Havemeyer Foundation

Proceedings of a Workshop on

INFLAMMATORY AIRWAY DISEASE: DEFINING THE SYNDROME

30th September – 3rd October 2002
Boston, USA

Editors: A. Hoffman, N. E. Robinson and J. F. Wade

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EDITORS' FOREWORD

The nomenclature of equine non-infectious airway disease is a source of puzzlement to many. The terms 'chronic obstructive pulmonary disease (COPD)', 'inflammatory airway disease (IAD)', 'chronic obstructive bronchitis (COB)', 'reactive airway disease', 'allergic bronchitis', 'heaves', 'broken wind' and 'small airway disease' confuse vets and other biomedical scientists. The plethora of terminology reflects a poor understanding of the pathogenesis of equine airway disease and leads to confusion as to the best way to treat and prevent airway disease.

For years, 'equine COPD' was used to describe any accumulation of neutrophils and mucus in the airways in the absence of active infection, eg the mature horse with severe heaves and the younger animal with reduced performance, increased airway neutrophils and mucus. However, the 2 conditions are very different and there is no evidence that the former is a consequence of the latter. A further source of confusion is the incongruous use of the term in human and veterinary medicine. In human medicine, COPD is a disease of aged smokers and the term would not be used to describe a young athlete with a cough, increased mucus production and reduced exercise ability. In the latter individual, smoking history, allergies, work environment and infection history would all be investigated; COPD would not be considered as a diagnosis. Equine and human COPD differ in many aspects. For example, the airway obstruction of human COPD is scarcely reversible whereas the obstruction of severe equine COPD is reversed by a change in environment, bronchodilator drugs or corticosteroids.

To address this confusion, a conference was held at Michigan State University in 2000. Delegates decided to eliminate the term 'COPD' and recommended the term 'heaves' to describe the severe but reversible airway obstruction seen in mature horses. Because of the natural recurrence of airway obstruction in heaves, and its

reversibility with drugs, 'recurrent airway obstruction (RAO)' was also deemed an appropriate term. By exclusion, all other forms of non-infectious airway disease are referred to as IAD, the topic of this workshop.

The Havemeyer Workshop series brings together investigators from a range of disciplines and locations to discuss topic of specific concern to the horse industry. IAD is such a topic and we therefore invited clinicians, epidemiologists, pathologists, microbiologists and physiologists to share knowledge with each other. Several important questions must be addressed before we can provide solid answers about IAD. First, we must decide what each of us means by the term IAD. Is the syndrome seen in young racehorses similar to that of the older 'cougher without heaves'? How important is infection? Does the culture of organisms from the trachea mean that these organisms are causing functional changes in the airways? Is inflammation regionalised in the lung? Does inflammation and mucus in the trachea indicate lower airway inflammation? What are the functional consequences of IAD and the clinical consequences to the horse? Finally, what recommendations for treatment and prevention can we make? Addressing these questions will require interdisciplinary studies and itt is our hope that this Workshop will stimulate the necessary interactions to make such studies a possibility. We are extremely grateful to Mr Gene Pranzo, President of the Havemeyer Foundation, for his support of this workshop and to Rachel Pepper at R&W Publications for the excellent organisational arrangements.

Ed Robinson
Michigan State University

Andy Hoffman
Tufts University

Havemeyer Workshop Organisers

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1983   **Third International Workshop on Lymphocyte Alloantigens of the Horse**
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October - Lexington, Kentucky, USA
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March - Gainesville, Florida, USA
Organiser: Dr M. M. LeBlanc

Trophoblast Differentiation
September - Edinburgh, Scotland
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January - San Diego, California, USA
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February - Sydney, Australia
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Allergic Diseases of the Horse
April - Lipica, Slovenia
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March - Naples, Florida, USA
Organiser: Dr M. M. LeBlanc

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July - Saari, Finland
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January - San Diego, California

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January - Santa Fe, New Mexico
Organiser: Dr D. P. Lunn

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June - Port Douglas, Australia
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International Equine Gene Mapping
July - Brisbane, Australia
Organiser: K. Bell

Second Meeting of the European Gamete Group (EEGG)
September - Loosdrecht, The Netherlands
Organiser: Dr T. A. E. Stout

Foal Septicemia III
October - Tufts University European Center, Talloires, France
Organiser: M. R. Paradis

Infectious Disease Programme for the Equine Industry and Veterinary Practitioners
October - Marilyn duPont Scott Medical Center, Morvan Park, Virginia, USA
Organisers: Drs J. A. Mumford and F. Fregin

From Epididymis to Embryo
October - Fairmont Hotel, New Orleans, USA
Organiser: Dr L. H-A. Morris

2002   **USDA International Plant & Animal Genome Conference**
January - San Diego, California

Comparative Neonatology/Perinatology
January - Palm Springs, California
Organiser: P. Sibbons

Stallion Behavior IV
June - Reykjavik, Iceland
Organisers: S. McDonell and D. Miller

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July - Pullman, Washington
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INFLAMMATORY AIRWAY DISEASE: DEFINING THE SYNDROME

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SESSION I:

Clinical evidence

Chairman:
    N. E. Robinson

INFLAMMATORY AIRWAY DISEASE: A CLINICIAN'S VIEW FROM NORTH AMERICA

B. R. Rush

Kansas State University, Manhattan, Kansas, USA

Inflammatory airway disease (IAD) describes a heterogeneous group of inflammatory conditions of the lower respiratory tract that appear to be primarily non-infectious. Poor exercise performance and excessive tracheal exudates on endoscopic examination are consistent clinical findings. Cytologic evaluation of bronchoalveolar lavage (BAL) fluid in horses with IAD will reveal one of the following inflammatory profiles: 1) mixed inflammation with high total nucleated cells, mild neutrophilia (15% of total cells), lymphocytosis, and monocytosis; 2) increased metachromatic cells (mast cells >2% of total cells); or 3) eosinophilic inflammation (5–40% of total cells). Therapeutic recommendations will be based on results of the BAL cytologic evaluation. The majority of cases will be treated with an anti-inflammatory preparation, bronchodilating agents, and avoidance of environmental irritants. Depending on cytological findings, immunostimulant or immunosuppressive therapy may be indicated to reduce pulmonary inflammation.

MAST CELL-RICH AND EOSINOPHILIC INFLAMMATION

Mast cell stabilising drugs and aerosolised corticosteroids are recommended for treatment of mast-cell rich IAD. Nebulisation of sodium cromoglycate (80–200 mg) improves clinical signs of respiratory disease and reduces histamine content in equine mast cells (Hare *et al.* 1994). Mast cell stabilising drugs also inhibit the release of inflammatory mediators from eosinophils and may be of potential benefit to horses with eosinophilic IAD, although therapeutic benefit has not been reported. Aerosolised corticosteroid administration improves clinical signs of disease and BAL findings in horses with mast-cell rich

IAD. Horses with eosinophilic pulmonary inflammation may have pulmonary parenchymal granulomas, peripheral eosinophilia, and evidence of extrapulmonary eosinophilic infiltration (skin, GI, urinary), necessitating systemic corticosteroid administration.

Bronchodilators do not address the primary inflammatory process. However, bronchodilator therapy is an important component of treatment of IAD because it provides immediate relief of airway obstruction and protects against irritant-induced bronchoconstriction. Administration of bronchodilators prior to exercise may prevent exercise-induced bronchoconstriction and improve performance of horses with mild to moderate airway obstruction. In addition, short-acting bronchodilators can be administered prior to administration of topically active mast cell stabilising drugs and corticosteroid preparations to improve pulmonary drug distribution and prevent irritant cough.

MAST CELL STABILISING DRUGS

Sodium cromoglycate (SCG) and nedocromil sodium (NS) inhibit mast cell degranulation and prevent the release of potent inflammatory mediators including histamine, leukotrienes and cytokines. In addition, nedocromil sodium has been shown to inhibit the release of inflammatory mediators from several other inflammatory cells (eosinophils, neutrophils, macrophages), and can block immediate vagal-mediated bronchoconstriction caused by irritant stimuli and exercise. Mast cell degranulation products may have an autocrine effect to induce mast cell hyperplasia, thus the proposed mechanism for reduced histamine content in equine mast cells recovered from horses with clinical IAD is reduction of histamine production with attenuated histamine

release. The therapeutic benefit of NS and SCG appears to increase with prolonged administration (Hoffman 1997). In humans, NS is approximately 10 times more potent than SCG with equivalent efficacy at equipotent doses. Coughing, throat irritation, and bronchoconstriction may be observed after administration, which is attenuated by precedent administration of a bronchodilator (Hoffman 1997). Neither sodium cromoglycate nor nedocromil sodium has direct bronchodilator or antihistamine properties. Therefore, these anti-inflammatory drugs are considered prophylactic medications and are labelled for the preventive management of asthma in human patients. Neither of these drugs has an effect on normal immunological defence mechanisms, nor any known systemic activity and there are no reports of toxicity.

CORTICOSTEROIDS

Aerosolised corticosteroids are effective in horses with mild to moderate airway obstruction with clinical signs ranging from exercise intolerance to horses with moderate increased effort of respiration at rest. Aerosolised corticosteroid preparations are short-acting glucocorticoids so prolonged therapeutic benefit is not anticipated from short-term administration. After the initial reduction in inflammation, administration once a day may be effective to maintain disease remission. There are 3 aerosolised corticosteroid preparations available in MDI formulation for administration to horses: fluticasone propionate, beclomethasone dipropionate, and flunisolide. The relative potency of these surface-active corticosteroids is fluticasone (relative potency = 18.0) > beclomethasone (13.5) > flunisolide (1.9; Barnes *et al.* 1998).

Fluticasone is the most potent and the most expensive of the aerosolised corticosteroids. Fluticasone is highly lipophilic and, consequently, has the longest pulmonary residence time. Because of its low oral bioavailability (<2%) and extensive first-pass metabolism (99%), fluticasone has the least potential for adverse systemic effects and the most favourable therapeutic index of all of the aerosolised corticosteroids. In heaves-affected horses, fluticasone (2,000 µg, BID, Equine AeroMask) reduces pulmonary neutrophilia, improves parameters of pulmonary function and reduces responsiveness to histamine challenge during an episode of airway obstruction (Viel *et al.* 1999).

Beclomethasone (500–1,500 µg, BID, Equine Aerosol Delivery Device) reduces pulmonary inflammation, improves parameters of pulmonary function and improves ventilation imaging of horses with recurrent airway obstruction (Rush *et al.* 1998a,b) There is no immediate (15 min) therapeutic effect but clinical signs and pulmonary function begin to improve within 24 h of administration (Rush *et al.* 1999). Using the Equine AeroMask, administration of beclomethasone (3,750 µg, BID) to horses with heaves improves parameters of pulmonary function and arterial oxygen tension for a 2-week treatment period (Ammann *et al.* 1998).

The timing of administration once a day has a pivotal effect on the safety profile and consequently the risk/benefit ratio of inhaled corticosteroids (Meibohm *et al.* 1997). Maximum adrenal suppression occurs with administration of aerosolised corticosteroids in the early morning hours, whereas endogenous cortisol production is least disrupted by administration in the afternoon. The longer the terminal elimination half-life of the drug, the earlier in the afternoon it should be administered. For example, the adrenosuppressive effects of flunisolide ($t^1/_2 = 1.5$ h) are minimised when a single daily dose is administered at 7 pm, whereas the optimum time for administration of fluticasone propionate ($t^1/_2 = 6$ h) is 4 pm. In addition to safety concerns, afternoon drug administration provides superior control of the clinical signs of nocturnal asthma. The safety and efficacy of administration of aerosolised corticosteroids once a day (afternoon/ evening) in horses has not been evaluated.

BRONCHODILATORS

Although horses with IAD do not have overt evidence of bronchoconstriction on physical examination, affected horses are hyper-responsive to irritant stimuli and may suffer from low-grade bronchoconstriction that impairs exercise performance. Short-acting bronchodilators provide immediate relief of airway obstruction. Administration prior to exercise may prevent exercise-induced bronchoconstriction and improve performance of horses with mild to moderate airway obstruction. In addition, short-acting bronchodilators can be administered prior to administration of anti-inflammatory medications to improve pulmonary drug distribution and prevent cough. Albuterol sulphate (360–900 µg,) is a potent, short-acting β_2 adrenergic agent with

rapid onset of bronchodilation (5 min) in horses (Derksen *et al.* 1999). The duration of effective bronchodilation is approximately 1–3 h. Albuterol improves parameters of pulmonary function by approximately 70% in horses with heaves during an episode of airway obstruction.

Long-acting bronchodilators are recommended for maintenance bronchodilation in horses with IAD (Hoffman 1997). Salmeterol is a long-acting β_2 adrenergic agent that is a chemical analogue of albuterol. In human patients, administration of salmeterol twice a day provides superior control of bronchoconstriction compared to regular (QID) or PRN administration of albuterol. In comparison to albuterol, salmeterol has higher lipophilicity, β_2 affinity, β_2 selectivity, and potency (10-fold). In addition to the bronchodilatory activity, salmeterol prevents irritant-induced bronchoconstriction and has anti-inflammatory properties such as inhibition of leukotiene release, prevention of histamine release, and reduction of eosinophil activity. Salmeterol (210 µg via Equine AeroMask) provides relief of clinical signs of airway obstruction for 6–8 h in heaves-affected horses (Henrikson and Rush 2001).

Ipratropium bromide is a synthetic, anticholinergic compound that produces bronchodilation, inhibits cough, and protects against bronchoconstrictive stimuli. Like atropine, ipratropium bromide is nonselective (M_1, M_2, M_3) muscarinic antagonist, and bronchodilation results from blockade of the M_3 receptor. Because of its quaternary ammonium structure, ipratropium is poorly absorbed from the respiratory system (6%) or gastrointestinal tract (2%). Therefore, ipratropium does not inhibit gastrointestinal motility and has minimal systemic adverse effects. In addition, ipratropium does not cause drying of respiratory secretions and does not inhibit mucociliary clearance. Ipratropium has a greater bronchodilatory effect on larger, more central airways. The onset of bronchodilation is approximately 15–30 min, and the effect lasts approximately 4–6 h.

MIXED INFLAMMATORY (NEUTROPHILIC) INFLAMMATION

Horses with neutrophilic inflammation often have a history of viral respiratory infection prior to the onset of IAD. Interferon-α (IFNα) has immunomodulatory and antiviral activity, and reduces pulmonary inflammation in horses with poor race performance and mixed inflammatory BAL fluid. The pathophysiology of mixed inflammatory IAD may be triggered by viral respiratory infection or environmental irritants (ozone). Low-dose (50–150 IU) natural, human IFNα reduces exudate in the respiratory tract, lowers total cell counts in BAL fluid, and converts the differential cell count to a non-inflammatory cytological profile (Rush Moore *et al.* 1996). Interferon-α administration is not effective in horses with mast cell-rich or eosinophilic bronchoalveolar lavage. In addition, IFNα is not beneficial in the treatment of acute, fulminate viral respiratory infection in horses. Oral administration of low-dose (0.22–2.2 IU/kg bwt) rIFNα-2a does not diminish the severity of clinical disease or duration of viral shedding in horses with experimental equine herpesvirus-1 infection (Seahorn *et al.* 1990).

IMMUNOSTIMULANT THERAPY

The indications for immunostimulant therapy are relatively specific; these compounds are not intended to treat a broad-spectrum of respiratory conditions. *Propionibacterium acnes* is recommended for treatment of chronic (probably infectious) respiratory disease in weanlings and yearlings that is unresponsive or transiently responsive to antibiotics. It is also recommended for prophylactic administration prior to stressful events that may impair pulmonary defence mechanisms, including weaning and long-distance transport (Evans *et al.* 1988; Kilmczak 1992). *Propionibacterium acnes* is considered adjunct to antibiotic therapy and is labelled for iv administration every 2–3 days for 3 treatments (Vail *et al.* 1990). In addition to treament of respiratory disease, *Propionibacterium acnes* is beneficial in the treatment of refractory papallomas and may provide adjunct therapy for treatment of sarcoid skin tumor.

Administration of *P. acnes* to healthy, yearling horses using the recommended dosage regimen increases the number of CD4+ lymphocytes, enhances lymphokine-activated killing activity, and increases expression of IFNγ and NK lysin (Flamino *et al.* 1998). Stimulation of systemic immunity can be documented for 4–5 days after administration, however, prolonged immunostimulant activity is not anticipated (Cox 1988). Fever, anorexia, and lethargy may occur 12–24 h after administration of the first or second injection, presumably due to

increased interleukin-1 production. Therefore, administration is not recommended immediately prior to an athletic event.

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INFLAMMATORY AIRWAY DISEASE: EUROPEAN CLINICIANS' PERSPECTIVE

P. M. Dixon, B. C. McGorum and R. S. Pirie

Department of Veterinary Clinical Studies, University of Edinburgh, UK

The term inflammatory airway disease (IAD) is not used widely in Britain at present because there is much uncertainty about which equine pulmonary disorder(s) this term describes. The International Workshop on Equine Airway Disease, held in Michigan State University in 2000, described IAD as a non-septic airway disease, particularly of young horses, which has an unknown relationship to heaves (Robinson 2001).

To date, IAD has been used by some clinicians as a 'dustbin' term for many pulmonary disorders that cannot be categorised into a more clear-cut diagnosis. The term IAD lends itself to this vague role, as almost all equine pulmonary diseases involve the airways and it is unclear how any airway disease could be anything except inflammatory in its pathogenesis, whether it be of an infectious, inhaled-irritant or hypersensitive aetiology. Even the extremely rare neoplastic equine pulmonary diseases have an inflammatory component.

In contrast to a North American referral caseload, in Britain cases of pleuropneumonia or other types of bacterial pulmonary disease in adult horses are seen relatively seldom and, thus, most of the referred caseload will be of chronic (>2 months duration) lower grade, diffuse pulmonary disease, with affected horses having excessive mucopurulent tracheal respiratory secretions present on bronchoscopy. At Edinburgh, workers have moved away from pulmonary function tests in the diagnosis of clinical cases of pulmonary disease, because of their insensitivity in cases with lower grade pulmonary disease. Historical, clinical, bronchoscopic and especially, tracheal respiratory secretions and bronchoalveolar lavage (BAL) fluid cytology findings are currently the mainstay for diagnosis.

The limitations of clinical examinations in the diagnosis of lower grade equine pulmonary disease cannot be overemphasised. Although an accurate history, and especially bronchoscopy, can confirm the presence of pulmonary disease, pulmonary cytology forms a mainstay for diagnosing the specific chronic pulmonary disease present; using previously described diagnostic criteria (Dixon *et al.* 1995). The use of both tracheal respiratory secretions (RS) and BAL fluid cytology is most helpful – eg in heaves (COPD) affected horses, both samples will have a permanent neutrophilia (>5% neutrophils in BAL fluid and usually 3–4 times this ratio of neutrophils in the tracheal RS). With infectious (post infectious) pulmonary diseases affected cases will have a transient (<2 weeks) BAL fluid neutrophilia, but a longer-term (ie for many months) tracheal RS neutrophilia, usually with a normal BAL fluid cytology.

After establishing a definite diagnosis in as many pulmonary cases as is possible, a significant number of horses are always left where no definitive diagnosis can be made, using current understanding and the available ancillary diagnostic techniques. Rather than artificially trying to manipulate these cases into some other diagnostic group, to date they have been simply classified as undifferentiated pulmonary disease. In many respects, including clinically (ie the presence of chronic, diffuse, low grade pulmonary disease); bronchoscopically (inflamed airways and excessive mucopurulent tracheal RS) and cytologically (tracheal RS neutrophilia and normal BAL fluid cytology) these undifferentiated cases are similar to the 'post infectious' pulmonary disease group - except that they have no history of immediately antecedent respiratory infection. Using current knowledge, this group of horses

with undiagnosed pulmonary disease could well be classified as suffering from IAD, as perhaps could some of the 'post infectious' pulmonary disease group – especially those that do not resolve with 6 weeks or so of environmental control, as do most of their cohorts.

Over the last 15 years, largely due to the work of Sasse (1971) and McPherson *et al.* (1978), there has been a major change in the use of ensiled grass versus hay for feeding indoor horses and to a slightly lesser extent, in the use of non-straw versus straw bedding in the UK. Almost all cases referred to Edinburgh are currently maintained in hay and straw-free environments. These management changes have resulted in a decrease in the incidence of classic heaves and severely affected (dyspnoeic) cases are rarely seen at present, in contrast to 15–20 years ago. Whilst these management changes have decreased the inhaled challenge to indoor horses, they have not eliminated it. It is interesting to speculate whether horses (possibly susceptible to heaves), which experience lung disease due to lower degrees of environmental inhalation challenge, could be classified as suffering from IAD?

As the authors' caseload is a selected referral population from a geographically distinct area, the opinion of other clinicians with different caseloads in other parts of Britain were also sought. P. Ramzan and M. Shepperd working with 2–3-year-old flat racehorses in Newmarket are now tentatively diagnosing IAD in this group of horses, especially in individual horses that develop a more chronic pulmonary disease following apparent respiratory infectious disease. Horses recently introduced to the indoor environment at the start of training appear to be at higher risk.

Tim Mair an equine practitioner in Kent, dealing primarily with sports and leisure horses, defined IAD as a non-heaves, chronic pulmonary disease of indoor horses of all ages, but did not frequently recognise this disorder in his patients.

IAD also appeared to occur in younger (1–2-year-old) horses in indoor environments and these cases appeared to respond to environmental control. Tim Brazil working in an equine practice in Oxford also recognised the disorder, including in National Hunt racehorses when first brought indoors for training at 4–5 years of age. The disorder was characterised by poor work, variable respiratory clinical signs, and the bronchoscopic detection of excessive mucopurulent tracheal secretions of neutrophilic character. The stress of competitive work, antecedent respiratory infection and adaptation to new indoor environments were believed to be risk factors for this syndrome.

Although no consensus was present, apparent agreement between the UK veterinarians was that IAD is:
• A disease of indoor horses.
• A diffuse, lower grade pulmonary disease.
Affected horses have:
• Increased volumes of mucopurulent secretions in their trachea (detected bronchoscopically).
• Tracheal respiratory secretions are neutrophilic in character.
• Antecedent respiratory infection is a risk factor.
• First coming indoors to training is a risk factor.
• Performing high speed exercise is a risk factor.

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INFLAMMATORY AIRWAY DISEASE: EFFECT OF ATHLETIC DISCIPLINE

M. Mazan and A. Hoffman

Lung Function Testing Laboratory, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, Massachusetts 01536, USA

Despite an increasing awareness of the importance of inflammatory airway disease (IAD) in limiting performance, the definition of the disease remains fluid. There appears to be a consensus that horses with IAD are: generally young, although middle-aged horses may be affected (Hare *et al.* 1994) and have impaired performance that may go unnoticed at rest or during light work. (Hare *et al.* 1994; Hoffman *et al.* 1998; Couëtil and Denicola 1999). Clinical signs which are reported are variable, including cough, nasal discharge and abnormal lung sounds (Morris 1991; Vrins *et al.* 1991; Viel 1997) as well as endoscopic evidence of tracheobronchial mucus accumulation (MacNamara *et al.* 1990; Vrins *et al.* 1991; Dixon *et al.* 1995a). Horses with IAD invariably have mild to moderate airway inflammation, which may involve neutrophils (Fogarty and Buckley 1991; Moore *et al.* 1995) mast cells (Vrins *et al.* 1991; Hare *et al.* 1994; Hoffman *et al.* 1998; Hoffman and Mazan 1999), eosinophils (Viel 1997) or lymphocytes (Moore *et al.* 1995). The horses may have normal lung function at rest, but show evidence of airway hyper-reactivity on exposure to non-specific agents, such as histamine (Klein and Deegen 1986; Hoffman *et al.* 1998), or evidence of flow limitations on forced expiratory manoeuvres (Couëtil *et al.* 2001). Although IAD is often thought of as a disease of younger horses which are expected to perform athletically, and as such is distinct from the picture of the dyspnoeic horse with overt recurrent airway disease (RAO), several workers have reported IAD in older athletic horses (Persson and Lindberg 1991; Hoffman *et al.* 1998; Hoffman and Mazan 1999; Couëtil *et al.* 2001).

One of the clinical findings most commonly reported in IAD is exercise intolerance, or decreased performance, with or without overt signs of respiratory disease. Horses appear to have remarkable respiratory reserve; thus subclinical airway disease may simply result in mild abnormalities on auscultation and endoscopy and occasional coughing (Bracher *et al.* 1991). The prevalence of coughing is hard to estimate, as many studies use this as an inclusion criterion (Vrins *et al.* 1991; Christley *et al.* 2000; Couëtil *et al.* 2001), whereas others report coughing less than 16–50% of the time (McKane *et al.* 1993; Moore *et al.* 1995). Other clinical signs which have been reported include prolonged respiratory recovery rate (Hare and Viel 1998), nasal discharge after exercise (Hare and Viel 1998), respiratory embarrassment at exercise (Dixon *et al.* 1995a; Hare and Viel 1998), worsening of signs during hot, humid weather and inability to perform work during collection (Hoffman *et al.* 1998).

The reported effect of IAD on performance has also varied. It seems to depend on the type of horse and the methods used by the individual investigator. Researchers looking at Standardbred racehorses (MacNamara *et al.* 1990) found that those with excessive tracheal mucus performed at a lower level than those with no mucus on endoscopy. Couëtil and Denicola (1999) and Nyman *et al.* (1999) found, in separate studies, that horses with IAD undergoing a treadmill stress test demonstrated more severe impairment of gas exchange during peak exercise than normal horses. Persson and Lindberg (1991) looking at Standardbred trotters and saddle horses with what was effectively IAD, found a different manifestation in racehorses to saddle horses. Not only were the saddle horses significantly older when airway disease was detected, but racehorses had decreased minute volume and an increased red blood cell to bodyweight ratio, whereas the affected saddle horses manifested tachycardia during exercise. Other studies have found that horses with obvious

TABLE 1: Signalment

Variable	Racehorse	Sport horse
Age (years)	4 ± 2.1	11.0 ± 4.9*
Thoroughbred (% of racehorses)	58	
Standardbred (% of racehorses)	42	
Gelding (% of total)	46	62
Mare (% of total)	41	27
Stallion (% of total)	9	6

*Significantly different from racehorses, $P<0.05$

TABLE 2: History

Variable (% positive responses)	Racehorse	Sport horse
Has turnout	33	86*
Eats hay	98	85
Affected primarily by hot, humid weather	69	59
Signs present > 6 months	20	77*
History of abnormal respirations at work	56	77*
History of cough	14	50*
History of lethargy	43	59
History of quitting	93	3*
History of EIPH or BAL evidence	35	7*

*Significantly different from racehorses, $P<0.05$

TABLE 3: Physical examination

Variable (% positive response)	Racehorse	Sport horse
Resting respiratory rate > 20/min	24	47*
Abnormal tracheal auscultation	21	64*
Abnormal lung sounds	24	54*
Nasal discharge present at physical examination	21	59*
Cough heard or elicited on PE	33	76*

*Significantly different from racehorses, $P<0.05$

TABLE 4: BAL

Variable	Racehorse (mean ± SD)	Sport horse (mean ± SD)
% neutrophils	5.1 ± 7.1	10.4 ± 11.8*
% mast cells	4.25 ± 2.8	3.4 ± 2.2
% macrophages	47.3 ± 17.9	43.1 ± 14.93
% lymphocytes	39.9 ± 15.9	43.0 ± 15.2
% eosinophils	0.59 ± 1.9	0.47 ± 1.7

*Significantly different from racehorses, $P<0.05$

TABLE 5: Lung function

Variable	Racehorse (mean ± SD)	Sport horse (mean ± SD)
$R_{RS\ 2HZ}$ (cmH$_2$O/L/s)	0.62 ± .31	0.59 ± 0.21
PC100R_{RS} (mg/ml histamine)	3.56 ± 2.2	4.28 ± 4.05

evidence of airway inflammation do not necessarily have a history of exercise intolerance (Sweeney *et al.* 1992). These differences in physiological and clinical response to exercise probably relate to the demands placed on racehorses compared to riding horses. This may also reflect the difficulty of diagnosing low-grade respiratory impairment and the trainer's failure to recognise poorer performance than nature intended, rather than the benign nature of the underlying disease.

The authors hypothesised that IAD is a progressive disease and, therefore, will present differently according to aerobic demands. Clinical records were reviewed of horses presented to the Large Animal Hospital at the Tufts University School of Veterinary Medicine from 1996–2002 with a primary complaint of lowered performance, and an eventual diagnosis of IAD based on abnormal bronchoalveolar lavage (BAL) cytology (>5% neutrophils (PMNs), >2% mast cells or >0.5% eosinophils; Hoffman *et al.* 1998). Horses were

excluded if they had signs of heaves at rest, a history of recent respiratory infection or recurrent increased respiratory effort when exposed to hay or pasture, compatible with RAO. Horses were placed into 2 categories: racehorses (RH, n=41) or other sport horses (SH, n=66), to determine if IAD presented differently in these 2 groups. The data are shown in Tables 1–5. The following variables were compared between the 2 groups: 1) signalment (age, sex distribution and breed); 2) history (access to turnout, hay feeding, effect of hot, humid weather, duration of signs and history of abnormal respirations at work, cough, lethargy, quitting or exercise-induced pulmonary haemorrhage); 3) physical examination (resting respiratory rate, abnormal tracheal or lung auscultation, nasal discharge or cough); 4) BAL (percentage of BAL cells classified as macrophages,

lymphocytes, neutrophils, mast cells and eosinophils by counting 500 cells stained with Wright-Giemsa [1,000 Xmag]); 5) lung function (baseline R_{RS} at 2 Hz and PC_{100} R_{RS2Hz} [provocative concentration of histamine that doubled R_{RS} at 2 Hz; Mazan *et al.* 1999]).

INFORMATION OBTAINED FROM THE STUDY

Signalment: There was a difference in age between the 2 groups, with SH being significantly older than RH (Table 1). There were no significant differences in sex distribution. *History* (Table 2): As expected, few RH had access to turnout, and most horses, both RH and SH, were given hay as roughage. As organic dust associated with a barn environment and hay feeding have been associated with lower airway inflammation (Sweeney *et al.* 1992; Holcombe *et al.* 2001) this finding may be important. A significant difference was found between the 2 groups with respect to duration of signs, with most RH having signs for less than 6 months, whereas most SH had signs for over 6 months. This may well reflect the level of exercise necessary to force a diagnosis through signs of exercise intolerance and, perhaps, the greater likelihood of a racehorse being examined endoscopically. It was also found that a significantly greater proportion of SH showed abnormal respiration at work and cough than did RH. Racehorses, on the other hand, were most likely to 'quit' during hard work. Again, SH are less likely to experience overt exercise intolerance as a consequence of IAD, due to the decreased aerobic demands of the sport. Both groups seemed to have more difficulty in hot, humid weather with equal frequency. This may reflect an increased growth of moulds and bacteria with consequent elevations in endotoxin levels, the effects of associated air pollution, or greater effects of inertance in moving heavier, more saturated air. Conversely, it may also reflect the greater likelihood of horses being exercised maximally during the warmer seasons.

When considering physical examination findings (Table 3), a significantly greater proportion of SH had an abnormally high resting respiratory rate, nasal discharge, cough or abnormal tracheal or lung auscultation, although these may be variably found in RH as well. This may be due to the progressive nature of IAD: SH tend to be older, have had signs for longer and have a lower athletic demand. It is likely that SH owners

TABLE 6: Occult disease

Variable (% positive response)	Racehorse	Sport horse
Occult disease – no nasal discharge or cough on PE or physical examination	54	17*
Occult disease – no nasal discharge, cough, or auscultation of lungs or trachea	40	10*

*Significantly different from racehorses, $P<0.05$

fail to notice incipient signs of IAD as it has a less profound impact on the work performed: only when more obvious signs appear, such as cough and nasal discharge, are these horses brought to a veterinarian's attention. Interestingly, cough was heard on physical examination of only 33% of RH, whereas 93% had quit at the $^3/_4$ mark. This underscores the need for more detailed methods of examination, including BAL and lung function testing, to confirm a diagnosis in these horses.

BAL findings varied significantly between groups (Table 4). Both groups had an elevated mast cell percentage, with no significant difference between them. There was, however, a significant difference between neutrophil percentages, with SH having double the percentage of the RH. This, rather than reflecting a fundamental difference between groups, is more likely to reflect a progressive inflammatory insult in the older horses of the SH group. Finally, although both groups had baseline R_{RS2Hz} within normal limits, they both experienced airway hyper-reactivity (Table 5). It should be noted that data at 2Hz were used in order to include the maximum number of IAD horses in this study, as some horses had missing data from R_{RS1-3}.

In summary, SH differ from RH in that they are older, have more obvious clinical signs and a greater degree of airway inflammation; probably reflecting a progression of disease. Racehorses present more of a diagnostic challenge as they are less likely to have easily detectable clinical abnormalities and more likely to present as an 'unknown' case of exercise intolerance. Indeed, if horses with cough and nasal discharge absent on history and physical examination are isolated, a striking difference is found between the 2 groups. If occult disease is defined as no cough or nasal discharge on history or physical examination, IAD

was occult in 54% of RH, but only 17% of SH (Table 6). If the definition is more stringent and includes the absence of abnormal chest or tracheal auscultation, occult disease still occurs in 40% of RH, but only 10% of SH. In addition to abnormalities on BAL, IAD in RH is most reliably detected through bronchoprovocation to demonstrate airway hyper-reactivity and underlying airway obstruction. This is discussed in more detail by Hoffman and Mazan (2003).

Inflammatory airway disease has thus far produced more questions than answers, and much work remains to be done. If the mechanism of airway hyper-reactivity, the stimulus for inflammation, and the role that genetics and environment plays in IAD can be discovered, it may ve possible to modify the development of disease in young horses and prevent its progression. The suspicion that IAD, left untreated, will eventually develop into the crippling disease, RAO, only heightens concern, and provides the stimulus to solve the many mysteries of IAD.

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MUCUS, COUGH, AIRWAY OBSTRUCTION AND INFLAMMATION

N. E. Robinson, C. Berney, H. DeFeijter-Rupp, A. M. Jefcoat, C. Cornelisse, V. Gerber, and F. J. Derksen

Pulmonary Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824, USA

Coughing, mucus accumulation and airway obstruction are features of inflammation of the airways, which occur to varying degrees depending on the pathogenesis of the inflammation and the functional and structural changes in the lungs. For example, in human asthma, much of the obstruction is due to bronchospasm, whereas in human chronic obstructive pulmonary disease (COPD), bronchospasm is much less important and mucus is the major cause of obstruction (Jeffery 2000).

In horses, cough is a highly specific sign of pulmonary disease. Coughing occurred in 71% of 300 horses referred to the equine clinic at Edinburgh University with suspected respiratory disease (Dixon *et al*. 1995a). In racehorses, cough is strongly associated with the presence of airway inflammation and accumulated secretions in the trachea (Burrell *et al*. 1996; Christley *et al*. 2001a,b). However, many racehorses have accumulated secretions in the absence of cough. For this reason, Burrell *et al*. (1996) concluded that coughing is a highly specific, but relatively insensitive sign of airway inflammation. Cough, which clears foreign materials from the larger airways, is initiated by activation of cough receptors located under and within the airway epithelium of the pharynx, larynx, trachea and bronchi (Widdicombe 1996). Therefore, although cough is a highly specific sign of airway disease, it is not specific for certain types of disease. Horses with airway foreign bodies, pulmonary oedema, heaves, infectious disease and lungworms all cough. Coughing is less common in horses with exercise-induced pulmonary haemorrhage (EIPH), a problem located within the alveoli and small, rather than large, airways.

Accumulation of airway secretions is also a non-specific sign of airway disease, and more than a few drops of mucus within the trachea is abnormal (Dixon *et al*. 1995b). Accumulated secretions are well documented in heaves-affected horses, both during clinical exacerbations and in remission (Dixon *et al*. 1995b; Jefcoat *et al*. 2001). Secretions also accumulate in the presence of inflammatory airway disease, infectious disease, and lungworms, but are a less consistent sign of EIPH (Dixon *et al*. 1995b). Secretions are composed of mucus and inflammatory cells. Mucus is produced and secreted by goblet cells in response to release of secretagogues such as neutrophil elastase.

Airway obstruction is clinically obvious in horses with heaves, in which it is associated with migration and accumulation of neutrophils within the airways (Robinson *et al*. 1996). Although obstruction is not clinically obvious in other airway diseases, measurements of lung function have demonstrated airway obstruction in horses with inflammatory airway disease in the absence of clinical signs. The majority of the resistance to breathing is located in the larger (nasopharynx, trachea, bronchi) rather than the smaller (bronchioles) airways. For this reason, there must be extensive bronchiolar obstruction before horses show respiratory distress which is obvious clinically. Small airway obstruction can lead to abnormal distribution of ventilation within the gas exchange region before the horse appears clinically ill. This abnormal ventilation distribution impairs oxygen exchange and may lead to poor performance.

The association among cough, accumulated secretions, airway obstruction and inflammation has not been extensively studied in horses. Dixon *et al*. (1995b) noted that there were greater volumes of tracheal secretions in coughing than in non-coughing horses. However, as noted above,

racehorses can have accumulated airway secretions containing increased numbers of neutrophils and not cough. In racehorses, coughing is associated with the presence of inflammatory cells, especially neutrophils in airway secretions.

Evaluation of the severity of airway inflammation is generally based on the proportion of inflammatory cells, especially neutrophils, in the tracheal secretions or in bronchoalveolar lavage (BAL) fluid (McGorum *et al.* 1993). The range of neutrophil ratios (NR) in the tracheal wash of horses without clinical signs of airway disease is very large but the range is smaller in BAL fluid. Cough and airway obstruction are generally associated with an increase in NR, but some stabled horses with no clinical signs of disease can have NR as high as those of some horses with heaves (Tremblay *et al.* 1993). However, depending on the total number of inflammatory cells in the airways, a given NR may be associated with a very high or a much lower total number of neutrophils. Total neutrophil count and neutrophil activity have never been investigated in association with cough, mucus, and airway obstruction in horses.

The relationship among cough frequency (counted over 4 h), accumulated tracheal secretions (range 0–5), airway obstruction (measured as maximal change in pleural pressure during tidal breathing [ΔPpl_{max}]), and BAL fluid cytology in 12 heaves-affected and 5 control horses was investigated recently (Robinson *et al.* 2003). When heaves-affected horses were stabled, cough frequency increased over 3 days. Cough frequency also increased transiently on Day 1 of stabling in control horses but then subsided. In heaves-affected animals, cough was initially sporadic with periods of several hours with no cough and then multiple coughs within a 15 min period. For this reason, it is important to count cough for long periods when attempting to evaluate cough severity. The judgement of owners or trainers on cough severity will be greatly coloured by when they were in the stable: during bouts of coughing or between bouts. Evaluation of the data from this goup of horses produced the following conclusions:

1. Horses that cough have accumulated airway secretions, but horses with accumulated secretions may not cough.

2. Horses that have measurable airway obstruction have accumulated secretions, but horses with accumulated secretions may not have measurable airway obstruction.

3. Horses that cough have measurable abnormalities of lung function, and most horses with abnormal lung function cough.

In heaves-affected horses, BAL fluid NR, total cell count and total neutrophil count all increased during stabling. In control horses, NR also increased during stabling, but the increase in total neutrophil count was much less than in heaves-affected animals. The relationship between BAL fluid and either cough frequency, accumulated secretions, or airway obstruction had a large amount of scatter. Whereas in heaves-affected animals, a BAL fluid NR greater than 10% was associated in general with the presence of cough, accumulated secretions and airway obstruction, greater than 10% neutrophils in control horses was not associated with cough, secretions or airway obstruction.

The relationship between inflammation and lung functions became much more apparent when cough, accumulated secretions, and airway obstruction were examined as a function of BAL fluid total neutrophil count. When neutrophil count was less than 30 cells/µl, there was very little accumulated airway secretion. Secretions increased between 30 and 100 cells/µl and appeared to plateau above 100 cells/µl. There was no measurable airway obstruction when neutrophil count was less than 50 cells/µl and obstruction increased between 100 and 1000 cells/µl. Coughing began when neutrophil count exceeded 100 cells/µl. These data explain why horses can have secretions and not cough but not vice versa. Accumulation of secretions begins at less severe levels of inflammation than those required to initiate cough. Cough is therefore a sign of quite severe airway inflammation. It is important to realise that the relationships described herein are derived from heaves-affected and control animals. It would be interesting to determine if similar relationships exist in other inflammatory airway diseases of the horse.

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RELATIONSHIP BETWEEN COUGHING AND AIRWAY INFLAMMATION IN YOUNG RACEHORSES

D. R. Hodgson, R. M. Christley, J. L. N. Wood*, S. W. J. Reid[†] and J. L. Hodgson

*Faculty of Veterinary Science, University of Sydney, New South Wales 2567, Australia; *Centre for Preventative Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK; [†]Veterinary Informatics and Epidemiology, University of Glasgow, Glasgow G61 1QH, Scotland*

INTRODUCTION

Coughing, an important problem in young racehorses, is a relatively specific indicator of lower airway disease. Potential causes in this group of horses include pneumonia and pleuritis, viral and bacterial respiratory infections, exercise induced pulmonary haemorrhage, chronic obstructive pulmonary disease and pharyngeal lymphoid hyperplasia. However, the most common cause of coughing among Thoroughbred racehorses is likely to be inflammatory airway disease (IAD), a syndrome resulting in mucopurulent tracheal discharge containing predominantly neutrophils. Despite this, risk factors for coughing in racehorses are unknown. Young racehorses represent a unique population, and differ from the general horse population in ways that may be relevant to the epidemiology of coughing. As well as the age factor, they tend to be involved in strenuous activity, are transported regularly, mix with other horses and, in general, are housed in stables.

Tracheal endoscopy and cytological evaluation of lower airway secretions are performed commonly in investigations of airway disease in horses. However, the value of the cytological assessment of tracheal wash samples, as an indication of lower airway pathology, has been questioned due to the wide variability in relative numbers of inflammatory cells in apparently normal horses. Therefore, a study was undertaken with 2 major aims: 1) to determine if coughing is a useful indicator of lower airway inflammation by investigating the association between coughing and tracheal inflammation, as measured by tracheal endoscopy and cytology; 2) to evaluate a number of potential risk factors for coughing in Thoroughbred racehorses in Sydney, Australia.

MATERIALS AND METHODS

To this end a matched case control study was conducted using 100 horses that coughed at least 4 times within minutes of commencing exercise and 148 controls, the latter being free of clinical signs of respiratory tract disease. Horses were identified from participating stables at 5 racetracks around Sydney by daily contact with the trainers and their consulting veterinarians. Cases were defined as Thoroughbred racehorses in training that coughed at least 4 times during a 10 min period of exercise. The case definition was kept simple because case identification relied on the observations of the trainer and the staff of the stable.

Controls were selected randomly from among non-cases in the stable from which the case was identified. A horse was defined as a non-case if it had not had a recognised episode fulfilling the selection criteria within the past 2 weeks.

Information relating to the recent history of each case and control was collected using a questionnaire. The investigators completed the questionnaire during discussion with the trainer or trainer's representative. Details recorded included the name, age and sex of the horse, the number of days since last transportation (1–7 days, 8–14 days, more than 14 days) and racing (never raced, 1–7 days, more than 7 days), and the stage of training. Stage of training was categorised as early (mostly trotting and cantering), mid (increasing fast exercise, up to 80% of top speed, usually over short distances), late (training included increasing amounts of exercise top speed gallop) or racing (has raced). Early, mid and late training phases usually lasted 4–5 weeks each.

Endoscopy of the upper and lower airways, and tracheal fluid collection were performed using

TABLE 1: Factors identified by conditional logistic regression model as being associated with the risk of coughing in Thoroughbred racehorses

	Cases	Controls	Unadjusted OR	**OR***	95%	CI*
Age (years)						
1 or 2	62	49	1*	1†		
3	21	34	0.3	0.5	0.2	1.7
4	10	40	0.1	0.2	0.04	0.7
>4	7	25	0.1	0.2	0.04	0.9
Recent transport (days)						
1–7	19	50	1*	1†		
8–14	18	33	1.9	3.5	0.9	13.6
>14	63	65	3.6	5.5	1.7	17.8
Stage of training						
Early	49	30	1*	1†		
Mid	18	20	0.8	0.6	0.2	1.6
Late	11	16	0.3	0.1	0.04	0.6
Trialed or racing	22	82	0.1	0.05	0.009	0.2
Previous racing						
Never	57	41	1*	1†		
1–7days	13	32	0.3	11.1	1.6	76.5
>7days	30	75	0.2	1.2	0.4	3.9

*Adjusted for age, stage of training and previous racing; †reference category

Darien Microbiology Aspiration Catheter using 12 ml of phosphate buffered saline (PBS). Prior to installation of PBS, the trachea was inspected and a score given for the following; nasopharyngeal mucus, pharyngeal lymphoid hyperplasia, lower respiratory tract mucus.

Slide preparations of tracheal aspirates were made by cytocentrifugation with 1 ml of undiluted sample. Following staining with Dif-Quik, an estimate of the relative numbers of alveolar macrophages, haemosiderophages, lymphocytes, neutrophils, giant cells and eosinophils was made by making counts of 100 cells in 3 different areas of the slide. The relative number of haemosiderophages was expressed as a proportion of all inflammatory cells.

As cases and controls were selected concurrently, each case was matched with its control horses on the basis of time and training stable. Therefore, analyses had to take account of this matching. Crude odds ratios were calculated for all variables using conditional univariable logistic regression, with coughing as the dependent variable. Dummy variables were generated for any categorical variable with more than 2 levels. Multivariable conditional logistic regression models were constructed through backward elimination using variables considered, *a priori*, to be potential confounders, and those which were significant at P<0.25 in the univariable screening. Variables remained in the model if removal of that variable resulted in a significant change (P<0.05) in the

likelihood ratio statistic, or if exclusion of the variable altered the estimate of effect of other variables by 5% or more. Biologically meaningful 2-way interaction terms between the independent variables were examined after identification of the reduced set main effects. The fit of the multivariable model was assessed by examining the delta-beta values. Horses with the greatest delta-beta values for each variable were sequentially excluded from the models. Models were judged as stable and robust when these exclusions did not cause significant effects. Conditional logistic regression was performed using LogXact version 2.1 (Cytel Statistical Software, Massachusetts, USA).

Correlation coefficients between stage of training and time since transportation were examined, following stratification by time since last race, to examine the relationships between these variables. Correlation coefficients were calculated using Statistica (StatSoft Inc, Oklahoma, USA).

RESULTS AND CONCLUSIONS

The hallmark of IAD is mucopurulent tracheal exudate containing neutrophils. Therefore, the results of the current study indicate that a majority of horses that cough during exercise have evidence of IAD. In this study, coughing was strongly associated with increases in tracheal mucus score and percent neutrophils in tracheal wash samples; 80% of cases had more than 20% neutrophils in their tracheal wash, compared to 20% of controls.

TABLE 2: Multivariable conditional logistic regression models of tracheal wash cytological findings after controlling for age. Only the results for the explanatory variable in each model are shown

	Univariable OR	Multivariable OR*	95%	CI*
Neutrophils (%)				P<0.001
0–20	1[†]	1[†]		
21–40	9.2	**10.7**	3.0	37.6
41–60	98.4	**140.6**	12.8	1546.7
61–80	109.8	**142.8**	8.7	2346.6
81–100	67.1	**103.7**	8.6	1254.0
Haemosiderophages (%)				P=0.3
0	1[†]	1[†]		
1–10	0.5	**0.8**	0.4	1.7
11–20	0.2	**0.8**	0.2	3.5
>20	0.1	**0.3**	0.05	1.3
Eosinophils (%)				P=0.2
0	1[†]	1[†]		
1–2	1.1	**1.0**	0.5	2.1
3–10	0.7	**0.7**	0.2	2.7
>10	8.4	**7.3**	0.7	73.7
Mast cells (%)				P=0.07
0	1[†]	1[†]		
1–2	0.6	**0.7**	0.3	1.4
3–4	0.3	**0.2**	0.05	0.8
>5	1.1	**0.8**	0.3	2.5
Ciliated epithelial cells (per ml)				P<0.001
0–20	1[†]	1[†]		
21–50	0.1	**0.2**	0.05	0.5
51–80	0.05	**0.06**	0.01	0.2
81–100	0.01	**0.01**	0.002	0.06
Bacteria				P<0.001
None	1[†]	1[†]		
Extra-cellular only	2.0	**2.6**	0.7	9.4
A few intra-cellular	10.3	**24.2**	2.1	285.3
Many intra-cellular	28.6	**41.7**	4.2	411.7

*Adjusted for age; †Reference category

Similarly, 94% of cases had at least some tracheal mucus, compared to 45% of controls. Because of the design of this study, the sensitivity of coughing as an indicator of IAD could not be calculated. As a result, the proportion of 'true' IAD cases identified using coughing as the only case criterion could not be determined. Assuming that the sensitivity of coughing, as an indicator of IAD, was as low as reported by others, a proportion of horses with IAD in the current study would not have been identified. Consequently, these horses may have been included in the current study as controls, which would tend to bias the measure of effect toward the null. Therefore, the association between IAD and clinical, endoscopic and clinicopathological findings may be greater than suggested by the current study.

Variables identified by multivariable conditional logistic regression as 'significantly associated with coughing' included age (decreased risk with increasing age), stage of training (horses in early training at greatest risk), time since last race (horses that had never raced at greatest risk) and time since transportation (horses transported over 14 days previously were more likely to cough than those transported in the last week; Table 1). Horses with cough were significantly more likely to have increased upper and lower tracheal mucus scores and increased pharyngeal lymphoid hyperplasia. In addition, the tracheal aspirates of coughing horses had increased odds of neutrophilic infiltration and were more likely to have bacteria observed intra-cellularly when compared to control horses (Table 2). However, despite this up to 20% of control horses had evidence of airway inflammation.

In summary, this study demonstrates a strong relationship between airway inflammation and coughing in racehorses during exercise. Management factors are the most likely contributors to this inflammatory process.

POOR PERFORMANCE: THE TRAINER'S PERSPECTIVE (SPORT HORSE)

C. Platz

PO Box 1240, Auburn, Maine 02411, USA

Although I am an equine veterinary practitioner, I wear a number of other hats. I am also a rider and trainer of dressage horses through the Federation Equestré Internationale (FEI) level and am one examination away from being United States Dressage Federation certified instructor. As a result of these endeavours, I share the perspective of both a consumer as well as a provider of equine veterinary services.

Most non-veterinary professional horsemen and women understand 4 different categories of respiratory disease:

1. Mechanical problems: eg laryngeal hemiplegia (roarer).

2. Infectious problems: pneumonia, influenza.

3. Heaves: an end-stage problem usually associated with old horses.

4. Allergies: a loose term applied to a horse that coughs a lot, sometimes for no apparent reason.

The idea that some horses have an innate respiratory sensitivity to environmental provocation that can worsen over time, the progression of which can be modified by management and medication, and which is often expressed by the horse in very subtle ways is not generally known by the majority of trainers and riders. This represents a significant gap between current veterinary science and the application of that science to the population of horses we serve.

ALTERNATIVE MANIFESTATIONS OF RESPIRATORY COMPROMISE

Most trainers and riders of dressage horses think that a horse expresses respiratory distress by coughing a lot. They do not become concerned unless they hear the horse coughing in his stall or throughout his work session. As a result, many hoses in the early stages of inflammatory airway disease are not identified and, therefore, do not benefit from prophylactic management that can help preserve airway function and prolong athletic usefulness.

While it is true that some horses with inflammatory airway disease do cough significantly, many do not. There are many other indications that a horse is experiencing some degree of respiratory distress. Early identification of affected individuals would benefit both the horse and those who have an interest in its well-being.

For example, some buyers of dressage horses know to avoid an individual that emphatically wants to carry his head 'long and low'. While this posture may indicate a desire to stretch the topline (which is a good thing in the dressage mount), it may also be a manifestation of the horse's need to straighten his airway to maximise airflow and, thus, an indicator of respiratory compromise (a bad thing in a dressage mount).

Other behaviour manifested by horses with inflammatory airway disease may be similarly misinterpreted as training issues, when in fact may be related to respiratory impairment. Horses are quite creative in developing strategies to evade that which causes them discomfort or stress. Horses that resist longitudinal flexion of the poll may do so in order to avoid respiratory distress. By keeping their throatlatch open, they can maximise airflow by maximising airway resistance.

Such horses may be quite soft and willing to yield their poll to the rein during warm-up and easier parts of their work session, but as the degree of difficulty increases, may abruptly jerk their heads and yank the reins from the rider's hands. By bringing themselves 'above the bit' (meaning

the profile of the face is above perpendicular to the ground) or carrying themselves 'long and low', the horses prevent the rein from decreasing the angle of neck and jaw, so the rider can no longer affect the configuration of their airway. After a period of recovery the horse may again yield to the bit, but when the physical demand re-escalates, the horse repeats its resistance by taking the reins away from the rider.

In at least one scenario of which I am personally aware, a non-coughing horse with severe inflammatory airway disease was sold to a buyer who was not informed of the horse's condition (selling the horse without details of his medical history and Aeromask no doubt increased the purchase price significantly). The gelding was placed in training with a professional. Without proper management the horse was unable to perform even lower level dressage with his head in the desired position. Since he did not cough, he was not identified as having respiratory disease.

The horse was by nature an extremely generous and accommodating individual but consistently refused to 'go on the bit', and was perceived to have a bad attitude. Various straps, gadgets and gimmicks were used to try to fix the problem to no avail and the horse eventually ended up in an Olympic rider's barn. By this time, the horse would deliberately run himself into a wall to avoid having to flex his poll, a behaviour intimidating to his junior rider. Hind limb lameness eventually resulted from the horse's struggle to keep his airway as open as possible. Ultimately the horse's mind and body were so damaged that he became unrideable at the age of 11, in addition to becoming a respiratory cripple.

Because of my experience with this and other horses with inflammatory airway disease, I think it is important that veterinarians, riders and trainers consider respiratory distress as part of the differential diagnosis for dressage horses exhibiting the following behaviours:

1. Exaggerated tendency to prefer long and low carriage of head and neck.
2. Reluctance to flex poll and yield in the jaw.
3. Inconsistency of willingness to flex in the poll and yield to the bit. This may vary from session to session (depending on environmental provocation of airway inflammatory) or within the work session (related to increased demands of collection or athletic effort).

4. Horse going behind the bit: that is, adopting false flexion that prevents the rider from asking the horse for genuine athletic effort. Usually over-flexing at the C-3/C-4 junction rather than at the poll/throatlatch.
5. Horse behind the leg, behind the aids or not through – strategies whereby the horse avoids using himself athletically and hence not increasing respiratory demand.
6. Horse exhibiting tension when athletic or flexion demands are increased; may be manifested by various means – tail swishing, teeth grinding, kicking at the leg, bucking and not going forward, stiffening in the back, neck, poll, etc.
7. Horse goes to the bit and then when asked to flex more in poll, backs off.
8. Lack of progress in training. While a drop in performance may be the indicator in some cases, some horses which hit a glass ceiling in performance may have reached the limit of their respiratory comfort zone. The demands of collection and flexion may be too great for what the horse can accommodate and still breath comfortably, so the horse does not progress in its work. These horses may give the impression of a benign resistance to working harder, eg 'lazy'. Rather than become 'air-hungry', horses may keep their athletic efforts within the limits of what they can do without running low on air.

As is shown by the list above, manifestations of respiratory compromise in dressage horses can easily be misunderstood or misinterpreted. There is a list of alternative explanations for these observations, including, but not limited to:

1. teeth and bitting issues;
2. vertebral column problems;
3. lameness;
4. saddle fit;
5. lack of expertise of rider, trainer.

Note: that lameness and back/neck pathology can easily result if the horse uses his body incorrectly in order to avoid respiratory stress. In my experience dressage horses will choose to evade, rather than work in a state of respiratory compromise.

In fairness to all concerned, it must be noted that determining the cause(s) of training and

soundness problems in horses is a complicated process; both knowledge and creativity are required. Back soreness, from incorrect positioning of the head and neck in an effort to maximise air flow, may provide and explanation for poor performance, which will recur unless the underlying respiratory condition is improved.

Not all, or even most horses with training problems, have airway disease, but some do and should be identified as early in the disease process as possible.

CLINICAL MANIFESTATIONS OF REACTIVE AIRWAY DISEASE–TRAINER'S PERSPECTIVE

Most non-veterinary equine professionals consider coughing to be the major indicator of respiratory problems. Most consider some coughing to be normal. When told that normal healthy horses generally do not cough without obvious reason, they are quite surprised. Other ways in which horses with reactive airways may manifest their condition include the following:

1. None – some horses with early documented airway inflammation do not cough at all. They give no indication of respiratory distress. They may not show increased respiratory rate or abdominal lift. Training problems may be the only indication that the horse is affected.

2. Excess snorting and blowing during warm-up, wanting to extend head and neck to do this.

3. Long, soft growling or moaning noises or gutteral grunting in rhythm to breathing at the onset of work, especially if not allowed to extend head and neck.

4. Horse sounds like its trying to 'clear its throat', culminates in part cough, part sneezing sound.

5. Few light coughs (1–12) which do not all resemble a COPD cough, but are light and sound like an irritation in the throat, rather than originating from the lungs.

6. All above signs are not usually random throughout the work, but are associated with the onset of a new level of activity, ie trot, canter, increased degree of engagement of hindquarters (which increases metabolic workload, hence increased demand on respiratory capacity).

7. Above signs may be associated with change of carriage of the horse, ie alteration in position of head and neck and, therefore, configuration of upper airway.

8. Nasal discharge – may be none, but may be unilateral or bilateral, greyish-white and egg white consistency, transient and episodic rather than copious, produced after trailering, demanding work session or on days when environmental provocation is particularly high. Appearance may be preceded by episode of snorting, groaning and attempting to 'clear the throat', sometimes coughing.

9. Sharp coughs after sudden exercise at liberty. Horses with affected airways are often quite tranquil and do not exert themselves much in turnout. They may gallop vigorously when provoked but may have a brief harsh coughing spell after such exertion. Does not sound like light cough under saddle (note: most normal horses WILL cough briefly after rolling).

10. Change in demeanour or behaviour in an unfamiliar allergenic environment – affected horses stabled at an indoor show or transported to a different part of the country may not show significant respiratory signs, but may seem subdued or even depressed. If in a competition situation, they may not perform with normal energy, sometimes misinterpreted as shipping fatigue or homesickness.

11. In my experience, horses that cough excessively when eating dusty hay do not necessarily have inflammatory airway disease. I have an unaffected horse who coughs easily from overt dust in his environment or feed, while his affected stable-mates do not respond to this challenge at all. It is almost as though the affected horses have an inappropriate response to appropriate stimuli!

It should be noted that subtle changes in demeanour, coughing or other mild respiratory signs may be indicators of early inflammatory airway disease. It is important to be aware of such indicators, because by the time more prominent signs are evident, the opportunity to intervene with the progression of airway disease may be lost.

MANAGEMENT STRATEGIES

The time-honoured approach of increasing ventilation and reducing dust and mould in the environment is the basis for managing horses with inflammatory airway disease. Not all horses will respond to increased access to turnout if the provocation for their symptoms comes from

outdoors – eg tree pollen. However, improving environmental air quality will decrease the respiratory burden on even pollen-sensitive individuals and should be a major goal of non-medical management.

Bedding

In addition to no bedding at all, peat, shredded paper, etc, new pelleted products such as 'Woody Pet' have been helpful for many horses. Lack of success with this product can usually be attributed to the fact that users do not understand how to use it, often because they do not read the directions on the bag. Keeping the proper amount of moisture in the bedding is essential. Woody Pet is excellent for trailering horses when normally shavings would be used. Straw is often too dusty or mouldy for ideal bedding.

Forage

Traditionally, Denji has been considered the gold standard for feeding forage to horses with inflammatory airway disease, but it has its drawbacks. It is expensive and contains molasses and alfalfa which may be problematic for some horses. Sometimes Denji is mishandled, stored improperly or has become contaminated and so becomes mouldy in some parts of the bale. The manufacturer has developed a new product called 'Totally Timothy' which avoids the alfalfa problem.

Hay cubes do not work well for aged horses with bad teeth, usually contain alfalfa, and are reported to case choke unless well-soaked.

I find soaked hay is the best forage for affected horses. Simply telling an owner to 'wet' the hay is often insufficient to remove dust and spores. Inadequately moistened hay may grow mould rather than protect the horse from allergens. It also has been know to cause gas colic.

Hay should be submerged for a prolonged period of time before feeding or, alternatively, should be fed submerged (such as in a tub of water with a weight on the hay to keep it below the water). Horses readily accept soaked hay and also adapt well to pulling the hay from underneath shallow water. I have fed hay that has been soaked up to 24 h in 90° weather in New England without any problems. Water used to soak hay should be replaced daily.

In cold climates hay can be stuffed by the section into heated water buckets, the bucket filled with water and then placed in the horse's stall. This has the added benefit of increasing water consumption in cold weather. Other strategies are to fill plastic wheeled bins with a bale of hay, cover with water and place a Styrofoam block on the top as insulation. The hay is then fed out in sections. This has been used in Massachusetts for a number of years.

Other dietary considerations

Horses with airway inflammation sometimes have multi-systemic sensitivities. Simplifying the diet by eliminating commercially formulated products in favour of simple grains with judicious use of supplements has resulted in apparent improvement in behavioural, digestive and skin problems in some horses. Alfalfa and molasses are especially troublesome for certain individuals.

SUMMARY

A gap exists between the scientific and lay community in the identification, understanding and management of reactive airway disease in horses. Most dressage trainers and owners are not enthusiastic about long-term medical treatment of respiratory conditions in their horses, but are quite receptive to management changes which will allow them to prolong their horses' working lives.

Horses affected with inflammatory airway disease should be identified as early as possible in their disease process to optimise successful intervention. As veterinarians and trainers become more aware of some of the subtle indications that a horse is experiencing respiratory impairment, affected individuals can be recognised and corrective measures taken to help protect their airways from further damage.

More work needs to be done to aid the non-veterinary equine professional in recognising early indications of inflammatory airway disease in horses, and in the development of devices for more convenient methods of soaking feed hay, as well as more satisfactory non-allergenic forage alternatives.

THE DIAGNOSTIC APPROACH TO CHRONIC COUGH IN PEOPLE

A. J. Ghio

Human Studies Division, United States Environmental Protection Agency, Chapel Hill, North Carolina, USA

Cough is cited frequently as the fifth most common symptom of patients presenting for medical care and accounts for 30 million office visits per year. Causes of cough and, therefore, the diagnostic approach, will vary with practice setting and the age of the patient population. Private practitioners will evaluate a different spectrum of disease compared to clinicians at a tertiary care centre. Similarly, paediatricians will diagnose asthma as the basis for cough with a greater frequency relative to physicians providing care to an adult population.

Cough is defined as an explosive expiration providing a protective mechanism for clearing foreign materials and tracheobronchial secretions. The afferent limb for the signal will involve cranial nerves V, IX, X and cervical nerves 2 and 3 originating from both rapidly adapting pulmonary stretch receptors and unmyelinated C fibres. Slowly adapting pulmonary stretch receptors may enhance the cough. The 'cough centre' is thought to be localised in the medulla. The efferent pathway includes the X and cervical nerves 3 and 5 to laryngeal and tracheal muscles, the diaphragm, and respiratory muscles.

There are several different criteria used to define an acute and a chronic cough. An acute cough is frequently defined as being less than 3 weeks in duration. Cough can be that symptom persisting longer than 3, 6 or 8 weeks. Irwin and Madison (2000) included a subchronic categorisation of cough as that between 3 and 8 weeks. An acute cough is most likely to be the result of infection including upper respiratory infection (eg pharyngitis), acute bacterial sinusitis, and pertussis. An acute cough can also reflect an exacerbation of chronic obstructive pulmonary disease, allergic rhinitis, asthma, congestive heart failure, pneumonia, aspiration, and environmental exposures (tobacco smoke being most prevalent). A cough persisting for 3–8 weeks most commonly is post infectious, but can also support a diagnosis of sinusitis and asthma.

The differential of cough persisting for weeks (ie the chronic cough) includes a much larger number of diseases. However, 3 diagnoses can account for 90% of all such patients: post nasal drip syndrome, asthma, and gastro-oesophageal reflux. In one study of non-smokers not on an ACE inhibitor having a normal chest X-ray, this number was 99.4%. Other diagnoses to be aware of are cough following a viral infection, a pertussive infection, and cough with ACE inhibitor therapy. Chronic cough can also be a presenting symptom of chronic bronchitis, interstitial lung disease, abscess, bronchiectasis, neoplasm and foreign body. Rare causes are psychogenic, impacted cerumen/hair, and foreign body. Approximately 25% of all patients presenting with cough will have more than one cause accounting for this symptom.

The most common cause of chronic cough in adults is the post nasal drip syndrome observed in allergic rhinitis, perennial non-allergic rhinitis, vasomotor rhinitis, nasopharyngitis and sinusitis. Secretions in the upper airway stimulate cough via stimulation of receptors within the pharyngeal/laryngeal mucosa. Common symptoms seen with this cough are nasal discharge, the sensation of liquid in the back of the throat and clearing of the throat. However, it may also be 'silent', which is a cough without any accompanying symptoms.

The second most common cause of chronic cough in adults is asthma. This can be noted with episodic wheezing and dyspnea but might also be 'silent'. Pulmonary function testing might reflect reversible airflow obstruction, but can be normal. If the latter occurs, airway hyper-reactivity can be

demonstrated using methacholine challenge. However, such a positive bronchoprovocation test does not prove asthma and can be falsely positive in 22% of these patients (bronchitis and bronchiectasis). To confirm the diagnosis, appropriate therapy must be employed with improvement resulting. Gastro-oesophageal reflux is cited frequently as the third most common cause of chronic cough, but was the most common in a recent study by Poe and Kallay (2003). Accompanying symptoms are heartburn and a sour taste in the mouth. Comparable to post nasal drip and asthma, reflux can be 'silent'. Cough results from a stimulation of receptors in the larynx, aspiration of gastric contents, and reflux of acid into the distal oesophagus. The most sensitive method for diagnosis is pH monitoring.

Cough following viral infections can persist for more than 8 weeks. Secretions stimulate receptors accounting for the symptom. Anthihistamine/decongestant may assist in some relief of the cough but, more frequently, it is related to airway inflammation and hyper-reactivity. A chronic cough with pertussis may be more common than thought in adults. Approximately one of 5 patients demonstrated positive serology for pertussis but such testing is not available to clinicians currently. Cough with ACE inhibitors ranges from 3–20% of all patients using the medication. It follows an accumulation of bradykinin. Onset can range from one week to 6 months while resolution requires 4 days to 4 weeks. Employment of an alternative ACE inhibitor does not help. Therapy is switching to an angiotensin II receptor antagonist.

The history of the patient is the first and the most important tool employed in the diagnostic approach to chronic cough. The existence of other symptoms and use of ACE inhibitors should be determined. Questions are directed at the characterising the cough: the onset, duration, frequency and alleviating and exacerbating factors. Smoking and occupational histories can be helpful

in diagnosis. The character and timing of the cough is queried, but usually is not helpful. Similarly, sputum production is of little assistance in differentiating the cause of chronic cough. A past medical history of heart, lung, thyroid, and gastrointestinal disease can contribute toward a diagnosis. Cobblestoning, secretions, stridor, rhonchi, wheezing, crackles and observing the quality of cough during the examination can also assist in diagnosis. Spirometry, both with and without bronchodilators, should also be obtained. If support for a specific diagnosis is found, therapy should be provided. If no support for a specific diagnosis is found or there is no improvement with initial therapy, an antihistamine, a decongestant, and a nasal steroid are provided. The patient should be re-evaluated in 2 weeks. If there is improvement, the post nasal drip syndrome is diagnosed. If there is none, sinus and chest X-rays should be considered. Chest X-rays might contribute to a diagnosis in cough with cancer, tuberculosis, sarcoidosis, and interstitial lung disease. In addition, methacholine challenge is done. If the last is positive, the patient should be treated for asthma with a combination of inhaled steroids and bronchodilators. The patient is again re-evaluated in 2 weeks. If there is no improvement on inhalers, the patient is placed on pH monitoring. If abnormal, therapy is directed toward reflux. Finally, if there is no improvement on this regimen of medication, bronchoscopy is considered. This procedure is infrequently of any value except in diagnosing the rare cancer.

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SESSION 2:

Aetiology

Chairman:
J. P. Lavoie

AETIOLOGICAL AGENTS: INDOOR ENVIRONMENT AND ENDOTOXIN

B. C. McGorum and R. S. Pirie

Department of Veterinary Clinical Studies, Easter Bush Veterinary Centre, University of Edinburgh, Roslin, Midlothian EH25 9RG, UK

Airborne dust in horse stables may contain high levels of bacterial endotoxins, over 50 species of moulds, large numbers of forage mites, plant debris and inorganic dusts. High levels of toxic gases such as ammonia are also present in some stables. Respirable and total airborne concentrations of organic dusts, endotoxin (McGorum *et al.* 1998; Malikides *et al.* 2000; Pirie *et al.* 2001) and β-D-glucan (an index of mould exposure; Malikides, unpublished data) in the breathing zones of horses in conventional horse stables may exceed the thresholds for induction of inflammation and hyper-responsiveness in healthy humans, and may exceed the air hygiene standards recommended for occupational exposure in man.

Inhaled organic dust and, in particular, inhaled endotoxin and moulds, have a well accepted role in the aetiology of heaves. Inhaled endotoxin is also an important cause of inflammatory airway disease in humans and, indeed, the airborne endotoxin concentration is the most important occupational exposure associated with the development and progression of airway disease in human agricultural workers (reviewed by Schwartz 2001). The pro-inflammatory properties of these airborne agents indicate that they also have the potential to contribute to the induction and/or persistence of inflammatory airway disease (IAD) in stabled horses. Consistent with this possibility, findings from a recent study into the role of airborne dust, endotoxin and β-D-glucan in IAD in young racehorses in Sydney, Australia indicate that inhalation of high concentrations of respirable endotoxin, but not β-D-glucan, is a strong risk factor for tracheal aspirate neutrophilia. Additionally, Burell *et al.* (1996) demonstrated that the duration of IAD, and the frequency of coughing, were significantly greater in horses that were housed in loose boxes and bedded on straw

(presumed high dust exposure) compared with those housed in American barns and bedded on paper (presumed low dust exposure).

Several other studies have also reported a relationship between stabling and inflammation of the upper and lower respiratory tracts in non-heaves-affected horses (Derksen *et al.* 1985; Clarke *et al.* 1987; Tremblay *et al.* 1993; Holcombe *et al.* 2001). The relationship between this stable-induced airway inflammatory response and IAD remains to be established. It could be argued that some of the horses in some of these studies may have had low-grade heaves (ie dust induced neutrophilic airway inflammation without significant airway dysfunction). However, this possibility could be eliminated in the study that included only horses considered to be too young to develop heaves (Holcombe *et al.* 2001). Interestingly, the ratios of neutrophils reported in airway samples from horses with stable-induced airway inflammation often exceeded 10%, and occasionally exceeded 30%, ratios that are commonly considered to support a diagnosis of IAD or heaves. While stabling was associated with airway neutrophilia, concomitant airway dysfunction or airway mucus hyper-secretion was not detected in these studies. The latter finding contrasts with IAD and heaves, which are both disorders characterised by significant airway mucus hyper-secretion.

Additional evidence supporting involvement of organic dust in IAD stems from short duration experimental inhalation challenges with hay dust suspension or soluble endotoxin (Pirie *et al.* 2001, 2002). These challenges induced a dose-dependent airway neutrophilia, in both heaves and control horses. The threshold exposures of dust or endotoxin required to induce airway neutrophilia in controls was higher than that for heaves-affected

horses, with control horses developing significant airway inflammation only following very high dust or endotoxin exposures. While these experimental challenges induced airway neutrophilia in control horses, mucus hyper-secretion, airway dysfunction and airway hyper-reactivity were not detected. It is possible that there may be significant variability among individual non-heaves-affected horses in their responsiveness to inhaled endotoxin, which was not identified in the aforementioned study, given the limited number of horses investigated. Consistent with this possibility, healthy, non-asthmatic humans have a broad range of stable and reproducible physiological responses to inhaled endotoxin, with only approximately 10–15% of the subjects developing either airflow obstruction after inhaling minimal amounts of endotoxin, or having a negligible airway response to high doses of inhaled endotoxin (Kline *et al.* 1999). Potential variability in responsiveness to inhaled agents complicates the investigation of these agents in horses, where subject numbers are limited. Investigation of the role of organic dust in IAD is complicated further by the additive or synergistic effect of co-exposure with other pro-inflammatory agents (Williams 1997). The pro-inflammatory effect of inhaled agents is also augmented by the presence of pre-existing airway inflammation, such as that induced by microbial agents. Additionally, as IAD is commonly observed in exercising horses, the pulmonary oxidative stress associated with exercise may augment the pulmonary response to these inhaled agents.

Horses with heaves show a consistent resolution of pulmonary neutrophilia and significant improvements in lung function following a reduction in organic dust exposure. In contrast, the authors are aware of some horses with IAD that had persistent pulmonary inflammation and associated clinical signs, despite being maintained fully at pasture for several months. This anecdotal observation suggests that factors other than inhaled organic dust are, at least in part, involved in perpetuating the pulmonary inflammatory response in horses with IAD. Nevertheless, the bulk of current evidence suggests that horses with IAD should be maintained in low dust environments.

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AETIOLOGICAL AGENTS: OUTDOOR ENVIRONMENT AND AIRWAYS

A. J. Ghio

United States Environmental Protection Agency, Chapel Hill, North Carolina, USA

Environmental agents that can potentially injure the airways of animals include ozone, nitrogen, sulphur dioxide and particles, the latter being of greatest concern. Most of the US population is exposed to ozone concentrations that exceed current standards. This is responsible for induction of inflammation in the airways, acute loss of pulmonary function, elevated airway reactivity, increased infection rate in the lung, decreased particle clearance and altered lung structure. Environmental sources of ozone include motor vehicles, power-generating plants and industrial combustion processes. Nitrogen oxides can cause shortness of breath, coughing and hyper-reactivity. Sulphur dioxide is produced by power plants and industrial processes. The highest levels, occurring in summer, can cause cough, phlegm and hyper-reactivity.

Air particles are a major environmental challenge to human airways. Inhalation of suspended particulate matter (PM) has challenged the lower respiratory tract in man for thousands of years. In the past century, episodes of extremely high levels of ambient air pollution particles in Europe and the USA have corresponded with acute elevations in human morbidity and mortality. This was instrumental in bringing about widespread monitoring and regulation of air quality from about 1970. However, approximately 10 years ago, several studies using time-series analysis linked exposure to ambient air pollution particles at levels currently observed in cities worldwide with indices of acute human morbidity and mortality (Samet *et al.* 2000). These findings were met with some scepticism but both re-evaluation of the initial studies and many new investigations confirmed their validity (Gamble and Lewis 1996; Krewski *et al.* 2000). The initial assumption was that this morbidity and mortality resulted from a lung injury, which was likely to be inconsequential in healthy individuals but significant in susceptible subjects.

Descriptions of haemorheological changes and acute elevations of morbidity and mortality attributable to cardiovascular disease have suggested that the biological effects of PM are not restricted to the lung. Also, although morbidity and mortality associated with ambient air PM levels is more common in the very young or old, and those with chronic cardiopulmonary disease, populations of all ages and health status seem at risk. In composition, ambient air pollution PM can range from crustal silicates and plant debris to carbonaceous products of combustion. Ambient air PM levels are relatively low usually (10–100 g/m^3), although this can approach 1,000 g/m^3 in particularly urban sites, representing constant exposure to what can be predominantly fine and ultra-fine particles.

Increased ambient air PM levels have been associated with a higher prevalence of respiratory symptoms, as well as hospitalisations for bronchitis. Increased incidence of wheeze and asthma have been correlated with elevated ambient air PM as have increased infections. An acute, reversible decrement in pulmonary function has been described following exposure to ambient air PM, although not in all studies (Goren *et al.* 1999). A chronic loss of pulmonary function has not been reported after exposure to elevated ambient PM. The presence of many particles can affect measurements of bronchial hyper-reactivity, and a challenge inhalation with particles has been proposed as an alternative to methacholine in the diagnosis of asthma (Cloutier *et al.* 1992). Investigations have not yet defined the effect of ambient air PM on bronchial hyper-reactivity. Inhalation of ambient air particles has been associated with an acute influx of neutrophils to the lower respiratory tract (Ghio *et al.* 2000). This is comparable to other particle-associated injuries, including cigarette smoking and diesel exposure.

The 2 chronic pathological processes noted on microscopic inspection of lungs from individuals chronically exposed to particles are emphysema and parenchymal fibrosis. These have distinct clinical and pathological characteristics and have been considered to be separate disorders but they have features in common and may be linked by a common mechanistic pathways such that an agent which normally produces a fibrotic injury can be modified to be associated with emphysema.

Collagen deposition and fibrosis in the human lung correlate with exposure to ambient PM (Pinkerton *et al.* 2000) which can elicit several changes in peripheral blood, including decreased red cells, elevated white blood cell counts and increased C-reactive protein, fibrinogen and blood viscosity. The last 2 changes contribute potentially to the association of ambient PM with thrombotic events. Currently, plasma fibrinogen levels appear the most sensitive systemic marker of exposure to ambient PM. Elevated plasma concentrations of this protein occur after exposure to many different particles, but the response is best described in smokers. There appears to be some dose-response relationship between particle exposure and the plasma fibrinogen level. High levels of plasma fibrinogen accurately reflect the severity of atherosclerosis and a hypercoagulable state. Elevations of this protein correspond to incidence of cardiovascular disease, vascular occlusive disease and failure of saphenous vein and prosthetic grafts. Therefore, increased levels of fibrinogen after particle exposures may predict an increase in cardiovascular disease. Inhalation of ambient air PM increases heart rate, decreases heart rate variability, and elevates arrhythmia rates. Hospital admissions for cardiovascular diseases are also associated with PM, and the incidence of myocardial infarction can be increased within 2 h of PM elevation and remaine elevated for 24 h.

Investigations have suggested carcinogenicity of ambient air PM but results can be conflicting as is also noted in investigations of cancer induction by mineral oxide particles (eg silica). It has been suggested that cancer may be triggered by changes in local pH as a result of acidic properties of the particle, contamination by biological agents including endotoxin and worsening of atopic disease by organic components. In addition, a fundamental property of many of these particles is the capacity to present an oxidative stress to the lung. There are numerous proposed pathways in which particles can directly support electron exchange with radical formation. Diminished concentrations of compounds with an ability to scavenge free radicals have also been reported and can affect oxidative stress after particle exposure (Al-Humadi *et al.* 2002). Finally, numerous particles effect a disequilibrium in iron metabolism in a tissue comparable to the effect of fibres (eg asbestos). A common feature found on light microscopy of the lung exposed to any particle is a ferruginous body. This histologic abnormality is a particle (or fibre) with accumulations of protein and iron and provides evidence of a local disruption of normal metal metabolism. The ferruginous body can reflect increased catalytically active iron in a tissue and therefore suggests oxidative stress. Features of the clinical presentation, physiological changes and pathology of individuals exposed to ambient air PM are common to many of the particle-related injuries.

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AETIOLOGICAL AGENTS: ALLERGY AND RELATED MEDIATORS

J. P. Lavoie

Faculty of Veterinary Medicine, University of Montréal, Quebec, Canada

Allergy is defined as a hypersensitive state acquired through exposure to a particular allergen. Allergic asthma (extrinsic) in humans is characterised by an early allergic response (EAR) that occurs within minutes of exposure to an allergen and the late allergic response (LAR), which develops 6–9 h later. EAR is initiated by the activation of cells bearing allergen-specific IgE, primarily mast cells, through IgE cross linkage and activation of the high affinity (FcεRI) receptors. Mast cell activation results in the release of preformed mediators, together with the synthesis and release of new mediators and cytokines. The release of preformed mediators is responsible for the initial contraction of airway smooth muscle, mucus secretions, vasodilation and microvascular leakage. Together, these changes result in the narrowing of the airway lumen and airflow obstruction characteristic of the EAR.

There is currently no evidence that horses with inflammatory airway disease (IAD) develop the acute respiratory signs, which would be expected with an EAR. However, the finding that a subset of horses with small airway disease have an increased number of mast cells in their bronchoalveolar lavage (BAL; Vrins *et al.* 1991), which is negatively correlated with airway hyper-reactivity (Hoffman *et al.* 1998b) and responds to mast cell stabilisers (Hare *et al.* 1994), supports the implication of these cells in IAD. Additional support for allergy being implicated in IAD, is the finding that blood basophils of horses with mild airway diseases exposed to various allergens had an increased basophil degranulation index and histamine release (Dirscherl *et al.* 1993).

The synthesis and secretion of mast-cell mediators, such as eicosanoid (leukotrienes, prostaglandins, PAF) and cytokines (IL-8, IL-5, RANTES, TNF-α), promotes the chemotaxis and activation of inflammatory cells, including eosinophils and neutrophils, into lung tissue, which amplify and sustain the airway obstruction taking place during the LAR. Tissue and BAL neutrophilia is commonly found in horses with IAD. BAL eosinophilia, however, is found only in a subset of horses with IAD (Moore *et al.* 1995; Hare and Viel 1998). Nevertheless, considering that tissue and BAL eosinophilia are not correlated in horses with inflammatory lung diseases (Winder *et al.* 1991), it is possible that infiltration of eosinophils in lung tissues is more common than suspected, based on BAL cytology results.

Cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) produced by mast cells and eosinophils can induce bronchial smooth muscle contraction, increased vascular permeability and increased mucus production. On a molar basis, LTD_4 in horses breathing spontaneously is 305–970 times more potent than methacholine in inducing smooth muscle contraction (Marr *et al.* 1998a). In a preliminary report, horses with IAD had increased levels of LTC_4 in BAL (Hoffman *et al.* 1998a). Cysteinyl leukotrienes do not appear to play an important role in heaves, as suggested by the inefficacy of a FLAP antagonist (Robinson *et al.* 1998), 5-lipoxygenase inhibitor (Marr *et al.* 1998b) and LTD_4 receptor antagonists (Stark *et al.* 2001; Lavoie *et al.* 2002). It remains to be determined whether these agents will also be ineffective in IAD.

Previously, it was believed that the inflammatory response, triggered by the mast cell release of inflammatory mediators, was a pre-requisite for the development of the LAR. However recent findings, using molecular tools and murine animal models, have highlighted the complex interactions involved in the modulation of allergic lung disease (Watanabe *et al.* 1997). Most cells present within the lung tissue including epithelial cells, myocytes, and nerve

endings, in addition to traditional inflammatory cells, contribute to this modulation. Among these, T cells (CD4+ cells, more specifically of Th2 cells) play a central role in allergic airway inflammation. Cytokines produced by Th2 cells, such as interleukin (IL)-4, IL-5 and IL-13 have been implicated in allergic inflammation. Th1 cells are important for cell-mediated immunity by their production of INF-γ and other cytokines. Using *in situ* hybridisation, it has been found that BAL cells from horses with heaves fed hay over a long period of time (months) have increased expression of IL-4 and IL-5 mRNA and decreased expression of INF-γ compared to controls (Lavoie *et al.* 2001). These findings are consistent with a predominant Th2 type cytokine response in heaves. In a follow-up study, it was found that the development of the Th2-type response corresponded in time with the development of airway obstruction and inflammation (Cordeau *et al.* 2002). IL-4 promotes the development and the growth of Th2 cell phenotype and is essential for the induction of B-cell isotypes switching to IgE antibody production. IL-5 production is typically associated with tissue eosinophil migration, which is not a common feature in heaves but has been found in some horses with IAD. Equine neutrophils express receptors for Th2-type cytokines, including IL-5 (Al-Dewachi *et al.* 2002) that provide a mechanisms by which IL-5 may contribute to neutrophil activation. However, using RT-PCR on BAL cells of horses with heaves (Giguere *et al.* 2002) or summer pasture associated COPD (Beadle *et al.* 2002), a significant increase in IL-4 was also found, but IL-5 mRNA was not detected in these studies. The contribution of Th2-type cytokine in IAD is currently being investigated.

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AETIOLOGICAL AGENTS: VIRUSES AND INFLAMMATORY AIRWAY DISEASE

J. L. N. Wood, J. R. Newton, K. C. Smith and D. J. Marlin

Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK

WHAT IS THE DISEASE DEFINITION USED IN THIS PAPER?

Inflammatory airway disease (IAD) is a poorly understood phenomenon and data from only a few population based studies are published. Direct comparison between studies is hampered by a lack of clear case definition and the absence of published studies comparing the usefulness of tracheal and bronchoalveolar lavage (BAL) in its diagnosis. IAD is defined here as a disease syndrome with neutrophilic inflammation detectable by tracheal or BAL in animals under 5 years old. Often, neutrophilia is accompanied by increased amounts of mucus visible endoscopically in the trachea shortly after exercise, although this is not regarded as essential to case definition. Although not specifically excluded in every report, classic heaves, or recurrent airway obstruction (RAO) is not included in this discussion.

STUDIES OF IAD AFFECTED HORSES

Workers at the Animal Health Trust have studied this disease syndrome in racehorses, in young pony foals and through experimental infections for over 20 years (eg Burrell 1985; Blunden *et al.* 1994; Burrell *et al.* 1996; Wood *et al.* 1997; Newton *et al.* 2001). Detailed clinical investigations of respiratory disease and loss of performance in British racing yards have demonstrated that IAD is present in over 65% of outbreaks, raising the suspicion that an infection, particularly with viruses, plays a key role in the aetiology. Either a history of prior IAD or IAD as a concurrent finding is common in young racehorses presented for loss of performance investigation within this clinic.

However, interpretation of data from case and outbreak investigations is complicated by the high incidence of infections in these young animals and the lack of appropriate control populations.

Detailed, prospective, longitudinal observational epidemiological studies have been undertaken to study respiratory disease in young British Thoroughbred racehorses. These studies give a basic description of the epidemiology. They also show how the disease incidence and prevalence vary between training yard, year and decrease with age (which is closely correlated with duration of stay in the training yard environment). The variation in prevalence between ages and calendar months is shown by Newton *et al.* (2003) and Wood *et al.* (1999).

One key finding from this research was that the average duration of disease incidents was around 9 weeks, but with a very long tail to the distribution. Some young racehorses, particularly in their '2-year-old' year, were affected for over 8 months, whereas the disease was not detected at the monthly examinations in 20% (all of which remained healthy during their '3-year-old' year). Such an effect will only be apparent in prospective studies following horses over long periods (Fig 1).

Although in these studies, horses were classified as having or not having IAD, an important consideration is that different clinical findings might be expected in a horse sampled in its first week of being affected with acute disease, compared to those in an 'average' horse in its 9th week or a badly affected animal in its 6th month of disease (perhaps in its 1st year in training). This is a important when comparing studies of naturally affected animals, particularly in relation to referral bias. Certainly in the UK, and probably elsewhere, trainers are much less likely to present horses for veterinary examination when in their 1st month of
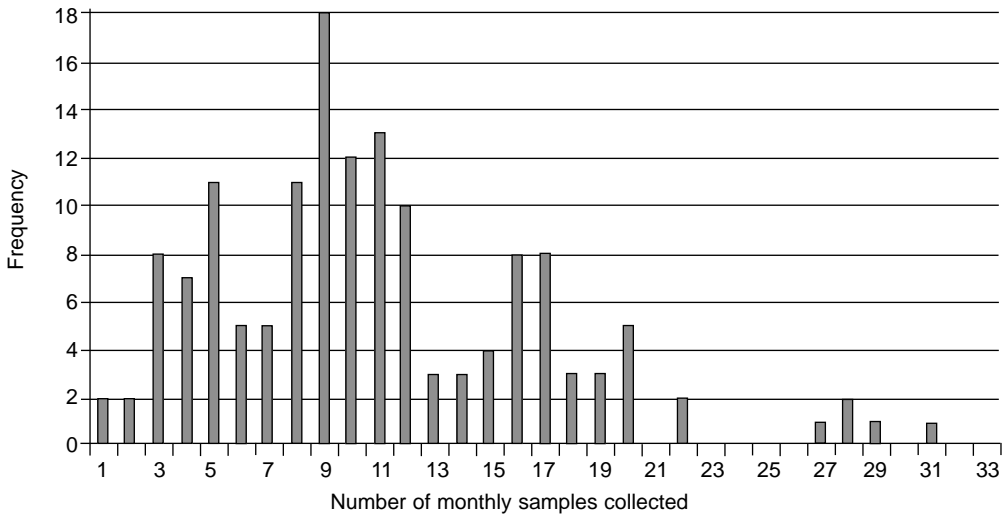
Fig 1: Number of months that individual horses were sampled over a 3 year study period (Wood et al. *1999).*

disease. Such an effect will be exaggerated by the fact that the disease, overall, is sub-clinical for >60% of the time and the likelihood of horses coughing is higher in the 2nd than the 1st month of disease (Burrell *et al.* 1996).

Studies referred to in the remainder of this paper are largely restricted to those in horses (mostly racehorses) and ponies less than 6 years of age that were affected naturally with the disease syndrome. Because there are few published studies that have used IAD as the case definition, studies of coughing (Christley *et al.* 2001), which is a fairly specific, if insensitive (Burrell *et al.* 1996), measure of IAD in young racehorses have also been considered.

Whenever multidisciplinary studies of IAD have been undertaken, they have provided clear evidence of a multifactorial aetiology (eg Burrell *et al.* 1996; Wood 1999). Considerable care is needed, therefore, to ensure that results from single factor studies (eg of stabling) are not confounded by unmeasured effects, such as of viral infection, which might be associated with the stresses of housing grazing animals.

HOW COMMON ARE THE DISEASE AND VIRAL INFECTIONS?

IAD is common in young racehorses. In a large scale study Wood *et al.* (1999) estimated the overall monthly prevalence to be 13.8% and the incidence to be 8.9 cases/100 horses/month. Criteria for

assigning causality in natural disease are reviewed by Newton *et al.* (2003), but it is clear that for viral infections to play a role in the aetiology of IAD, they must: a) occur in affected animals; and b) their presence must be associated with occurrence of disease. Few studies have assessed the role of viral infections in IAD and those that have been identified are shown in Table 1. For prospective, or longitudinal studies, incidence rates are presented, but for case control studies, prevalences of infection in cases are included. It is clear from the data in the table that incidence rates of different viral infections were generally lower than the incidence of IAD and the prevalence proportions in cases (where reported) was low (<10%). The exception was the reported association between lung disease in foals and active equine herpesvirus-2 (EHV-2) in the lung (Murray *et al.* 1996). On the basis of these results, it seems unlikely that these viruses are key to the aetiology of IAD in young racehorses.

WHAT ARE THE ASSOCIATIONS BETWEEN VIRAL INFECTION AND IAD?

In the study of Burrell *et al.* (1996), no viral infection was associated with IAD, although upper respiratory tract disease was statistically associated with EHV-1/4 infection. However, considerable care is needed when interpreting

TABLE 1: Summary of rates of viral infections in studies of naturally occurring IAD

Study	Rate of infection (/100 horses/month or % if incidence)					
	Influenza	EHV	ERV-1	ERV-2	Adenovirus	EHV-2
Burrell *et al.* (1996)	0	10.9	5.5	N/A	0.8	N/A
Wood (1999)	0.8	4.4	2.2	0.8	1.5	N/A
Thomson (1978)	N/A	3.3	N/A	N/A	N/A	N/A
Hoffman *et al.* (1993)	0%	4%	N/A	N/A	0%	N/A
Christley *et al.* (2001)	0%	9%	8%	2%	N/A	N/A
Murray *et al.* (1996)	N/A	N/A	N/A	N/A	N/A	100% in blood, 66% in lung
Moore *et al.* (1995)	N/A	N/A	N/A	N/A	N/A	N/A

N/A: Not assessed or not reported

statistically non-significant findings, as the lack of significance can occur due to lack of effect, or insufficient sample size to detect any effect.

In a larger study undertaken in 7 training yards over 3 years, EHV (1 and 4) was the most common viral infection, with an overall incidence of 4.4 cases/100 horses/month (Wood *et al.* 1999). This was the only viral infection statistically associated with IAD and horses with EHV-1 or EHV-4 infections that month (diagnosed serologically) were around 5 times as likely to be affected with IAD than those that had not experienced it (odds ratio=4.9, 95% confidence interval 1.4–17.4). However, it only occurred at 7.5% of sampling points, and was detected in only 7.5% of incident cases (Wood 1999). A similar prevalence proportion for EHV-1 or EHV-4 was reported by Hoffman *et al.* (1993: 4%) and Christley *et al.* (2001: 10%) and neither of these authors found EHV to be associated with disease.

These and other case control studies of respiratory disease or coughing in British or Australian racehorses (Newton *et al.* 2001) found no association of any virus with disease, other than a minor one for influenza in the British horses (Newton *et al.* 2001).

In contrast, Moore *et al.* (1996) reported that the BAL fluid differential cytology in horses with IAD supported the hypothesis of persistent viral respiratory tract infection as a potential cause of IAD, but no specific viral investigations were undertaken. However, in the authors' experience the inflammatory response and cytological picture in IAD associated with bacteria, allergy and viruses may be very difficult to differentiate. Subsequent investigations demonstrated that human interferon alpha had significant efficacy in treating IAD in young racehorses, after a mean duration of 3.1 months (Moore *et al.* 1996, 1997).

The results of this work are hard to evaluate in terms of the primary aetiology of IAD, but they provide an interesting insight into the more chronic disease.

Contrasting results were also produced for foals by Murray *et al.* (1996), who found a strong statistical association between the presence of EHV-2 in tracheal aspirates and clinical signs of lower respiratory disease (although no mention was made of bacteriological or cytological evaluation). The virus was isolated from 20/30 cases but only 1/20 controls (although it was present in the blood of almost all cases and all controls).

DISCUSSION

Studies of the association between acute viral infections and IAD have largely been consistent in demonstrating that known equine viruses do not play a substantial role in the aetiology or pathogenesis of IAD in the racehorse. In particular, there is evidence that EHV-1 and EHV-4 (which serologically cross react) play only a small role in causing the disease. The size of this role reflects the incidence of infection in the horse populations in question. There is consistency in the effect that EHV-1/4 appear to have (when they do occur) in that a transient neutrophilia was induced by EHV-1 infection in one reported experimental study (Kydd *et al.* 1996) and during some of the authors' (unpublished) investigations into outbreaks of respiratory disease in racehorses, a TW neutrophilia (often in the absence of increased mucus) has frequently been observed in outbreaks of EHV respiratory disease.

The disease that the influenza virus causes in susceptible animals undoubtedly involves airway inflammation but, other than in outbreaks in well vaccinated populations, usually occurs in distinct

outbreaks and is not confused with the endemic IAD that affects most groups of young racehorses in training.

If strong statistical associations between viral infection and IAD had been demonstrated by many workers, as for bacterial infections, it would be necessary to consider whether or not the associations were confounded by other effects. However, the lack of evidence of significant causation means that confounding should not be considered a major issue in judging whether or not the role of acute viral infection is important.

There are questions that remain around the role of EHV-2 infection, and perhaps that of other, unidentified viral infections, in IAD. One study has demonstrated a strong association between detection of EHV-2 in tracheal aspirates and disease in young foals, but we have been unable to detect this virus in similar samples collected from British racehorses, using appropriate cultural methods (unpublished observations). More advanced molecular diagnostic techniques might help to resolve the role that this ubiquitous infection plays in several disease syndromes, including IAD.

It is essential in all future studies of the aetiopathogenesis of IAD in horses, that the stage of disease in each animal be considered before over-optimistic statements are made about cause and role.

CONCLUSIONS

To conclude, there is a growing body of evidence to suggest that the role of viral infection in the aetiology of IAD is small, although further work needs to be done on chronic, low grade infections such as EHV-2.

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NATURAL HISTORY OF EQUINE INFLUENZA

H. G. G. Townsend

Department of Large Animal Clinical Studies and Veterinary Infectious Disease Organisation, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B4

EPIDEMIOLOGY

Equine influenza (EI) is a cause of inflammation of the respiratory tract and is the most commonly diagnosed viral respiratory disease of the horse. The virus is highly infectious and contagious with transmission occurring via direct, aerosol and fomite exposure. Outbreaks occur most often in groups of racehorses, with those 2–3 years of age suffering the highest morbidity (Newton and Mumford 1995; Morley *et al.* 2000a). Subclinical infection is probably common in horses older than one year of age (unpublished data).

CLINICAL SIGNS

The signs of EI seen most frequently are fever, cough and mucopurulent nasal discharge. Affected horses may also experience malaise, inappetance, dyspnea, weight loss and loss of athletic performance (Gross *et al.* 1998). In general, signs of fever and coughing resolve by about Day 10 post challenge, but close examination will reveal evidence of mucopurulent nasal discharge for at least 21 days in some animals. Serial endoscopy of experimentally infected foals (Sutton *et al.* 1997) provided visual evidence of inflammation of the respiratory tract, including findings consistent with pharyngeal lymphoid hyperplasia, hyperaemia and oedema of the mucosa of the large airways and mucopurulent debris in the trachea. Detailed serial ultrasonographic examination of 8 horses following experimental challenge showed evidence of pulmonary consolidation, oedema and fluid filled airways by Day 7 post challenge, with resolution of these signs by Day 14 (Gross *et al.* 1998).

PATHOLOGY

Infection of equine fetal tracheal explants is followed by viron budding at the base of microvilli of infected tracheal epithelium 12 h post infection. Virons were visible in the interstitial space by 24 h, scattered loss and clumping of cilia were evident in samples taken between 24 and 72 h post infection (O'Niell *et al.* 1984). Logical consequences of these lesions are decreased rate of mucociliary clearance (Willoughby *et al.* 1992) and secondary bacterial infections of the respiratory tract (Sarasola *et al.* 1992; Newton *et al.* 1999).

Following experimental infection, the virus replicates in the epithelial cells lining the respiratory tract. Peak death and shedding of these cells occurs around Day 2 and continues for up to 8–10 days post challenge. Viral antigen associated with cellular debris can be found in bronchoalveolar lavage (BAL) fluid samples 21 days post infection (Sutton *et al.* 1997). Subsequent to experimental infection of pony foals, immunoperoxidase staining was used to detect the presence of viral antigen in cells collected from the nasopharynx, trachea and mainstem bronchus using cytological brushes passed through an endoscope, and in samples obtained by bronchoalveolar lavage (Sutton *et al.* 1997). The antigen was particularly obvious in exfoliated ciliated columnar epithelial cells that were found in BAL fluid samples.

Numbers of neutrophils in BAL fluid samples were significantly increased in samples collected on Days 3 and 7 after experimental challenge of pony foals, with half the animals having high neutrophil counts in BAL fluid until Day 21 (Sutton *et al.* 1997). Sequential BAL fluid samples

following experimental challenge of horses showed increases in the percentage of neutrophils until the end of data collection 13 and 28 days post challenge (Gross *et al.* 1998; Kastner *et al.* 1999). The results of complete blood counts performed during both of these studies were also consistent with a systemic inflammatory response (increased neutrophil count and plasma fibrinogen concentration) persisting for at least 21 days post challenge.

Gross *et al.* (1998) observed intra-cellular bacteria in 25% of transtracheal aspirates from challenged horses on Days 4, 8 and 14 post challenge. Kastner (1999) isolated bacteria from BAL fluid samples up to 13 days post challenge and in another study large numbers of *Streptococcus zooepidemicus* were isolated from primary culture of all nasal swabs obtained from 30 influenza challenged horses 10 and 14 post challenge (H.G.G. Townsend, unpublished data).

Equine influenza has been described as an inflammation of the upper respiratory tract. However, infection and death of epithelial lining cells of the respiratory tract and alveolar macrophages provides evidence for widespread infection of the lower respiratory tract. Clumping and loss of cilia occurs and this provides a logical explanation for the measured decreases in mucociliary clearance and proliferation of bacteria in the respiratory tract. Exposure of the basal lamina occurs along the length of the trachea and bronchi. Infection and death of epithelial cells and subsequent proliferation of respiratory tract bacteria explain the signs of inflammatory airway disease observed following natural and experimental equine influenza infections. Although the clinical signs of disease may resolve within a few days, there is ample evidence to show that inflammation of the respiratory tract may persist in many animals for at least 21 days and in some for more than 28 days. Detailed studies following animals beyond 28 days post challenge have not been published. Information from such studies would be helpful in assessing any long term consequences of this disease.

RELATIONSHIP BETWEEN INFLUENZA AND OTHER IMPORTANT CAUSES OF IAD

Clearly, infection with influenza virus causes inflammation of the respiratory tract. In most situations the epidemiology, clinical history, clinical signs and laboratory findings of EI should allow differentiation of this disease from IAD (inflammatory airway disease) and heaves. Although influenza and IAD are both common causes of airway inflammation in 2–3- year-old horses, there is good epidemiologic evidence showing that most cases of IAD of young racehorses are not directly associated with previous EIV infections (Christley *et al.* 2001). It is possible that pre-existing IAD may influence the clinical severity of concurrent EIV infections. However, there appear to be no published reports addressing this issue.

Thorsen *et al.* (1983) reported high levels of haemagglutination inhibiting activity against influenza A1, but not A2, in mucus samples from horses with chronic obstructive pulmonary disease. However, well-designed studies aimed at detecting a causal relationship between EI virus infection and heaves have not been published. Although there is insufficient data to discount this hypothesis, there are arguments against any association between EI and the subsequent development of heaves. Importantly, the peak incidence of influenza occurs in young horses (Morley *et al.* 2000b) several years before the peak incidence of heaves. Also, there are no published reports of increased incidence or prevalence of heaves following natural outbreaks of influenza, nor are there any reports of increased incidence of heaves among the thousands of horses that have been experimentally infected with influenza virus.

Although it appears that viral respiratory disease of horses is not a pre-disposing factor in the development of IAD or heaves, it is possible that both of these diseases could be exacerbated by concurrent infection with influenza virus. Presently, there appear to be no reports of field or experimental studies on the impact of acute viral infections in animals with pre-existing IAD or heaves, even though there is no reason why this should not occur on a regular basis. An exploration of the clinical effects of influenza in animals with pre-existing IAD or heaves, could prove very enlightening.

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AETIOLOGICAL AGENTS: BACTERIA

J. R. Newton, J. L. N. Wood, K. C. Smith, D. J. Marlin and N. Chanter*

*Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU *Intervet UK Ltd, Walton Manor, Walton, Milton Keynes, Buckinghamshire MK7 7AJ, UK*

Inflammatory airway disease (IAD), characterised by mucus and a neutrophilic inflammatory response in the distal airways, is an important form of respiratory disease in both young Thoroughbred racehorses and Welsh Mountain ponies, despite the marked differences in the management of these 2 equine populations.

The role of bacteria in the aetiology of IAD remains controversial due to the potentially mixed nature of infections, and the possibility of contamination of the lower airways from bacteria of upper respiratory tract origin during endoscopy. However, there is increasing evidence for a causal involvement of bacteria in IAD in young horses.

A study in racehorses in the UK demonstrated, after allowing for the effects of trainer, age, season, equine herpesvirus (EHV) infection, disease the previous month and random variation between horses, that IAD was still significantly associated with lower airway infection with a limited number of bacterial species, particularly *Streptococcus zooepidemicus*, *Actinobacillus/Pasteurella* spp. and *Streptococcus pneumoniae* (Wood 1999). The seasonal variation in prevalence of IAD among racehorses linked to the introduction of yearlings in the autumn and the decreasing amount of disease with increasing age and time in training, are all consistent with acquisition of immunity against an infectious aetiology (Fig 1).
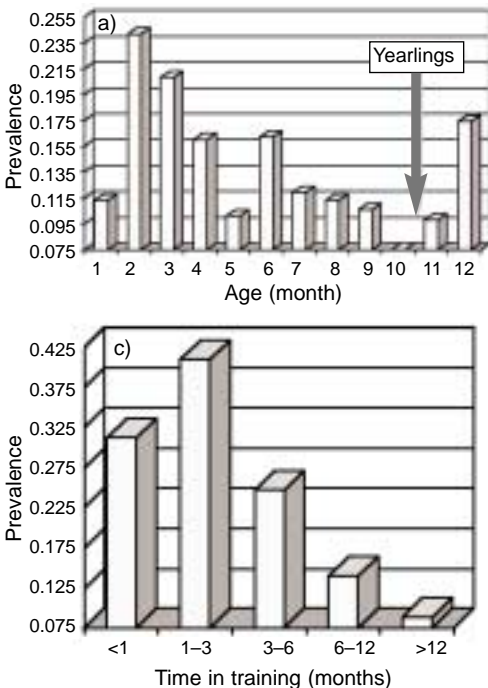

Fig 1: Variation in prevalence of IAD in young racehorses with: a) month of the year; b) age; and c) time in training.
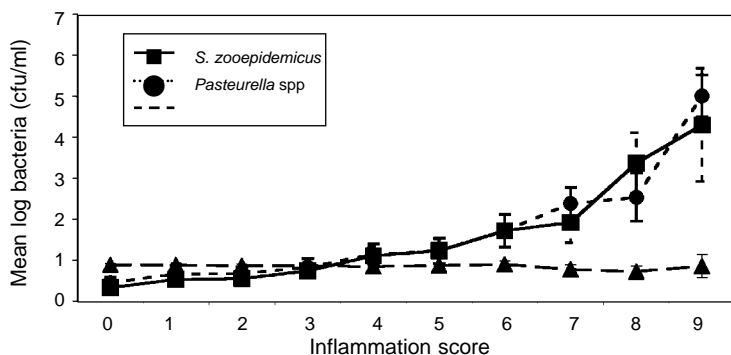
Fig 2: Variation in mean log_{10} cfu/ml of 3 bacterial species with inflammation score in young racehorses.

The traditional use of Koch's postulates (recovery of organism from natural cases, reproduction of signs with inoculation and recovery of organism from induced lesions) to confirm a causal association between disease and bacteria is problematical for IAD. This is because of the multiple nature and high prevalence of natural infections and absence of specific pathogen free animals. However, use of the 9 Bradford-Hill criteria (Hill 1965) to assess whether the epidemiological associations between these bacteria and IAD are likely to be causal, does suggest that bacteria cause lower respiratory tract disease in horses (Wood and Chanter 1994).

Several studies have confirmed a reasonable strength of association (measured as relative risk) and a significant biological gradient for these bacteria and IAD, with increasing numbers of bacteria having a greater strength of association with disease (Burrell *et al.* 1986, 1996; Wood *et al.* 1993; Wood and Chanter 1994; Wood 1999; Chapman *et al.* 2000). Examination of tracheal wash data from young Thoroughbred racehorses, using a 0 to 9 ordinal scoring of airway inflammation, demonstrates a significant biological gradient for increasing mean log colony forming units per ml of *S. zooepidemicus* and *Actinobacillus/Pasteurella* spp. and increasing inflammation score (Fig 2). There was no such gradient for *Staphylococcus* spp.

The associations with IAD are also specific for the three bacterial species of *Streptococcus zooepidemicus, Actinobacillus/Pasteurella* spp. and *Streptococcus pneumoniae* and are consistent between different studies (Burrell *et al.* 1986, 1996; Wood *et al.* 1993; Wood and Chanter 1994; Wood 1999; Chapman *et al.* 2000). Bacterial infections as causes of IAD in young horses are biologically plausible, are coherent as the results do not conflict with current knowledge, and are analogous with

similar and identical bacteria being the cause of respiratory disease in other species. Furthermore, *S. equi*, a subtype of *S. zooepidemicus* (Chanter *et al.* 1997), causes 'strangles' in horses and is generally accepted as a primary bacterial equine pathogen. There is limited experimental evidence that intra-tracheal inoculation of *S. pneumoniae* causes IAD and pneumonia in young pony foals, with the organism being recovered from the trachea and pneumonic lesions (Blunden *et al.* 1994). A temporal relationship between bacterial infection and IAD is difficult to confirm due to the possibility of an earlier pre-disposing viral infection. However, results of a longitudinal study of IAD failed to show that the association between bacteria and IAD is dependent on prior infection with any of the known equine viruses (Wood 1999).

The use of endoscopy to examine and sample the distal airways has been criticised for introducing upper respiratory tract bacteria, consequently precluding meaningful evaluation of tracheal wash bacteriology results and their association with IAD. However, a large proportion (62%; Wood *et al.* 1993) of endoscopically recovered samples remain bacteriologically sterile during routine examinations. There is a highly significant trend towards lower proportions of sterile washes with increasing airway inflammation (P<0.00001; Wood *et al.* 1993; Newton 2002). Furthermore, analyses of associations with IAD restricted only to horses with certain bacteria recovered from the nasopharynx (Table 1) show highly significant trends in association with increasing inflammation specifically for *S. zooepidemicus, Actinobacillus/ Pasteurella* spp. and *S. pneumoniae* (P<0.0006). There are no such trends for other potential pathogens found in the nasopharynx such as *Staphylococcus* and *Acinetobacter* spp. (P>0.6).

Novel approaches have been used to

TABLE 1: Linear trend in proportions of tracheal washes positive for different bacterial species among only horses from which the same organism was isolated from the upper respiratory tract

Case/control	Inflammation score	TWs (%) positive for bacterial spp.*	Total TWs examined (100%)	Odds ratio
Streptococcus zooepidemicus				
Control	0	21 (28%)	74	1.00 (ref)
Case	0	3 (30%)	10	1.08
Control	1	28 (45%)	62	2.08
Case	1	6 (60%)	10	3.79
Control	2	30 (83%)	36	12.6
Case	2	20 (71%)	28	6.31
$\chi 2$ for linear trend = 33.25 (P≤0.00001)				
***Pasteurella/Actinobacillus* spp.**				
Control	0	19 (22%)	85	1.00 (ref)
Case	0	4 (31%)	13	1.54
Control	1	25 (32%)	77	1.67
Case	1	6 (43%)	14	2.61
Control	2	16 (80%)	20	13.9
Case	2	13 (76%)	17	11.3
$\chi 2$ for linear trend = 30.28 (P≤0.00001)				
Streptococcus pneumoniae				
Control	0	7 (24%)	29	1.00 (ref)
Case	0	1 (33%)	3	1.57
Control	1	9 (32%)	28	1.49
Case	1	2 (67%)	3	6.29
Control	2	11 (69%)	16	6.91
Case	2	7 (70%)	10	7.33
$\chi 2$ for linear trend = 11.85 (P=0.00058)				
***Staphylococcus* spp.**				
Control	0	110 (43%)	253	1.00 (ref)
Case	0	8 (28%)	29	0.50
Control	1	88 (45%)	197	1.05
Case	1	15 (45%)	33	1.08
Control	2	32 (48%)	67	1.19
Case	2	12 (29%)	42	0.52
$\chi 2$ for linear trend = 0.238 (P=0.63)				
***Acinetobacter* spp.**				
Control	0	8 (12%)	67	1.00 (ref)
Case	0	1 (14%)	7	1.23
Control	1	7 (11%)	63	0.92
Case	1	1 (11%)	9	0.82
Control	2	10 (14%)	74	1.15
Case	2	1 (5%)	21	0.37
c2 for linear trend = 0.07 (P=0.80)				

Case = horse showing clinical signs (nasal discharge, coughing or pyrexia)
Control = horse NOT showing clinical signs (nasal discharge, coughing or pyrexia)

investigate variability, both in susceptibility to bacterial infection among horses and in infection by different *S. zooepidemicus* subtypes (Newton 2002).

Transferrin, an iron-binding host protein, may act as a source of iron for pathogenic respiratory bacteria such as *Actinobacillus* spp. and *Pasteurella* spp., which have been shown to chelate iron from transferrin using transferrin-binding proteins (Ratledge and Dover 2000). To this end the authors

have examined whether different equine transferrin haplotypes are associated with differences in measures of disease and infections of the respiratory tract for 2 distinct forms of respiratory disease in 2 separate equine populations.

Analyses examining the effects of genetic haplotypes of iron-binding transferrin on IAD in racehorses demonstrated a significantly protective effect of the D haplotype. In transferrin haplotype D positive horses there was a reduced risk of
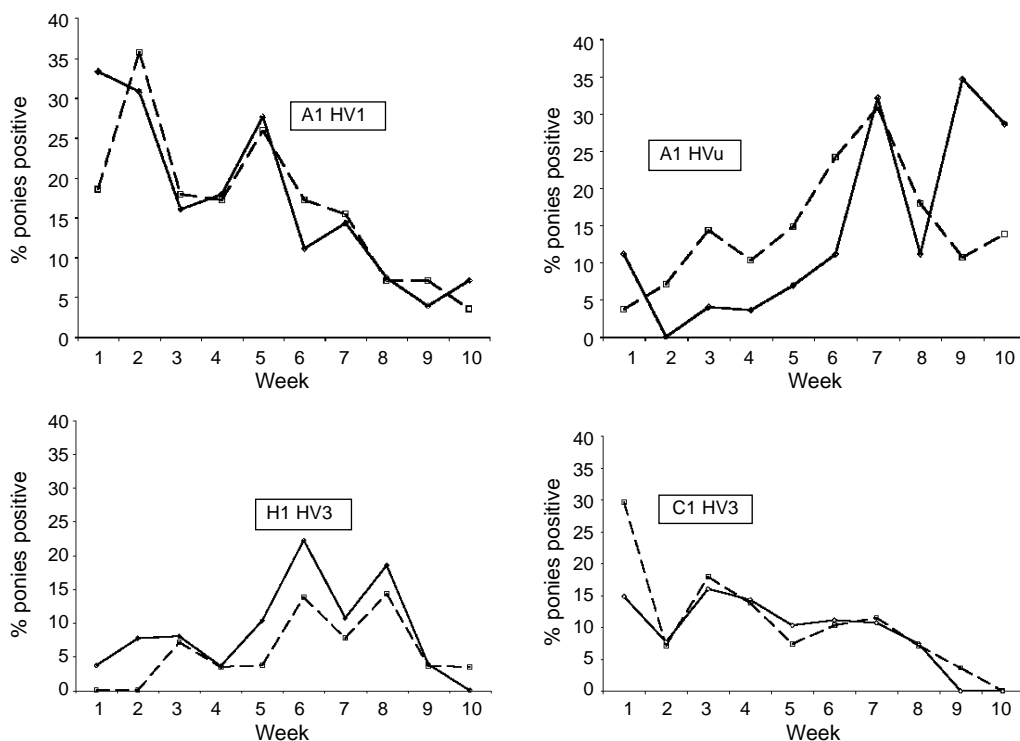
Fig 3: Proportions of ponies positive by week for tracheal wash (TW – solid line) and nasopharyngeal (NP – hatched line) isolates of 4 subtypes of S. zooepidemicus.

IAD and reduced counts of *S. zooepidemicus, S. pneumoniae, S. equisimilis* and *Mycoplasma equirhinis.* In addition, horse-level analyses demonstrated that horses with haplotype D suffered a significantly lower overall prevalence of IAD during months of repeated examination in a longitudinal study, and that this was primarily due to significantly shorter mean duration of IAD episodes. These results were consistent with the direction of effect of this haplotype for clinical respiratory disease and *S. zooepidemicus* infection in Welsh Mountain pony foals.

Pony-level analyses from a blinded, randomised, controlled trial of a bacterial vaccine showed good correlation between clinical scores and mean *S. zooepidemicus* counts ($R^2=0.46$, $P<0.001$), although there were no significant differences in clinical measures or infectious scores between genders or vaccine groups (Newton 2002). There were, however, significant differences in clinical ($0.0002<P<0.025$) and *S. zooepidemicus* infection measures ($0.0007<P<0.019$) between animals possessing different transferrin haplotypes. Ponies with transferrin D haplotype demonstrated

significantly less clinical disease and *S. zooepidemicus* infection than those that did not possess this transferrin type. Ponies with the F2 haplotype, had significantly higher measures of disease and infection.

Further work is required to investigate whether the effects of transferrin observed in these 2 separate equine populations are directly attributable to differences in transferrin or are due to a genetically closely linked mechanism.

To estimate the prevalence of different *S. zooepidemicus* types and investigate the association of types with respiratory disease, tracheal and nasopharyngeal isolates from Welsh Mountain pony foals and Thoroughbred racehorses were typed by 2 different PCR assays (Chanter *et al.* 1997; Walker and Timoney 1998). PCR of the 16S-23S RNA gene intergenic spacer of *S. zooepidemicus* could identify 8 distinct types (A1&2, B1&2, C1&2, D1&2; Chanter *et al.* 1997), and PCR of the M-protein hypervariable region could classify 5 types (HV1-5) 12, giving 40 total possible types.

A group of 29 recently weaned Welsh

Mountain ponies on the bacterial vaccine trial were assessed clinically and sampled weekly by tracheal wash and nasopharyngeal swab for 10 consecutive weeks, and again 16 weeks later (n=319 samplings). *S. zooepidemicus* was isolated from >90% of all pony samples (Newton 2002). From 538 typed isolates, 39 different types were identified including previously unidentified HV types (HVu) and polymorphic forms (eg A1/D1HV1/2). Of typed isolates, 89% were of 12 types and 52% were of only 4 types (A1HV1, A1HVu, A1HV3, C1HV3). More types were isolated from the trachea than nasopharynx and 24% of HV types were polymorphic compared to 6% of intergenic spacer types. At the pony population-level the prevalence of types varied over time, with evidence for clonal succession as nasopharyngeal types predicted tracheal types well (Fig 3; Newton 2002). There was a significant positive correlation between numbers of colonies of specific types and clinical respiratory score.

In addition, Thoroughbred racehorses in 3 Newmarket training yards were sampled approximately monthly between February and September 2000 with 761 tracheal washes (3 yards) and 225 nasopharyngeal swabs (2 yards) submitted. Of these, 22% of samples were positive for *S. zooepidemicus*. From 217 typed isolates, 23 different types were identified and >50% of tracheal and nasopharyngeal isolates were of only 5 and 3 types, respectively.

In conclusion, despite issues over sampling techniques and the current lack of experimental reproduction of disease, there remains considerable credible evidence for a causal association between IAD and a limited number of specific bacterial infections of the lower respiratory tract in young horses. New approaches are providing better insights into the complex interplay of host and bacteria. This may lead to a realistic means of targeted control of IAD in these young animals.

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DYSREGULATION OF INFLAMMATION

F. Bureau and P. Lekeux

Department of Functional Sciences, Faculty of Veterinary Medicine, University of Liege, Bat B42, Sart Tilman, 4000 Liege, Belgium

Chronic or recurrent airway disorders, such as heaves and inflammatory airway disease (IAD), are directly or indirectly related to an exaggerated inflammatory reaction. It is important, therefore, to understand why the inflammatory process is dysregulated in these diseases.

Chronic or recurrent airway inflammations are associated with overexpression of inflammatory proteins involved in both immune and inflammatory responses. Atopic asthma is a human inflammatory disorder of the airways which resembles heaves from an aetiological and patho-physiological point of view (Snapper 1986; Robinson *et al.* 1996). Reports state that asthmatic inflammation is characterised by overexpression of many inflammatory genes (Barnes 1996). These genes encode: 1) pro-inflammatory cytokines, including interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), which amplify pulmonary inflammation; 2) chemokines, such as interleukin-8 (IL-8), macrophage inflammatory protein-1α (MIP-1α), macrophage chemotactic protein-3 (MCP-3), RANTES (regulated on activation normal T- cell expressed and secreted) and eotaxin, which are chemotactic for leukocytes; 3) adhesion factors, including inter-cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, which play a cardinal role in leucocyte recruitment, margination, diapedesis and transepithelial migration; and 4) inflammatory enzymes, such as cytosolic phospholipase A2 (cPLA2), inducible nitric oxide synthase (iNOS), which generate inflammatory mediators (Barnes and Adcock 1998). Protein overexpression depends on increased gene transcription, suggesting that activation of some transcription factors underlies asthma pathogenesis. Most of the inflammatory genes overexpressed in asthma contain κB sites for Nuclear Factor-κB (NF-κB) within their promoter (Baeuerle and Baichwal 1997). This suggests that these genes are controlled predominantly by NF-κB, which could be of particular importance in the initiation and the perpetuation of allergic airway inflammation.

The NF-κB family is composed of 5 structurally related DNA-binding proteins: p50, p52, p65/RelA, c-Rel/Rel, and RelB (Siebenlist *et al.* 1994). The most common form is a heterodimer composed of p50 and p65 subunits, although the different family members can associate in various homo- or heterodimers through a highly conserved N-terminal sequence, called the Rel homology domain. Dimerisation of various NF-κB subunits produces complexes with different DNA-binding specificities and transactivation potentials. In most cell types, inactive NF-κB complexes are associated with inhibitory proteins of the IκB family, which sequester NF-κB in the cytoplasm. The members of the IκB family are IκB-α, IκB-β, IκB-ϵ, p100, p105 and Bcl-3; the most common IκB protein is IκB-α (Siebenlist *et al.* 1994; Whiteside *et al.* 1997). p105 and p100 are the precursors of p50 and p52, respectively. Following various stimuli, eg viruses, bacteria, pro-oxidants, and pro-inflammatory cytokines, IκB proteins are phosphorylated, ubiquitinated and rapidly degraded by the proteasome, allowing NF-κB nuclear translocation and transcriptional initiation of NF-κB-dependent genes (Karin and Ben-Neriah 2000).

Increased NF-κB activity has been demonstrated in macrophages of induced sputum and in bronchial epithelial cells of patients with stable asthma compared to normal subjects. This reinforces the assumption that persistence of NF-κB activity could be particularly important in the pathogenesis of chronic inflammation in asthma and suggests that increased NF-κB activity could

play a role in other allergic airway disorders such as heaves (Hart *et al.* 1998).

Studies of molecular mechanisms responsible for the inappropriate inflammatory reaction associated with heaves, showed that NF-κB is more active in the bronchi of heaves-affected horses than healthy horses (Bureau *et al.* 2000a). Furthermore, NFκB activity was correlated closely to the pulmonary dysfunction associated with the disease and to the expression of ICAM-1 in the airways (Bureau *et al.* 2000a). Bureau *et al.* (2000a) also observed that 21 days after removal of the allergen, NF-κB activity is maintained at high or moderate levels in the bronchi of most heaves-affected horses, suggesting that perpetuation of NF-κB activity could contribute to the persistence of bronchial inflammation when the allergen is evicted.

NF-κB induces: i) expression of pro-inflammatory cytokines which, in turn, activate NF-κB, initiating positive autoregulatory feedback loops; and ii) expression of inhibitory proteins of the inhibitor of NF-κB family, thus initiating negative autoregulatory feedback loops. Bureau *et al. (*2000b) showed that positive autoregulatory feedback loops involving IL-1β and TNF-α develop in the airways of heaves-affected horses following allergen-induced crisis. Usually, negative loops dominate, allowing transient, rather than persistent, NF-κB activity. Therefore, the authors of the present paper investigated whether such negative feedback loops were present in the airways of heaves-affected horses. It was observed that IκB-α, the most common inhibitor of NF-κB, is not expressed in the bronchial cells of healthy or heaves-affected horses. Surprisingly, the prominent IκB protein present in the airways of healthy or heaves-affected horses was IκB-β, which is not under the dependence of NF-κB for its expression. IkB-ß expression was fainter in the airways of heaves-affected horses compared to healthy subjects. These observations indicate that negative regulatory mechanisms are deficient in bronchial cells of heaves-affected horses and provide an explanation for the persistent NF-κB activity found in the bronchi of these horses.

These studies also showed that apoptosis is significantly delayed in lung neutrophils from heaves-affected horses. This delay is dependent on autocrine production of granulocyte/monocyte colony-stimulating factor (GM-CSF) and could also be involved in the persistence of inflammation observed in heaves (Turlej *et al.* 2001).

Although some cases of IAD either resolve spontaneously or respond to antibiotic treatment, a proportion of IAD-affected horses show persistent inflammation, sometimes in the absence of detectable pathogens. The molecular mechanisms by which inflammation persists have not yet been elucidated. Resolution of whether aberrant NF-κB activity and/or increased granulocyte survival are involved in the maintenance of inflammation in horses with IAD should provide valuable information regarding pathogenesis and could assist the development of rational IAD therapies.

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SESSION 3:

Diagnostic measures of inflammation

Chairman:
> *J. L. Hodgson*

SIGNIFICANCE OF TRACHEAL INFLAMMATION

J. L. Hodgson

University Veterinary Centre Camden, Faculty of Veterinary Science, University of Sydney, New South Wales 2006, Australia

Although collection of tracheal aspirates (TAs) is considered a routine procedure for evaluation of the respiratory tract in young performance horses, a number of controversial issues remain associated with their interpretation. Most notable are the relative numbers of inflammatory cells that are normally found in TAs, the significance of increased amounts of mucus and numbers of inflammatory cells, especially in relationship to their effect on performance and the interpretation of bacterial cultivation.

Inflammation in the lung can be regionally localised and may be restricted to the large airways, extend into smaller airways, or involve the pulmonary parenchyma. Tracheal aspirates 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. As such, they provide a non-homogenous sample and are not representative of any one segment of the lung. In contrast, a bronchoalveolar lavage (BAL) samples cells and secretions from the distal, smaller airways and commonly from the right caudo-dorsal lung lobes. There is no correlation between cytological findings between these 2 methods of sample collection. Therefore, a cytological diagnosis of inflammatory airway disease (IAD) will be influenced by the method of sample collection.

A number of factors may influence results of TA including long distance transportation or exercise prior to sample collection, coughing during sample collection and the sampling technique. These factors must be taken into consideration when evaluating samples and when comparing studies in which TAs were used to determine the presence of lower airway inflammation.

Interpretation of the amount of mucus within a TA is best performed in conjunction with endoscopic evaluations of the lower airways. This will facilitate accurate estimation of the amount of mucus present in the airways, as opposed to the amount of mucus collected by TA. The amount of mucus in TAs increases when pulmonary irritation occurs, such as in cases of IAD. However, the point at which mucus accumulation and/or airway inflammation become clinically significant, especially for different levels of performance, is currently contentious. This is particularly the case in horses not exhibiting overt signs of respiratory disease.

The information derived from TAs most commonly used for diagnosis of IAD is the differential cell count and, in particular, the relative % of neutrophils. The dilemma with interpretation of the relative numbers of neutrophils 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 and between studies. In addition, poor correlation between the relative number of neutrophils in TA and pulmonary histopathology has been described. 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, in studies of younger, more homogeneous populations of horses, smaller variations in neutrophil ratios in normal horses have been found. For example, studies investigating airway inflammation in young racehorses demonstrated that in 73–80% of clinically normal racehorses, neutrophils do not exceed 20% of the inflammatory cells found in TA samples (Sweeney *et al.* 1992; Christley *et al.* 2001). Furthermore, in a study where 1,235 TA samples were obtained

from 724 horses in race training, almost 90% of horses sampled had relative percent neutrophil values <10%. A strong statistical association between presence of >20% neutrophils in TA specimens and signs of respiratory disease (ie coughing) in young racehorses and the likelihood of isolating significant numbers of bacteria has been reported (Newton *et al.* 1999; Chapman *et al.* 2000; Christley *et al.* 2001). Therefore, it is likely that that >20% neutrophils in TAs from young racehorses is abnormal, representing a significant inflammatory process and a strong risk for clinical respiratory disease.

The absolute numbers of cells present (TNCC) should also be taken into account when interpreting the significance of the relative proportions of neutrophils in TA as they indicate more accurately a shift in cell populations. Unfortunately, the absolute number of cells is frequently not determined for TAs, reflecting the difficulties that may be encountered during enumeration of cells due to the entrapment of cells within mucus, the presence of clumps of epithelial cells, and the variable dilution of secretions by saline during collection. Estimates of the cellularity of samples have been advocated to help overcome this deficiency and may be incorporated in an inflammation score to further evaluate shifts in cell populations.

Other inflammatory cell types may also be evaluated in TAs from young performance horses to determine the presence of lower airway inflammation. However, less is known about the significance of the relative changes of these cell types in relation to IAD. Pulmonary alveolar macrophages (PAM) are the most abundant type of inflammatory cell present in TAs from normal horses and their presence is a prerequisite to assess the adequacy of a TA. Although PAMs are the most common inflammatory cell type in normal horses, increased numbers are rare in horses with IAD and their significance is unknown.

Lymphocytes are present in low numbers in normal TAs and may be difficult to differentiate accurately from other cell types present such as small macrophages, and epithelial cells. 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.

Eosinophils are usually present in very low numbers in TA from normal horses. Large increases in the relative numbers of eosinophils may be observed in cases of ascarid migration and lung worm infestation, whereas smaller elevations (>1%) are interpreted as evidence of a type I hypersensitivity response to inhaled allergens.

Mast cells are rare, or present in low numbers, in TAs from normal horses. This is in contrast to samples obtained by BAL, in which higher numbers of mast cells may be observed. There is little information on alterations in mast cell numbers in TAs. Furthermore, in a study investigating the relative numbers of mast cells in TAs and BALs obtained sequentially from racehorses it was found that BALs had significantly higher proportions of mast cells than TAs (Hughes *et al.* 2002). Therefore, TA may be less sensitive than BAL for detection of alterations in relative numbers of mast cells.

Epithelial cells may also be evaluated when assessing TAs. The epithelial cells present in TAs are predominantly ciliated columnar epithelial cells from the trachea and bronchi, but cuboidal cells from the smaller airways should also be observed. Squamous epithelial cells should not be present in TAs from normal horses and, if observed, represent oropharyngeal contamination. Mild changes to epithelial cells may be seen in normal horses including nuclear changes and the loss of some cilia. These changes probably represent normal wear and tear or turnover of cells. Pathological changes to epithelial cells (epithelial atypia) result from inflammation. However, there are many causes of airway inflammation and there are no epithelial changes that are specific for a particular diagnosis or aetiology. The exception is ciliocytophthoria, which has been reported in horses with acute respiratory viral infection.

Results of microbial cultivation assist in the interpretation of tracheal inflammation, but 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 and include fever, anorexia, coughing and nasal discharge. However, absence of these signs does not preclude bacterial infection, especially in milder cases of IAD.

Cytological evaluation of TAs 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 intra-cellular bacteria. 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.

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 aetiology, usually cultivate $<10^3$ bacteria (cfu)/ml and, frequently, no bacteria at all. If $>10^3$ cfu/ml are cultivated, it is likely that these bacteria are contributing to the disease process and identification of the bacteria involved will assist in interpretation of their significance. Bacteria that are most commonly isolated from IAD include *Streptococcus* spp., *Pasteurella* spp., *Actinobacillus* spp and occasionally *Bordatella bronchiseptica* and *Mycoplasma* spp. *Escherichia coli*, *Klebsiella* and strictly anaerobic bacteria are rarely isolated from uncomplicated cases of IAD, but may be isolated if antimicrobial therapy has been initiated or pleuropneumonia or lung abscessation has evolved. 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 as they are rarely significant. Isolation of non-pathogenic bacteria (coagulase negative *Staphylococcu*s spp., *Corynebacterium* spp., *Bacillus* spp.) indicates contamination at the time

of sampling.

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. Elevated percentages of neutrophils are associated with signs of respiratory disease (coughing) and increased numbers of bacteria. In addition, elevated percentages of neutrophils are a common finding in racehorses presented for poor performance. However, there have not been any studies which have evaluated directly the significance of cytological changes in TAs with any measurable variable of lung function.

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SIGNIFICANCE OF BRONCHOALVEOLAR CYTOLOGY IN INFLAMMATORY AIRWAY DISEASE OF HORSES

L. Viel

Department of Clinical Studies, University of Guelph, Guelph, Ontario N1G 2W1, Canada

INTRODUCTION

Based on limited, large epidemiological studies of the racetrack environment, respiratory conditions are the second most important factor contributing to poor performance in horses (Bailey *et al.* 1999). Known causes often associated with reduced performance are upper airway obstruction, possibly exercise-induced pulmonary hemorrhage, and lower airway inflammation. Young athletic horses suffering from non-infectious inflammatory airway disease (IAD) often demonstrate a gradual decline in their level of performance accompanied by variable clinical signs including cough, nasal discharge and prolonged recovery post exercise. IAD is currently believed to be distinct from the Heaves syndrome (Robinson 2001), and is characterised by the presence of inflammatory cells in the bronchiolar airways, with minimal accumulation of mucopurulent secretions. The presence of inflammatory cells is accompanied by airway hyper-reactivity in response to environmental stimuli.

BRONCHOALVEOLAR LAVAGE (BAL)

In human asthma, it was originally believed that cells collected by sputum samples mirrored the inflammatory cells trafficking the airways. However, Busse and Wenzel (2000) suggested that inflammatory cells may appear in the airway lumen in response to certain stimuli but then return to the subepithelial milieu. The latter was speculated because active remodelling observed at the level of the small airways is often not present in the larger cartilaginous airways. Therefore, cells collected from the lower trachea may not be an accurate reflection of inflammatory activity in the bronchiolar airways. Collection of cells directly from the peripheral (bronchiolar) airways by BAL may thus provide a more representative sample for the study of IAD (O'Byrne and Postma 1999).

For the BAL procedure in horses, an endoscope (10–13 mm outer diameter with a length of 1.8–2 m or greater) or a specialised naso-tracheal tube is used. The infusion of 60–100 ml pre-warmed lidocaine solution (0.3%) to desensitise the irritant cough receptors in the lower trachea and large bronchi is recommended to ensure animal comfort and reduced stimulation of excessive coughing. Following secure wedging of the endoscope in a sub-segmental bronchus, 250–300 ml pre-warmed (37°C) sterile 0.9% saline or phosphate buffered saline is instilled into the lung as 1–3 smaller boluses. Each bolus is followed by aspiration of the fluid. The volume of lavage fluid retrieved usually ranges from 40–60% of the original infusate, but this volume may decrease with increasing severity of airway inflammation. The gross appearance (colour, turbidity, presence of flocculent debris) of the pooled fluid retrieved contributes to the clinical pathologist's overall interpretation. Samples of collected fluid must be kept on ice if processing for cytology is not possible within 1 h after collection. Routine processing of BAL fluid consists of determining the total number of leucocytes recovered as well as the differential cell count. The total cell count can be obtained using either a Neubauer, Malassez or Fuchs-Rosenthal haemocytometer or an automated counting method such as a Coulter counter. In the latter, special care should be taken particularly when the samples contain clumps of mucus, which can obstruct the aperture causing substantial underestimation of the total cell number. The total cell count is usually expressed as either the total number of cells recovered from the lavage yield or as the

TABLE 1: BAL cytology of normal horses (italics) and young athletic horses exhibiting signs of poor performance (typical cytological abnormalities are in bold text)

Age (years)	Mac (%)	Lymph (%)	PMN (%)	MC (%)	EO (%)	Author
2.7–3.5	*60–67*	*28–32*	*0.5–7*	*0.2–1*	*0–1*	Hare *et al.* (1994)
	(64)	*(30)*	*(3.4)*	*(0.5)*	*(0.3)*	Hare and Viel (1998)
						Fogarty and Buckley (1991)
						Rush Moore *et al.* (1995)
4.3 ± 1.9	64 ± 15	23 ± 11	**13 ± 12**	0.3 ± 0.7	0.1 ± 0.3	Fogarty and Buckley (1991)
3.7 ± 0.3	48 ± 2	36 ± 2	**10 ± 1**	2 ± 1	4 ± 1	Rush Moore *et al.* (1995)
	55 ± 11	34 ± 11	**12 ± 7.7**			Couëtil and Denicola (1999)
3.3 ± 0.7	33 ± 10	**60 ± 17**	4 ± 1	2 ± 0.5	0.2 ± 0.07	Doucet (1994)
3.4 ± 1.1	22 ± 10	**73 ± 10**	1.5 ± 1.3	1 ± 0.8	1.4 ± 3.8	Viel *et al.* (2000)
3.4 ± 1.6	57 ± 12	36 ± 14	4 ± 3	**4 ± 2**	0.5 ± 0.3	Hare *et al.* (1994)
8 ± 0.3	46 ± 1	49 ± 2	3 ± 0.3	**3 ± 0.4**	0.2 ± 0.05	Hoffman *et al.* (1998)
3.7 ± 0.3	48 ± 2	36 ± 2	10 ± 1	2 ± 1	**4 ± 1**	Rush Moore *et al.* (1995)
2.6 ± 0.9	59*	26*	0.8*	1.4*	**12***	Hare and Viel (1998)

Mac = macrophage, Lymph = lymphocyte, PMN = neutrophils, MC = mast cell, EO = eosinophils
*Data expressed as median value

concentration of cells per millilitre of recovered lavage fluid. With the known total cell count and the percent cell differential, the absolute number of each cell type recovered per unit volume of fluid can be quantified. However, the accuracy and reliability of this method requires standardised volumes of infusate and lavage fluid recovery. If available, cytospins can be made to facilitate the differential cell count. Following centrifugation of samples (600–800 *g* for 15 min), air-dried smears should also be made. Slides can be stained with a routine cytological stain such as Wright-Giemsa for the cellular differential count.

BAL FLUID CYTOLOGY

Bronchoalveolar lavage (BAL) fluid may display a variety of distinct inflammatory cytological profiles which differ significantly from those of healthy horses. One or more inflammatory cell types may be elevated in the BAL fluid, including mast cells, globule leucocytes, eosinophils, neutrophils, lymphocytes, macrophages or exfoliated epithelial cells (Table 1). For example, BAL fluid recovered from young horses with poor performance may show a significant increase in the mast cell population with very few eosinophils, or mast cells accompanied by a marked increase of the eosinophil population (Hare *et al.* 1994; Hoffman *et al.* 1998). Recent studies in such horses have demonstrated a strong correlation between increase of either eosinophils and/or mast cells in

the BAL fluid and airway hyper-reactivity (Hare *et al.* 1998; Hoffman *et al.* 1998). BAL fluid with large numbers of non-toxic neutrophils tends to contain more flocculent debris representing clumps of mucopus, and occasionally casts of inspissated mucous plugs from the terminal airways (commonly known as Curschmann's spirals) are seen (Fogarty and Buckley 1991; Rush Moore *et al.* 1995; Couëtil and Denicola 1999). More recently, an additional feature of BAL fluid of some poorly performing young horses is an elevated proportion of lymphocytes with no clinical indication of an active infectious airway process (Doucet 1994; Viel *et al.* 2000). Lastly, BAL fluid of young athletic horses with respiratory viral infection shows a massive exfoliation of mucosal columnar epithelial cells accompanied by numerous free cilia and detached ciliated tufts. Sutton *et al.* (1998) clearly demonstrated that this characteristic ciliocytophtheria includes broken ciliated plates containing numerous virus particles, supporting evidence of virally-induced epithelial injury. In such horses, the total number of alveolar macrophages and lymphocytes are significantly elevated during the active infectious process and are accompanied by a gradual increase in the neutrophil population from Days 3–15 post infection. This probably corresponds with the onset of secondary bacterial infection. Further, significant airway hyper-reactivity persists for as long as 8 weeks post infection in horses with either influenza or herpesvirus, which may well be a

consequence of the effects of sustained elevations in neutrophil and mast cell populations.

CONCLUSIONS

There is no doubt that recent literature on BAL fluid cytology, which has been collected using generally standardised BAL techniques, has permitted a more reliable comparison of the various cytological profiles involved in IAD in young athletic horses with poor performance. Specifically, IAD appears to be distinct from the typical neutrophilic airway inflammation observed in recurrent airway obstruction (heaves). From the examples provided above, it can be hypothesised that the variety of inflammatory cell profiles in IAD may ultimately reflect different aetiologies and immune responses to airway insult. Therefore, IAD should not be viewed as a distinct entity, but rather as a syndrome resulting from a myriad of possible causes.

RECOMMENDATION

- Bronchoalveolar lavage should be adopted as the method of choice to obtain representative cell populations from the lower airways.
- BAL sampling procedures should continue to be standardised (endoscope/tubing size, volume of infusate, volume of recovered fluid) in order to permit comparison between studies.
- Control (normal) BAL total cell counts and percent cell differential counts should not exceed 5% for neutrophils, 2% for mast cells and less than 1% for eosinophils. The macrophage:lymphocyte ratio should be approximately 60:30%.
- Studies on BAL cytology in young athletic horses should report total cell counts, cell differential (%) counts and absolute cell differential counts.
- It is paramount that some form of quality control on cell differential interpretation be established to allow reliable comparison between studies.
- Epidemiological longitudinal studies need to be performed to determine the significance of the various cytological profiles on pulmonary health and performance.
- Contribution of inflammation to the structural remodelling of airways should be investigated and correlated with BAL cytology.

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CYTOLOGY OF INFLAMMATORY AIRWAY DISEASE

K. C. Smith, J. R. Newton, S. M. Gower, S. M. Cade, D. J. Marlin,
C. M. Deaton and J. L. N. Wood

Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK

When inflammatory airway disease (IAD) is defined as described by Wood *et al.* (2003), the condition is the most important form of respiratory disease in young racehorses in training (Burrell *et al.* 1996; Wood 1999). The condition affects 25–30% of horses in training in the United Kingdom and United States of America. Epidemiological studies indicate a strong association with infection by *Streptococcus zooepidemicus*, *Streptococcus pneumoniae* and *Actinobacillus/Pasteurella* spp. (Wood *et al.* 1993; Wood 1999). The incidence and prevalence of IAD decline with age, suggesting the development of acquired immunity to infection (Chapman *et al.* 2000; Newton *et al.* 2003).

Endoscopic tracheal wash (TW) is the main method used by the authors for diagnosis of IAD, and is used routinely by racehorse practitioners in Newmarket and Lambourn. The method is less invasive than trans-tracheal aspiration, allows visualisation of the tracheal lumen, and is generally well tolerated by young horses in training (Whitwell and Greet 1984). Endoscopic TW samples can also be used for quantitative bacteriology, and the fact that a large proportion of endoscopically recovered samples are sterile suggests that contamination of samples from the upper respiratory tract is not a common problem (Wood *et al.* 1993). TW samples should be collected after exercise, and the horse should not be fed prior to endoscopy. It is essential to ensure that the endoscope and catheter are sterile, and to minimise the procedure time. Once the endoscope is positioned in the trachea approximately 10 cm from the carina, and the appearance of the mucosa and quantity of mucopus have been recorded, the technique involves infusion of 30 ml normal or phosphate buffered saline, which is then withdrawn. The tracheal aspirate is mixed thoroughly then aliquoted into an unfixed sample for quantitative bacteriology and a fixed sample for cytology (10% neutral buffered formalin).

Normal TW fluid contains a predominance of non-degenerate ciliated and non-ciliated epithelial cells and macrophages. Mucus is scanty and not inspissated. Neutrophils generally comprise less than 20% of the nucleated cell population (Beech 1975). The principal endoscopic and cytological findings in IAD are the presence of visible tracheal mucopus on endoscopy, and the presence of neutrophilic inflammation on TW cytology. Degenerate neutrophils generally predominate in bacterial infection, which accounts for a high proportion of IAD cases in young horses, whereas non-degenerate neutrophils usually predominate in hypersensitivity disorders of the recurrent airway obstruction (RAO) group seen in older horses. It is critical that tracheal wash cytology, with particular reference to recognition of airway neutrophilia, is correlated with microbiology to distinguish pathogenic from commensal or contaminating micro-organisms. Toxic degenerative changes and phagocytosed bacteria can often be found in neutrophils in cases of bacterial infection, and macrophages may also contain phagocytosed micro-organisms in some cases. Microbiological and serological investigations may reveal evidence of infection with other pathogens, such as viruses or *Mycoplasma* spp., in a small minority of IAD cases (Wood 1999; Wood *et al.* 2003).

While neutrophils are the most important inflammatory cell type encountered in IAD, other non-epithelial cell types that may be found in TW fluid include macrophages, eosinophils, lymphocytes, mast cells and plasma cells (Beech 1975; Whitwell and Greet 1984). Macrophages are the predominant non-epithelial cell type in normal TW samples, and may be resting or activated. The
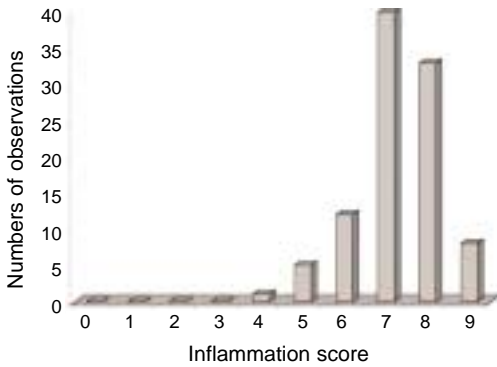
Fig 1a: Application of 9-point tracheal wash scoring system to horses previously scored as 3 on 3-point scale.
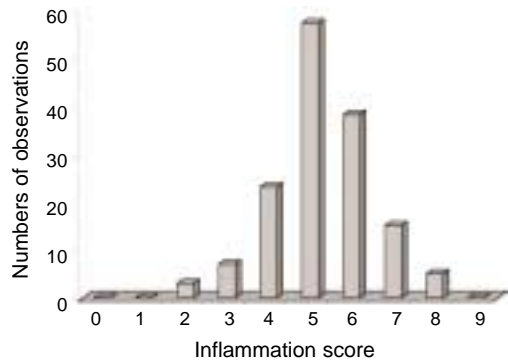
Fig 1b: Application of 9-point tracheal wash scoring system to horses previously scored as 2 on 3-point scale.
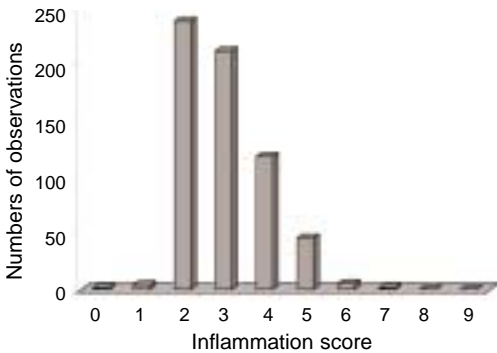
Fig 1c: Application of 9-point tracheal wash scoring system to horses previously scored as 1 on 3-point scale.
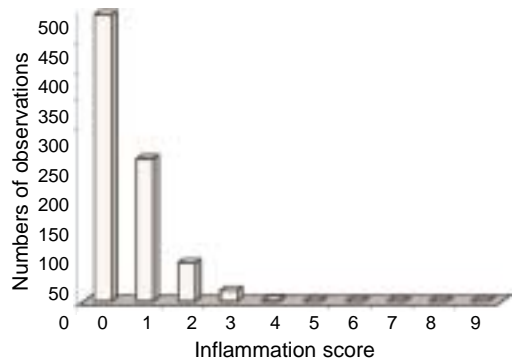
Fig 1d: Application of 9-point tracheal wash scoring system to horses previously scored as 0 on 3-point scale.

nature of any phagocytosed material is important in establishing a diagnosis of infection (bacterial or fungal infection in particular), and in assessing the grade and chronicity of airway haemorrhage. Eosinophils are rare in normal airways, but may occur in small to moderate numbers in TW samples collected from horses with hypersensitivity reactions or endoparasitism. The cells may be intact or degranulated. Studies in Canada using bronchoalveolar lavage (BAL) samples from young horses suggest that pulmonary eosinophilia and airway hyper-responsiveness represent early subclinical events in progression to RAO (Hare and Viel 1998), and indicate the importance of long-term monitoring of young racehorses with TW eosinophilia.

Lymphocytes may increase in number in viral airway disease, with concurrent epithelial cell degeneration (Freeman *et al.* 1993) but viral infection that has not become complicated by bacterial infection is rarely sampled diagnostically.

Mast cells increase in number in cases of type 1 hypersensitivity, but are more easily visualised in equine BAL than TW samples, where correct sample fixation and processing is critical in preserving intact mast cell granules. Plasma cells are recovered occasionally from TW samples of horses with chronic airway inflammation. More usual cytological changes associated with increasing duration of IAD are a progressive increase in the ratio of macrophages to neutrophils; appearance of multinucleated macrophages; development of mucous casts (Curschmann's spirals); and hyperplasia or dysplasia of epithelial cells (Whitwell and Greet 1984).

TW scoring systems should permit: 1) rapid screening of large batches of samples; 2) assessment of response to therapy; and 3) pattern recognition (Freeman *et al.* 1993). A 3-point scoring system was introduced in Newmarket in the 1980s (Whitwell and Greet 1984). More
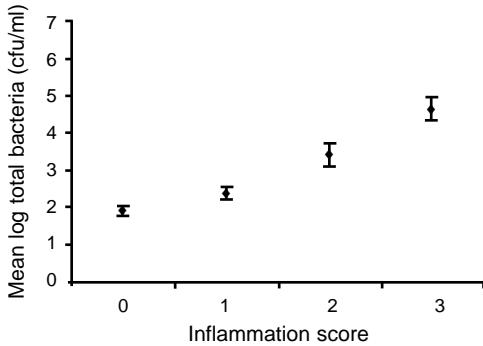
Fig 2a: Correlation of total bacterial count with 3-point tracheal wash score.
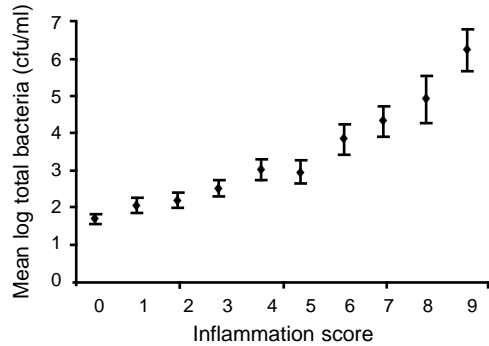

Fig 2b: Correlation of total bacterial count with 9-point tracheal wash score.

recently, this scoring system has been refined into a 9-point score (authors' unpublished data). The 3-point score is derived as follows: moderate or profuse tracheal mucopus 1 point; total nucleated cell count over 1,000 cells/mm^3 1 point; and neutrophils predominant or present in moderate numbers 1 point. TW 9-point scoring involves: 1) assessment of tracheal mucopus: score 0 (absent) to 3 (profuse); 2) assessment of smear cell density: score 0 (low) to 3 (high); and 3) assessment of neutrophil proportion: score 0 (absent or occasional) to 3 (predominant). Using the 9-point score, scores of 0–2 are interpreted as normal, or within acceptable limits. Scores of 3–9 are interpreted as indicating inflammatory disease of increasing severity.

The 3-point scoring system is simple and robust, but may underestimate mild inflammation. The 9-point scoring system is more sensitive and particularly useful in diagnosing mild inflammation or assessing changes in degree of inflammation over time. Figure 1 illustrates the increased sensitivity of the 9-point over the 3-point score, through application of both scoring systems to a large series of TWs collected prospectively from young Thoroughbred racehorses in 7 training yards in Newmarket, Lambourn and Epsom that were sampled regularly between 1993 and 1996, generating a total of 1,689 samples. Both TW scoring systems described in this abstract concentrate on changes in mucus quantity and neutrophil proportions. The 9-point score correlates well with total and species-specific bacterial counts (Fig 2, Newton *et al.* 2003), and with other measures of airway inflammation such as hydrogen peroxide in breath

condensate (C. Deaton, unpublished data; Marlin *et al.* 2003).

BAL is not used routinely in young Thoroughbreds in Newmarket, so most large scale screening by this group has been based on TW. Studies in Australia have established parameters for normal BAL cytology in Thoroughbred horses in training (aged 1–7 years of age) with particular reference to diagnosis and grading of execise-induced pulmonary haemorrhage (McKane *et al.* 1993). Prospective collection of paired TW and BAL samples from young racehorses is likely to be valuable in establishing the relative utility of the 2 techniques in diagnosis and prognosis of IAD. Lung biopsy is rarely used in racehorse practice, but may provide additional information on pathology and prognosis (Savage *et al.* 1998). Previously published post mortem data suggest an incomplete correlation between tracheobronchial cytology and pulmonary histopathology (Larson and Busch 1985), although detailed post mortem studies of the respiratory system of young horses are rare (Blunden and Gower 1999).

CONCLUSION

1) IAD is characterised cytologically by moderate to marked airway neutrophilia; 2) use of a 9-point scoring system incorporating assessment of tracheal mucopus, smear cell density and proportion of neutrophils permits IAD to be graded, and response to therapy evaluated; and 3) concurrent evaluation of TW cytology and quantitative bacteriology indicates a strong association with bacterial infection in young horses.

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QUANTIFYING AND CHARACTERISING MUCUS IN THE AIRWAYS

V. Gerber, A. M. Jefcoat, J. A. Hotchkiss* M. King[†] and N. E. Robinson

*Pulmonary Laboratory, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan 48824, USA; *Toxicology, Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, USA; [†]Pulmonary Research Group, University of Alberta, Edmonton, Alberta, Canada*

Mucus accumulation is a hallmark of recurrent airway obstruction (RAO) and inflammatory airway disease (IAD; Burrel 1985; MacNamara *et al.* 1990; Moore 1996). Secretions may accumulate due to increased production/secretion and/or decreased clearance, but little is known of the mucus apparatus in IAD. This paper reviews methods of quantifying and characterising mucus, describes findings in the horse and proposes questions for investigation. The terms 'mucus (accumulation)' and '(airway) secretions' are used interchangeably to describe gel-like secretions and their constituents in airways.

HOW CAN WE QUANTIFY GROSS MUCUS ACCUMULATION?

In one study, the amount of secretions aspirated transtracheally (without prior infusion of saline) was measured directly. Four control horses had no, or <1 ml, aspirable mucus and 18 of 22 horses with varying degrees of lower airway disease had 1– >5 ml of tracheal secretions (Schatzmann *et al.* 1972).

Various systems have been used to grade the amount of secretions in the trachea endoscopically. Although 'no mucus or a few single mucus droplets' in the trachea can be regarded as normal, large variations are observed in asymptomatic horses. Horses with IAD usually have mild to intermediate accumulations of mucus in their trachea. The amount varies and can overlap with observations in both asymptomatic and RAO-affected horses (Dieckmann 1987; Gerber *et al.* 2001; V. Gerber, unpublished results). In a large clinical study, Dixon *et al.* (1995) demonstrated the non-specific nature of mucus accumulation, which increased in all categories of lower airway disease. They also noted that mucus scores were significantly higher in coughing than non-coughing horses, an association found in other populations (Hodgson *et al.* 2003; Robinson *et al.* 2003). Interestingly, mucus accumulation is only weakly associated with airway inflammation assessed by bronchoalveolar lavage (BAL) fluid, does not increase with stabling in asymptomatic horses (but BAL fluid neutrophils do) and is not increased in older compared with younger asymptomatic horses (Gerber *et al.* 2001). Increased accumulation of mucus (not correlated with exercise-induced pulmonary haemorrhage) is associated with poor performance in racehorses (MacNamara *et al.* 1990). Sport horses with increased mucus, however, may perform well in dressage and show-jumping (Gerber *et al.* 2001). It is unclear what intensity and duration of exercise may increase endoscopically visible mucus accumulation and for how long (Luft 1987).

It is difficult to compare studies because of inherent variability and subjectivity (eg a horse may cough and clear its trachea from mucus, and a report of 'a few' vs 'many' mucous flakes may depend on the observer). Therefore, the authors have developed a standardised grading system and are testing its repeatability both within an individual horse and between observers. However, as it is possible only to assess the amount and appearance of secretions in the large airways, the limitations of endoscopic observation requires other, complementary measures of mucus.

HOW CAN WE CHARACTERISE AND QUANTIFY THE COMPONENTS OF MUCUS?

Mucus is a physiologically heterogeneous mix of water (~95%), electrolytes (1%), lipids (1%), proteins (2–3%) and variable amounts of epithelial and inflammatory cells. Cells may contribute directly to mucus volume and their contribution could be estimated by methods assessing airway inflammation. However, mucins (high molecular weight glycoproteins) constitute the network of the mucus gel and can greatly influence the total volume by drawing and retaining water in the gel.

The major gel-forming mucins expressed in human and rodent airways are MUC2, MUC5AC and MUC5B. They contain non-repetitive cystein-rich regions, believed to allow intra- and intermolecular cross-linking through disulphide bond formation. MUC5AC is mainly produced in goblet cells, whereas submucosal glands secrete MUC5B (in 2 distinct glycoforms). Together they form the bulk of the 'normal' human mucus-gel. MUC2 is predominantly an intestinal mucin.

Gerber *et al.* (2002) identified 2 equine homologues of gel-forming mucin genes and demonstrated by RT-PCR that MUC5AC, but not MUC2, is expressed in horse airways, and MUC5AC mRNA up-regulation is a potential primary mechanism for mucus hypersecretion in RAO. These findings are complemented by those based on reactivity with antibodies raised against human MUC5B and MUC5AC, demonstrating equine versions of these mucins in respiratory tract secretions (Walley *et al.* 2001) and MUC5AC in goblet cells (V. Gerber, unpublished data).

Jefcoat *et al.* (2001), to investigate carbohydrate side chains of mucins, used carbohydrate-specific enzyme-linked lectin assays (ELLA). Of the oligo-saccharides tested, α-1, 2 fucose discriminates best between control and RAO-affected horses (even in remission), is associated with mucus cells and can be measured in BAL fluid by ELLA using Ulex europaeus agglutinin I. Therefore, it may be possible to use α-1, 2 fucose levels as an indirect measure of mucin accumulation in BAL fluid. α-1, 2 fucose is associated with secreted mucins produced by airway goblet cells. Sialyl Lewis X (a tetrasaccharide structure associated with membrane bound mucins) has been identified in equine BAL fluid and may impart specific binding activity toward structures such as bacterial adhesin molecules.

Mucus is mainly produced by goblet cells, since submucosal glands are rare in horse airways (Kaup *et al.* 1990). Goblet cell hyper- and metaplasia is a histopathological characteristic of RAO (Kaup *et al.* 1990) and one of the first changes in chronic small airway disease. Surprisingly, initial morphometric measurements of the volume of stored mucins in the epithelium showed no difference between control and heaves-affected animals (Hotchkiss 1998). These contradictory findings highlight the problem of defining control groups. However, they may point to functional, rather than structural, changes in mucus apparatus. Because the orientation of the airway epithelium must be maintained, morphometric studies require large biopsy or post mortem samples.

HOW TO INVESTIGATE PHYSICAL PROPERTIES, RHEOLOGY, CLEARABILITY AND CLEARANCE OF MUCUS?

Studies on healthy horses have shown that mucociliary clearance rate is influenced more by physical properties of mucus than by variations of ciliary beat frequency. Based on physical appearance only, Schatzmann *et al.* (1972) proposed that mucus viscosity is proportional to severity of clinical signs in horses with lower airway disease, and Pietra *et al.* (2000) linked mucus viscosity with the proportion of neutrophils in tracheobronchial secretions. Gerber *et al.* (2000) used magnetic micro-rheometry on samples obtained by bronchial brushing to measure rheology of mucus from airways of animals with no obvious mucus accumulation. Viscoelasticity was normal in RAO-horses in remission, but during exacerbation increased 3-fold compared with healthy controls. Predicted mucociliary and cough clearability in RAO-affected horses were reduced significantly. Using the same method, horses with IAD showed surprisingly low average viscoelasticity (V. Gerber, unpublished data). Based on these incomplete data, as no direct comparison of IAD vs. healthy or RAO horses has been performed, it is proposed that mucus clearance could be normal or even improved in IAD.

It is important to note that other physical properties of mucus, such as spinnability and adhesivity, also influence its clearability. The phospholipid content and composition of mucus affect its adhesivity. In addition, surfactant increases mucus transport velocity, is present in amounts sufficient to significantly reduce surface tension in the trachea of healthy horses, but may be deficient in RAO, and after long transports.

Finally, mucociliary clearance rates can be measured by endoscopic and scintigraphic marker methods. Decreased clearance in RAO has been reported (Coombs and Webbon 1987; Turgut and Sasse 1989), but others found no difference between healthy and RAO horses (Willoughby *et al.* 1991). Although no studies have investigated mucociliary clearance rate in IAD it is interesting that viral infections (influenza and herpes, but not rhinovirus) can compromise mucociliary clearance.

CONCLUSIONS AND QUESTIONS

Currently, few of the available tools have been used to investigate mucus in IAD. The amount of secretion in the trachea increases in some horses

with IAD, but this is a non-specific finding. The authors propose that the functional significance of mild to moderate mucus accumulations (and the syndrome of IAD) depends on the degree of performance expected from the horse. This also points to the difficulty of defining IAD and control groups of 'healthy' horses. Another question to be addressed is the association of large vs. small airway inflammation with mucus accumulation. To address such questions, a standardised endoscopic scoring system and complementary tools such as mucin markers in BAL fluid are needed. Measurements of mucin production, storage and secretion are available to investigate functional vs. structural changes of the mucus apparatus leading to the cause(s) of increased mucus accumulation in IAD. Decreased clearability seems less likely to play a major role, because IAD affected horses appear to have favourable mucus rheology. However, viscoelasticity remains to be directly compared to normal horses, and complemented by other measures of mucus clearability.

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BREATH CONDENSATE MEASURES OF AIRWAY INFLAMMATION

D. J. Marlin, C. M. Deaton, J. R. Newton* and K. C. Smith*

*Centres for Equine Studies and *Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK*

There are a wide range of techniques and approaches for the diagnosis and investigation of pulmonary disease, including auscultation, radiography, scintigraphy, conventional pulmonary function testing and exercise tolerance. In human medicine and research, endoscopy of the respiratory tract is considered a highly invasive procedure and is only undertaken in circumstances where there is a clear and specific indication. In contrast, in clinical veterinary medicine, endoscopy of the respiratory tract and, in particular, the trachea with collection of tracheal wash, is probably the most common diagnostic approach now used. Bronchoalveolar lavage (BAL) has also been used extensively in research studies into the causes and mechanisms of pulmonary disease in horses such as recurrent airway obstruction (RAO) and exercise-induced pulmonary haemorrhage (EIPH).

Endoscopy of the respiratory tract has many advantages over other techniques. It allows direct visualisation of the larger airways and specific sampling of different regions of the respiratory tract (eg tracheal wash, BAL, transbronchial biopsy, bronchial brushing). These samples may then be used for a wide range of *ex vivo* analyses, including cytological examination, investigation of gene expression, biochemical and immunological assay and bacterial culture. The fact that sampling is regionally specific can be both an advantage and a disadvantage, depending on the specific disease process or research question. For example, in the case of diffuse lung conditions such as RAO, a single BAL sample from the dorso-caudal lung may be representative of the lung as a whole. However, a positive bacterial culture in a tracheal wash may occur in parallel with negative culture in BAL, depending on the precise region of lung affected in a focal infection. BAL must also be conducted under sedation and the use of local anaesthesia of the airways is desirable to reduce coughing. This precludes the use of BAL close to competition in many racing or competition horses. BAL also causes changes locally including a transient inflammatory response, which lasts up to around 48 h (Sweeney *et al.* 1994) and changes in airway lung fluid biochemistry (Deaton *et al.*, unpublished data). Whilst it is possible to sample different regions of the lung in a sequential order, there is no clear evidence that the lung responds homogeneously to all challenges or insults, or that the lung is always homogenous at the start of an experiment.

The fact that endoscopy of the upper airway may be harmful in man led to the development of less invasive techniques to monitor airway inflammation, such as sputum induction (Jones 1968), which has only gained significant popularity as a diagnostic technique in human respiratory medicine in the last 10–15 years. For example, in 2002, there were over 200 papers published relating to the use of this technique. Sputum induction does result in a mixed sample which includes saliva and secretions and cells from all levels of the airway. Furthermore, whilst sputum induction has been shown recently to be both diagnostically useful and safe in adults (Vlachos-Mayer *et al.* 2000), there is evidence that it may be associated with a greater incidence of adverse effects in children (Jones *et al.* 2001) and is unsuitable for use in neonates and infants.

More recently, even less invasive techniques have been applied to investigate pulmonary inflammation. The technique of analysing gases (eg ethane, pentane, carbon monoxide, nitric oxide) or substances dissolved in moisture within the breath (exhaled breath condensate or EBC) such as hydrogen peroxide has become widely used. This approach offers the advantage of being totally non-
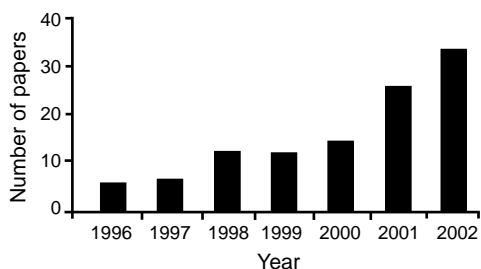
Fig 1: Number of publications on exhaled breath condensate by year.

invasive, does not require administration of drugs (eg sedative, local anaesthetic) and is repeatable more often than techniques such as endoscopy. Analysis of exhaled gases and mediators in EBC also has the advantage that measurements can be repeated on a frequent basis to follow the time course of inflammation, for example, following an inhaled allergen challenge. It has the disadvantage or advantage, depending on the precise circumstances, of being global rather than regional. In humans, contamination of exhaled breath by nasal or oral secretions or gases has been problematic (Griese *et al.* 2002).

Commercial systems for collecting exhaled breath condensate from human subjects have been developed (eg Ecoscreen from Jaeger). However, custom systems have also been developed. In all systems described, the principle involves directing exhaled breath only by use of a mask, mouthpiece or nosepiece and valve system through a cooled collecting vessel. As the warm and saturated

exhaled breath is cooled, its capacity to hold water vapour is reduced and droplets of EBC form on the surface of the vessel.

The interest in analysis of markers of inflammation in EBC for diagnosing and monitoring airway inflammation in human medicine and in animal studies has increased markedly in recent years (Fig 1).

The presence of a wide range of substances has been reported in EBC of man and different animals, including hydrogen ions, nitrite (NO_2), nitrate (NO_3), NH_4^+, H_2O_2, electrolytes, 3-nitrotyrosine, S-nitrosothiols, LTB_4, LTC_4, LTD_4, LTE_4, LTF_4, cys-LTs, PGE_4, 8-isoprostane, IL-6, IL-4, IL-8, IL-10, IFN, HGF, MDA, TBARS and arachidonic acid. Of these different mediators, the most commonly measured is hydrogen peroxide and the rest of this review will, therefore, concentrate on the measurement of H_2O_2 in EBC.

There is clear evidence that EBC H_2O_2 is elevated in human pulmonary inflammatory conditions such as asthma, adult respiratory distress syndrome (ARDS) and human chronic obstructive pulmonary disease (COPD; Table 1). In addition, EBC H_2O_2 has been shown to be increased following cigarette smoking (Nowak *et al.* 1996), during acute upper and lower respiratory tract infections (Dohlman *et al.* 1993) and in bronchiectasis (Loukides *et al.* 1998), cystic fibrosis (Jobsis *et al.* 2000) and sepsis (Sznajder *et al.* 1989).

As noted above, the measurement of mediators in EBC has been contentious. There have been marked differences of opinion as to the source of many substances present in EBC. It is clear that the formation of breath condensate from evaporation of water from the lung surface can be

TABLE 1: Examples of hydrogen peroxide concentration in exhaled breath condensate (EBC) of controls and patients with asthma, COPD and adult respiratory distress syndrome (ARDS)

| Author	Condition	Control (µmol/l)	Disease (µmol/l)	Ratio
Jobsis *et al.* (1997)	asthma	0.15	0.60	4
Antczak *et al.* (1997)	asthma	0.01	0.26	26
Antczak *et al.* (1999)	asthma	0.01	0.18	18
Emelyanov *et al.* (2001)	asthma	0.02	0.13	7
Loukides *et al.* (2002)	asthma	0.20	0.95	5
Dekhuijzen *et al.* (1996)	COPD (human)	0.03	0.21	7
Dekhuijzen *et al.* (1996)	COPD (human)	0.03	0.60	20
Nowak *et al.* (1998)	COPD (human)	0.05	0.55	11
Nowak *et al.* (1999)	COPD (human)	0.04	0.48	12
De Benedetto *et al.* (2000)	COPD (human)	0.12	0.50	4
Baldwin *et al.* (1986)	ARDS	0.34	1.68	5
Sznajder *et al.* (1989)	ARDS	0.72	2.34	3
Wilson *et al.* (1992)	ARDS	0.01	0.55	55
Kietzmann *et al.* (1993)	ARDS	0.25	1.50	6
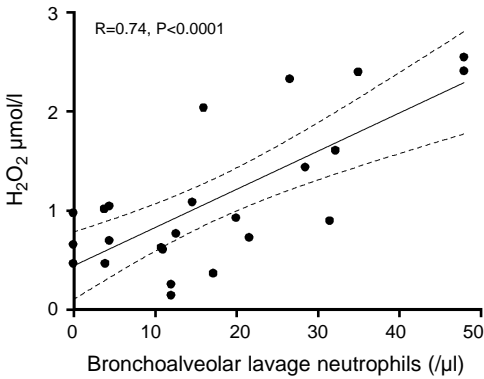
Fig 2: *EBC H_2O_2 concentration as a function of BAL neutrophil count in healthy control horses and in horses with mild to moderate airway inflammation. Dotted lines represent 95% confidence interval. Deaton* et al. *unpublished data.*
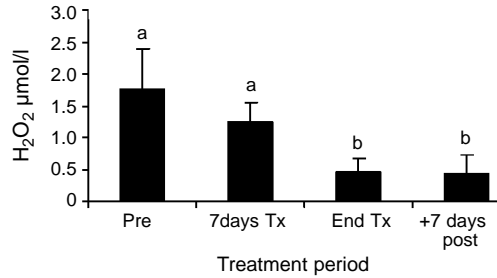

Fig 3: *Mean concentrations of hydrogen peroxide (H_2O_2) in expired breath condensate (EBC) prior to treatment in horses with inflammation (pre), after 7 (7 days Tx) and 14 days of treatment (End Tx) with inhaled fluticasone propionate (3.5 µg/kg SID) and 7 days after the termination of treatment (+7 days post). Columns with different letters are significantly different (P<0.05). Marlin* et al. *(2002).*

'contaminated' with larger droplets of airway secretions that are aerosolised during breathing. The type of collection system employed determines to what extent contamination of the final 'EBC' sample occurs. For example, in collection systems with a short conducting tube going directly into the condensing and collecting chamber and valves distal to the collecting vessel, aerosol contamination is likely to occur.

To study the time course of inflammation in experimental studies a system for collecting EBC from conscious, unsedated horses at rest has been developed and described previously (Deaton *et al.* 2001). The system consists of a facemask and non-rebreathing valve. Expired breath only is directed along a 250 cm long, 5 cm diameter heated tube into a 5-litre u-shaped stainless steel condensing tube which is held in a reservoir of ice and water at ~4°C. After the horse breathes through the system for around 10 min, ~4 ml of EBC can be recovered from the steel collecting tube. The authors have also successfully applied a modified EBC collection technique for use in conscious, unsedated cats.

A technique involving sedation and intubation of the upper airway and trachea has also been described (Fey *et al.* 2001) LTB_4 and bradykinin were measured in airway secretions collected using this technique. EBC has also been collected from calves (Reinhold *et al.* 2000).

Hydrogen peroxide, hydrogen ions, nitrite, nitrate and electrolytes are all detectable in the exhaled breath condensate of horses. Healthy, normal horses at pasture have EBC H_2O_2 in the range of 0.1–0.8 µmol/l EBC. The coefficient of variation for 3 sequential repeated measurements is less than 7%. The technique appears to be sensitive and can detect an increase in inflammation in stabled horses compared to horses maintained at pasture. In RAO affected horses with marked inflammation but without clinical signs of dyspnoea, EBC H_2O_2 is increased to around 2–3 µmol/l. In the human studies on asthma, COPD and ARDS cited in Table 1, the mean increase in the patients over the controls ranged from 4–55-fold. However, the median increase for all studies was 7-fold, which is similar to the increase seen in RAO horses. High ratios in human studies appear to be due most commonly to low H_2O_2 concentrations in EBC of controls rather than excessively high concentrations in patients.

EBC H_2O_2 concentration is closely correlated to BAL neutrophil numbers in the horse (Deaton *et al.* unpublished data; Fig 2) and is also reduced following treatment of non-infectious airway inflammation with inhaled corticosteroids (Fig 3).

The concentration of H_2O_2 in EBC is also increased in naturally occurring airway inflammation (allergic or bacterial). In acute challenges in experimental situations with either ozone or allergen, there is an increase in EBC H_2O_2 within 1–2 h of exposure, which does not occur following nebulisation of saline.

In conclusion, measurements of mediators such as H_2O_2 in EBC are not a replacement for endoscopy of the respiratory tract, but allow monitoring on a more frequent basis without causing disturbance to the airways and aid in the identification of appropriate sampling times with more invasive procedures such as BAL.

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SESSION 4:

Functional significance

Chairman:

 F. J. Derksen

LUNG FUNCTION FOR BEGINNERS

F. J. Derksen

College of Veterinary Medicine, Michigan State University, Veterinary Medical Centre, East Lansing, Michigan 48824-1314, USA

The primary function of the lung is gas exchange. Air moves in and out of the lung with a respiratory frequency and tidal volume appropriate for the animal's level of activity. This results in an arterial oxygen tension of about 100 mmHg and an arterial carbon dioxide tension of 40 mmHg. Tests of pulmonary function can be divided in tests of gas exchange and tests of pulmonary mechanics (Leff and Schumacher 1993).

Blood gas tensions reflect the gas exchange efficiency of the lung. In the diseased lung, ventilation and perfusion are poorly matched, a portion of the cardiac output is shunted through the lung, and gas diffusion is impaired. All these factors result in hypoxaemia. However, arterial blood gas tensions are also affected by factors unrelated to the gas exchange efficiency of the lung. These factors include hypoventilation and inhalation of low oxygen tension gases, such as occurs at higher altitudes. Therefore, investigators often report alveolar-arterial oxygen differences (Wagner and Gillespie 1989; Nyman and Bjork 1999). The difference between the alveolar and arterial oxygen tensions truly reflects the gas exchange efficiency of the lung.

For efficient gas exchange to occur, blood flow to the lung and ventilation must be carefully matched. Tests of ventilation-perfusion matching, such as the multiple inert gas elimination technique (Nyman and Bjork 1999) and lung scintigraphy (Votion and Ghafir 1999), are particularly useful because with these tests the mechanisms of hypoxaemia can be determined.

Ventilation is achieved when respiratory muscles generate pressures in the thoracic cavity that result in expansion or contraction of the lung. When the lung is diseased, so that airways are narrowed or the lungs are stiffer, more pressure is needed to ventilate the system. Accordingly, measurement of intra-thoracic pressure is a simple test of lung mechanics. Intra-thoracic pressure is best approximated by pleural pressure (Derksen and Robinson 1980).

The pressure that moves air in and out of the respiratory system is used to overcome resistance to air flow and to stretch the lung. In a normal standing horse, respiratory system resistance resides primarily in the upper airways. Indeed, approximately 50% of the total respiratory system resistance resides in the nose, and another 30% in the larynx and trachea. Only 20% of the total respiratory system resistance resides within the lung (Art and Serteyn 1988). Inside the lung, the majority of the resistance to flow occurs in the trachea and large bronchi. The small airways provide little resistance to air flow. Therefore, tests of resistance are sensitive to upper and major lower airway obstruction, but insensitive to small airway obstruction.

Small airway obstruction causes redistribution of ventilation and hypoxaemia. Therefore, small airway obstruction can be affected by tests of gas exchange and by tests of pulmonary mechanics that are sensitive to redistribution of ventilation, such as dynamic compliance. Another test of pulmonary mechanics, forced expiratory flow-volume curves, is also sensitive to small airway obstruction. Forced expiratory flow volume curves can be generated in standing horses (Couëtil and Rosenthal 2001). In horses with recurrent airway obstruction, flows are reduced, especially during the last portion of the forced vital capacity.

Normal airways contract in response to irritants. When airways are inflamed, this normal response is exaggerated, ie the airways are hyper-responsive. Airway hyper-responsiveness is a characteristic feature of human asthma, recurrent airway obstruction and inflammatory airway

disease of horses (Derksen and Robinson 1985; Hoffman and Mazan 1998; Sterk 2002). It can be detected by measuring airway function as horses breathe increasing concentrations of irritants such as histamine.

The ultimate effect of airway obstruction is poor oxygen exchange, which leads to hypoxaemia. Normal horses exercising vigorously become hypoxaemic and hypercapnic. Exercise-induced hypoxaemia occurs because blood traverses the lung so rapidly that there is insufficient time for complete gas exchange (Wagner and Gillespie 1989). Airway obstruction, is likely to exaggerate exercise-induced hypoxaemia, by causing decreased minute ventilation and mismatching of ventilation and perfusion.

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AIRWAY OBSTRUCTION AND HYPER-REACTIVITY IN HORSES WITH SIGNS OF INFLAMMATORY AIRWAY DISEASE

A. Hoffman and M. Mazan

Lung Function Testing Laboratory, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, Massachusetts 01536, USA

FUNCTIONAL DISTURBANCES OF INFLAMMATORY AIRWAY DISEASE (IAD)

The last decade has seen exciting new developments in the understanding of early lung disease in the horse. Investigators of IAD have disclosed the clinical, endoscopic, radiographic, and cytological changes associated with this loosely defined syndrome (Mazan 2002). However, the evidence that horses with the signs of IAD, possess a *functional disturbance* has only recently surfaced. One hint that IAD is associated with lung dysfunction came from Klein and Deegen (1986) and later Doucet and Vrins (1991), who demonstrated greater airway reactivity in horses exhibiting mild respiratory signs, without heaves. Before these studies were performed, it was assumed that only horses in crisis from heaves exhibit airway obstruction and airway hyper-reactivity (Derksen and Robinson 1985; Armstrong and Derksen 1986; Hoffman 2002a). Now it is clear that either: 1) highly sensitive methods of lung mechanical measurements; or 2) provocation tests are necessary to detect the functional disturbances associated with the syndrome of IAD.

FORCED EXPIRATORY MANOEUVRES FOR THE DETECTION OF FLOW LIMITATION IN IAD

Forced manoeuvres was first employed in the horse by Gillespie (1974) and later refined for use in standing, sedated horses by Couëtil and Rosenthal (2001). Couëtil showed that horses with a history of IAD (chronic cough, exercise intolerance) had significantly lower values for forced expiratory flows (at 75–95% exhaled vital capacity) compared to controls and to horses with heaves in remission. In the same horses, they were able to see higher pulmonary resistance by conventional methods (oesophageal pressure-flow data), confirming that horses with IAD had significant flow limitation. Accompanying this functional data, was bronchoalveolar lavage (BAL) cytology showing higher neutrophil % and total cell count (TCC) in the IAD group (polymorphonuclear [PMN] = 20. 4 ± 9.3%, TCC = 535 ± 187 cells/µl) vs controls (PMN = 6.8 ± 2.7%, TCC = 321 ± 100 cells/µl), although these were not significantly different due to the small numbers of horses with IAD (n=5). Forced expiratory manoeuvres causes an effort-independent constriction of small airways by sudden imposition of a gradient between intra-bronchial (negative) and alveolar (less negative) pressure (Couëtil and Rosenthal 2000). Other studies from Couëtil and Denicola (1999) have shown that horses similarly defined as IAD have exercise intolerance, so there is a clear association between flow limitation, airway inflammation, and exercise intolerance.

OSCILLOMETRY FOR THE DETECTION OF AIRWAY OBSTRUCTION PATTERN AND AIRWAY REACTIVITY

Oscillometry is the measurement of the pressure-flow relationship (impedance) derived from imposed oscillatory perturbations of the respiratory system, rather than measurement of endogenous pressure-flow data. The magnitude and phase relationship between pressure and flow dictate impedance. Oscillometry reveals how airway obstruction is distributed anatomically in the lung (peripheral vs central, homogeneous vs. heterogeneous peripheral distribution) and serves to gauge its severity

(Lutchen and Hantos 1988; Young and Tesarowski 1994; Lutchen and Suki 1996; Lutchen and Gillis 1997). Using this technique, low-grade airway obstruction was demonstrated in horses with signs of IAD with concurrent abnormal BAL cytology, with values for resistance of the respiratory system intermediate between controls and horses with a history of heaves (Hoffman 1999).

For the purpose of the IAD workshop, the authors re-examined oscillometry and bronchoalveolar lavage (BAL) cytologic data from outpatients, submitted to the laboratory for testing between 1997–2001 (Table 1). Horses (n=107) were categorised according to BAL cytology as follows: control, n=23 (normal BAL cytology), IAD, n=55 (>10% PMN, >2% mast cells, or >0.5% eosinophils), or heaves (history of clinical heaves, but currently in remission). The cutpoint for PMN that distinguished IAD from controls, was increased from >5 to >10%. This was done in light of previous work (Hoffman and Mazan 1998; Hoffman 1999)

that failed to show a relationship between PMN (<25%) and airway obstruction or airway reactivity, and with regard to previous observations that PMN increases even in normal horses following exposure to mouldy hay (Tremblay and Ferland 1993). The authors hypothesised that using this new system for grouping horses, there would be significant differences between IAD and controls with regard to respiratory system resistance at various oscillatory frequencies (as before).

The following variables were compared between control, IAD, and heaves (during remission): age, sex distribution, resistance -- R_{RS} (from 1–5 Hz), reactance -- X_{RS} (1–5Hz), slope of R_{RS} between 1–3 Hz assuming a 3rd order polynomial, $PC_{100}R_{RS}$ (provocative concentration of histamine that doubled R_{RS} at 1 Hz), $\log_{10}PC_{100}R_{RS}$, percentage of BAL cells classified as macrophages, lymphocytes, PMN, mast cells, and eosinophils by counting 500 cells stained with Wright-Giemsa (1,000 xmag).

TABLE 1: Clinical signs, pulmonary function data and bronchoalveolar lavage cytology from control horses with IAD or RAO (in remission)

Variable	Control (n=23)	IAD (n=55)*	RAO (n=29)[†]
Age	5.6 ± 0.68	6.3 ± 0.65	16.3 ± 1.0 [a,b]
Age range	(2–14 years)	(2–24 years)	(5–29 years)
Sex distribution (Mare,Gld,Stln)	47.8, 21.7, 30.4	49, 34, 17	41.4, 58.6, 0 [a,b]
Oscillometry			
R_{RS}			
1 Hz (cm H_2O/l/s)	0.581 ± 0.047	0.719 ± 0.054	0.962 ± 0.103 [a,b]
2 Hz (cm H_2O/l/s)	0.503 ± 0.033	0.602 ± 0.039	0.773 ± 0.069 [a,b]
3 Hz (cm H_2O/l/s)	0.524 ± 0.035	0.600 ± 0.036	0.744 ± 0.058 [a,b]
4 Hz (cm H_2O/l/s)	0.570 ± 0.040	0.622 ± 0.034	0.698 ± 0.053 [a]
5 Hz (cm H_2O/l/s)	0.685 ± 0.056	0.650 ± 0.045	0.763 ± 0.099 [a,b]
X_{RS}			
1 Hz (l/cm H_2O)	-0.330 ± 0.021	-0.369 ± 0.019	-0.466 ± 0.079 [a,b]
2 Hz (l/cm H_2O)	-0.067 ± 0.014	-0.092 ± 0.016	-0.227 ± 0.061 [a,b]
3 Hz (l/cm H_2O)	-0.101 ± 0.015	0.071 ± 0.016	0.074 ± 0.057
4 Hz (l/cm H_2O)	0.228 ± 0.015	0.199 ± 0.015	0.097 ± 0.039
5 Hz (l/cm H_2O)	0.240 ± 0.30	0.223 ± 0.036	0.073 ± 0.056
Resonant frequency (Hz)	2.77 ± 0.1	2.84 ± 0.08	3.67 ± 0.29 [a,b]
Slope R_{RS} from 1–3 Hz	0.098 ± 0.018	0.163 ± 0.022	0.236 ± 0.081 [a]
Airway reactivity			
$PC_{100}R_{RS}$ (mg/ml histamine)	11.31 ± 1.84	3.622 ± 0.462 [c]	3.21 ± 0.497 [c]
$\log_{10}PC_{100}R_{RS}$ (mg/ml histamine)	0.942 ± 0.08	0.419 ± 0.050 [c]	0.377 ± 0.078 [c]
BAL			
Mac (%)	46.4 ± 2.6	44.1 ± 1.5	28.2 ± 2.5 [c]
Lymph (%)	49.3 ± 2.6	46.6 ± 1.4	36.0 ± 3.3 [c]
Neutrophils (%)	2.91 ± 0.4	5.24 ± 0.81	33.1 ± 5.1 [c]
Mast cells (%)	1.37 ± 0.15	3.39 ± 0.27 [c,d]	2.12 ± 0.28
Eosinophils (%)	0.05 ± 0.02	0.47 ± 0.13 [a]	0.22 ± 0.09

*IAD defined as clinical history of cough and/or exercise intolerance/decline in performance, not heaves and abnormal BAL cytology (>10% PMN, >2% mast cells, >0.5% eosinophils). [†]In remission from 'heaves' (RAO) as defined clinically by Robinson (2000). One Way ANOVA; significant difference (P<0.05) from [a]control and/or [b]IAD group; significantly different (P<0.001) from [c]control or [d]heaves. Values expressed as mean ± SEM.
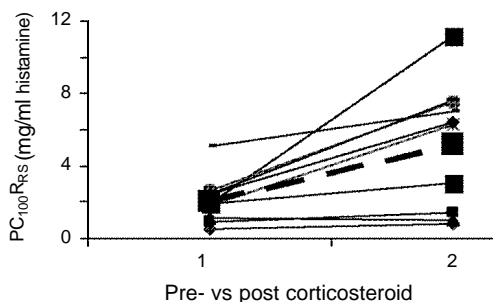
Fig 1: Pre- and post corticosteroid treatment (30 days).

The following observations were made. There were no significant age differences between IAD and control horses, but horses with a history of heaves were significantly ($P<0.005$) older. The age range of controls was narrower than for IAD or heaves, with some horses exhibiting only cough or exercise intolerance and abnormal BAL (ie IAD) as old as 24 years, but as young as 2 years old. Stallions were missing from the heaves-in-remission group, but more prevalent in the control and IAD group that contained racehorses. Whether some of the horses in the RAO group raced at an earlier time, was not disclosed by the study.

The R_{RS} of the IAD group was higher (between 1–4 Hz) but only a trend ($P=0.055$) was found. This differs from a previous study (Hoffman 1999) where significant ($P<0.01$) increases in R_{RS} were observed in horses with IAD vs controls. The difference lies in the re-categorisation of IAD (>10% PMN vs >5% in earlier studies). Hence, the exact criterion used to group horses clearly affects the outcome. The heaves group had significantly greater R_{RS} across frequencies, and X_{RS} was similarly elevated, a feature not observed in IAD. Hence, airway obstruction (increased R_{RS}) and airway wall shunting or increased dynamic elastance (increased X_{RS}) are features of heaves (Young and Tesarowski 1997), but many horses with IAD do not exhibit these abnormalities at baseline if measured with oscillometry. Resonant frequency, the frequency where all impedance is due to resistance, was shifted to a significantly greater value in horses in remission from heaves. This is further evidence that the degree of airway obstruction is significantly more severe in heaves horses in remission vs controls, or even horses with IAD. This data is supported by Couëtil and Rosenthal (2001).

Frequency dependence of R_{RS} was quantified as the slope of the 3rd order polynomial (ie fitting a parabola) that modelled R_{RS} from 1–5 Hz. A value above zero denoted frequency dependence, and a value of zero a lack of frequency dependence, which is considered normal from 1–5 Hz. Horses presented in remission from heaves but presented for coughing and/or exercise intolerance, had significantly greater frequency dependence. Values for IAD were intermediate between controls and heaves. Frequency dependence denotes either: 1) heterogeneity of ventilation (time constants) in the lung; and/or 2) generalised increase in peripheral airway disease. This supports the notion that small airways were preferentially affected in IAD and heaves, although large airways were also obstructed in some cases.

The most important difference in lung function tests between IAD and controls, was airway reactivity to histamine. The dose of histamine that doubled R_{RS} (1 Hz) was significantly ($P<0.001$) lower in IAD and in the heaves group. This expresses a fundamental difference between IAD and controls with respect to the tendency of airways to constrict. Either histamine evoked airway constriction of *greater magnitude* (eg due to pre-existing airway wall inflammation and mucus blockage), or the pattern of constriction evoked by histamine was more heterogeneous (Lutchen and Jensen 2001). The histamine response results in greater frequency dependence as well as a pattern of increased R_{RS} at all frequencies (Mazan and Hoffman 1999), indicating that small and large airways are constricting simultaneously. Peripheral airways constrict to a greater extent than central airways in response to histamine, hence oscillometry coupled with provocation can give insight into the relative response of the small vs large airways.

With the importance of airway reactivity to the pathophysiology of IAD, efforts have been focused on the development of a non-invasive field test of non-specific airway reactivity (Hoffman *et al.* 2001). This system will allow the practitioner to screen horses with suspected IAD on the basis of airway reactivity, and permit large scale testing in order to study risk factors that lead to this condition (Hoffman 2002b).

Finally, a subset of horses presented to Tufts University (n=10) with chronic cough and found to have airway hyper-reactivity ($PC_{100}R_{RS}$ mean 2.08 mg/ml, range 0.57–5.03 mg/ml) and elevated BAL mast cell % (>2%) were retested after a course of treatment with oral prednisone (1 mg/kg BID 7 days, 0.8 mg/kg BID 7 days, 0.6 mg/kg

BID 7 days and 0.4 mg/kg BID 7 days) without change in their environment or feeding practices. Figure 1 shows the change in $PC_{100}R_{RS}$ for these horses. Whether it was an inadvertent change of seasons, environment, or whether it was the effect of the drug prednisone, these horses had a significant (P=0.002) increase in $PC_{100}R_{RS}$ from 2.08–5.23 mg/ml, or just over one log_2 dose of histamine. However, only 7 out of 10 horses showed improvement. This finding is interpreted as an indication that airway hyper-reactivity is in part an indication of an inflammatory process, but there are other factors that cause airway hyper-reactivity, which may be mechanical in nature. The data also support the notion that horses with IAD exhibiting cough, are presented with sub-optimal lung function. Re-testing makes it possible to eventually disclose a horse's best lung function.

In conclusion, these data delineate a clear pattern of airway hyper-reactivity in horses referred to our hospital with persistent signs of IAD, such as cough, exercise intolerance, and mucus in the airways. In contrast to previous studies (Hoffman 1999), a change in grouping criterion for IAD (from >5% to >10% neutrophils on BAL) resulted in a loss of statistical significance for airway obstruction. This suggests that neutrophilic inflammation criteria are relevant to airway obstruction, and a lower cut-point for 'normal' ($\leq$5%) is a useful refinement. In the future, field and referral programs for diagnosing IAD should include pulmonary function tests in conjunction with BAL or other cytological methods. After a suitable period of treatment, pulmonary function tests can be used to gauge the progress of individuals in the context of their clinical and performance trends.

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INFLAMMATORY AIRWAY DISEASE AND GAS EXCHANGE

G. Nyman

Department of Large Animal Clinical Sciences, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

Impairment of pulmonary gas exchange and oxygenation of the arterial blood accompany respiratory disease in horses. The common contributors to alveolar-arterial oxygen tension differences are alveolar hypoventilation, shunt, uneven distribution of alveolar ventilation and perfusion (V_A/Q) and/or alveolar-capillary diffusion limitation of oxygen. A possibility to study pulmonary gas exchange and particularly the ventilation-perfusion distribution in more detail is to use the multiple inert gas elimination technique (MIGET), developed by Wagner *et al.* (1974a). From the results of using this technique it was suggested that an uneven distribution of V_A/Q ratios was the mechanism of hypoxaemia in humans with asthma or chronic obstructive pulmonary disease (Wagner *et al.* 1977). As the technique has been used for evaluation of anaesthesia-induced hypoxemia in horses in Uppsala it was interesting to determine the gas exchange in horses with inflammatory airway disease.

The results presented and discussed in this abstract are based on research in riding horses and Standardbred trotters with chronic obstructive bronchiolitis (recurrent airway obstruction, RAO) or inflammatory airway disease (IAD). The disease was diagnosed by clinical examination and verified with lung biopsy taken through the 10th intercostal space about 20 cm above the ventral lung border. Since bronchoalveolar lavage (BAL) was not introduced in the clinic at the time of the studies, lung biopsies were used to provide a morphological diagnosis of the case material. Tracheal washes were taken to exclude the presence of bacterial infections. Pulmonary gas exchange was assessed by conventional blood gas variables, ie arterial and mixed venous blood gases, and the V_A/Q distribution was estimated

with MIGET adapted for use in the standing horse (Hedenstierna *et al.* 1987) and during exercise in Standardbred trotters (Nyman *et al.* 1995)

When MIGET is performed, 6 gases (sulphur hexafluoride, ethane, cyclopropane, enflurane, diethyl ether and acetone) inert in the sense of being chemically inactive in blood, are dissolved in isotonic sodium chloride solution and infused into the jugular vein at a rate of 30 ml/min in the standing horse and 120–550 ml/min during exercise. When a steady state condition is recorded, arterial and mixed venous blood samples are drawn, and mixed expired gas is collected from a heated mixing box connected to a nose mask.

Gas concentrations in the blood samples and expirate are measured by the method of Wagner *et al.* (1974b). The arterial/mixed venous and mixed expired/mixed venous concentration ratios (retention and excretion, respectively) are calculated for each gas and their solubility coefficient in blood are measured in each horse by a 2-step procedure. These data are then used for deriving the distribution of ventilation and blood flow in a 50-compartment lung model with each compartment having a specific V_A/Q ratio ranging from 0 to infinity. Ventilation and blood flow in healthy subjects have a log normal distribution against V_A/Q ratios. This means that the distributions of ventilation and of blood flow against V_A/Q ratios cross each other at a V_A/Q of 1, and that perfusion exceeds ventilation in regions with V_A/Q ratios below one, and that ventilation exceeds perfusion in compartments with V_A/Q ratios above one. For the information obtained concerning the V_A/Q distribution, data are presented for the mean and standard deviation of the blood flow log distribution (Q mean and log SDQ, respectively), shunt (Qs; perfusion of lung

regions with $V_A/Q<0.005$), the mean and standard deviation of the ventilation log distribution (Vmean and log SDV, respectively). All subdivisions of blood flow and ventilation are expressed in percent of cardiac output and expired minute ventilation, respectively. Diffusion limitation can be calculated since the calculation of PaO_2 from the V_A/Q distribution assumes diffusion equilibrium between end-capillary blood and alveolar gas.

THE HEALTHY HORSE

In the healthy horse, a good matching between ventilation and perfusion results in an overall good oxygenation of the blood (Fig 1). The narrow distribution of perfusion, with absence of low V_A/Q regions but a minor shunt is measured. On the other hand, a high V_A/Q mode is frequently seen. This high V_A/Q appears to be connected with low pulmonary artery pressure, which suggests poor perfusion of upper lung regions. It has been suggested that the regulation of the perfusion distribution is highly efficient and that collateral ventilation is less important for the optimal matching of the ventilation and perfusion in the resting horse.

PULMONARY GAS EXCHANGE IN HORSES WITH MODERATE TO SEVERE RAO

Eight adult horses (4–15 years, 382–490 kg) with clinical signs of moderate to severe chronic bronchiolitis, were studied with regard to
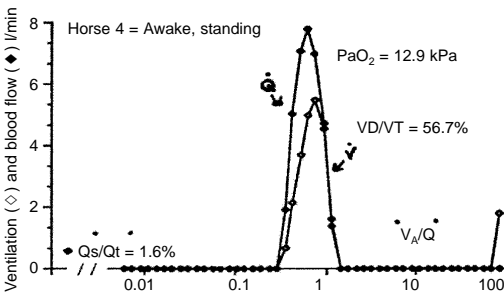

Fig 1: V_A/Q distribution in a healthy standing horse. Note the narrow major V_A/Q mode centered on a ratio of one, and a small mode within high V_A/Q regions. Ventilation-perfusion distribution (V_A/Q) alveolar ventilation as open circles (V_A) and perfusion as filled circles (Q); shunt in per cent of cardiac output (Qs/Qt); arterial oxygen tension in kPa (PaO_2); dead space ventilation (VD/VT).

ventilation/perfusion relationships and lung morphology. On biopsy, all horses showed prominent inflammatory, hyperplastic and metaplastic bronchiolitis. In addition, diffuse acinar hyperinflation was evident at necropsy performed in 4 of the horses. There was a significant agreement between the extent of bronchiolar epithelial hyperplasia in necropsy specimens of lungs and the degree of ventilation of high V_A/Q regions and dead space.

The arterial oxygen tension (PaO_2) was significantly decreased despite an increased respiratory rate and minute ventilation. Maintenance of normal arterial carbon dioxide tension was achieved by the considerable increase in ventilation. Cardiac output was in the normal range, but pulmonary arterial pressure was significantly increased.

The major gas exchange disturbance was a widened major V_A/Q mode producing a certain amount of hypoxaemia (examples shown in Fig 2). The increased scatter of V_A/Q ratios was caused by an uneven distribution of alveolar ventilation and capillary blood. The considerably increased dead space ventilation and ventilation of 'high V_A/Q regions' ($V_A/Q >10$) caused by a relative increase in alveoli, which are overventilated in comparison to their perfusion, created a loss or 'wasted ventilation' of about 75% of the total minute ventilation. The ventilation of high V_A/Q regions and dead space might be explained by hyperinflation, with subsequent compression of the pulmonary vascular bed and impeded regional perfusion. This produces high V_A/Q regions if ventilation is normal or is less affected than perfusion. High V_A/Q regions have been shown to be caused by a Zone I, ie a zone in the lung where the alveolar pressure exceeds capillary pressure and compresses the vessels, except for residual blood flow in capillaries at the junction between alveolar septa (corner vessel blood flow; Hedenstierna *et al.* 1979). These vessels participate in gas-exchange, as indicated by high V_A/Q ratios. A similar finding of high V_A/Q-regions has been made in asthmatic children, and assumed to be due to regional hyperinflation and impeded perfusion (Freyschuss *et al.* 1984). Neither shunting ($V_A/Q=0$) nor perfusion of very poorly ventilated lung regions (low V_A/Q) was found. In view of the abundance of luminal mucus and epithelial hyperplasia in bronchioles, likely to cause small airway obstruction, the minimal shunt may appear unexpected. However, it could be an
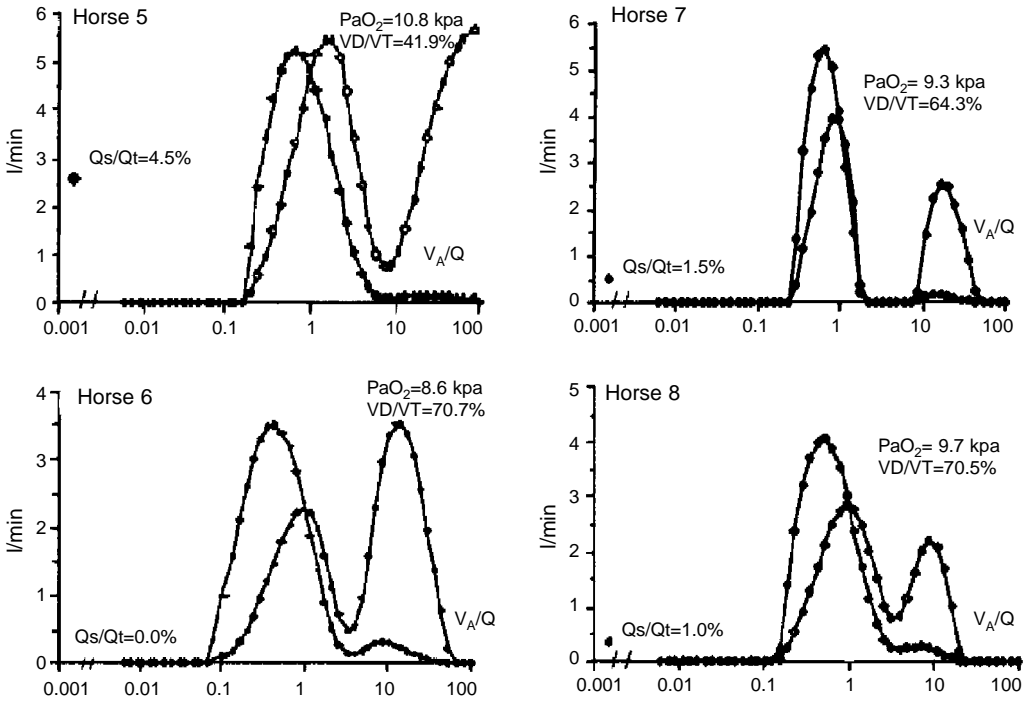
Fig 2: V_A/Q distribution in RAO Horses 5–8. Note the much broader distributions, and larger mode within high V_A/Q regions than in healthy horses (cf Fig 1). Ventilation-perfusion distribution (V_A/Q); alveolar ventilation as open circles (V_A) and perfusion as filled circles (Q); shunt in percent of cardiac output (Qs/Qt); arterial oxygen tension in kPa (PaO_2) and dead space ventilation (VD/VT).

indication of compensatory mechanisms, such as collateral ventilation, or efficient hypoxic pulmonary vaso-constriction distributing the blood flow away from poorly ventilated or non-ventilated lung regions. Diffusion limitation can be excluded as a cause of hypoxaemia since the measured and the calculated PaO_2 were similar.

Interestingly, some human patients with obstructive pulmonary disease showing normal $PaCO_2$ and 'high V_A/Q-regions', classified by Wagner *et al.* (1977), analogous to the horses in this study. Wagner *et al.* (1977) also showed that other human COPD patients were characterised by high $PaCO_2$ and large amounts of 'low V_A/Q -regions'. Although no increase in venous admixture (shunt and low V_A/Q -regions) has been measured in RAO horses at the author's department, analogous classifications may exist in equine RAO patients, which might explain the different findings of shunt fraction reported by different authors.

From these findings it is hypothesised that the major functional disturbance is hyperinflation of the lung, increasing the burden both on circulation and ventilation.

PULMONARY GAS EXCHANGE AND LUNG PATHOLOGY IN HORSES WITH RAO BEFORE AND AFTER CONTROLLED ENVIRONMENT

Pulmonary gas exchange and lung pathology in seven client owned horses with RAO were evaluated at crisis (C) and in clinical remission (R; own unpublished data). No medical treatment was given to the 7 horses during the study period. After the change from a conventional management system to paper bedding, grass silage and housing in a well ventilated stable a clinical improvement was noted in all horses. After 3–12 months, the respiratory rate was significantly reduced and stable on a day to day basis, cough was no longer present and the tracheal mucus was minor or no longer present. At this stage pulmonary gas exchange, MIGET and lung biopsies were repeated.
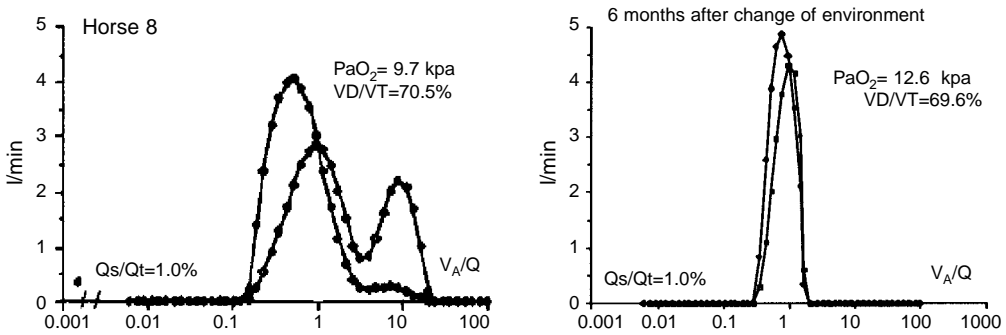
Fig 3: The major improvement in pulmonary gas exchange after the horses had been stabled in controlled environment was a reduced scatter of V_A/Q ratios. Log SDQ, the logarithmic standard deviation of the perfusion distribution, decreased and a significant increase of perfusion to normal V_A/Q regions was seen. Minimal shunt and no 'low V_A/Q regions' were seen either in RAO crisis or in clinical remission. Ventilation-perfusion distribution (V_A/Q), alveolar ventilation as open circles (V_A,) and perfusion as filled circles (Q); shunt in percent of cardiac output (Qs/Qt); arterial oxygen tension in kPa (PaO$_2$) and dead space ventilation (VD/VT).

The total minute ventilation and respiratory rate were significantly reduced in all horses compared to C. Epithelial hyperplasia and goblet cell metaplasia seen on lung biopsy, had abated in 4 of 7 horses after a period of 3–6 months. After 6 months of treatment, 2 horses were euthanised and one horse was re-evaluated after 12 months. At the end of the study, bronchiolitis, seen as epithelial hyperplasia and goblet cell metaplasia, was reversed in 5 of 7 horses.

The pulmonary arterial mean blood pressure was significantly reduced but as no differences in heart rate and cardiac output were measured during environmental control, a reduced vasoconstriction is assumed. The major improvement in pulmonary gas exchange in R was a reduced scatter of V_A/Q ratios (Fig 3). Log SDQ was significantly decreased and a significant increase of perfusion to normal V_A/Q regions was seen in R. Minimal shunt and no 'low V_A/Q regions' were seen both before and after the horses had been stabled in a controlled environment. A slight improvement in arterial oxygenation was measured but there were individual variations within the group.

An interesting and unexpected observation was that the relative distribution of ventilation to regions with high V_A/Q and dead space did not change simultaneously with the decrease in total minute ventilation. Thus, ventilation of dead space and high V_A/Q regions remained on average 66% of the total minute ventilation. Pulmonary resistance has been reported to be reduced following administration of bronchodilatators to

RAO horses, but is nevertheless greater than in control horses. These findings indicate a residual cause of obstruction and Robinson *et al.* (1996) suggested that airway obstruction persisted in peripheral airways due to mucus and inflammatory changes in the airway wall. The findings described in the present abstract indicate that a V_A/Q mismatch still exists in horses with RAO in R. Whether regional hyperinflation and impeded perfusion of the lung persisted in the asymtomatic RAO horses, despite the decrese in total minute ventilation, remains to be answered. Another possible explanation of the remaining V_A/Q mismatch is reduced regional perfusion caused by destruction of lung tissue, with loss of pulmonary capillary network in areas of emphysema (Gillespie and Tyler 1969).

From these findings it is hypothesised that even though a clinical remission was seen in horses with RAO, distribution of ventilation remained altered compared to lung-healthy horses and pathological lesions were not improved in all horses after six months in a low dust environment. These findings suggest that destruction of pulmonary capillaries and/or emphysema may still be present in RAO horses in R.

GAS EXCHANGE DURING EXERCISE IN STANDARDBRED TROTTERS WITH IAD

Most investigations on RAO in horses have been carried out on middle-aged to older horses during rest. Although IAD is quite common in young performance animals, and possibly can represent

an early stage of RAO, the significance in pulmonary function and gas exchange still remains unclear.

Standardbred trotters, 3–7 years old, were examined during a graded exercise with the highest workload at 95% of VO_{2max}. All horses had mucus present in the airways and a morphological diagnosis of mild bronchiolitis (considered as IAD) with either peribronchiolitis or bronchiolitis with luminal mucus and/or goblet cell metaplasia, and bronchioloar epithelial hyperplasia was determined in one horse. The Standardbred trotters with IAD could achieve an adequate pulmonary gas exchange during a graded exercise test compared to healthy Standardbreds (Nyman *et al.* 1999). In line with several studies, exercise-induced hypoxemia and hypercapnia developed at the highest work load. From these findings it is suggested that the airflow was relatively unaffected in trotters with mild bronchiolitis during exercise, but as no data on respiratory mechanics was evaluated in this study an increased respiratory resistence can not be ruled out. Although there was no difference in log SDQ between healthy and IAD horses during exercise (indicated by ANOVA), a tendency to an increased distribution of perfusion was seen at the highest workload in horses with IAD. The question whether the increased total red cell volume and haematocrit in IAD compared to healthy Standardbreds was a compensatory mechanism for an insufficient oxygen delivery in relation to tissue demand, or reflects that horses with IAD were in very good racing condition, remains unanswered. Recently, Harmegneis *et al.* (2002) used scintigraphy to study pulmonary perfusion distribution in 'heavey' horses during exercise. They reported an impaired gas exchange and more heterogeneous perfusion distribution at the highest workload in these horses, either if they were clinically affected or in remission.

In line with previous work, the exercise-induced arterial hypoxemia was induced by a considerable diffusion limitation of oxygen, some ventilation-perfusion mismatch and only minor hypoventilation, responsible for 61%, 35% and 4%, respectively, of the alveolar-arterial oxygen tension difference at the highest work load (Table 1).

TABLE 1: General cardiopulmonary variables and inert gas data in horses at rest with recurrent airway obstruction (RAO) or Standardbred trotters during exercise with inflammatory airway disease (IAD)

	Healthy	RAO	RAO crisis	RAO remission	Healthy	IAD
Number of horses	8	8	7		7	7
Speed m/s	Rest	Rest	Rest		8.8 ± 0.3	8.7 ± 0.4
Hb g/l rest, Hct %			131.1 ± 18.1	119.6 ± 8.2*	53.7 ± 2.6	55.5 ± 5*
V_E l/min	65.2 ± 10.1	118.6 ± 32.8*	133.1 ± 26.5	90.9 ± 10.3*		
V_A ml/ (min x kg)					2460 ± 574	2328 ± 370
RR breaths/min	13.6 ± 4.5	29.1 ± 8.2*	25.1 ± 8.3	16.4 ± 2.3*	109 ± 8	108 ± 5
Qt l/min	40.2 ± 5.6	35.8 ± 2.8	41.2 ± 6.8	36.2 ± 8.2		
Qt ml/ (min x kg)					633 ± 53	642 ± 136
HR beats/min	41.3 ± 8.0	34.6 ± 4.1	43.4 ± 6.8	40.3 ± 7.0	212 ± 8	212 ± 13
PAP mmHg	26.3 ± 5.4	33.6 ± 7.9*	33.1 ± 6.6	25.6 ± 5.6*	83 ± 11	98 ± 15*
PaO_2 mmHg	94.5 ± 9.0	73.5 ± 10.5*	79.5 ± 13.5	89.3 ± 10.5*	60.8 ± 3.2	59.3 ± 9.0
$PaCO_2$ mmHg	42.7 ± 4.5	43.5 ± 2.3	45.7 ± 2.3	44.3 ± 1.5	50.3 ± 5.0	50.3 ± 7.3
Mean V_AQ for Q	0.77 ± 0.22	0.70 ± 0.13			3.47 ± 0.66	3.51 ± 0.71
Log SDQ	0.41 ± 0.13	0.74 ± 0.19*	0.59 ± 0.23	0.38 ± 0.09*	0.46 ± 0.16	0.62 ± 0.25
Mean V_A/Q for V	1.58 ± .96	2.90 ± 1.37*			4.31 ± 1.16	4.34 ± 0.6
Log SDV	1.10 ± 0.72	1.62 ± 0.29	0.7 ± 0.6	1.0 ± 0.5	0.39 ± 0.08	0.44 ± 0.10
Shunt, % of Qt	1.2 ± 1.1	1.8 ± 1.5	1.1 ± 0.1	0.9 ± 0.2	0.5 ± 0.3	0.3 ± 0.2
High V_A/Q, %of V	6.0 ± 7.6	10.8 ± 7.1	2.5 ±	1.2 ±		
Dead space, %of V	48.4 ± 14.9	63.3 ± 10.0*	63.2 ± 4.9	65.3 ± 4.3		

Statistical differences * from the previous value are indicated for rest (*t*-test) and during exercise (ANOVA). All values are presented as mean ± SD. Haemoglobin concentration (Hb), haematocrit (Hct), minute ventilation (V_E), alveolar ventilation (V_A), cardiac output (Qt), heart rate (HR), mean pulmonary arterial pressure (PAP), arterial oxygen and carbon dioxide tensions (PaO_2, $PaCO_2$)

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FUNCTIONAL IMAGING OF INFLAMMATORY AIRWAY DISEASE

D. Votion

Department of Functional Sciences, Faculty of Veterinary Medicine, University of Liege, Sart Tilman B42, 40000 Liege, Belgium

INTRODUCTION

In recent years, lung scintigraphy has centred on the functional modifications encountered in heaves-affected horses. Several functional tests were found to be valuable for: 1) the early detection of the condition (Votion *et al.* 1999a); 2) the assessment of environmental and medical management of the disease (Votion *et al.* 1999a,b); and for 3) the study of the disease process at the cellular level (Fairbairn *et al.* 1993; Fairbairn *et al.* 1996). However, these scintigraphical tests have never been performed on horses affected with inflammatory airway disease (IAD) and might also contribute to a diagnosis of the condition, to assess treatment and to improve knowledge about the pathogenesis of IAD.

Following a brief introduction to the nuclear functional imaging, this paper describes several pulmonary function studies that may be of interest in IAD-affected horses.

NUCLEAR FUNCTIONAL IMAGING

Scintigraphy is a nuclear imaging technique that uses gamma-emitting radioactive tracers to visualise and quantify functions of the body. Depending on the pulmonary function to be studied, the tracer may be inhaled by the horse or given by iv injection. The distribution of the radioactive compound within the body is monitored with a gamma-camera. This gamma-camera is connected on-line to a data-processing system that builds-up a 2-dimensional image of the 3-dimensional tracer distribution. In this image, the colours vary proportionally to regional radioactivity.

PULMONARY FUNCTION STUDIES

A consensual definition of IAD is not yet adopted. Nevertheless, it is agreed in general that mild pulmonary inflammation and decreased performance characterise this respiratory tract disease. The scintigraphical tests that enable detection and follow up of the inflammatory process will be reviewed and it will be shown how scintigraphy might contribute to the understanding of exercise intolerance.

Study of inflammatory process

Several scintigraphical possibilities exist for the study of the inflammatory process: scintigraphy with radioactively labelled cells of the blood, scintigraphy with radioactively labelled antibodies and, the scintigraphical determination of the alveolar-capillary barrier permeability.

Blood cell labelling: Sites of inflammation can be detected using radiolabelled white blood cells. In human medicine, the use of labelled granulocytes has become an established means of diagnosing a variety of inflammatory conditions in which neutrophil migration is a prominent pathological feature. With small doses of radioactivity, granulocytes are not damaged and the cells keep their physiological properties. Lymphocytes, however, are strongly sensitive to radiations. Because lymphocytes are long-lived cells, the risk of malignant transformation cannot be excluded due to chromosomal damage. Therefore, the labelling of lymphocytes is not a common procedure in human medicine even if the technique is feasible. On the other hand, it might

be tested on experimental horses suffering from IAD.

In heaves-affected horses, labelled leukocytes were used to follow the time course accumulation of inflammatory cells into the lung of allergic horses exposed to dusty hay. Using this technique, the contribution of neutrophils, eosinophils and platelets to the pathogenesis of heaves was investigated (Fairbairn *et al.* 1993, 1996). Sub-populations of blood cells were separated by density centrifugation and the *ex-vivo* labelled cells were administered iv to heaves-affected horses exposed to mouldy hay. Scintigraphy confirmed that airway inflammation in heaves is characterised by a predominant neutrophilic inflammation (Fairbairn *et al.* 1993). In addition, the active migration of neutrophils from the blood compartment to the air space can be demonstrated by collecting tracheal secretions. Indeed, the tracheal aspirate of heaves-affected horses showing clinical signs of the disease was radioactive, whereas no radioactivity was found in control horses or in heaves-affected horses during clinical remission of the disease (Votion *et al.* 2000).

Labelled antibodies: Monoclonal antibodies directed against a specific sub-population of leukocytes have become commercially available. Using a monoclonal antibody directed against neutrophils, it was possible to demonstrate the active migration of neutrophils in heaves-affected horses showing clinical signs of the disease (Votion *et al.* 2000).

A novel approach to the detection of inflammation is based on the use of radiolabelled monoclonal antibodies directed against adhesion molecules. Adhesion molecules promote the migration of leukocytes into inflamed tissue by allowing leukocytes to be successfully attached to the endothelium. Activation of these adhesion molecules is a very early event in inflammation; consequently, these radioactive antibodies might be used as an early radionuclide detector of acute inflammation.

Alveolar-capillary barrier permeability: The scintigraphical study of the alveolar-capillary barrier permeability enables 2 other events of the inflammatory process to be followed: 1) the vascular leakage due to increased permeability of the endothelium; and (2) the modification of the alveolar epithelium permeability that increases

because the inflammatory reaction damages this epithelium.

Endothelial permeability: During an acute inflammatory process, local vasodilatation and increased endothelial permeability result in the extravasation of plasma. This vascular leakage may be measured using a double isotope dilution technique. In the double isotope method, 2 tracers are injected iv:

- the first tracer is made up of radioactive proteins that have a molecular weight near that of albumin;

- the second consists of radioactive red blood cells labelled with another radioactive element. The lung radioactivity obtained following administration of both tracers is compared. Presuming that red blood cells stay in the vascular bed, additional radioactivity found in the lung compartment is attributed to pulmonary oedema.

Epithelial permeability: The alveolar epithelium permeability is measured by studying the clearance from lung to blood of a small hydrophilic compound labelled with radioactivity. Hydrophilic compounds deposited into alveoli by nebulisation slowly diffuse through the intercellular junctions of alveolar cells. Once in the interstitium, they are cleared rapidly by the blood flow because the intercellular junctions of the endothelial cells are wider. When an inflammatory process alters the alveolar epithelium, the clearance of the compound from the alveoli is accelerated. Therefore, a more rapid clearance from the lung indicates alteration of the alveolar epithelium.

The scintigraphical determination of alveolar clearance rate demonstrated a significantly accelerated clearance of the radioactive tracer from alveoli when heaves-affected horses with clinical signs of the disease were compared to healthy horses. After 2 months at pasture, these heaves-affected horses were free of signs of the disease. During this remission, horses had normal pulmonary function tests (ie mechanics of breathing and arterial blood gas analysis). Furthermore, the mean alveolar clearance rate was similar to that of healthy horses. After 2 months stabled in a controlled environment (ie poor in allergens responsible for clinical signs), heaves-affected horses in remission remained free of

symptoms and presented normal pulmonary function tests. However, they showed an mean alveolar clearance rate intermediate between healthy and clinically-affected horses that suggested the existence of mild inflammation. Scintigraphy was more sensitive than pulmonary function tests in the diagnosis of this mild respiratory condition (Votion *et al.* 1999a). These results suggest that alveolar clearance rate determination might be of value for IAD detection.

Regional lung function

Definition of the ventilation-perfusion relationship and determination of the exercise-induced pulmonary perfusion redistribution (EIPPR) might contribute to the understanding of decreased performance in IAD-affected horses.

Ventilation-perfusion relationship: With scintigraphy, regional analysis of the ventilation-perfusion relationship may be performed. For that purpose, it is necessary to compute an image of the ratio between both parameters. To this aim, the ventilation image is divided by the perfusion one. From the computed ventilation to perfusion ratio (V/Q) image, the lung regions 'more ventilated than perfused', 'more perfused than ventilated' or 'equally ventilated and perfused' may be identified clearly (Votion *et al.* 1997). This V/Q image enables determination of the normal V/Q gradient as well as identification of local dysfunction.

Using V/Q images analysis, it was demonstrated that heaves induced respiratory function impairment and that respiratory dysfunction may be still present following medical treatment despite clinical recovery (Votion *et al.* 1999b).

Exercise-induced pulmonary perfusion redistribution: The creation of exercising to resting perfusion ratio images enables the visualisation of the EIPPR. These images demonstrated that exercise redistributes blood flow to the dorso-caudal regions of the lungs. This particular redistribution may contribute to location of bleeding sites in exercise-induced pulmonary haemorrhage (O'Callaghan *et al.* 1987). Variation in EIPPR was found to occur depending on the

clinical status: higher heterogeneity in blood flow distribution from rest to exercise was associated to the heaves condition (Harmegnies *et al.* 2002).

CONCLUSIONS

The use of scintigraphy to scrutinise cellular and physiological changes induced by IAD might help to improve knowledge of the disease and may lead to a better definition of the syndrome.

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INFLAMMATORY AIRWAY DISEASE AND CLINICAL EXERCISE TESTING

L. Couëtil

Purdue University, West Lafayette, Indiana 47907, USA

The purpose of exercise testing is to evaluate the physiological response of the horse in a controlled way to detect and, if possible, quantify the effect of inflammatory airway disease (IAD) on performance. The latter may be assessed objectively in racehorses by comparing finishing position, racing times, and earnings in affected and control horses. However, many confounding factors may influence race performance such as track condition and design, jockey or driver, and length of the race.

In other athletic horses, the effect of IAD on performance is more subjective. The negative impact of IAD on performance is suggested by a study of Standardbred racehorses (n= 965) revealing that horses finishing in the last two positions were 5.8 times more likely to have mucopurulent exudate during post-race endoscopy of the trachea than horses finishing first or second (P<0.0001; MacNamara *et al.* 1990). Another study found that Thoroughbred racehorses exhibiting marked decrease in performance had a significantly increased percentage of neutrophils in bronchoalveolar lavage (BAL) fluid (Fogarty and Buckley 1991). Furthermore, 41% of horses with IAD returned to previous level of performance after improving ventilation and decreasing the amount of air born dust in the horses' stall.

Horses with IAD pose a diagnostic challenge for the clinician because they are clinically asymptomatic at rest, except for increased respiratory exudate visible by endoscopy in the majority of cases and mild intermittent cough in only a 3rd of the cases. Poor performance may also result from a variety of other causes, such as upper airway obstruction, lameness, exertional rhabdomyolysis, and cardiac and neurologic diseases. More importantly, it is common to

diagnose several concomitant problems in the same horse (Morris and Seeherman 1991). Clinical exercise testing should be tailored to the horse's fitness level and type of activity to allow a safe and consistent evaluation of respiratory, cardiovascular, and musculoskeletal function. The key is standardisation, whereby test results from one horse may be compared to results from healthy controls matched for age and fitness level. Alternatively, test results collected from one horse can be compared before and after therapy. Such testing may be conveniently conducted on a high-speed treadmill. Some field tests have also been used. However, they only allow a limited range of measurements and currently, are less suitable than treadmill exercise testing for evaluation of respiratory function. Based on test results, the clinician needs to assess if the degree of IAD is likely to affect performance and to eliminate other possible causes of poor performance.

The main role of the respiratory system is to transport oxygen through the conducting airways to the alveolar-capillary gas-exchanging surface of the lung and to allow diffusion of oxygen into the blood. In return, carbon dioxide will be transported in the opposite direction. Several studies have shown that healthy fit horses develop hypoxemia and hypercapnia during strenuous exercise mainly due to diffusion limitation and ventilation/ perfusion mismatch and, to a lesser extent insufficient ventilation (Wagner *et al.* 1989; Nyman *et al.* 1995; Hopkins *et al.* 1998). Consequently, even mild respiratory disease affecting conducting airways or gas exchange areas of the lung may significantly impair oxygen delivery to exercising muscles and result in decreased performance.

A clinical exercise testing study of Standardbred racehorses found that horses with
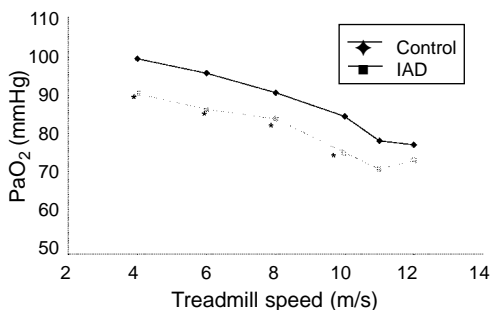
Fig 1: Partial pressure of oxygen in arterial blood (PaO$_2$) of healthy racehorses (control) and horses with inflammatory airway disease (IAD) during a standardised treadmill test. *Data significantly different from control values (P<0.05).
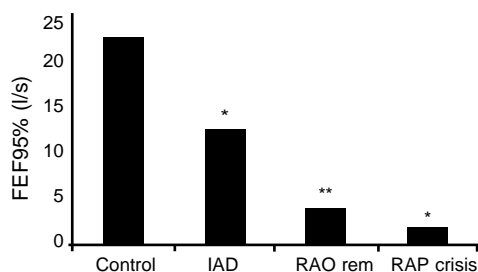

Fig 2: Mean forced expiratory flow after 95% of the vital capacity has been exhaled (FEF95%) in healthy horses (control), horses with inflammatory airway disease (IAD), and horses with recurrent airway obstruction or heaves during period of disease exacerbation (RAO crisis) or clinical remission (RAO rem). *Data significantly different from control values (P<0.001). **Data significantly different from control and IAD values (P<0.01).

IAD exercising submaximally maintained lower arteriovenous oxygen content difference and exhibited increased red cell volume (RCV/kg bwt) and pulmonary artery pressure in comparison to healthy controls (Nyman *et al.* 1999). These findings suggested a compensatory response to exercise-induced hypoxemia (Persson 1967; Persson 1983) even though significant differences in PaO$_2$ between IAD and control horses were not found. The elevated pulmonary artery pressure was thought to result from increased vascular resistance. Elevation in RCV/kg BW or packed cell volume has been shown to correlate with increase in pulmonary blood pressure and vascular resistance (Funkquist *et al.* 1995; Davis and Manohar 1988). Also, horses with more severe airway disease such as heaves have significantly elevated pulmonary artery pressure (Eberly *et al.* 1966; Nyman *et al.* 1991). Others have shown that racehorses with IAD exhibit a more pronounced exercise-induced hypoxemia than healthy controls during a standardised run to fatigue (Fig 1; Couëtil and DeNicola 1999). These findings are consistent with data in horses with heaves where exercise-induced hypoxaemia was worse during periods of maximum airway obstruction (disease crisis) than during periods of disease remission where airway obstruction was minimum (Art *et al.* 1998). The degree of exercise-induced hypoxemia is more pronounced as the level of training and VO$_{2max}$ increase (Christley *et al.* 1997). Therefore, assessment of the significance of exercise-induced hypoxemia is dependent on control data matched for horses' age and fitness level.

Another investigation evaluating gas exchanges and lung biopsy parameters in Standardbred racehorses showed that horses with IAD had impaired performance potential compared to healthy controls, based on lower minute ventilation at a speed corresponding to a heart rate of 200 bpm (V$_{E-200}$/kg bwt) and increased RCV/kg BW (Persson and Lindberg 1991). In addition, the severity of lung biopsy score was negatively correlated with V$_{E-200}$/kg bwt, suggesting that bronchial epithelial hyperplasia of the small airways was probably causing airflow obstruction. These findings are consistent with the fact that small airway obstruction is evident in horses with IAD when using sensitive methods such as forced expiration or forced oscillatory mechanics (Fig 2; Hoffman and Mazan 1999; Couëtil *et al.* 2001). Also, a significant negative correlation has been reported between forced expiration indices and percentage of neutrophils in BAL fluid, indicating that as airway inflammation becomes more severe, small airway obstruction is more pronounced (Couëtil *et al.* 2001).

In athletic horses with IAD other than racehorses, the degree of airflow obstruction is usually more pronounced. This may be evident clinically as a mildly increased respiratory rate and efforts, and sometimes wheezes are heard during thoracic auscultation with a rebreathing bag. Clinical exercise testing is rarely performed on these individuals because of a lack of reference values for horses with different fitness levels and aerobic capacities. In such cases, BAL fluid cytology is valuable and the degree of airflow obstruction may be quantified by lung function testing.

Clinical exercise testing is also a useful way of assessing response to IAD treatment. In
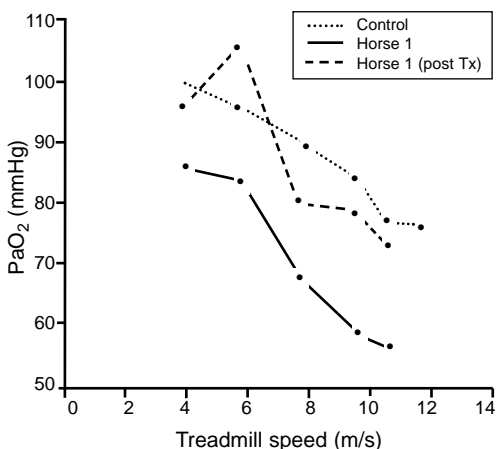
Fig 3: Partial pressure of oxygen in arterial blood (PaO$_2$) of healthy racehorses (control), horses with inflammatory airway disease (IAD), and a horse (Horse 1) with IAD before and 2 weeks after therapy (post Tx) during a standardised treadmill test.

racehorses, an improvement in PaO$_2$ may be seen during a standardised treadmill test in conjunction with normalisation of BAL fluid cytology (Fig 3). In horses with detectable airflow obstruction, resolution of flow limitation is commonly observed within 1–2 weeks of therapy. Resolution of airway inflammation and hyper-responsiveness usually takes more time.

In conclusion, IAD in athletic horses results in mild impairment of lung function in comparison to heaves. However, it may lead to poor performance by compromising delivery of oxygen to the blood. The degree of lung dysfunction may be objectively assessed during clinical exercise testing and also serve as a baseline to evaluate response to treatment.

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WORKSHOP SUMMARY

WORKSHOP SUMMARY

In July 2000 a workshop addressed the confusing issue of terminology used to describe equine lower airway disease (ie tracheobronchial disease) (Robinson 2001). At that workshop the syndrome of 'heaves' (also known as recurrent airway obstruction or RAO) was defined. Participants in that workshop recognised that many horses have a chronic, lower grade, diffuse form of airway inflammation that is not accompanied by clinical signs of airway obstruction, and they agreed to name that syndrome inflammatory airway disease (IAD). The present Havemeyer Workshop was arranged to review the current understanding of IAD. The following statements represent the general consensus about IAD reached by participants in the Havemeyer Workshop.

CLINICAL DEFINITION OF THE SYNDROME

- The clinical presentation of IAD may include cough, accumulation of secretions in the trachea, nasal discharge, poor performance, and prolonged recovery after exercise.

- Affected animals do not have increased respiratory effort at rest (ie they are not depressed, inappetant, or febrile).

- Affected horses are not systemically ill.

- The CBC, blood chemistry, and fibrinogen concentration of these horses are within normal limits.

- Horses with IAD have increased proportions of inflammatory cells, in particular neutrophils, mast cells, and/or eosinophils in airway secretions.

CLINICAL FINDINGS

- Horses with IAD can have airway inflammation and lung dysfunction without overt clinical signs.

- Horses with IAD may have accumulated secretions in the trachea without a cough.

- Cough is an insensitive indicator of IAD.

- In racehorses, IAD is most common in young horses (2–4 years of age) and diminishes with time in the training environment.

- IAD in racehorses has been shown to be associated with poor performance.

ENDOSCOPIC OBSERVATIONS

- The clinical significance of mucus accumulation depends on the level of performance expected from the horse.

- In order to chart the progression of the disease and response to therapies, gross observations of mucosal appearance and the thickening and blunting of the bronchial bifurcations should be noted, and the amount of tracheal secretions should be quantified using a standard 5-grade scoring system.

EPIDEMIOLOGY

- IAD can occur in horses of all ages and disciplines.

- In the majority of young racehorses, IAD resolves but it remains persistent in a minority.

- IAD in racehorses has been studied most extensively in young animals in the UK and Australia, where up to 80% of horses are affected at some point in the first year of training.

- In non-racehorses, IAD is usually diagnosed at an older age than in racehorses.

- Studies in sport horses maintained permanently indoors indicate that 70% of horses or greater may be affected at one time.

- Non-racehorses presenting for evaluation at referral centers tend to have more obvious clinical signs of respiratory disease than do young racehorses. This is probably because racehorses put great aerobic demands on their lungs and so dysfunction is more readily apparent.

AETIOLOGY

- The aetiology of IAD probably involves many factors that act either synergistically or sequentially to initiate and/or prolong airway inflammation.

- The air that horses breathe in many conventional stables contains particle levels known to cause airway inflammation in other species.

- Conventional stabling is associated with airway inflammation in apparently healthy (non-heaves-affected) horses.

- Respirable endotoxin concentration is a risk factor for increased neutrophils in airway secretions.

- Viral infection has been detected in only a small proportion of cases of IAD.

- In some of the racehorses with IAD, the presence of detectable tracheal secretions is associated with the presence of bacteria that are likely to be pathogenic in the horse.

- In the UK and Australia, increased numbers of bacteria (predominantly *Streptococcus, Actinobacillus* and *Pasteurella)* are frequently isolated from the trachea of young racehorses with IAD.

- The role of allergy in IAD is unknown.

- The relationship between IAD and heaves is currently unknown.

LABORATORY FINDINGS

- The CBC, blood chemistry, and fibrinogen concentration of these horses are within normal limits.

- Both tracheal aspirates and bronchoalveolar lavage fluid should be collected to fully evaluate the lower respiratory tract.

- Tracheal aspirates and bronchoalveolar lavage

sample different areas of the lower respiratory tract and, therefore, their cytological interpretations are not interchangeable.

- The presence of neutrophils in tracheal secretions or BALF does not *per se* signify the presence of infection.

TRACHEAL ASPIRATES

- In young healthy racehorses, current information suggests that there should be <20% neutrophils and <1% eosinophils in tracheal aspirates.

- In 2–3-year-old performance horses, the presence of >20% neutrophils in tracheal aspirates is associated with increased mucus and increased risk of coughing.

- Tracheal aspirates for bacterial isolation should be collected endoscopically with a guarded catheter using 10–15 ml of sterile saline 1–2 h after exercise and a minimum of 24 h after transportation.

BRONCHOALVEOLAR LAVAGE

- In clinically healthy horses, current information suggests that there are on average 60% macrophages, 35% lymphocytes, <5% neutrophils, <2% mast cells, <0.1% eosinophils and occasional or no epithelial cells in bronchoalveolar lavage (BAL) fluid.

- An increased percentage of mast cells or eosinophils in BAL fluid is associated with airway hyper-reactivity.

- Bronchoalveolar lavage fluid should be collected and processed according to the recommendations of the International Workshop on Equine Chronic Airway Disease (Robinson 2001).

LUNG FUNCTION

- Conventional measurements of pulmonary function (maximal change in pleural pressure during tidal breathing (ΔPpl_{max}), pulmonary resistance, and dynamic compliance) are unaltered in horses with IAD.

- IAD-affected horses evaluated at referral centres for cough or poor performance commonly have airway hyper-reactivity and/or

airway narrowing.

RECOMMENDATIONS FOR FUTURE INVESTIGATIONS

- Standardise the scoring systems for the variables commonly used to characterise IAD in order to enable comparison between studies.

- Determine the prevalence of IAD in different populations of horses around the world.

- Investigate the pathological changes in horses with IAD and determine the relationship between airway remodelling, BAL fluid cytology, and lung function.

- Determine the relationships between tracheal lavage cytology, bronchoalveolar lavage cytology, mucus accumulation, pulmonary function at rest and during exercise and performance.

- Conduct controlled studies of currently recommended therapies of IAD.

- Determine the effect of treatment on the airway inflammation, mucus accumulation, pulmonary dysfunction, and exercise intolerance present in IAD.

- Evaluate the effects of particulate and endotoxin exposure in young horses.

- Characterise the immunological mechanisms of IAD.

- Investigate the possible genetic predisposition to IAD.

REFERENCE

Robinson, N.E. (2001) Chairperson's introduction: International Workshop on Equine Chronic Airway

LIST OF PARTICIPANTS

Laurent Couëtil, United States

Frederik Derksen, United States

Paddy Dixon, United Kingdom

Vincent Gerber, United States

Andrew Ghio, United States

David Hodgson, Australia

Jennifer Hodgson, Australia

Andrew Hoffman, United States

Jean-Pierre Lavoie, Canada

Nicholas Malikides, Australia

David Marlin, United Kingdom

Melissa Mazan, United States

Bruce McGorum, United States

Richard Newton, United Kingdom

Gorel Nyman, Sweden

Rachel Pepper, United Kingdom

Candice Platz, United States

Gene Pranzo, United States

Ed Robinson, United States

Bonnie Rush, United States

Ken Smith, United Kingdom

Hugh Townsend, Canada

Laurent Viel, Canada

Dominque Votion, Belgium

James Wood, United Kingdom

***Back row** (**left-right**): Richard Newton, Hugh Townsend, Ed Robinson, Jean-Pierre Lavoie, James Wood, Fred Derksen, Andy Hoffman, Dominique Votion, David Marlin, David Hodgson, Laurent Couëtil, Chris Deaton, Bruce McGorum*

***Front row** (**left-right**): Rachel Pepper, Ken Smith, Nick Malikides, Jenny Hodgson, Melissa Mazan, Bonnie Rush, Laurent Viel, Vince Gerber, Paddy Dixon.*

Dr Gorel Nyman also attended the Workshop but had to leave before the picture was taken.

AUTHOR INDEX