DEVELOPMENT AND RESOLUTION OF PULMONARY ARTERIAL HYPERTENSION IN RAO HORSES

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TITLE

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(ABSTRACT)

Equine recurrent airway obstruction (RAO) is associated with airway inflammation and bronchoconstriction in clinically affected horses. Horses demonstrating severe pulmonary compromise develop concurrent secondary pulmonary hypertension. The development of pulmonary hypertension is well documented in RAO affected horses, however, it is not known how rapidly increases in pulmonary artery pressure occur after the onset of RAO. It is also not known if pulmonary hypertension resolves concurrently with resolution of RAO. The goal of this study was to measure pulmonary artery pressure in RAO affected horses during the development and resolution of RAO. To accomplish this, three RAO affected and three normal horses were placed in a challenge environment where clinical parameters, pulmonary function, right heart and pulmonary artery pressures were measured on day 1, 3 and 5. After evaluating horses on day five, their environment was modified to reduce exposure to respirable debris and anti-inflammatory medication (dexamethasone) was initiated. Identical clinical parameters were measured on days 7 and 9. In our study, the arterial oxygen content in RAO horses was significantly less that that of control horses from day 1 through day 9. A concurrent increase in pulmonary artery pressure also developed on day 3 in RAO affected horses, and persisted through day 5. While some trend towards a difference between groups was noted, no other significant differences were observed between RAO and normal horses. These findings suggest that horses with severe RAO also develop significant increase in pulmonary artery pressure, which rapidly resolves with appropriate management of RAO.

DEDICATION

This work is dedicated to my father, Dr. Robert A. Martin, who lovingly encouraged me to pursue this Master's program at Virginia Tech. I appreciate his guidance, support and wisdom as a father and as a professional.

My wife, Lisa Z. Martin, deserves considerable praise. She loves me unconditionally and has provided unspeakable motivation with her constant encouragement and love. She is my best friend.

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AHR	airway hyperresponsiveness
BALF	bronchoalveolar lavage fluid
C_{dyn}	dynamic compliance
COPD	chronic obstructive pulmonary disease
$ETCO_2$	end tidal carbon dioxide tension
f	respiratory frequency
FiO_2	fraction inspired oxygen
HETE	hydroxyeicosatetraenoic acid
iNANC	inhibitory nonadrenergic noncholinergic
LT	leukotrienes
MCR	mucocilliary clearance rate
NANC	nonadrenergic noncholinergic
O_2sat	oxygen saturation
$paCO_2$	arterial carbon dioxide tension
paO_2	arterial oxygen tension
$p_{(A-a)}O_2$	alveolar to arterial oxygen tension difference
p_AO_2	alveolar oxygen tension
PAP	pulmonary arterial pressure
ΔPpL_{max}	pleural pressure difference between end exhalation and end inhalation
Q_S/Q_T	physiologic shunt fraction
RAO	recurrent airway obstruction
R_L	airway resistance
TX	thromboxane
V_A/Q	alveolar ventilation to perfusion ratio
V_D/V_T	alveolar dead space fraction
V_T	tidal volume
Wb	work of breathing

CHAPTER 1: LITERATURE REVIEW

Normal Equine Anatomy

Lungs

Two pleural sacs line the thoracic cavity and meet in the median plane to form the mediastinum. The mediastinum contains the heart and all other thoracic organs except the lungs which reside within the pleural sacs. [1] In the regions where the mediastinum contains no organs it is relatively thin and may be fenestrated as a result of an underdeveloped or degenerated lamina propria that separates the two pleural layers. Fenestrations are seen mainly caudal to the heart. This part of the mediastinum is pushed far over to the left because of the greater mass of the right lung. [1]

The lungs reflect the general shape of the thoracic cavity and are long and laterally compressed cranially. The root of the lung lies opposite the 6^{th} rib and includes the principal bronchus and the pulmonary artery and veins. The right lung is larger than the left because it includes the centrally located accessory lobe. Inter- and intralobar fissures are absent so that cranial and caudal lobes are separated only by the wide cardiac notch. The lobation of the equine lungs is indistinct. [1]

The tracheobronchial tree begins distal to the larynx and ends at the level of the respiratory bronchioles. These conducting airways deliver air to the alveolar ducts and alveoli where gas exchange occurs. The tracheobronchial tree provides a frictional resistance that opposes airflow and must be overcome by the work of the respiratory muscles. [2] It also forms the anatomic dead space that does not participate in gas exchange. The tracheobronchial tree also protects the lung from inhaled irritants such as dusts and pollutant gases, from antigens and from infectious agents. [2] The equine tracheobronchial tree branches in a monopodal manner and has up to 24 branches. [2,3] In each lung region corresponding to a lobe, there is one major bronchus, which divides multiple times. With each branch the daughter airway is smaller than its parent, which progresses almost directly to the periphery of the lung. [2]

Airways are lined by a mucous membrane, consisting of the epithelium and lamina propria, under which are varying amounts of smooth muscle, and cartilage. [4] Firm support to the airway walls greater than 1-2mm in diameter is provided by cartilage. [2] U-shaped cartilage rings form most of the tracheal wall with smooth muscle bridging the space between the cartilage tips dorsally. In the bronchi, cartilage plates encircle most of the airway but gradually become thinner and disappear toward the periphery. Smooth muscle simultaneously encircles the airway until there is no cartilage, which generally is less than 1 mm in diameter. Then smooth muscle completely surrounds the airway. [2] The pull of the surrounding alveolar septa keeps these small airways, lacking cartilage, patent.

The epithelium in the trachea is predominantly pseudostratified-columnar changing to mostly cuboidal in the bronchi. The epithelial lining contains mucus secreting goblet cells, ciliated cells, and cells with microvilli that participate in fluid and electrolyte exchange. [4] Submucosal glands and goblet cells contribute to mucus secretion that blankets the large airways. The mucociliary clearance mechanisms are provided by the ciliated epithelial cells. [2] The ciliated epithelial cells beat spontaneously and continuously to transport the mucous layer, containing debris, toward the larynx to be swallowed or expelled. The ciliary rate of beating is regulated by changes in the intracellular concentrations of cAMP and Ca^{2+}. A change in the epithelial structure due to disease can severely decrease the ability of the mucociliary clearance mechanism to function.

In the bronchioles, the epithelium is simple cuboidal. There are no goblet cells in the bronchiolar epithelium where the Clara cells function as the secretory cell. Ciliated cells become more sparse as bronchioles near termination. [5] A variety of sensory and motor nerves and blood vessels penetrate the lamina propria to just beneath the epithelium. [2,3] The blood vessels are a plexus of the bronchial circulation, provide nutrients to the airway wall and participate in the inflammatory response in airway disease. [2]

Heart

The heart is divided into four chambers: two atria, which act as priming chambers, and two ventricles, which are the main pumps. The equine heart is cone shaped, but has a blunt apex when relaxed during diastole. The heart is positioned almost vertically on the sternum, (with the long-axis in a dorso-ventral direction) [6] making direct contact with the ribs through the cardiac notches of the lungs from the 3rd to the 4th space on the right. More dorsally there is lung tissue between the heart and chest wall. [1] The weight of the heart depends on the amount of training, in the Thoroughbred the weight of the heart relative to body weight is about 1%. [6]

Blood returns to the right atrium from the caudal vena cava, cranial vena cava and the coronary sinus. From the right atrium blood flows through the tricuspid valve into the right ventricle. The pulmonary circulation is characterized by an extensive capillary network providing surface area for gas exchange and low vascular resistance. Pulmonary veins feed the left atrium with blood returning from the lungs which travels through the mitral valve to the left ventricle and out through the aortic valve to the systemic circulation. Circulation to the conducting airways, interlobular septa and pleura is supplied by the bronchial arterial vascular bed. [6]

In most species, there is a significant pressure difference between the systemic circulation and pulmonary circulation. In humans, a normal systemic arterial pressure mean is about 100 mmHg, while a normal pulmonary arterial mean is about 15 mmHg. [7] This means that the average pressure in systemic circulation is about six times higher than that of pulmonary circulation. This pressure difference is reflected by much thicker walls in the systemic arteries. Pulmonary arteries contain relatively little smooth muscle and look very similar to systemic veins.

The equine cardiac chambers are designed to accommodate the high and low pressure systems found in the systemic and pulmonary circulation. The left ventricle drives blood through a high pressure, high resistance systemic circulation. It, therefore, has a cylindrical shape suited for high pressure pumping. The right ventricle has a large surface area in comparison to its volume and is more suited to low pressure pumping. [6]

Methods of Measuring Right Heart and Pulmonary Arterial Pressures

Cardiac catheterization is a procedure that has been used for decades to determine cardiac and pulmonary pressures. In order to record right-sided hemodynamics, a catheter is passed through the jugular vein and into the right heart and pulmonary artery. Location of the catheter tip may be ultrasound guided or distinguished by the characteristic pressure wave form of the right atria, ventricle or pulmonary artery. [8,9]

Measurement of intravascular pressure in horses is commonly performed by using a fluid-filled catheter system. [10] In this system the intravenous catheter is filled with fluid (ex. heparinized saline) and mounted to an external transducer that measures the pressure exerted by the blood on the fluid-filled system. This is an easy and economical method. However, drawbacks such as electrical noise, signal attenuation, temperature dependent baseline drift, and resonance which requires damping are commonly encountered. [10] Another consideration when using the fluid-filled catheter system is the location of the transducer to establish a zero position, which corresponds with the level of the right atrium where the pressure is approximately zero. One study suggested that several cardiac pressure measurements made in the 1960's and 1970's produced conflicting reports because of selecting different locations of the zero position. [10] Therefore, studies by various workers, using the fluid-filled catheter system to report intracardiac pressures, may differ due to undefined hydrostatic pressures caused by the lack of a universally accepted baseline.

Some problems associated with fluid-filled catheter systems may be overcome by using a microtransducer mounted at the tip or within the catheter. [11] Recent studies that measured equine pulmonary arterial pressure used the microtip transducer within the lumen of the catheter. [9,12] The results are improved especially when a pressure signal needs to be differentiated. [11] However, these systems are expensive and fragile requiring careful manipulation.

Risks are associated with pulmonary arterial catheterization resulting in serious complications and occasionally death. [13] For this reason, non-invasive techniques have been developed in man to measure pulmonary arterial pressure. The most widely accepted noninvasive method for estimating right ventricular and pulmonary arterial pressures has been use of Doppler echocardiography to measure tricuspid regurgitation peak systolic velocity. [14] A simplified version of the Bernoulli equation can then be used to calculate the pressure gradient across this valve.

Bernoulli equation (modified)

$$P = 4 \times V_2^2$$

Where
P = the pressure gradient across the narrowing tricuspid valve
V_2 = the maximum velocity in the narrowing

Optimal recording of peak velocities may not be possible in all cases. Also, significant expertise is required to obtain a complete velocity spectra in patients with minimal tricuspid regurgitation. [14] Currently, there is limited information on the reliability and repeatability of Doppler estimates of the atrial-ventricular pressure difference in horses because it is difficult to obtain an adequate view of the tricuspid valve to permit accurate measurement of tricuspid regurgiation. [15]

Hemodynamics

Equine pressure pulse contours were obtained by Brown and Holmes [10] using micro-manometers. These pulse contours describe the characteristic wave form for the right and

left heart. During cardiac catheterization, the wave forms are often used to verify the location of the catheter as it is manipulated. [11,12,16] This study utilized this method to identify the location of the catheter tip. An idealized pulmonary arterial pressure waveform and a measured pulmonary arterial waveform from one of the control horses is listed in figure1.

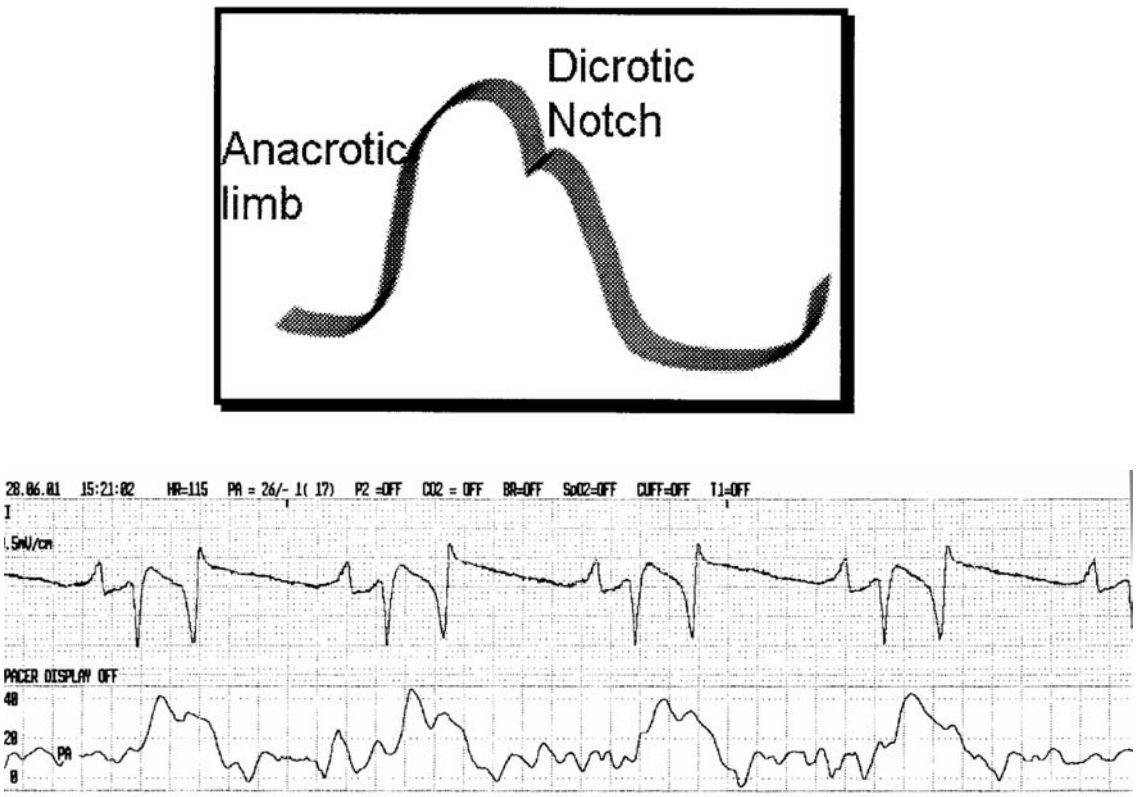

Figure 1Waveforms

This figure demonstrates an idealized pulmonary arterial pressure waveform (top picture) and an actual pulmonary arterial waveform measured from one of the control horses in this study.

For the purpose of research investigation pressures in the right heart and its outflow tracts are measured. Direct measurements of these pressures are made using a catheter. Some normal values of intracardiac and outflow tract pressures in the horse are presented in table 1. [17,18]

Table 1 *Normal Intracardiac and Outflow Tract Pressures in the Horse*

	Arterial (systemic)	Right Ventricle	Pulmonary Artery	Left Ventricle
Systolic (mmHg)	**111.8 ± 13.3**	**45.84 ± 5.76**	**34-48**	**140-148**
Diastolic (mmHg)	**67.7 ± 13.8**	**5.76 ± 3.49**	**14-22**	**15-17**

Ventilation and Perfusion

Ventilation is not uniformly distributed throughout the normal equine lung. Intrapleural pressure changes are not uniform over the thoracic cage, and, because of gravitational effects, pressure is more negative in the dorsal than in the ventral part of the lung. [19] As a result, the dorsal alveoli are more distended, less compliant, and receive less air during inspiration at any ventilatory rate. Also, lack of ventilatory uniformity may be due to the occurrence of inequalities in the regional small airways and alveoli compliance where inhaled air preferentially enters areas of the lung with low resistance and high compliant alveoli. [20] Limitation of the non-uniform changes in regional ventilation may be caused by the interdependence between adjacent lung regions where the mechanical interactions are the result of a network of interconnecting elastic and collagenous tissue fibers. [21]

The equine lungs are perfused by the pulmonary artery, which supplies the gas exchange regions. The distribution of blood flow throughout the lung is influenced by the pressure difference between the pulmonary arteries and the pulmonary veins, and also by the vascular resistance and gravitational forces. Pulmonary vascular resistance is low being estimated at one sixth of the systemic circulation and is equally distributed between the pre- and the post-capillary vessels. Pulmonary vascular resistance may be altered by exercise, lung volume, alveolar hypoxia, emboli, hypotension, vasoactive agents and autonomic regulation. [20,22]

Most equine lung regions have a ventilation/perfusion ratio (V_A/Q) of 0.8. [23] Efficient gas exchange occurs in the lung regions where this ratio is 0.8 to 1.0. In horses at rest, uniformity of regional V_A/Q ratio is demonstrated [23,24] while there is only a mild mismatch in exercising horses. [25] When ventilation is impaired, the V_A/Q ratio decreases, and when perfusion is inadequate the V_A/Q ratio increases. The vertical gradient of pulmonary perfusion in the horse allows for preferential perfusion and decreased V_A/Q ratio in the basal portion of the lung with increased V_A/Q ratio in apical lung regions. This vertical gradient of pulmonary perfusion depends upon the balance between pulmonary arterial, venous, alveolar and interstitial pressures. [23] The proportion of pulmonary venous return not involved in gas exchange due to shunting of mixed blood can be estimated by the physiologic shunt fraction (Q_S/Q_T) which is described by the following equation:

$$Q_S/Qt = (Ci_{O2} - Ca_{O2})/ (Ci_{O2} - Cv_{O2})$$

Where Q_S/Qt refers to physiologic shunt and Ci_{O2} equals the O_2 content of blood draining from capillary beds associated with well-ventilated alveoli. Ca_{O2} is arterial blood oxygen content and Cv_{O2} is venous blood oxygen content. [7]

Each respiratory cycle moves a volume of gas, the tidal volume (V_T), from the environment into the respiratory system. Tidal volume is reported to be 12 ml/kg in horses. [20] The remaining lung volume after exhalation of normal expiration is the functional residual capacity, and after maximal expiration is called residual volume. [22] The product of tidal volume (V_T) and the respiratory frequency (f) is equal to minute ventilation (V_E) and consists of dead space ventilation of conducting airways (V_D) plus alveolar ventilation (V_A) where gas exchange occurs. [20,22]

An overall evaluation of the pulmonary gas exchange can be provided by measurement of arterial blood gas tensions, i.e. PaO_2 and $PaCO_2$. Hypoxemia (PaO_2 lower than 80-84

mmHg) may be the result of limitation at the level of ventilation, perfusion, diffusion or ventilation-perfusion matching. [20] Another factor influencing PaO_2 values is alveolar partial pressure of oxygen. Alveolar partial pressure in O_2 can be evaluated by measuring the end-tidal fraction of O_2 in the expired gas with a mass spectrometer or by using the equation:

$$P_{AO2} = (BP - P_{H2O}) \times F_{IO2} - P_{aCO2}/R$$

where BP is the barometric pressure (around 760 mmHg at sea level), P_{H2O} is the water vapor pressure which varies with the temperature, F_{IO2} is the fractional concentration of O_2 in inspired air (0.21), P_{aCO2} is the partial arterial pressure of CO_2 and R is respiratory exchange ratio which is usually considered to be 0.8. [7,20]

Recurrent Airway Obstruction

Etiology

Equine recurrent airway obstruction (RAO, also know as chronic obstructive pulmonary disease or COPD) is a syndrome results from exposure to one or more components of moldy hay, bedding, or stable dust. [26,27] Equine RAO is considered a multifactorial disease with a genetic component [28] and severity increasing with age (6-10 years). [29,30] Stabling RAO-susceptible animals and feeding them poor quality hay, rich in dust and mold, can repeatedly induce airway obstruction. [28,31] When exposed, RAO susceptible horses respond to the inhaled irritants [32-35] by developing airway inflammation and airway obstruction. [28] The percentage of neutrophils in the BALF of diseased horses is also increased 4-6 fold over healthy horses. [32] Airway obstruction results from bronchospasm and the accumulation of mucus and exudates in the airway lumen associated with inflammation. [36,37] While there is no curative therapy for equine RAO, acute episodes are reversible. Removal of the RAO-susceptible horse from the environmental stimulus, or administration of bronchodilators or corticosteroids leads to clinical remission. [28,38]

The environment of horses is rich in particulate matter that originates in hay, straw, and stable dust. [39] Dust in horse stables contain over 50 species of molds, large numbers of forage mites, endotoxins and inorganic compounds. [36] Herbage stored with 20-30% moisture content is rich with spores of molds and actinomycetes such as *Aspergillus fumigatus, Faenia rectivirgula, and Thermoactinomyces vulgaris*. [28,40] *Micropolyspora faeni* is another predominant etiological antigen found in the northern hemisphere. [31,41] Clinical exacerbations of RAO can be produced by aerosol provocation tests using antigens derived from *M. faeni*, *Aspergillus* or other fungi. [26] Tachypnea, increased minute ventilation, and increased breathing effort is observed in RAO-susceptible horses [28] while control horses are unaffected. [29,40,42] The clinical disturbances can occur within 1-2 hours of exposure, but is more typically noted within 4-10 hours after challenge. [31] Since only RAO-susceptible horses are affected by the natural and specific antigen challenge it is believed that RAO is a pulmonary hypersensitivity to specific antigens rather than a nonspecific response to dusts and irritants in the stable environment. [27,28] Presently, the relative importance of each of these agents in the etiology of RAO is unknown, and it is probable that the pathogenicity of some agents (e.g. molds) is potentiated by the presence of others (e.g. endotoxins). [34]

Clinical signs include mucopurulent nasal discharge, dyspnea, tachypnea, coughing, exercise intolerance and increased expiratory effort. [28,36,43] Wheezes and expiratory crackles are heard upon thoracic auscultation. Weight loss, cachexia and hypertrophy of the external abdominal oblique muscles may occur in severe cases. [43,44] Clinical classification of RAO separates affected horses into stages one to five. Stage 1 includes horses with a history of RAO, but no clinical signs; Stage 2 horses have occasional cough and seasonality of clinical signs; Stage 3 horses exhibit coughing and exercise intolerance with some seasonal improvement of clinical signs; Stage 4 horses have persistent respiratory compromise and effort visible at rest; Stage 5 horses have severe respiratory compromise, tachycardia and poor body condition. [43]

Airway Inflammation

Inflammation is commonly understood to be the basis of almost all the lung changes that occur in chronic airway diseases indicating that all horses with clinical signs of heaves have pulmonary inflammation. [45,46] Inflammation in equine RAO patients is characterized by pulmonary neutrophilia. [46,47] A study of 300 horses, by Dixon et al. [48], found elevated neutrophil ratios in BALF from RAO-affected horses compared to the control horses. Derksen [46] reported that normal horses and RAO-susceptible horses in clinical remission had few neutrophils in bronchoalveolar lavage fluid. While exposure to hay challenge did not affect the neutrophil numbers in BALF fluid obtained from normal horses, the neutrophil numbers markedly increased in horses affected with heaves. [46,49] This increase in pulmonary neutrophils was accompanied by an increase in pulmonary resistance and hypoxemia. [46]

Inhalation of allergens by heavey horses leads to several inflammatory events in the airways, such as neutrophil recruitment and activation. [47] Neutrophil recruitment takes place within a few hours of natural antigen challenge. [50] The neutrophils washed from airways of heavey horses are strongly activated. [51] A number of studies show that the number of neutrophils in the BALF fluid and in tracheobronchial secretions correlates with the severity of the airway obstruction in horses. [47,52-54]

Excessive stimulation of lung macrophages changes the cell's primary function from phagocytosis to the secretion of strong neutrophilic chemo-attractants like IL-8 and MIP-2 [52] and LTB4. [33] Neutrophils migrate into the bronchoalveolar space to improve the elimination of inhaled particles and release and activate proteases, nucleases, peroxidases, nitric oxide, hypochlorite and reactive oxygen species. [52] Strong toxic oxidants, particularly, HOCl, cause tissue damage directly or indirectly through the activation of proteases and the inactivation of protease inhibitors. [55] In the human lungs,

this is considered the most important determinant for tissue injury at sites of neutrophilic inflammation. [56,57]

In a study of 300 horses, by Dixon and coworkers, eosinophils were absent from the tracheal respiratory secretions of 93% of the RAO cases. It is accepted that neutrophilia, not eosinophilia, is a defining feature of inflammation in equine RAO. [52] However, an increase in IgE has been measured in airway secretions from RAO horses, which may suggest a role for mast cells. [42]

During the inflammatory cascade, cytokines and mediators are released that have a variety of effects in the airways. Histamine, one of the known mediators, contracts airway smooth muscle, increases the sensitivity of airway sensory receptors, facilitates neurotransmission at airway autonomic ganglia, and augments the response of smooth muscle to acetylcholine that is released from parasympathetic nerves. [37] Histamine, which is released from pulmonary mast cells, is shown to be in higher concentrations in fluid from pulmonary epithelium of RAO horses following exposure to *Aspergillus fumigatus*, *Alternaria tenuis* and calcium ionophore A23187 in vitro. [58] Although important, the role of histamine in equine RAO is thought to be minor due to the ineffectiveness of treatment of advanced RAO horses with antihistamines. [59]

Allergen inhalation activates the arachodonic acid cascade in the airway mucosa [60] with a significant shift in the lipid mediator profile. [47] This shift results in an increase in proinflammatory mediators such as thromboxane, 15-HETE, leukotrienes [33] and platelet-activating factor. [50,60] Cyclooxygenase blockade prevents the increase in TXB_2 , but it does not affect the changes in pulmonary function and airway reactivity. [28] This suggests that TXB_2 and cyclooxygenase products of arachodonic acid metabolism are not responsible for airway hyperreactivity and alterations in pulmonary function in RAO-affected ponies. [60] Increased mucus secretion and smooth muscle contraction in RAO-

affected horses may be due to an increased production of 15-HETE by airway epithelium and decreased leukotriene production blocking LTB4-mediated neutrophil chemotaxis. [61]

Arachidonic acid metabolites, LTD4 and LTB4, are pro-inflammatory lipids which may play a role in the pathogenesis of RAO. It has been shown that normal horses respond to inhalation of LTD4 with increased pleural pressure, suggesting bronchoconstriction. [33] However, inhaled LTB4 is a potent neutrophil chemotaxant that shows no great effect on bronchomotor tone in the horse. [33] Asymptomatic RAO horses exhibit a reduced effect to these lipids possibly because of a desensitization due to chronic exposure to these leukotrienes. [33]

While the neutrophil and its products are integrated with equine airway inflammation, the importance of their role is currently under criticism. According to Olsweski, long-term airway effects may be caused by the products of neutrophils but the cholinergic component of airway obstruction is probably neutrophil independent. [47] During acute exacerbation of RAO, histamine, LTD4 and 5-HT may explain the mechanisms of increased cholinergic airway tone. These mediators are traditionally associated with a type I allergic reaction that is mast cell derived. [47] However, $ß_2$-sympathomimetics that are potent mast cell stabilizers do not inhibit the inflammatory response to antigen challenge.

Bronchospasm

Bronchospasm is excessive contraction of airway smooth muscle and is a major cause of airway obstruction in equine RAO. Administration of bronchodilators to RAO-affected horses results in a rapid decrease in pulmonary airway resistance (R_L). [28,62,63]
The bronchospasm associated with equine RAO may have multiple causes. Serotonin (5-HT), [47] histamine [45] and leukotrienes C4, D4 and E4 [36] contract airway smooth muscle via activation of their specific muscle receptors. Antihistamines are not useful in the treatment of heaves indicating histamine may not be significant in equine bronchospasm.

[37] Equine bronchial smooth muscle is potently contracted by leukotrienes but they have no effect on tracheal smooth muscle. [64] Mediators like 5-HT, LTD4 and histamine increase the response of smooth muscle to endogenous acetylcholine (Ach). [36,47] Acetylcholine release from parasympathetic nerves is augmented by 5-HT and histamine [47] however, there is no support for an increased airway response to Ach by airway smooth muscle in in-vitro studies. [65,66]

Defective inhibitory control of airway smooth muscle could also participate in the etiology of bronchospasm. Prostaglandin E_2 is a potent inhibitor of smooth muscle contraction. [62] RAO-affected horses show a decline in production of PGE_2 in the airway mucosa [61,65] possibly potentiating the contractile effect of Ach on smooth muscle. [37] The observation that airway mucosal production of PGE_2 is reduced and plasma thromboxane B_2 is elevated suggests that prostaniod production is shifted to favor bronchospasms in RAO-affected horses. [65] The inhibitory nonadrenergic-noncholinergic (iNANC) nervous system, in vitro, is also dysfunctional in RAO horses. [62,65] In control horses, the smooth muscle-inhibitory function of the iNANC system is restricted to the trachea and larger bronchi. [65] Consistently, there is a lack of iNANC function in the larger bronchi of horses acutely affected with RAO. [28,62] Nitric oxide, a smooth muscle relaxant and key component to the iNANC system, [65] is quickly inactivated by reactive oxygen species during the inflammatory process. However, the underlying cause of the dysfunction of the iNANC system remains to be determined.

Bronchospasm does not develop in normal horses when 0.1mg/ml of histamine is inhaled but a RAO-affected horse develops severe airway obstruction upon inhalation of this solution. [28] This reaction is described as airway hyperresponsiveness (AHR) and is usually a non-specific response to the various agents that cause inflammation and bronchospasm. [45] The exact mechanism is unclear, but *in vitro* studies have shown the basis for AHR in horses with heaves involves several mechanisms, including altered acetylcholine release, defective iNANC responses and decreased inhibitory function of

prostanoids. [62,65] Hyperresponsiveness is important because it perpetuates and compounds airway obstruction and also indirectly stimulates release of inflammatory mediators.

Equine pulmonary inflammation is often associated with airway hyperresponsiveness which is defined as excessive active airway narrowing in response to a variety of agonists. Inflammation contributes to airway obstruction through the actions of mediators like histamine, methacholine, and citric acid [28] on neuromuscular regulation, and to structural changes in the airway wall that amplify the effects of bronchospasm. [37] Following a natural challenge RAO-affected horses exhibit nonspecific airway hyperresponsiveness during acute exacerbation via the challenge. [45,50] Hyperresponsiveness develops within 24 h of antigen exposure [28] and may persist for at least 72 h until it wanes with removal of challenge and the resolution of inflammation. [37] The mechanism of this hyperresponsiveness is currently unclear.

Ultrastructure and Histology

Lung

The pathology of equine RAO is not always clearly identifiable. This is possible because the severity and the types of lesions are variable in horses with clinical signs of RAO and because at the level of the bronchioles and the alveoli, the lesions are not homogeneously distributed. Therefore, pathologic lesions may have surrounding regions of tissue that do not exhibit pathologic change. [59]

The principal pathological lesion in RAO-affected horses is bronchiolitis marked by accumulation of intraluminal neutrophils and peribronchial inflammatory cells, predominantly lymphocytes. [28] Other lesions of the lung in RAO-affected horses include degeneration, necrosis and exfoliation of Clara cells, goblet cell metaplasia, intraepithelial lamellar inclusion bodies and gas trapping in affected airways. Destruction of ciliated surface epithelial cells is also seen with mucus metaplasia, alveolar fibrosis,

submucosal and intercellular edema, increased pores of Kohn and necrosis of Type I cells with replacement of Type II alveolar epithelium. [28,36]

In approximately 50% of horses with RAO the severity of the clinical signs agreed with the morphological findings within the large conducting airways. [67] The principal finding is the change within the ciliated cells, which is associated with a corresponding degree of epithelial hyperplasia. Lost ciliated cells are replaced with an undifferentiated type containing numerous apical microvilli. This results in a disturbance in the mucociliary clearance mechanism and an increased mucosal turnover rate. [68] In addition to the loss of ciliated cells, ciliary changes include lack of orientation, absence of microtubules, extra microtubules, swollen cilia, irregular arrangement, and compound cilia. Horses appear to display a greater heterogeneity of ciliary ultrastructure than other species suggesting that ciliary ultrastructural changes may be difficult to interpret. [69] Under light microscopy epithelial hyperplasia and desquamation was detected in large airways along with goblet cell hyperplasia, intraepithelial mucosal cysts, mucosal deposits, pronounced intercellular clefts with an accumulation of mononuclear cells.

Inhalation challenge to RAO-affected horses causes changes in mucus quality and quantity. The mucus secreting goblet cell plays a major role in excess mucus production in the horse due to hyperplasia, whereas in most human chronic pulmonary diseases submucosal gland hyperplasia occurs. [70] It is also believed that stimulation of the goblet cells and submucosal glands by inflammatory mediators such as leukotrienes, histamine and protaglandins along with increased autonomic tone increases mucus production. Changes in mucus quality are due to the destruction of leucocytes and respiratory epithelial cells thus increasing viscosity. The mucopurulent secretions in RAO are not as viscous or as purulent as the secretions of other equine pulmonary infections. [70]

There are various reports concerning the mucociliary clearance rates (MCR) in RAO-affected horses. Normal horses exhibit a MCR of about 2 cm/min. [69] According to this

study by Willoughby, who used scintigraphy after the injection of Tc-99m sulfide colloid into the tracheal lumen, the rates of control versus RAO horses are not significantly different. This differs from the findings of Coombs and Webbon [71] who found the MCR of RAO horses to be 1.39 and 1.14 (cm/min) respectively. Willoughby explains that technical and/or disease processes may account for the different results found in previous studies.

Tight junctions are the main site of hydrophilic solute permeation in the alveolar-capillary bed. They control passive diffusion of solutes and fluid across the membrane and maintain gradients created by active transcellular mechanisms. The tight junctions of the alveolar cells are tighter than that of capillary endothelium indicating the rate of movement of hydrophilic solutes depends upon alveolar cell integrity. [72] No anatomically identifiable changes in the tight junctions or in the sub-epithelial basement membrane were seen. [67] However, the standard method of assessing the airway epithelium membrane permeability is by scintigraphical clearance of nebulized 99m-Technetium from the lung into pulmonary circulation. RAO horses have a significantly faster alveolar clearance rate of 99m-Technetium (4.17%/min) than normal horses (1.8%/min). [73] This is functional evidence of an increase in epithelial permeablity possibly due to epithelial damage caused by the inflammatory response observed in RAO as suggested by BALF results.

Terminal airways are regarded as the starting point of chronic obstructive pulmonary disease. [67] Changes within the terminal airways are distinctly focal in nature, so that in severe cases some areas show no changes. [74] The loss of Clara cell granules was associated with an increase in the endoplasmic reticulum and in severe cases Clara cells were replaced by goblet cells or cells with lamellar intracytoplasmic deposits. Light microscopy of the alveolar region show emphysema, alveolar fibrosis in areas of peribronchiolar inflammation, focal dilatation of alveolar septa due to edema, collagen, elastic fibers and connective tissue cells. [74] Electron microscopy displayed an increase in

Kohn's pores, marked alveolar dilatation, and degenerative changes in type II alveolar cells characterized by fatty change, necrosis and in some cases accumulation of lamellar bodies. [74] Contrary to the large airways, there is a good relationship between clinical diagnosis and morphologic changes found in terminal airways. [74]

Right Heart

Cor pulmonale is defined as right ventricular hypertrophy secondary to chronic pulmonary disease associated with sustained pulmonary hypertension. Cor pulmonale is a common sequelae in human chronic obstructive pulmonary disease with an incidence of right ventricular hypertrophy in 40% of the cases. [75] Severely affected RAO horses consistently develop pulmonary hypertension, but do not develop right ventricular hypertrophy. [37,76,77] Apparently, there are only two descriptions of cor pulmonale in horses with RAO. Salutini [76] noted that RAO-affected horses could die due to cor pulmonale. In the same year, Sporri and Schlatter [78]found on post mortem examination right ventricular hypertrophy of two horses with RAO marked by pulmonary hypertrophy. In general, the literature describes a very low incidence of cor pulmonale in RAO horses although it is unclear if this is due to lack of observation or a truly low incidence.

An anatomical study, by Dixon, of 17 affected RAO horses revealed no gross evidence of right ventricular dilatation or right ventricular hypertrophy. Left ventricular: right ventricular (LV:RV) wall thickness ratios were measured, using the same location for each horse, and no significant difference was found between RAO and control horses. [76] No evidence of cardiac dilatation was seen which is consistent with the low-recorded incidence of cor pulmonale. However, the aortic: pulmonary artery (AO:PA) circumference ratio for controls was 1.04 ± 0.10 and 0.89 ± 0.11 for the RAO affected group. [76] The dilated pulmonary artery in RAO horses is likely attributable to pulmonary hypertension.

Long term studies have shown pulmonary arterial pressures quickly revert to normal limits during remission phases of the disease. [76] The absence of continual pulmonary hypertension may not provide a constant increase in RV workload. This factor may influence the low incidence of RV hypertrophy and development of clinical signs of cor pulmonale in equine RAO. [76] Persistent pulmonary hypertension in human COPD leads to secondary muscular hypertrophy and arteriosclerotic changes in pulmonary arterioles. RAO-affected horses do not display these anatomical changes. [76]

Changes in Pulmonary Function

Compared to controls, horses with clinical RAO have increased respiratory frequency (f), pulmonary resistance (R_L), maximum transpulmonary pressure difference (ΔPpl_{max}), maximum inspiratory and expiratory flow rates, work of breathing (W_b), and expiratory to inspiratory time ratio (E_T:I_T), with decreased dynamic compliance rates and arterial oxygen tension (PaO_2). Tidal volume, in RAO-affected horses, may be decreased [45] or be equivalent to controls. [44,45,79,80] This is possible because RAO horses have a shorter inspiratory and expiratory time as compared to controls. RAO-affected horses recruit the respiratory muscles prior to the peak of the passive expiratory airflow thus smoothing out the normal biphasic tidal breathing flow volume loops found in control horses. [80]

Administration of bronchodilators decreases R_L in RAO horses due to smooth muscle relaxation, [81] but an inconsistent effect on C_{dyn} suggests persisting peripheral airway obstruction attributable to pulmonary inflammation. After environmental change, time to remission positively correlates with age, duration of clinical signs, ΔPpl_{max} and W_b, and negatively correlates with C_{dyn}. [44]

During remission, traditional pulmonary function testing is insufficiently sensitive to detect subclinical airway disease. [82] RAO-affected horses have airway hyper-reactivity to methacholine and significantly faster scintigraphical alveolar clearance rates of 99m-technetium than during remission at pasture, but intermediate values are found when

horses are stabled in a controlled environment. However, clinical examination and pulmonary function testing cannot differentiate between remission at pasture or in a controlled stabled environment. [72]

In the horse, C_{dyn} and R_L are relatively insensitive measurements, despite established use as indicators of pulmonary obstruction, because they rarely fall outside the normal value range until obstruction is so severe that it is clinically apparent. This is particularly true when assessing small airway function. Although small airways comprise most of the lung airway they normally are responsible for less than 20% of R_L. [36] Therefore, in diseases like RAO where most of the pathological change is in the small airways, obstructive lesions are often extensive before significant changes in R_L can be detected. C_{dyn} is an insensitive measurement because it is influenced by body size, lung volume, and breathing frequency and is, therefore, subject to considerable variation. [36]

Hypoxemia in RAO-affected horses is frequently reported. [3,83,84] In horses, the most common mechanism of hypoxemia in pulmonary disease is thought to be an uneven distribution of V_A/Q ratios. [25] Hypoxemia, in horses with RAO, results from ventilation to perfusion (V_A/Q) mismatch and diffusion impairment caused by airway obstruction, increased venous admixture, thickening and fibrosis of the alveolar-capillary membrane, and, in severe cases, alveolar destruction. [83] A study by Nyman and colleagues [83] revealed the presence of high V_A/Q regions and increased dead space, with little or no shunt or perfusion of poorly ventilated regions. Usually right to left shunts are not increased in RAO horses. This is probably the result of increased numbers of pores of Kohn allowing collateral ventilation. [28,74] Much of the increase in minute ventilation is due to an increase in airflow to the deadspace and regions with high V_A/Q ratios. [28] Hypoxic pulmonary arteriole vasoconstriction, inflammatory mediator inducted vasospasm and capillary destruction due to emphysema contribute to the V_A/Q mismatch and reported pulmonary arterial hypertension. [28]

Although airway obstruction results in an increased work of breathing, hypercapnia is not a consistent finding, [45,83,85] but has been reported in RAO horses. [86,87] Hypercapnia can occur with hypoventilation, V_A/Q mismatching or severe diffusion impairment. [86,88] Hypoventilation and V_A/Q mismatch are the two most common causes of hypercapnia in humans with chronic airway disease. [22]

An increase in respiratory rate is noted in COPD-affected horses accompanied by a change in the breathing pattern. [28,80] In severe cases, horses have a short-duration peak of very high expiratory flow followed by low flow at end-exhalation. [80] Because of hypoxemia the horse has an increased respiratory drive resulting in an increased frequency of breathing without a change in tidal volume. [80] Therefore, airflow rates must increase to compensate for the shorter period between breaths. In RAO-affected horses high flow rates occur at the end of inspiration and the beginning of exhalation. The low flows and the increase in pulmonary resistance that occurs toward the end of exhalation may be due to dynamic compression of airways and flow limitation during increased breathing effort. [80]

Hypertension

The pulmonary vascular bed is a high flow, low-pressure circulation system. Six studies reporting pulmonary arterial pressures in normal horses were summarized by Littlejohn and are listed in the following table 2. [8]

Table 2 Pulmonary Arterial Pressures in Normal Horses

Author (Date)	***Systolic (mmHg)***	***Diastolic (mmHg)***
Fisher et al. (1963)	39	16
Eberly et al. (1966)	Mean pulmonary pressure 34 mmHg	
Gall (1967)	36	21
Beltran (1973)	33	10,6

Bergsten (1974)	45	22
Dixon (1978)	45	22

Pulmonary hypertension can occur as a consequence of a variety of diseases such as persistent fetal circulation, left heart disease and chronic obstructive pulmonary disease. [89] When pulmonary hypertension cannot be attributed to any other underlying cause it is termed primary pulmonary hypertension. In humans, this is fatal without heart-lung transplantation but the incidence of primary pulmonary hypertension is low. [89] Secondary pulmonary hypertension is much more common and defines the pulmonary hypertension seen in horses with RAO. Increased pulmonary vascular resistance, abnormally high left atrial pressure and increased blood flow are broad contributing factors that cause pulmonary hypertension. Regardless of the cause, the definitive diagnosis of pulmonary hypertension requires right heart catheterization and direct measurement of PAP. [89] Because cardiac output does not differ from control horses and RAO-affected horses the pulmonary hypertension seen in RAO-affected horses is the result of increased vascular resistance. [83]

Regulation of pulmonary vascular resistance is by alveolar oxygen tension, potassium channels and a variety of locally produced and circulating vasoactive factors. [90] The vasoconstrictor response of pulmonary vessels to moderate hypoxia is a characteristic that distinguishes them from vessels in the systemic circulation. This vasoconstriction is pronounced in the pulmonary arterioles and is also observed in some pulmonary arteries. Voltage-gated potassium channels are implicated in the constrictor response to hypoxia in pulmonary arterial smooth muscle. [90] When these channels are inhibited voltage-gated calcium channels open due to an increase in membrane potential. This results in a rise in cytosolic calcium levels and subsequent vasoconstriction. Respiratory disease also promotes changes in blood concentrations of pulmonary vasoactive factors. Factors produced locally include adrenomedullin, endothelins and nitric oxide. [90] Changes in blood concentration of these and other circulating factors, such as natriuretic peptides and

5-hydroxytryptamine, alter the vasomotor tone of the pulmonary smooth muscle. Ongoing research is working to elucidate the importance of these factors as they relate to pulmonary vascular resistance and secondary pulmonary hypertension.

Increased pulmonary arterial pressure has been consistently described in RAO-affected horses, [8,11,28,31,37,76] the magnitude of hypertension increasing with the severity of disease. [37] Using a fluid-filled catheter system, P.M. Dixon measured the pulmonary arterial pressures in control horses and RAO-affected horses during exacerbation of airway disease and remission. [16] These horses were obtained via referral over a three-year period in which classification of RAO-affected horses was determined by criteria of McPherson and colleagues. [85] Positioning of the catheter was judged by the pulse contours and pressure values observed during manipulation of the catheter. The results of the pulmonary pressure values are listed in Table 3. [16]

Table 3 *Pulmonary Pressures (control and RAO)*

	Number of cases	***Systolic mmHg***	***Diastolic mmHg***	***Mean mmHg***
Control	20	33.77 (± 3.19)	15.08 (± 4.91)	23.54 (± 2.98)
RAO cases symptomatic	25	65.45 (±19.85)	31.01 (±15.61)	44.56 (±13.84)
RAO cases asymptomatic	10	42.25 (± 4.60)	19.25 (± 4.64)	28.13 (± 4.37)

These results are very similar to the reported values recorded by other authors using different catheterization methods. [8]

The pathogenesis of pulmonary hypertension, primary or secondary, is poorly understood even in human patients. [91] The pulmonary hypertension which accompanies lung disease is thought to be the result of at least two processes; the mechanical limitation of flow in

the vascular bed of the diseased lung [8,76] and the pulmonary vasoconstriction induced alveolar hypoxia. [76,89,92,93] There are numerous potential mechanisms involved in these processes such as, alveolar hyperinflation compressing capillaries, endothelial factors, vascular remodeling, inflammatory mediator-induced vasospasm and hypoxic vasoconstriction. [91]

The effects of alveolar hypoxia in chronic lung disease is a major contributor to the development of pulmonary hypertension in man [94,95] due to an uneven distribution of V/Q ratios. [83] In comparison to normal horses, RAO-affected horses have an increased scatter of V/Q ratios and the magnitude of V/Q inequality correlates with the clinical signs and severity of bronchiolitis. [83] Right to left shunts are not usually increased, possibly because of the increased number of pores of Kohn allows collateral ventilation. [28,96] Also, RAO horses have a considerable increase in dead space ventilation and ventilation of high V/Q regions which can lead to as much as 75% loss of the effective alveolar ventilation. [83] Alveolar hyperinflation may be one reason for increased ventilation of dead space. Hyperinflation and disruption of alveolar septa increased pulmonary pressures by compressing or eliminating capillaries in the alveolar bed. [83]

To optimize gas exchange, pulmonary vasoconstriction occurs as a common response to alveolar hypoxia by diverting blood flow away from the most hypoxic lung regions and ensuring that ventilation is matched with perfusion. [12] However, hypoxia-induced pulmonary vasoconstriction (HIPV) may become pathological and lead to an increase in pulmonary arterial pressure. The pulmonary endothelium, among other factors, controls the tone of the vascular smooth muscle through the production of vasoactive mediators. In man, there is an increased production of endothelin-1, a vasoconstrictive peptide, by the pulmonary epithelium in response to hypoxia accompanied by a decrease in production of relaxing factors like PGI_2. [94] Elevated systemic and pulmonary endothelin levels are reported in horses during exacerbation of RAO. [97] However, endothelin has been shown not to be a mediator of HPV in response to acute hypoxia but may be a

modulator in the slower (>10 minutes) phase of HPV. [12] Currently, further study is required to determine the role of endothelin in pulmonary hypertension of the equine RAO patient.

Recent studies in man indicate that pulmonary hypertension in chronic obstructive pulmonary disease should not be regarded as a simple complication of alveolar hypoxia because the remodeling of pulmonary vessels appears to be the principal pathological change. [93] This is suggested because pulmonary hypertension is not resolved when hypoxemia is corrected with acute or chronic oxygen administration. [93] Equine RAO patients do not follow this pattern. Oxygen administration readily reverses pulmonary hypertension in horses to a level close to the control group. [16] Even with partial reversal of hypertension this implies that reduction of hypertension is mainly due to pulmonary vascular hypoxic responses to alveolar hypertension rather than to structural vascular changes. Remission of clinical signs also brings about a reduction in pulmonary hypertension in equine RAO patients. [16,76]

Prolonged pulmonary hypertension may lead to the development syndrome known as cor pulmonale. [76] Cor pulmonale is defined as right ventricular hypertrophy secondary to chronic pulmonary disease associated with sustained pulmonary hypertension. Cor pulmonale is a common sequelae in human chronic obstructive pulmonary disease with an incidence of right ventricular hypertrophy in 40% of the cases. [76] Severely affected RAO horses consistently develop pulmonary hypertension, but do not develop right ventricular hypertrophy. [37,76,77] Apparently, there are only two descriptions of cor pulmonale in horses with RAO. Salutini [76] noted that RAO-affected horses could die due to cor pulmonale. In the same year, Sporri and Schlatter [76] found on post mortem examination right ventricular hypertrophy of two horses with RAO marked by pulmonary hypertrophy. In general, the literature describes a very low incidence of cor pulmonale in RAO horses although it is unclear if this is due to lack of observation or a truly low incidence.

Management

Dust in horse stables contains over 50 species of moulds, large numbers of forage mites, endotoxins and inorganic components. [36] Conventional stabling environments utilize hay feed and straw bedding whereas, a new recommended environment uses wood shaving bedding and a complete pelleted diet. [98] Airborne dust concentration measured in these two different management systems showed that airborne dust concentrations containing *Micropolyspora faeni*, *Aspergillus fumigatus*, and mite allergens, was significantly higher in the conventional method versus the recommended method. [98] Even "good quality" hay contains large amounts of fungal spores. These spores can be airborne for hundreds of miles making elimination of fungal spores impossible from the environment. [99] Therefore, the most favorable long term method of controlling RAO essentially entails minimizing the horse's exposure to the etiological antigens. Remission of clinical signs can be achieved by keeping horses at pasture or a controlled environment. [31,35,44,100] Several studies have demonstrated that environmental changes alone cause improvement in pulmonary lung function and resolution of airway inflammation. [49,100,101] A controlled environment (i.e., bedding horses on shredded paper and feeding a completely cubed diet) caused symptomatic RAO horses to become asymptomatic within 8.4 ± 4.8 days (mean, s.d.). In addition, their pulmonary function values were not significantly different from control horses. [44] In a well-ventilated 4-stall barn, changing the environment of one stall was sufficient to improve lung function within 3 days in RAO patients. Further improvement of lung function and resolution of airway obstruction occurred over the subsequent 30 days on pasture. Thus, any specific horse may experience varying degrees of airway obstruction is possible depending upon the environmental conditions. [100]

Therapy

Owners may have difficulty-keeping animals out of poorly ventilated barns. As a result, drug therapy for RAO-affected horses is essential. However, therapeutic efforts are nearly always unsuccessful unless the environment is altered to minimize exposure to

aerosol antigens. Combination drug therapy and environmental control is the most effective form of RAO management. [59]

Anti-inflammatory drugs are the first pharmacological choice when environmental management is insufficient. As yet, there is nothing more effective than corticosteroids, which, when administered in adequate doses, improves lung function and resolves clinical signs in RAO-affected horses. [58,102] It is important to note that airway inflammation will return following cessation of corticosteroid therapy if exposure to antigens is not minimized or eliminated. [59]

Several mechanisms are involved in the beneficial effects of corticosteroids, and because many of the effects involve gene expression and protein synthesis effects of corticosteroids are not immediate. Administration of corticosteroids decreases the number of circulating lymphocytes, inhibits the cellular migration of neutrophils into the equine lung, [103] and increases the number of peripheral blood neutrophils by increasing release from bone marrow and diminishing removal from the circulation. They also inhibit cytokine production such as IL-2, , inhibit phospholipase A which prevents the production of metabolites, including prostaglandins and leukotrienes, from arachodonic acid, decrease the expression of leukocyte adhesion factors, and inhibit IgE-dependent histamine release. [103] Important effects of corticosteroids on B-adrenoceptors include increasing B-adrenoceptor numbers and their coupling to adenylyl cyclase so that the B-agonist-induced concentration of cAMP is increased in airway smooth muscle. [104] Corticosteroids potentiate the action of beta-2-receptor agonists to improve bronchodilation and inhibit mediator release from inflammatory cells. [59]

Daily administration of i.v. dexamethasone (0.1 mg/kg) to RAO challenged horses caused a significant reduction of airway obstruction within 3 days with improvement persisting through the 10-day stabled treatment period. [102] A significant increase in C_{dyn}

and decreases in R_L and ΔPpl_{max} were observed indicating a dilatation of both large and small airways. Dexamethasone I.V solution also had significant effects of the BALF cell count. By day 10 of this study, the percentage of BALF neutrophils significantly decreased while increasing the percentage of lymphocytes and mast cells compared to that of horses receiving no treatment. [102]

As powerful inhibitors of the inflammatory response, corticosteroids may present undesirable side effects. For example, laminitis, a potentially serious complication, may become apparent weeks after initiation of corticosteroid therapy. [59] Caution should be used with the therapeutic aim being an optimal response with minimal side effects. It is reported that the corticosteroid, dexamethasone, is very effective in relieving airway obstruction in severe RAO-affected horses and during a 10-day treatment period no side effects were observed. [102] In contrast, during the same 10 day trial, no significant clinical effect was observed associated with treatment with prednisone. This observation suggests that prednisone is not an effective treatment of RAO and other inflammatory diseases in horses. [102]

With increased doses, corticosteroids suppress the immune response resulting in increasing susceptibility to respiratory or other infections. Although not common, prolonged use may result in Cushing-like signs including depression, hyperglycemia, polydipsia and polyuria. Also, corticosteroids depress ACTH release from the posterior pituitary. With prolonged use, sudden withdrawal may result in adrenal insufficiency. Prevention of corticosteroid dependency is minimized by an appropriate therapeutic regimen. [59]

In human asthma, aerosolized beclomethasone diproprionate improves clinical signs of airway obstruction, decreases airway hyperresponsiveness, improves peak expiratory flow, increases forced expiratory volume, and often eliminates the need for bronchodilators. [105] In RAO challenged horses, aerosolized beclomethasone improves

clinical signs of disease and pulmonary function test responses. A hand-held metered dose delivery system was designed to effectively provide a uniform pulmonary distribution of drugs. However, the duration of benefit was more prolonged after parenterally administered dexamethasone than aerosolized beclomethasone. [103] This result may be, in part, due to insufficient delivery of aerosolized drug to the lower airway of severe RAO-affected horses.

Aerosolized beclomethasone attenuated pulmonary neutrophilia during a 7-day treatment period to a degree comparable to that of parenterally administered dexamethasone during the same treatment period. [106] However, the BALF fluid neutrophil count did not return to baseline values after corticosteroid treatment. In this population of horses, the pulmonary function response to corticosteroid administration was more complete than the neutrophilic response. [103] Other studies have identified a divergence between severity of pulmonary neutrophilia and pulmonary function abnormalities in RAO-affected horses. [49,107] It appears that pulmonary neutrophilia is resistant to corticosteroid therapy if horses remain in an allergen-challenged environment. [103]

In severe cases, more potent steroids are used. One dose of triamcinolone (20-40 mg/500kg IM) will decrease airway obstruction for several weeks. [104] Dexamethasone is administered at 0.1 mg/kg IV or IM for 2 days then gradually reducing the dose. Prednisone or prednisolone may be considered for use of continued maintenance once obstruction is relieved because prolonged use of dexamethasone or triamcinolone is associated with undesirable side effects, particularly laminitis. [104] Recall, that the efficacy of prednisone or prednisolone is questioned in horses.

Resolution of airway inflammation has been attempted with mast cell stabilizers like sodium cromoglycate. This compound, when given by inhalation, will inhibit both the immediate and late phase inflammatory response to antigen challenge in the susceptible equine lung. [36] The mechanism of action is unknown. The belief that sodium

cromoglycate is simply a mast cell stabilizer is currently being questioned. This is because some $ß_2$ sympathomimetics that are more potent mast cell stabilizers do not display the properties of sodium cromoglycate. It is thought that a likely mechanism is the inhibition of platelet activating factor, which is involved in both early and late phases of pulmonary inflammation. [36] Sodium cromolyn is most beneficial if given to asymptomatic RAO-susceptible horses. It can, however, be given to horses during acute exacerbation of the disease. The clinical effects can last for several days, whereas, cromolyn last a few hours in human patients.

Because bronchodilators improve pulmonary function and clinical signs within minutes of administration they comprise the first-line emergency treatment of acute severe airway obstruction. Bronchodilators effectively relieve bronchoconstriction but do not alter the inflammatory process and duration of response is only a few hours. [81,103] Failure of bronchodilators to fully resolve alterations in pulmonary function likely reflects continued airway obstruction attributable to pulmonary inflammation. [81] The most marked effect of bronchodilators is the reduction in R_L and ΔPpl_{max} but values are not reduced to those found in remission states.

Justification

Since only RAO-susceptible horses are affected by the natural and specific antigen challenge, it is believed that RAO is a pulmonary hypersensitivity reaction to specific antigens, rather than a nonspecific response to dusts and irritants in the stable environment. [27,28] The subsequent inflammation is characterized by pulmonary neutrophilia within a few hours post antigen challenge. [45,47] The most predominant inflammatory response in RAO-affected horses occurs in peripheral airways resulting in bronchoconstriction and airway inflammation. [51] Concurrent mucus plugging also occurs. These events result in restricted ventilation of the small airways and subsequent development of alveolar hypoxia. It is presumed that associated capillary beds constrict, and in the most severely affected horses, pulmonary artery pressures become elevated.

Pulmonary arterial hypertension is consistently described in RAO-affected horses with the magnitude of hypertension increasing with the severity of disease. [37]

The most important treatment for RAO patients is modification of the environment in order to reduce exposure to respirable debris that originates from hay and bedding. [41] Such a change can improve airway function within three to seven days. When environmental management is insufficient, administering anti-inflammatory drugs, particularly corticosteroids, is the most preferred treatment. Corticosteroids, when administered in adequate doses, improve lung function and resolves clinical signs in RAO-affected horses. [58,102] It is likely that the resolution of pulmonary hypertension coincides with resolution of clinical RAO, but the dynamics of this association have not been closely examined. The goal of this study was to repeatedly measure pulmonary artery pressures in RAO affected horses during development and resolution of clinical disease.

Specifically, we tested two hypotheses:

- **An increase in pulmonary arterial and right heart pressures occurs concurrently with onset of severe, acute RAO.**
- **Institution of environmental change and anti-inflammatory medication results in rapid resolution of both RAO and pulmonary arterial hypertension.**

CHAPTER 2: EXPERIMENT

Summary

Pulmonary arterial hypertension is a documented component of equine RAO. This study evaluates the treatment of Recurrent Airway Obstruction (RAO) with the institution of environmental change and anti-inflammatory medication by observing the dynamics of pulmonary pressures and clinical signs of respiratory disease.

A block schedule (days 1-9) was designed with two treatment groups of three RAO-susceptible horses and three controls. Both treatment groups were exposed to a natural hay challenge in a closed barn for a period of 4 days. Each horse was subject to physical exam, measurements of gas exchange, and pulmonary arterial catheterization on days 1, 3, 5, 7, 9. Data was collected on day 1 prior to natural challenge. Arterial and venous blood samples were collected for the purpose of recording blood-gas values. Beginning on day 5, horses were moved to a "reduced" challenge environment and treated with dexamethasone.

The environmental challenge exacerbated the airway diseases in RAO-susceptible horses but not controls. During the challenge, respiration rate and heart rate increased in RAO-affected horses. Compared to controls, the mean pulmonary arterial pressure was significantly elevated in RAO-affected horses on days 3 and 5. Upon administration of the treatment protocol RAO-affected horses demonstrated significant clinical improvement over the following five day period. Heart rate and respiration rate returned to baseline by day seven. The mean pulmonary arterial pressure of RAO-affected horses decreased in accordance with remission of clinical signs. By day nine, the mean pulmonary arterial pressure of RAO-affected horses returned to levels equal to that prior to the natural challenge. The hypercapnia, hypoxia and acidosis also resolved over the five day remission period.

These results indicate that pulmonary arterial pressures increase with clinical signs of RAO in response to environmental challenge. Also, resolution of pulmonary arterial hypertension may be quickly achieved, in an acute phase of disease, following a common treatment protocol of environmental change and dexamethasone administration.

Introduction

Increased pulmonary arterial pressure is consistently described in horses with recurrent airway obstruction (RAO), [8,11,28,31,37,76] with the severity of hypertension increasing proportionally with the severity of clinical signs. [37] As a result of primary lung disease, increased pulmonary arterial pressures occurs secondarily in horses with RAO (also known as chronic obstructive pulmonary disease or heaves). The pulmonary hypertension, which accompanies lung disease, is the result of two primary processes: the mechanical limitation of flow in the diseased vascular bed and the pulmonary vasoconstriction induced by hypoxemia. [7] There are numerous potential mechanisms involved in these processes such as hypoxic vasoconstriction, alveolar hyperinflation compressing capillaries, endothelial factors, vascular remodeling, and inflammatory mediator-induced vasospasm. [37,91]

Hypoxic vasoconstriction is a contributor to increased pulmonary arterial pressures in the horse. [12] However, recent studies in man indicate that pulmonary hypertension in chronic obstructive pulmonary disease should not be regarded as a simple complication of hypoxemia because the remodeling of pulmonary vessels appears to be the principal cause. [93] This is suggested because pulmonary hypertension is not resolved when hypoxemia is corrected with oxygen administration. [93] Equine RAO patients differ because they exhibit partial resolution of pulmonary hypertension with oxygen administration. Dixon and his coworkers, investigated the role of hypoxic vasoconstriction in RAO-affected horses by insufflating these patients with oxygen. [16] Pulmonary arterial pressures (PAP) readily decreased after oxygen administration, but not to the level observed in control horses. This implies that in the acute phase of disease

the hypertension is at least in part due to pulmonary vascular hypoxic responses rather than to structural vascular changes. However, the incomplete resolution of pulmonary hypertension to the level of control horses suggests that other factors like inflammatory mediators causing vasospasm or pulmonary vascular structural changes may contribute to the pulmonary hypertension.

Modification of the environment to reduce exposure to etiological antigens can improve airway function in RAO horses within three to seven days. [44] When environmental management is insufficient corticosteroids and bronchodilators are commonly administered. As yet, there is nothing more effective than corticosteroids, which, when administered in adequate doses, improve lung function and resolve clinical signs in RAO-affected horses. [58,102]

The clinical signs associated with pulmonary hypertension may overlap with those associated with severe RAO. An increase in respiratory effort and exercise intolerance are often observed in humans with primary pulmonary hypertension. [108] Changes in pulmonary function consistent with mild airflow obstruction have also been reported as a sequel of severe pulmonary hypertension. Since increased PAP is a documented component of RAO, it is possible that pulmonary hypertension contributes to the clinical signs associated with RAO.

The purpose of this study was to define the dynamics of pulmonary arterial pressure changes in RAO horses over a period of nine days during which a natural challenge (days 1-5) and a standard treatment protocol of environmental change and dexamethasone administration (days 5-9) was instituted.

Materials and Methods

Study population

Six horses, 5 mares and 1 gelding, were obtained from the VMRCVM research herd. Three control and three RAO-susceptible horses with ages ranging from 8 to 19 years old were included, and horses in each group were age matched within 3 years. Breeds included two Thoroughbreds, two American Quarter Horses, one Saddlebred and one Walking Horse. The diagnosis of RAO was based upon history of disease, physical examination findings, evaluation of a complete blood count, thoracic radiographs and ultrasound, and cytological evaluation of transtracheal and bronchoalveolar lavage fluid. Response to a natural challenge environment consisting of an enclosed barn, straw bedding, and alfalfa hay diet, also contributed to the diagnosis of RAO.

Experimental Design

Two study groups were assigned, RAO-susceptible and control horses, in a generalized block design. Block one consisted of two control and two RAO-susceptible horses with one control and one RAO-susceptible horse in block two. Each block began on day 1 and ended on day 9 with data sampling on days 1, 3, 5, 7, and 9. The block was divided into a challenge period (days 1-5) and a resolution period (days 5-9). The challenge period began after data sampling on day 1. The natural challenge period was designed to induce respiratory disease in RAO-susceptible horses. The challenge was initiated by decreasing ventilation in the barn, bedding all horses on straw and feeding moldy hay twice daily on days 1-4 and the morning of day 5. The resolution period began on day five at 12 noon, after data collection was complete. The resolution period was designed to resolve the respiratory signs by opening doors and windows, changing their diet to a complete pelleted feed BID (Triple Crown Senior) and bedding to low dust shavings. Also,

corticosteroid (dexamethasone 0.1mg/kg IV, SID) therapy was initiated on day 5 and administered for the remainder of the study period.

Assessment of Horses

Prior to initiation and daily during the study period, a complete physical examination of each horse was performed. This examination included measurements of body temperature, heart rate, peripheral pulse quality, assessment of mucous membrane color and capillary refill time, respiratory rate, respiratory character, auscultation of trachea, thorax, and abdomen. Changes in nostril flare, abdominal push, nasal discharge along with coughing were also subjectively assessed observations that were used to determine clinical RAO in susceptible horses versus control horses.

Measurements of gas exchange

Measurements of gas exchange were evaluated on days 1, 3, 5, 7, and 9. Venous and arterial blood gases samples were collected from the jugular vein (venous) and the carotid or facial artery (arterial) in heparinized 3cc syringes, using a 1½ inch, 22G needle. Immediately after collection, the samples were capped to prevent exposure to air, and placed on ice. Samples were analyzed within ten minutes of collection using a portable I-Stat blood gas analyzer (Heska, Fort Collins, CO).

End tidal CO2 was measured by placing a reverse Aeromask (Aerovet, London, Ontario) over the horse's muzzle, and attaching a portable capnograph (Protocol Systems, Inc. Model # 204-EL). Values of end tidal CO2 were recorded for twelve breaths, and the average of the twelve readings were used as that day's value.

The calculated value, alveolar-arterial oxygen tension difference ($P_{(A-a)}O2$), was evaluated using the equation:

$$P_{AO2} = (BP - P_{H2O}) \times F_{IO2} - P_{aCO2}/R$$

where BP is the barometric pressure (around 760 mmHg at sea level), P_{H_2O} is the water vapor pressure which varies with the temperature, $F_{I_{O2}}$ is the fractional concentration of O_2 in inspired air (0.21), P_{aCO2} is the partial arterial pressure of CO_2 and R is respiratory exchange ratio.

Measurements of right heart and pulmonary artery pressure

Prior to the study, a Doppler echocardiogram was performed on each horse to rule out any pre-existing cardiac abnormalities. Right heart catheterization and mean pressure measurements of the right atrium, right ventricle, and pulmonary artery were performed as previously described (Littlejohn 1980). The lower fifth of the jugular groove was clipped and prepared for sterile procedure. A local anesthetic (2 % lidocaine) bleb was injected subcutaneously and a final sterile scrub was performed. A 10G introducer (MILA International, Covington, KY) was placed through the skin and into the jugular vein. A sterile equine cardiac catheter (240 PE tubing) was introduced into the lower fifth of jugular vein, through the right atrium, right ventricle, and into the pulmonary artery. Pressures were measured in each chamber or vessel before the catheter was advanced further. Proper catheter placement was confirmed by the presence of previously described characteristic pressure waveforms. [10] An external pressure transducer was placed at the point of the shoulder, which is considered to be the level of the right atrium. This placement was measured on day one and was the same throughout the study. The transducer was attached to the proximal (external) end of the cardiac catheter with an appropriate sterile extension set filled with heparinized saline. The transducer was then attached to a Propaq CS Vital Signs Monitor (Welch Allyn, Beaverton, Oregon) to display pressure waves, systolic, diastolic, and mean pressures, and cardiac electrical activity. The transducer was then zeroed while exposed to atmospheric pressure. An ECG was attached to the horse using a three lead format. Pulse pressure tracings and pressure measurements were recorded continuously over a 30 second period (no less than 20 cardiac cycles) and pressure values were the average of twenty measurements while exposed to atmospheric pressure.

Statistical Analysis

A generalized block design was used to compare two treatment groups; RAO versus control. Repeated measures Analysis of Variance was performed using The Mixed Model Procedure of the SAS System. Each response variable was tested for main effects of treatment by day interactions. Treatment by day interactions evaluate significance between groups on a single day, not between days. Significant interaction effects were further analyzed using Bonferroni-corrected multiple comparisons. All comparisons were declared significant at $\alpha \leq 0.05$.

Results

All RAO horses showed subjective evidence of clinical disease within 48 hours of being placed in the challenge environment. These signs included increased respiratory effort, abdominal push, nostril flare, coughing, and audible respiratory crackles and wheezes, which worsened with time. Clinical signs of RAO rapidly diminished after day 5, when horses were moved to the remission environment, and treatment with dexamethasone was initiated. In contrast, there were no significant differences between groups in heart rate, respiration rate and rectal temperature. (Table 4)

Compared to controls, horses showing clinical signs of RAO had significantly higher mean pulmonary arterial pressure on day 3 ($p < 0.0001$) of 43.5 mmHg and day 5 ($p < 0.0001$) of 47.6 mmHg. (Figure 2) By day 7, (2 days after initiation of the treatment protocol) and through day 9, there was no significant difference between in PAP between RAO and control horses. The PAP pattern of control horses displayed no significant change for the duration of the block and had an average mean PAP of 20.7 mmHg.

Mean right atrial and ventricular pressure for both treatment groups was normal (<5 mmHg). However, one control subject consistently had right atrial pressures above this

normal reference range. This horse began the study with mean RAP of 8.0 mmHg and did not vary more than 3 mmHg throughout the study.

Horses with RAO had a significantly lower partial pressure of arterial oxygen than control horses starting on day 1 of the study, which persisted through day 7. On day 9, there was no significant difference between these groups.

There was no significant difference in any of the other values that were measured although a trend towards an increase in the $PaCO_2$ and $P(A\text{-}a)O_2$ was noticed in RAO affected horses during the challenge period. (figures 3, 4) These trends reversed when treatment was initiated. Horses with RAO also trended towards a decrease in end tidal CO2, (figure 5) which also reversed with treatment, but these changes were not significantly different from the control group.

Table 4 Physical Exam Data for Control and Diseases Horses

Days	Heart Rate (bpm) Mean +/- SE		Respiration Rate (bpm) Mean +/- SE		Temperature (°F) Mean +/- SE	
	Control	RAO	Control	RAO	Control	RAO
1	35± 5.33	40± 5.33	17± 3.51	19± 3.51	99.4±0.49	98.7±0.49
3	37± 5.33	49± 5.33	26± 3.51	35± 3.51	99.4±0.49	100.1±0.49
5	36± 5.33	58± 5.33	20± 3.51	29± 3.51	99.2±0.49	99.5±0.49
7	39± 5.33	44± 5.33	19± 3.51	26± 3.51	98.8±0.49	98.7±0.49
9	37± 5.33	37± 5.33	19± 3.51	21± 3.51	98.7±0.49	98.7±0.49

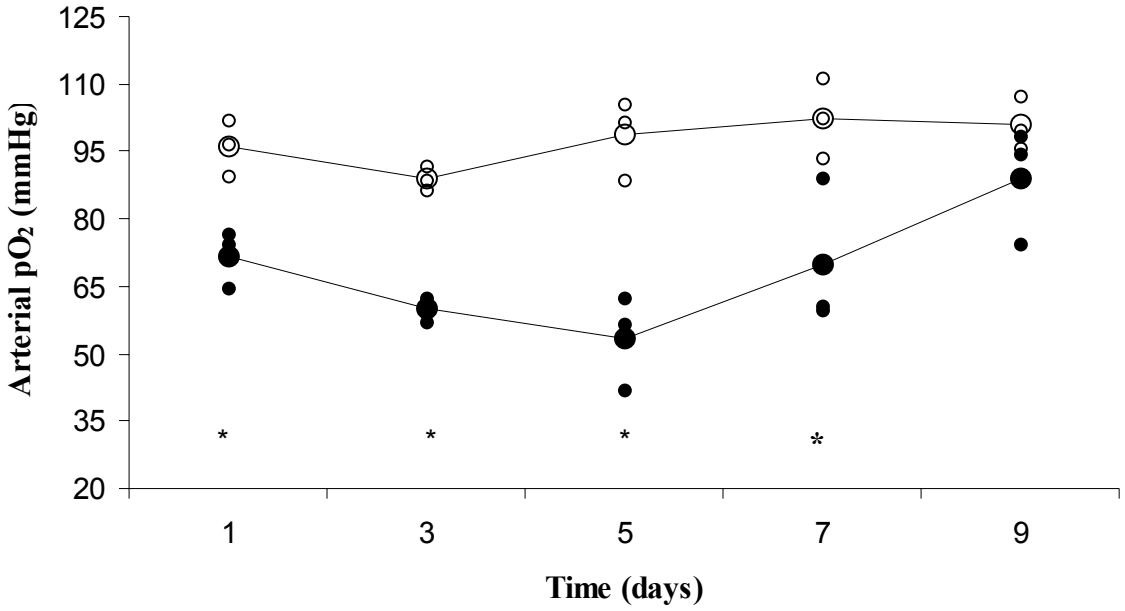

Figure 2 Arterial pO_2 vs. Time

The arterial pO_2 was measured for treatments days 1,3,5,7,9. Horses were maintained in the challenge environment from day 1 to day 5. After horses were evaluated on day 5, the horses environment was changed to minimize aerosolized challenge, and treatment with dexamethasone was initiated. Horses remained in this environment and were treated daily with dexamethasone for the remainder of the experimental period (day 5 thru 9) Values for each RAO horse (•) are given with a line connecting the RAO treatment mean (●). Individual values for controls (○) are listed with a line connecting the control mean (○). The asterisk (*) identifies a significant treatment by day interaction.

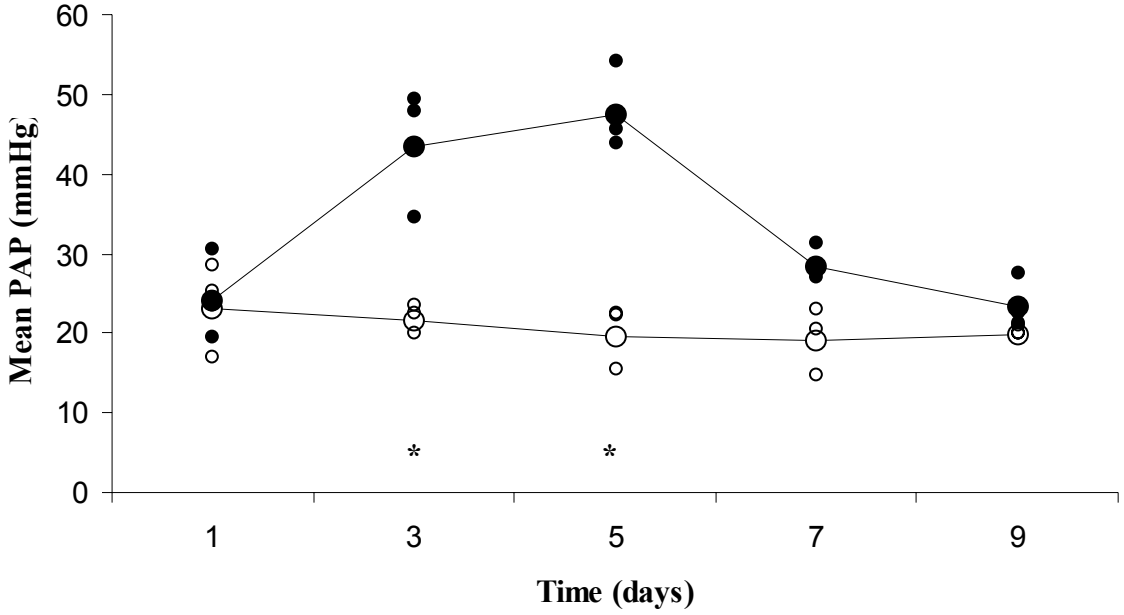

Figure3 *Mean Pulmonary Arterial Pressure vs. Time*

The mean pulmonary arterial pressure was measured for treatments days 1,3,5,7,9. Horses were maintained in the challenge environment from day 1 to day 5. After horses were evaluated on day 5, the horses environment was changed to minimize aerosolized challenge, and treatment with dexamethasone was initiated. Horses remained in this environment and were treated daily with dexamethasone for the remainder of the experimental period (day 5 thru 9) Values for each RAO horse (•) are given with a line connecting the RAO treatment mean (●). Individual values for controls (○) are listed with a line connecting the control mean (○). The asterisk (*) identifies a significant treatment by day interaction.

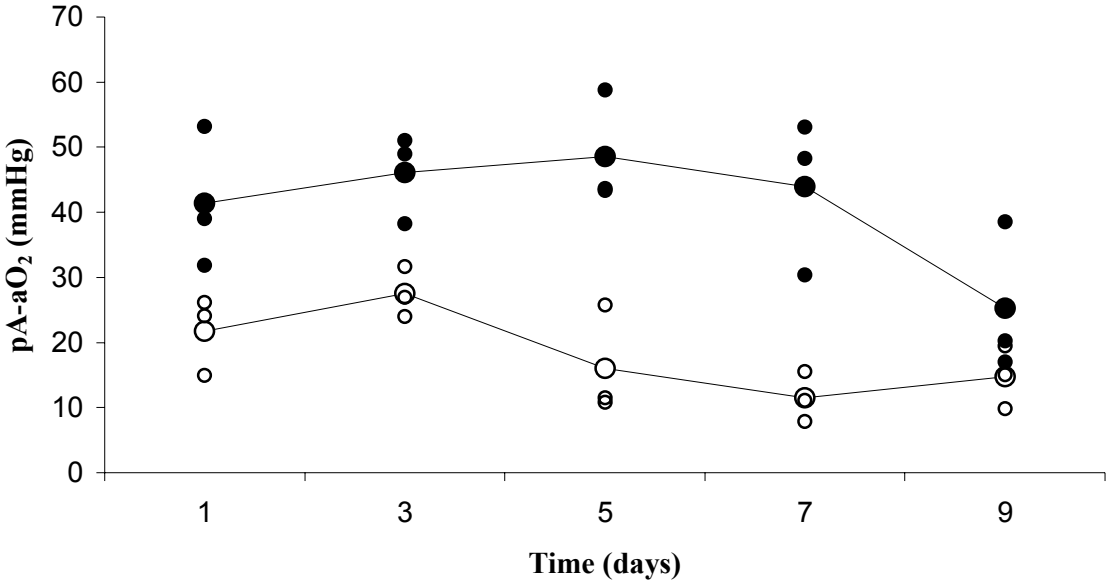

Figure 4 Alveolar-arterial oxygen tension difference vs. Time

The alveolar to arterial oxygen tension difference was measured for treatments days 1,3,5,7,9. Horses were maintained in the challenge environment from day 1 to day 5. After horses were evaluated on day 5, the horses environment was changed to minimize aerosolized challenge, and treatment with dexamethasone was initiated. Horses remained in this environment and were treated daily with dexamethasone for the remainder of the experimental period (day 5 thru 9) Values for each RAO horse (•) are given with a line connecting the RAO treatment mean (●). Individual values for controls (○) are listed with a line connecting the control mean (○).

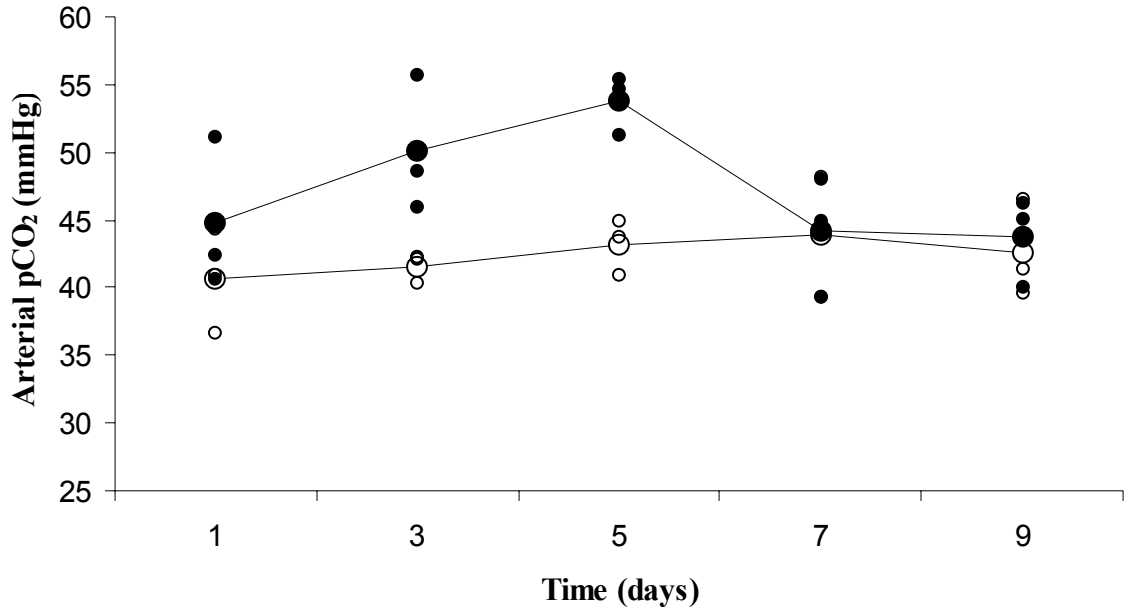

Figure 5 Arterial pCO_2 vs. Time

The arterial pCO_2 was measured for treatments days 1,3,5,7,9. Horses were maintained in the challenge environment from day 1 to day 5. After horses were evaluated on day 5, the horses environment was changed to minimize aerosolized challenge, and treatment with dexamethasone was initiated. Horses remained in this environment and were treated daily with dexamethasone for the remainder of the experimental period (day 5 thru 9) Values for each RAO horse (•) are given with a line connecting the RAO treatment mean (●). Individual values for controls (○) are listed with a line connecting the control mean (○).

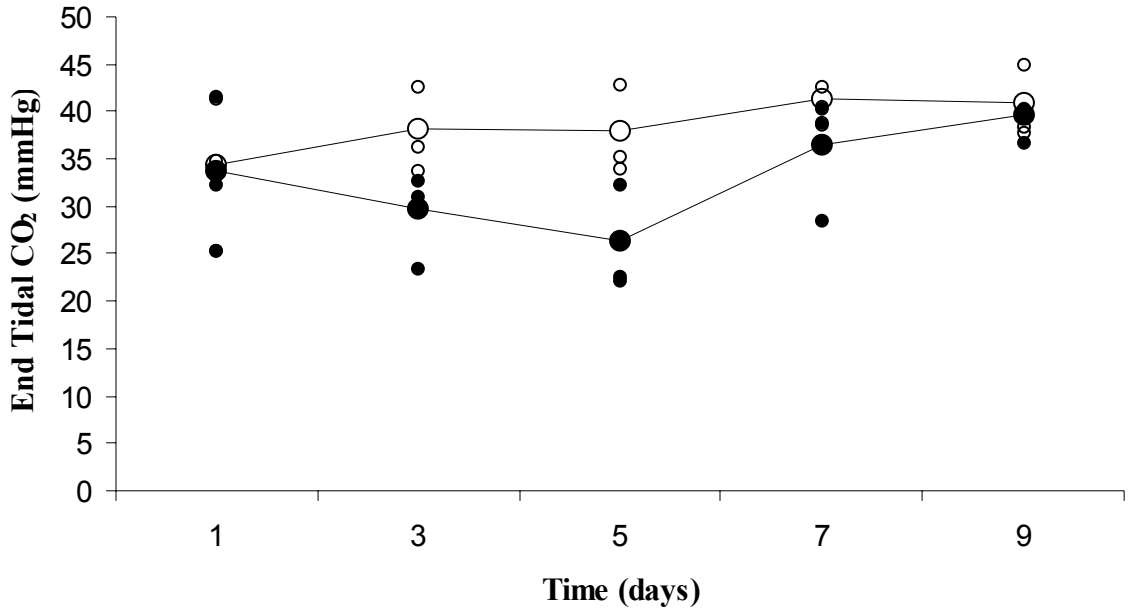

Figure 6 End Tidal CO_2 vs. Time

The end tidal CO2 was measured for treatments days 1,3,5,7,9. Horses were maintained in the challenge environment from day 1 to day 5. After horses were evaluated on day 5, the horses environment was changed to minimize aerosolized challenge, and treatment with dexamethasone was initiated. Horses remained in this environment and were treated daily with dexamethasone for the remainder of the experimental period (day 5 thru 9) Values for each RAO horse (•) are given with a line connecting the RAO treatment mean (●). Individual values for controls (○) are listed with a line connecting the control mean (○).

Discussion

The results of this study confirm that pulmonary hypertension develops rapidly with the onset of acute equine RAO. Even in this small sample population, by day 3, RAO horses demonstrated a significant increase a significant increase in pulmonary arterial pressure as compared to normal horses. With the initiation of environmental change and the administration of dexamethasone, clinical signs of RAO rapidly diminished, and pulmonary artery pressure no longer differed significantly from the control horses. The pulmonary arterial pressures measured in this study during clinical signs of RAO were very similar to values reported by Dixon [16] and Littlejohn [8] for clinically affected RAO horses. Pulmonary arterial pressure of control horses was also consistent with literature. [16] The mean pulmonary arterial pressure of RAO horses in this study is compared to values found by Dixon in table 5.

Table 5 Mean PAP Values Compared with Literature

	Martin (mmHg)	***Dixon (mmHg)***
Control	21.50	23.54 (± 2.98)
RAO asymptomatic		
Day 1	24.14	28.13 (± 4.37)
Day 9	23.37	
RAO symptomatic		
Day 5	47.53	44.56 (± 13.84)

This study is one of the few that measured PaO_2, pulmonary arterial pressures and clinical signs repeatedly. On day 1, RAO-susceptible horses, when compared to controls, had significantly lower PaO_2 levels, and the mean PaO_2 concentration (72 mmHg) was below the lower limit of the normal reference range (82 mmHg). [26] In contrast, there was no significant difference in PAP between groups on day 1. By day 3, horses with RAO developed a significant increase in PAP when compared to controls. The mean PaO_2 also decreased on day 3 to 60 mmHg. While this observation is based on a small

number of horses, it suggests, when PaO_2 concentration approaches approximately 60 mmHg, affected RAO horses may experience increased PAP. A correlation between increased pulmonary artery pressure and decreased PaO_2 has previously been reported in RAO affected horses. [84] Our results are suggestive of a similar relationship.

Previous studies have documented that hypoxia contributes to the development of pulmonary hypertension in equine patients. Dixon discovered oxygen administration partially reverses pulmonary hypertension in RAO affected horses. With cessation of oxygen administration, pulmonary hypertension resumed within minutes allowing the authors to conclude hypoxia was a major factor in its etiology. [16] Dixon measured arterial partial pressure of oxygen and documented an association between increased PAP and decreased PaO_2. Inversely pulmonary arterial pressure returned to near normal as PaO_2 values increased to levels of that prior to the study. Since PAP decreased in response to oxygen therapy, these findings suggest that at least part of the pulmonary hypertension observed in RAO affected horses is due to alveolar hypoxia and vasoconstriction of associated pulmonary capillary beds. [12] This association is presumed to minimize ventilation-perfusion mismatch by shunting blood towards ventilated regions of the lung and maximizing gas exchange. [11] In this study, the PaO_2 in RAO horses was significantly lower when compared to control horses, while there was no difference between pulmonary artery pressures between the two groups. Changes in PAP were not observed until the mean PaO_2 in the RAO group was less than approximately 60-65 mmHg. The concurrent development of hypoxemia and pulmonary hypertension suggests that significant proportion of the lower airways were no longer being effectively ventilated. These findings imply that horses with RAO are more likely to experience concurrent pulmonary hypertension when PaO_2 values reflect significant hypoxemia (PaO_2 <65mmHg)

The partial resolution described in Dixon's article indicates that other factors are involved in the etiology of pulmonary hypertension. These factors are likely to include

inflammatory mediator induced vasospasm and capillary destruction due to alveolar over inflation and emphysema. [28] Another factor shown to shown to sensitize blood vessels to the actions of catecholamines is dexamethasone administration. Therefore, excess glucocorticoids may provoke hypertension. [109] The treatment in this study was aimed at decreasing pulmonary inflammation with appropriate dexamethasone administration plus environmental change.

Dexamethasone administration decreases inflammation associated with equine RAO which is primarily characterized by pulmonary neutrophilia. [36] Neutrophil recruitment and activation takes place within a few hours of natural antigen challenge. [50] Several studies indicate that neutrophilia in the BALF fluid and in tracheobronchial secretions correlates with the severity of the airway obstruction. [47,52] In this study, no attempt was made to document resolution of airway inflammation. However, by products of inflammation have been shown to affect airway smooth muscle function. During the inflammatory cascade, cytokines and mediators are released that have a variety of effects in the airways. Histamine, one of the known mediators, contracts airway smooth muscle, increases the sensitivity of airway sensory receptors, facilitates neurotransmission at airway autonomic ganglia, and augments the response of smooth muscle to Ach that is released from parasympathetic nerves. [37] Histamine, which is released from pulmonary mast cells, is shown to be in higher concentrations in fluid from pulmonary epithelium of RAO horses following exposure to *Aspergillus fumigatus*, *Alternaria tenuis* and calcium ionophore A23187. [58] Although not measured, it is expected that the RAO-horses in this study exhibited a decreased concentration of these inflammatory by-products during the resolution phase as a result of dexamethasone administration.

Inhalation of allergens activates the arachidonic acid cascade in the airway mucosa [60] with a significant shift in the lipid mediator profile. [47] This shift results in an increase in proinflammatory mediators such as 15-HETE and leukotrienes. [50,60] Increased mucus secretion and smooth muscle contraction in RAO-affected horses may be due to an

increased production of 15-HETE by airway epithelium. [61] Arachidonic acid metabolites, LTD4 and LTB4, are pro-inflammatory lipids which may play a role in the pathogenesis of RAO. It has been shown that normal horses respond to inhalation of LTD4 with increased pleural pressure, indicating bronchoconstriction. Lung parenchyma is potently contracted by LTD4 but tracheal smooth muscle does not. Inhaled LTB4 is a potent neutrophil chemotaxant that shows no great effect on bronchomotor tone in the horse. [33] Asymptomatic RAO horses exhibit a reduced response to these lipids possibly because of a desensitization due to chronic exposure. [33]

Resolution of pulmonary inflammation was the primary goal of the treatment protocol. Our results indicate that this treatment protocol concurrently resolves the pulmonary hypertension in RAO horses to levels found in remission and very close to control subjects.

Physical exam findings, including heart rate, respiration rate and temperature, did not correlate with changes in gas exchange and pulmonary arterial pressure in this study. While heart rate and respiration rate increased during the challenge period no significant difference was found between treatment groups. The lack of a statistically significant difference in these parameters between groups may be due to individual variation, and the small number of animals in each group. These findings also suggest that heart rate, respiratory rate, and temperature are insensitive indicators of RAO severity.

While mean right atrial pressure did not differ between groups throughout the study, one horse in the control group consistently had a high mean RAP (8-11 mmHg) while the all other subjects were less than 5 mmHg. This horse began the study with mean RAP of 8.0 mmHg and did not vary more than 3 mmHg throughout the study. A Doppler echocardiogram performed on all horses hearts prior to the study showed no abnormalities. To measure intracardiac pressures, an external pressure transducer was placed at the point of the shoulder, which is considered to be the level of the right atrium.

This placement was measured on day one and was the same throughout the study. Pressures measured throughout this study may be inaccurately influenced by the initial vertical placement of the transducer. If the estimated height at which the transducer was placed was not an accurate assessment of the level of the right atrium, then pressure measurements throughout the study may have been erroneously elevated. Since right atrial pressures are the lowest measured in this study, they would be most dramatically affected by this inaccuracy. The horse displayed no other clinical signs of cardiac dysfunction, so this was considered the most likely cause of the horse's persistently elevated mean right atrial pressure

Although the sample size in this study is very small, significant changes in measured parameters were found. The pattern of pulmonary hypertension, and hypoxemia correlated with severity of RAO and this is consistent with what is reported in the literature. [16,36] In this study, pulmonary hypertension was severe during the natural challenge in RAO-affected horses suggesting that it may be part of the clinical problem associated with airway obstruction. Pulmonary hypertension in equine RAO patients is not routinely treated. Our study showed that treatment with anti-inflammatory drugs and environmental modification rapidly reversed the pulmonary hypertension and RAO clinical signs, suggesting that there is no real need to treat pulmonary hypertension specifically. In humans, hypertension does not always resolve when airway function improves, so it becomes a separate disease that requires therapy. [93] Prolonged pulmonary arterial hypertension in equine patients is not significant because of the reversible pattern observed. Therapy for equine RAO directed at reducing pulmonary inflammation and environmental change resolves the consequences of pulmonary hypertension and the clinical signs of RAO.

At the beginning of the study, RAO-susceptible horses appeared to be in clinical remission. However, PaO_2 values were significantly different between controls and the RAO group on day 1, and persisting until day 9 thus suggesting some level of subclinical

disease. This is evidence that clinical signs are insensitive indicators of subclinical RAO. To determine if RAO-susceptible horses are truly in remission, it may be necessary to include evaluation of blood gas parameters or other parameters of pulmonary function as part of the selection criteria.

In summary, this study demonstrates pulmonary arterial hypertension develops concurrently with clinical signs of RAO. With initiation of a treatment protocol involving dexamethasone administration (0.1 mg/kg IV SID) and environmental change pulmonary arterial hypertension rapidly resolves. Also, complete resolution of pulmonary hypertension may be achieved demonstrating pulmonary arterial pressures equal to values found in remission. Specific therapy aimed at resolving pulmonary hypertension in RAO horses is not needed because of the rapidly reversible nature of pulmonary hypertension.

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