile. *Am J Respir Crit Care Med* 2001; 164:1410-1413.
91. Hare JE, Viel L, O'Byrne PM, et al. The effects of sodium chromoglycate on light racehorses with elevated metachromatic cell numbers on bronchoalveolar lavage and reduced exercise tolerance. *J Vet Pharmacol Ther* 1994; 17:237-244.
92. Hoffman AM, Mazan MR, Ellenberg S. Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. *Am J Vet Res* 1998; 59:176-181.
93. Mazan MR, Hoffman AM. Effects of aerosolized albuterol on physiologic responses to exercise in standardbreds. *Am J Vet Res* 2001; 62:1812-1817.
94. Couetil LL. Aerosol medications for the management of inflammatory airway disease (IAD). In: Proceedings of the Am Col Vet Int Med 2002; 20:716-718.
95. Robinson NE, Olszewski MA, Boehler D, et al. Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. *Equine Vet J* 2000; 32:393-400.
96. Bailey CJ, Rose RJ, Reid SW, et al. Wastage in the Australian Thoroughbred industry: a survey of Sydney trainers. *Aust Vet J* 1977; 75:64-66.
97. Couetil LL, Denicola DB. Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease. *Equine Vet J Suppl* 1999; 30:77-82.
98. Malikides N, Hughes KJ, Hodgson DR, et al. Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses 2: evaluation of the relative percentage of neutrophils. *Aust Vet J* 2002 (in press).
99. Bracher V, von Fellenberg R, Winder NC. An investigation of the incidence of chronic obstructive pulmonary disease (COPD) in random populations of Swiss horses. *Equine Vet J* 1991; 23:136-141.
100. Christley RM. Studies of the epidemiology of respiratory disease in Thoroughbred racehorses in Sydney, Australia. PhD dissertation, University of Sydney, 1998.
101. Dixon PM. Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. *Vet Rec* 1992; 131:229-235.
102. Dixon PM. Ancillary diagnostic techniques for the investigation of equine pulmonary disease. *Equine Vet Educ* 1997; 9:72-80.
103. Gerber V. Mucus in equine lower airway disease. In: Proceedings of the 2nd World Equine Airways Symp and 19th Comp Respir Soc Meet, CD-ROM, 2001; 1-11.
104. Moore BR, Krakowka S, Cummins JM, et al. Changes in airway inflammatory cell populations in Standardbred racehorses after interferon-alpha administration. *Vet Immunol Immunopathol* 1996; 49:347-358.
105. Beech J. Cytology of tracheobronchial aspirates in horses. *Vet Pathol* 1975; 12:157-164.
106. Roberts CA. The relationship between visible mucus, EIPH, age and finishing position in Thoroughbred racehorses in

- the UK. In: Proceedings of the 1st World Equine Airways Symp and 16th Comp Respir Soc Meet, 1998; 4.
107. Wisner ER, O'Brien TR, Lakritz J, et al. Radiographic and microscopic correlation of diffuse interstitial and bronchointerstitial pulmonary patterns in the caudodorsal lung of adult Thoroughbred horses in race training. *Equine Vet J* 1993; 25:293-298.
108. Mazan MR, Hoffman AM, Vin R. Do radiographic changes correlate with lung mechanics, airway reactivity, or BAL cytology in horses with inflammatory airway disease. In: Proceedings of the Comp Respir Soc Symp; 1999; 17:74.
109. Robinson NE, Derksen FJ, Jackson CA, et al. Management of heaves. *Equine Vet Educ* 2001; 13:247-259.
110. Derksen FJ, Brown CM, Sonea IM, et al. Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. *Equine Vet J* 1989; 21:23-26.
111. Mair TS, Sweeney CR. Advances in the diagnosis of equine lung disease: sampling from the lower airways. *Equine Vet J* 1990; 22:147-148.
112. Larson VL, Busch RH. Equine tracheobronchial lavage: comparison of lavage cytologic and pulmonary histopathologic findings. *Am J Vet Res* 1985; 46:144-146.
113. Mair TS. Diagnostic techniques for lower respiratory tract diseases. In: Robinson NE, ed. *Current Therapy in Equine Medicine*. Philadelphia: WB Saunders Co, 1993; 299-303.
114. Christley RM, Hodgson DR, Rose RJ, et al. Comparison of bacteriology and cytology of tracheal fluid samples collected by percutaneous transtracheal aspiration or via an endoscope using plugged, guarded catheter. *Equine Vet J* 1999; 31:197-202.
115. Sweeney CR, Sweeney RW, Benson CE. Comparison of bacteria isolated from specimens obtained by use of endoscopic guarded tracheal swabbing and percutaneous tracheal aspiration in horses. *J Am Vet Med Assoc* 1989; 195:1225-1229.
116. Beech J. Techniques of tracheobronchial aspiration in the horse. *Equine Vet J* 1981; 13:136-137.
117. van den Berg JS, Guthrie AJ, Meintjes RA, et al. Water and electrolyte intake and output in conditioned Thoroughbred horses transported by road. *Equine Vet J* 1998; 30:316-323.
118. Martin BB, Beech J, Parente EJ. Cytologic examination of specimens obtained by means of tracheal washes performed before and after high-speed treadmill exercise in horses with a history of poor performance. *J Am Vet Med Assoc* 1999; 214:673-677.
119. Davis MS. Exercise-induced bronchoconstriction in man and animals. In: Proceedings of the Comp Respir Soc Symp 1999; 17:88 .
120. Clark CK, Lester GD, Vetro T, et al. Bronchoalveolar lavage in horses: effect of exercise and repeated sampling on cytology. *Aust Vet J* 1995; 72:249-252.
121. Raidal SL, Love DN, Bailey GD. Effect of a single bout of high intensity exercise on lower respiratory tract contamination in the horse. *Aust Vet J* 1997; 75:293-295.
122. Blunden AS, Mackintosh ME. The microflora of the lower respiratory tract of the horse: an autopsy study. *Br Vet J* 1991; 147:238-250.
123. Mair TS, Stokes CR, Bourne FJ. Cellular content of secretions obtained by lavage from different levels of the equine respiratory tract. *Equine Vet J* 1987; 19:458-462.
124. Roszel JF, Freeman KP, Slusher SH. Equine pulmonary cytology. In: Proceedings of the Annu Conv Am Assoc Equine Pract 1985; 31:171-181.
125. Hewson J, Viel L. Sampling, microbiology and cytology of the respiratory tract. In: Lekeux P, editor. *Equine Respiratory Diseases: IVIS*; 2002.
126. Fogarty U. Evaluation of a bronchoalveolar lavage technique. *Equine Vet J* 1990; 22:174-176.
127. McGorum BC, Dixon P, M, Halliwell REW, et al. Comparison of cellular and molecular components of bronchoalveolar lavage fluid harvested from different segments of the equine lung. *Res Vet Sci* 1993; 55:57-59.
128. Fogarty U, Buckley T. Bronchoalveolar lavage findings in horses with exercise intolerance. *Equine Vet J* 1991; 23:434-437.
129. Couetil LL, Rosenthal FS, Denicola DB, et al. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am J Vet Res* 2001; 62:538-546.
130. Sutton GA, Viel L, Carman PS, et al. Pathogenesis and clinical signs of equine herpesvirus-1 in experimentally infected ponies in vivo. *Can J Vet Res* 1998; 62:49-55.
131. McKane SA, Rose RJ, Evans DL. Relationships between bronchoalveolar lavage cytology and gas exchange during exercise in racehorses presented for poor racing performance. In: Proceedings of the Int EIPH Conf 1993; 13-14.
132. Willoughby RA, McDonnell WN. Pulmonary function testing in horses. *Vet Clin North Am Large An Pract* 1979; 1:171-191.
133. Doucet MY, Vrins AA, Fort-Hutchinson AW. Histamine inhalation challenge in normal horses and horses with small airway disease. *Can J Vet Res* 1991; 55:285-293.

134. Mazan MR, Hoffman AM, Manjerovic N. Comparison of forced oscillation with the conventional method for histamine bronchoprovocation testing in horses. *Am J Vet Res* 1999; 60:174-180.
135. O'Callaghan MW, Pascoe JR, O'Brien TR, et al. Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VI. Radiological/pathological correlations. *Equine Vet J* 1987; 19:419-422.
136. Lakritz J, Wisner ER, Finucane T, et al. Morphologic and morphometric characterization of lung collagen content in clinically normal adult Thoroughbreds in race training. *Am J Vet Res* 1995; 56:11-18.
137. Persson S, Lindberg R. Lung biopsy pathology and exercise tolerance in horses with chronic bronchiolitis. *Equine Exerc Physiol* 1991; 3:457-464.
138. Taylor RH, Lerman J, Chambers C, et al. Dosing efficiency and particle-size characteristics of pressurized metered-dose inhaler aerosols in narrow catheters. *Chest* 1993; 103:920-924.
139. Robinson NE. Pharmacology of the equine tracheobronchial tree. In: *Proceedings of the Annu Conv Am Assoc Equine Pract* 1997; 43:95-105.
140. Rush BR, Raub ES, Rhoads WS, et al. Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. *Am J Vet Res* 1998; 59:1039-1043.
141. Jackson CA, Robinson NE, Berney CEA, et al. Prednisone - is it really effective in the treatment of chronic obstructive pulmonary disease? In: *Proceedings of the Annu Conv Am Assoc Equine Pract* 1999; 45:304-305.
142. Ammann VJ, Vrins AA, Lavoie J-P. Effects of inhaled beclomethasone dipropionate on respiratory function in horses with chronic obstructive pulmonary disease (COPD). *Equine Vet J* 1998; 30:152-157.
143. Rush BR, Raub ES, Thomsen MM, et al. Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction. *J Am Vet Med Assoc* 2000; 217:359-364.
144. Kelly HW. Comparison of inhaled corticosteroids. *Ann Pharmacol* 1998; 32:220-232.
145. Flood-Page P, Barnes NC. What are the alternatives to increasing inhaled corticosteroids for the long term control of asthma? *BioDrugs* 2001; 15:185-198.
146. Derksen FJ, Olszewski MA, Robinson NE, et al. Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. *Am J Vet Res* 1999; 60:689-693.
147. Tesarowski DB, Viel L, McDonnell WN, et al. The rapid and effective administration of a beta2-agonist to horses with heaves using a compact inhalation device and metered-dose inhalers. *Can Vet J* 1994; 35:170-173.
148. Rush BR. Immune therapy in respiratory disease. *Vet Clin North Am Equine Pract* 1997; 13:531-548.
149. Viel L, Hewson J, Parsons D, et al. Tracheal aspirates and bronchoalveolar lavage fluid differentials in poor performance racing horses. In: *Proceedings of the Comp Respir Soc Symp* 2000; 18:57.
150. Robinson NE, Derksen FJ. COPD: newer concepts. In: *Proceedings of the 1st World Equine Airways Symp and 16th Comp Respir Soc Meet, CD-ROM, 1998.*
151. Nyman G, Bjork M, Funkquist P. Gas exchange during exercise in standardbred trotters with mild bronchiolitis. *Equine Vet J Suppl* 2001;30:96-101.

All rights reserved. This document is available on-line at www.ivis.org. Document No. B0316.0802.

