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ETIOPATHOGENESIS OF RAO

RAO is considered to be a “hypersensitivity” reaction to inhaled organic dusts (mites, endotoxin) and/or molds (*Asp. fumigatus* and *F. rectivirgula*) found in poorly cured hay.1 This condition is typically found in horses in the Northeastern and Midwestern region of the US as well as in the UK and Europe. (Note that a similar pulmonary disorder called summer pasture associated obstructive pulmonary disease (SPAOPD) develops in certain horses in the southern U.S. and the UK which are exposed to organic dusts or molds while on pasture. While the clinical signs are similar (attenuated breathing effort, airway neutrophilia), this review focuses primarily on RAO). The exact immunological mechanisms that contribute to the airway neutrophil influx, excessive mucus production and bronchoconstriction are not completely known. Data exist that both support and refute RAO as a typical allergic (Ig-E mediated or Th-2) response.2-7 What most researchers agree upon is that many different cell types including airway luminal cells (macrophages, lymphocytes, neutrophils), airway epithelial cells and smooth muscle cells, are activated and involved at different time points in the development of the disease. It has also been recognized that certain families of horses develop RAO. However, the mode of inheritance of RAO, considered to be a polygenetic trait, is not completely defined (? autosomal dominance, autosomal recessive) and is under investigation.8,9

CLINICAL SIGNS OF RAO

Horses with RAO are usually afebrile and exhibit a cough (especially when worked), a mucopurulent nasal discharge and an accentuated expiratory effort that is typically evident in the resting horse. The respiratory rate of affected horses may be normal or increased (tachypnea). Hypertrophy of the external oblique and rectus abdominis muscles may be evident in chronic cases giving rise to a “heave line.” On thoracic auscultation, inspiratory or expiratory wheezes, crackles, or tracheal “rattles” may be detected although in some horses, adventitious sounds are restricted to one lung region. Auscultation may detect an expanded lung field. Severely affected horses exhibit exercise intolerance, weight loss, nostril flaring and cachexia and are reluctant to move. Despite the development of pulmonary hypertension in affected horses, signs of right heart failure (cor pulmonale) such as tachycardia, jugular distention, jugular pulsation and ventral edema rarely occur in horses with RAO.10 Horses in remission from episodes of RAO are indistinguishable from healthy non-affected cohorts although some alterations in their pulmonary function tests remain.

DIAGNOSIS OF RAO

A tentative diagnosis is based upon the clinical signs and a history of the horse becoming affected after husbandry alterations (such as being fed hay, being stabled or being maintained in a dusty lot) have been implemented. RAO-affected horses are also
more likely to become symptomatic in hot and humid weather. Endoscopic examination of the lower respiratory tract reveals excessive mucopurulent exudate within the trachea that originates from the right and left lung lobes as the disease is diffuse. Cytological evaluation of either the tracheobronchial secretions or of the bronchoalveolar lavage samples reveals a high percentage of intact (non-degenerate) neutrophils trapped in a background of mucus. Note that in clinically healthy horses, the BALF contains approximately 60% macrophages, 35% lymphocytes, < 5% neutrophils, < 2% mast cells and < 1% eosinophils.11 Intracellular or extracellular bacteria are rarely seen (hence an aseptic exudate) but pollen, fungal hyphae, and Curschmann’s spirals (inspissated mucus plugs) may be visible. The presence of fungal hyphae should not be confused with fungal pneumonia. A CBC and serum chemistry panel (especially serum globulins and fibrinogen levels) are usually completely normal in RAO-affected horses suggesting that the “inflammatory response” is compartmentalized to the lungs.12 Imaging studies are rarely performed on field cases because the diagnosis is relatively straightforward. Ultrasonographic evaluation of the thoracic cavity of RAO-affected horses confirms the existence of an expanded lung field. Small hypoechoic areas of irregularity on the lung surface may be detected but airway inflammation itself is not imaged.13 Thoracic radiographic examination is recommended in cases that fail to respond to conventional therapeutic interventions (outlined below). Radiographs are useful in confirming the diagnosis of RAO (bronchointerstitial pattern); in detecting the existence of bronchiectasis (dilation/deformation of the bronchi or bronchioles that infrequently occurs in severe cases of RAO)14 and in ruling out other pulmonary disorders that might “mimic” RAO such as neoplasia, equine multinodular pulmonary fibrosis or other causes of interstitial pneumonia.

Some advocate intradermal skin testing for the diagnosis of RAO. Theoretically, if RAO is an IgE-mediated disorder, affected horses should react if they are sensitized to a given allergen. However, results of skin testing in RAO cases have been disappointing: In some studies, clinically normal horses have positive skin test reactions to allergens while affected horses lack positive reactions.15-20 In other studies, the percentage of RAO-affected horses exhibiting positive skin reactions (to any antigen) exceeds that of controls or the response of RAO-prone horses to histamine challenge (measured at 0.5 h post injection) or to Aspergillus antigens (measured at 24 h post injection) is enhanced.18,19 Thus, positive skin tests and increased concentrations of serum precipitins to fungal and thermophilic actinomycete antigens are found in many normal as well as in RAO-affected horses and probably reflect a level of exposure of the horse rather than a susceptibility to RAO. In general, intradermal skin testing does not allow one to identify RAO-affected horses or the specific inhaled particulate that is inciting the inflammation.

**TREATMENT OF RAO**

Environment management changes—eliminating or reducing mold or dust exposures—remain the most important aspects of therapy but are also the most difficult to permanently implement.12 If pasture is not available, affected horses should be transitioned over a 3-5 day period to a diet consisting of pelleted feeds or hay cubes. Although some horses improve if the hay is soaked in water, this practice reduces the nutrient value of the hay and is usually forsaken during freezing weather. Unless pasture exposure exacerbates the condition, horses should be outside as much as possible with access to a clean, well-ventilated three-sided shelter that protects horses from inclement weather. In chronically-affected horses that are turned out to pasture, clinical remission and normalization of conventional pulmonary function tests takes 4-8 weeks depending upon weather conditions. Interestingly, recent data suggest that when RAO-susceptible horses are maintained in a low-dust environment (outside, no hay)
for years, they still exhibit reduced forced expiratory flows despite the absence of clinical signs.\textsuperscript{21} This finding suggests the existence of irreversible airway remodeling in affected animals.\textsuperscript{21,22} If the horse must be stabled, the stall and those in the immediate vicinity should be bedded with shavings. In a study from our laboratory of 12 RAO-affected horses that were stabled and fed a pelleted ration, the pulmonary function tests and BALF neutrophil percentages improved within 4 weeks of implementing these changes. However in 4 of the 12 horses, pulmonary health, as assessed by BALF neutrophil percentages, maximum pleural pressure changes and inflammatory cytokine profiles of the BALF cells, had not returned to normal by the conclusion of the study. This demonstrated that reducing inhaled dust loads while horses remain stabled is not sufficient for all cases.\textsuperscript{23}

**MEDICAL MANAGEMENT**

Corticosteroids, as opposed to nonsteroidal anti-inflammatories, are very efficacious in reducing the pulmonary inflammation. Corticosteroid “treatment failures” probably result from either inadequate dosing schedules and/or failure to make concurrent environmental alterations. Corticosteroids may be contraindicated in horses predisposed to laminitis (e.g. horses with endocrinopathies) or those with gastritis or gastric ulceration.

One potential dosing regimen is to administer prednisolone (not prednisone) at 2.2 mg/kg PO q 24 hours for 7 to 10 days and then to reduce the dose to 1.1 mg/kg q 24 hours PO for 7 to 10 days; to 0.50 mg/kg PO q 24 hours for 7 to 10 days and then to 0.50 mg/kg PO every other day (EOD) for 7 to 10 days. Alternatively, parenteral dexamethasone may be given. This approach is especially indicated if the horse is in respiratory distress as an improvement in its breathing efforts should be evident within 6-8 hours of steroid administration. One dexamethasone protocol involves administering 0.1 mg/kg IM (or IV) EOD for 3 to 4 treatments and then 0.025 mg/kg IM (or IV) EOD for 3 to 4 treatments. A similar dosing schedule may be used if the parenteral dexamethasone formulation is given orally except that the initial dosage is increased to 0.15 mg/kg PO EOD for 3 to 4 treatments to account for the reduced bioavailability of dexamethasone. As in above, the dose is decreased incrementally by 25%. In horses that remain stabled and fed a pelleted feed while receiving this oral dexamethasone protocol, normalization of pulmonary function and a significant reduction in airway epithelial and luminal cell inflammatory gene expression (IL-8, IL-1\(\beta\)) occurred within 4 weeks of implementing the regimen.\textsuperscript{23}

Inhaled steroids have also been used to treat the pulmonary inflammation of RAO but may not provide a therapeutic benefit for 24 to 72 hours. Thus for horses in respiratory distress, inhaled steroids should not be the main route of glucocorticoid administration.\textsuperscript{24} A metered dose inhaler containing fluticasone can be attached to the Aeromask,\textsuperscript{2} the EquineHaler,\textsuperscript{5} or Aerohippus\textsuperscript{c} and administered to the horse during inspiration. The recommended dose of fluticasone is 2 mg q 12 hours\textsuperscript{5} but doses as high as 6 mg have been administered for several weeks without inducing laminitis in research horses.\textsuperscript{24} In RAO-affect ed horses that were still fed hay, fluticasone administration was associated with a marked improvement in pulmonary function, a normalization of BALF neutrophil counts and a reduction in inflammatory mediators in the BAL cells.\textsuperscript{5} Another topical steroid that has been used is becomethasone dipropionate (1-3 \(\mu\)g/kg q 12 hours). Note that inhaled steroids are still absorbed systemically in sufficient quantities to suppress endogenous cortisol production in a dose-dependent fashion. Bronchodilators may also be used (in conjunction with corticosteroids) to treat RAO. Of the three types of compounds used to relax airway smooth muscle, the beta-2 adrenergics (\(\beta_2\)-agonists) such as clenbuterol and albuterol are more widely used than the methylxanthines (aminophylline and pentoxifylline) and the anticholinergics (atropine, ipratropium bromide).
Experimental investigations suggest that clenbuterol administered by mouth at 0.8 μg/kg q 12 hours may alleviate some of the signs of RAO. In addition to a direct smooth muscle effect, clenbuterol may stabilize mast cells, increase mucociliary clearance, improve airway secretions and decrease inflammatory cytokine production.\textsuperscript{25,26} If beneficial effects are not obtained at the 0.80 μg/kg dosage, then the dosage may be increased step-wise by 25% increments. Adverse effects included tachycardia, tachypnea and sweating. Other β\textsubscript{2}-agonists such as albuterol (short-acting), salmeterol or fenoterol (longer-acting) are also administered as metered dose inhalers (MDIs) attached to either the Aeromask,\textsuperscript{a} the Equinehaler,\textsuperscript{b} or the Aerohippus.\textsuperscript{c} Suggested dosing protocols include: albuterol 600-720 μg q 3-4 hours; fenoterol 1 to 2 mg q 6-8 hours or salmeterol 500 μg q 6-8 hours. Administration of the β\textsubscript{2}-agonist before inhaled corticosteroid administration enhances the deposition of the latter in the smaller airways. Long-term use of β\textsubscript{2}-agonist has been associated with a down-regulation of the β\textsubscript{2}-receptors in human beings, but whether this occurs in horses is unknown. Thus some clinicians recommend that the bronchodilators be used in conjunction with steroids to prevent down-regulation of β\textsubscript{2}-receptors.

With regard to the parasympatholytics, atropine (0.01 mg/kg IV once) generally is used for emergency relief of airway obstruction but it causes airway secretions to become more viscous and may cause colic. Glycopyrrolate has been reported to be efficacious at a dose of 0.005 mg/kg (IV, IM), but it too may also cause colic. Ipratropium bromide is administered by inhalation at a dose of 0.5-3 μg/kg q 8 hours with a low risk of inducing systemic side effects.\textsuperscript{26} However, it may take an hour for a beneficial effect to be observed thus it is not the bronchodilator of choice for “rescue”.

### OTHER THERAPIES

In horses that develop respiratory distress due to the bronchospasm, airway inflammation and hypoxemia, additional therapies are necessary. Administering nasal oxygen at flow rates as low as 5 L/min improves arterial oxygen tensions by as much as 30 mmHg in some severely-affected horses.\textsuperscript{27} As the total nasal oxygen flow increases, so too will the mean arterial oxygen tensions, although not to the same degree that occurs in healthy horses. Flow rates of 30 L/min (delivered by 2 nasal cannulae at 15 L/min) are associated with coughing and gagging in the horse so it is suggested to not exceed flow rates of 10 L/min per nostril. Nasal oxygen supplementation does not reduce breathing frequency suggesting that stimulation of vagally-mediated afferents—perhaps responding to inflammatory mediators— is responsible for the tachypnea. Furosemide (1.0 mg/kg IV or nebulized) provides beneficial effects to RAO-affected horses within 20 minutes of its administration by decreasing lung resistance (R\textsubscript{L}) and increasing lung compliance (C\textsubscript{dyn}) without affecting PaO\textsubscript{2}.\textsuperscript{28} The beneficial effects appear to be mediated by prostaglandin E\textsubscript{2}, derived from either the renal or airway epithelium: PGE\textsubscript{2} promotes smooth muscle relaxation. **Prior treatment with the cyclooxygenase inhibitor, flunixin meglumine, prevents the furosemide-induced bronchodilation.**\textsuperscript{29} (Nonsteroidal anti-inflammatories have not been found to be beneficial in the treatment of RAO).

### PROGNOSIS

RAO-prone horses are likely to develop acute exacerbations of the disease when husbandry practices lapse or when weather conditions become hot and humid. Horses do not “out-grow” the disorder, and in fact, some clinicians believe that the inflammation becomes more difficult to manage as the horse ages. The basis for the lack of clinical response is unknown but may be the result of lung remodeling or potential down-regulation of the glucocorticoid receptor. In a follow-up survey...

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\textsuperscript{a} Aeromask, Trudell Medical, London, Ontario, Canada.

\textsuperscript{b} EquineHaler, Equine Health Care Inc, Jorgensen Laboratories, Loveland, CO.

\textsuperscript{c} Aerohippus, Trudell Medical, London, Ontario, Canada.
conducted by Naylor of RAO-affected horses that had been examined and treated at a referral center 2-4 years previously, it was found that 20% of the original cases (3/15) had been euthanized, that 33% were still receiving bronchodilators on an as-needed basis and that the athleticism had not decreased in 92% of the horses.30 Similarly, in a follow-up survey conducted by Aviza and colleagues,12 13% of the horses that had been diagnosed with RAO 3-4 years earlier had been euthanized. However, in contrast to Naylor’s findings, Aviza noted that more than 50% of the respondents in that survey stated that the athletic performance of the horse had been compromised by the disease. Perhaps this difference between the two surveys simply reflected a failure of the owners to comply with environmental management recommendations. When queried about the husbandry practices, 77% of the respondents still stabled the horse for part of the day and 84% still fed dry hay. Because the effects of repetitive episodes on the development of irreversible changes in lung structure have yet to be determined, clients should be encouraged to implement effective husbandry changes that minimize the recurrences of RAO.

WHAT IS IAD AND HOW DOES IT DIFFER FROM RAO?

In 2007, a consensus statement was released by the American College of Veterinary Internal Medicine in an effort to clarify the differences between IAD from RAO.31 Following exposure to a dusty hay challenge, an RAO-susceptible horse develops an accentuated expiratory effort, exhibits maximum pleural pressure changes (from peak inspiration to peak expiration) exceeding 15 cm H2O and develops airway neutrophilia characterized by BALF neutrophil % > 25%. In contrast, the horse with IAD when exposed to dusty hay, often develops a cough but usually fails to show overt changes in its resting breathing pattern or demonstrable changes in conventional pulmonary function tests (ΔPpl\text{max}, Cdyn). Interestingly, in one study of IAD affected horses, cough was more prominent in older horses (mean age = 10 years) which had a mild BALF neutrophilia (mean % PMN = 9).32 In that same study, IAD affected horses with increased BALF eosinophil or mast cell percentages exhibited increased airway hyperreactivity to inhaled methacholine or histamine challenge.33,34 However, one of the limitations of the airway hyperreactivity test is that some control horses also display increased airway hyperresponsiveness in the absence of airway inflammation.

ETIOPATHOGENESIS

IAD, also referred to in the literature as small airway disease, is found in young to middle-aged athletic horses whose chief presenting complaint is poor performance and/or exercise intolerance. It is unknown whether IAD is a separate disease from RAO or whether it will progress to RAO. Affected horses lack systemic signs of infection such as fever, hematological abnormalities and increases in serum fibrinogen and globulin. Endoscopically, excess tracheal exudate (mucus and inflammatory cells) is often seen and cytological analysis of the bronchoalveolar lavage fluid reveals abnormal cell distributions. (NB: equine researchers have found a poor correlation between cytological profiles of equine tracheal aspirates and BALF and recommend BALF analysis for the diagnosis of pulmonary inflammatory disorders. Recall also that the BALF contains approximately 60% macrophages, 35% lymphocytes, < 5% neutrophils, < 2% mast cells and < 1% eosinophils in healthy horses.11 Horses with IAD (Table 1) exhibit increased total nucleated cell counts characterized by (1) a mild neutrophilia, a lymphocytosis and monocytosis;35 (2) increased percentages of mast cells36 or (3) eosinophilia.37 Based upon these different inflammatory cell profiles, it has been suggested that various pathogenic mechanisms are associated with each phenotype but this hypothesis remains to be proven. Suggested etiological agents include persistent low-grade viral infections (based upon serological data and
response to oral human interferon alpha therapy);38,39 inhaled organic dusts, endotoxin or fungal cell components;40 inhaled cold air during exercise;41 exposure to air pollutants42 and/or pulmonary hemorrhage.43,44

**DIAGNOSIS**

Diagnosis of IAD is thus based predominantly upon the identification of a non supplicative inflammation in horses coupled with the clinical signs. Nevertheless, some researchers will diagnose IAD in horses lacking cytological alterations in BAL based upon performance criteria from standardized exercise testing protocols (completion of test, degree of arterial hypoxemia during exercise). Thoracic radiographs are not useful in the diagnosis of IAD as no correlation exists among radiographic changes, airway hyperresponsiveness and BALF cytology.45 However, radiographs are useful in ruling out pneumonia, lung abscesses or EMPF. Ultrasonographic examination may demonstrate pleural artifacts but overt airway (luminal) inflammation will not be detected. Note that in horses with IAD secondary to EIPH, pleural changes (comet tails, artifacts) may be worse in the 14-17th intercostal spaces, especially along the dorsal aspect of the lung.46

**TREATMENT OF IAD**

Therapeutic approaches will be similar to those used for RAO and include environmental changes aimed at reducing inhaled dusts. Such measures include wetting the hay, using hay cubes and/or pelleted feed in place of hay; replacing straw bedding with shavings; keeping the horse outside as much as possible (especially during stall/barn cleaning) and wetting down the riding arenas. Airway inflammation, regardless of the predominant cell type, can be controlled by glucocorticoid therapy either systemic or inhaled for 4-6 weeks. (See RAO discussion for dosages). In horses

<table>
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<tr>
<th>Table 1. Mean (SEM) Total Nucleated Cell Counts and Percentages in BALF of Horses with IAD</th>
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<tbody>
<tr>
<td><strong>BALF tncc</strong></td>
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<tr>
<td>IAD 366</td>
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<tr>
<td>Control 153</td>
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<tr>
<td>IAD 590</td>
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<td>Control 530</td>
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<td>IAD 650</td>
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<td>Control 360</td>
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<tr>
<td>IAD 535 (85)</td>
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<td>Control 321 (33)</td>
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* Total nucleated cell counts per μL

Experimental populations
Ref 35: 32 STB racehorses (mean age 3.7 yrs) with poor performance (> 3 months) and endoscopic evidence of inflammation; controls were 2.7 years with adequate performance.
Ref 36: 12 IAD-affected horses (10 STB, 2 TB) with a mean age of 3.5 yrs, had been in full training or racing condition and exhibited exercise intolerance, prolonged respiratory recovery, cough and muco-purulent nasal discharge following post-exercise at least 2 wks prior to clinical examination.
Ref 37: 11 STB horses in full training or racing condition were studied: 5 (mean age 2.6 yrs) had IAD and clinical signs of respiratory disease, poor performance at least 2 wks prior to referral. Controls were 3.5 years of age.
Ref 48: 5 IAD horses (4 research herd, 1 client owned) mean age 17 years had a 6 month history of coughing and endoscopic evidence of increased mucopurulent respiratory secretions.
REFERENCES


