Evaluation of Clinical Methods of Pulmonary Gas Exchange Assessment in the Standing Horse

by

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(ABSTRACT)

There are limited methods of assessing pulmonary function in horses at rest. In this study, we developed clinical techniques to measure gas exchange efficiency in horses. These techniques were then used to evaluate horses with varying degrees of lower respiratory disease. Three groups of horses (Group 1: asymptomatic, n=6; Group 2: symptomatic only with rebreathing, n=11; Group 3: symptomatic at rest, n=9) were selected based on physical exam, transtracheal aspirate, and thoracic radiographs. Blood samples were obtained from the transverse facial artery and jugular vein. Maximal end-tidal CO₂ tension (E₇₆CO₂) was measured by an infrared capnograph through a facemask. Alveolar O₂ tension, alveolar dead space fraction (VₑDB/VₑT), and physiologic shunt fraction (Qₛ/QₑT) were calculated using standard formulas. Horses with both mild and severe signs of lower respiratory disease had significant (p<0.05) differences in gas exchange indices at rest compared to asymptomatic horses.

Albuterol was administered to seven of the Group 2 horses from a metered-dose inhaler through an equine facemask at a dose of 90 μg per 100 kg. Blood samples and tidal gas samples were obtained 15 minutes post-treatment, and Qₛ/QₑT and VₑDB/VₑT were calculated. Albuterol caused significant (p<0.05) hypoxemia 15 minutes following inhaled administration. This was accompanied by a significant increase in Qₛ/QₑT, suggesting that the hypoxemia was due to increases in which the ratios of ventilation to perfusion were decreased.
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List of Abbreviations

$P_{\text{BAR}}$ = Barometric pressure
$p_{\text{H2O}}$ = Water vapor pressure
$paO_2$ = Arterial partial pressure of oxygen
$pAO_2$ = Alveolar partial pressure of oxygen
$pvO_2$ = Venous partial pressure of oxygen
$pmvO_2$ = Mixed venous partial pressure of oxygen
$FiO_2$ = Inspired fraction of oxygen
$paCO_2$ = Arterial partial pressure of carbon dioxide
$EtCO_2$ = End-tidal partial pressure of carbon dioxide
$Hgb_x$ = Hemoglobin concentration of sample $x$ (in gm/dl)
$\%Sat_x$ = Percent oxyhemoglobin in sample $x$
$Q_s/Q_T$ = Physiologic shunt fraction
$V_{DB}/V_T$ = Alveolar dead space fraction
$Cxo_2$ = Oxygen content of sample $x$
$R$ = Respiratory exchange ratio
$EIPH$ = Exercise Induced Pulmonary Hemorrhage
$COPD$ = Chronic Obstructive Pulmonary Disease
INTRODUCTION

Lower respiratory disease is a common clinical finding in the horse. In one study, 54% of randomly selected horses had chronic obstructive pulmonary disease (CCPD) apparent on physical examination.\(^1\) In the same study, only 10% of the horses examined using routine clinical diagnostic techniques had no evidence of lower respiratory disease. The incidence of exercise-induced pulmonary hemorrhage (EIPH) in racehorses has been cited as 10-15% when diagnosed by physical examination,\(^2\) 80% when diagnosed by endoscopy,\(^3,4\) and 90% when diagnosed by microscopic cytology of respiratory secretions.\(^5\) In these examples, the reported incidence of disease increased with the use of more sensitive diagnostic techniques.

The primary function of the lung is the exchange of oxygen and carbon dioxide between the environment and the body.\(^6\) The normal resting horse is quite efficient at this exchange, and this allows the horse to increase the volume of gas exchange in response to exercise. Impairment of the efficiency of gas exchange, on the other hand, can lead to exercise intolerance.

The efficiency of gas exchange is in part due to precise ventilation-perfusion (\(V_l/Q\)) matching.\(^7\) Ventilation-perfusion mismatching is the most
common and important mechanism in gas exchange impairment in lower respiratory disease, and can be demonstrated in horses with subclinical lung disease. Unfortunately, current techniques used to document these conditions are expensive and time-consuming, and are not suitable for routine clinical use.

The physiologic shunt fraction (\(Q_S/Q_T\)) and alveolar dead space fraction (\(V_{DB}/V_T\)) are calculated indices designed to detect changes in ventilation/perfusion matching. \(Q_S/Q_T\) is designed to detect oxygen transfer deficits due to areas of \(V/Q < 1\), extrapulmonary shunting, and diffusion impairment. \(V_{DB}/V_T\) is designed to detect areas of \(V/Q > 1\). Both indices have been used in human respiratory medicine to assist in the detection and quantification of lower respiratory disease.

In this study, we demonstrate clinical techniques for the calculation of \(Q_S/Q_T\) and \(V_{DB}/V_T\) in unsedated horses. The results of these calculations are then compared to the severity of lower respiratory disease found using routine clinical methodology in these horses. To illustrate the usefulness of these indices in the monitoring of treatment, horses with suspected bronchospasm were treated with inhaled albuterol, and the changes in these indices following treatment are reported.
PART 1: EQUINE LOWER RESPIRATORY ANATOMY AND

PHYSIOLOGY

I: Pulmonary Anatomy and Physiology

A. Central Nervous System Control of Ventilation

The central nervous system controls ventilation through a network of neurons within the reticular substance of the brain stem. The inspiratory center is a group of neurons which possess a degree of intrinsic excitability. Impulses from this center increase in a crescendo-like pattern, recruiting inspiratory muscle fibers via the phrenic nerve. This crescendo is offset by inhibitory stimuli from the pneumotaxic center in the pons, and stretch receptors in the pulmonary parenchyma (Figure 1). Inhibitory signals resulting in the end of inspiration may also result from the apneustic center in the pons.

The impulses created in the inspiratory center are transmitted to the phrenic nerves and then to the diaphragm, the primary inspiratory muscle. Electromyography in

Figure 1: Central Control of Breathing
(from Nunn, JF: "Applied Respiratory Physiology")
conscious standing ponies\textsuperscript{10, 11} and horses\textsuperscript{12} has demonstrated electrical activity in the costal diaphragm with each inspiration, and electrical activity in the abdominal muscles coincided with expiration. The latter coincided with the second phase of the biphasic expiratory pattern recognized in horses.

The ventilatory responses to changes in arterial oxygen and carbon dioxide are quite different. Hypoxemia results in preferential stimulation of the diaphragm, and a decrease in stimulation to the expiratory muscles.\textsuperscript{12,13} Hypercapnia results in a greater increase in expiratory muscle activity than inspiratory muscle activity.\textsuperscript{13,14} Differences in the pattern of respiratory muscle activation, with the resultant variation in forced exhalation, may produce variation in the pattern of expiratory gas flow and content.

Environmental temperature and humidity and metabolic heat production can have a short-term effect on the rate and pattern of breathing.\textsuperscript{15} No differences were found in the ventilatory parameters between ponies acclimatized to different environments, but significant increases in both minute ventilation and respiratory rate were observed when ponies that were abruptly moved to different environments. Blood gas values remained constant, and the changes in minute ventilation were primarily due to changes in dead space ventilation.
B. Pulmonary Mechanics

Expansion of the thoracic cavity creates negative intrathoracic pressure, which is relieved by expansion of the lung through the inflow of air.\textsuperscript{16} This requires transmission of the negative intrathoracic pressure from the intrapleural space to the alveoli through the pulmonary parenchyma. The tendency of the pulmonary parenchyma to transmit this force is expressed by the formula $C = \Delta V / \Delta Ppl$, termed compliance.\textsuperscript{17} Tissue with low compliance transmits only a small portion of the pressure applied to it to the airways, resulting in lower volume of air moved per unit of force applied. Lung compliance is the result of interstitial elasticity and alveolar surface tension forces.\textsuperscript{16}

Differences in regional compliance is in part related to the amount of surface tension in the alveoli. According to the Law of LaPlace ($PR = 2T$; in which $P$ is the pressure offsetting the collapse of a sphere, $T$ is the surface tension, and $R$ is the radius of the sphere), an aqueous film will exert a greater surface tension, and require a greater offsetting force to prevent collapse, as the radius of the structure decreases. Due to the properties of alveolar surfactant, the opposite is true in the lung. The effect of surfactant in reducing the surface tension of the watery film on the surface of the alveolus is greatest as the size of the alveolus decreases. This results in a narrow range of surface tensions across a wide range of alveolar sizes.\textsuperscript{17}
The mean alveolar diameter is larger in the dorsal lung, in part due to greater negative pleural pressure in the dorsal lung regions compared to the ventral lung regions.\textsuperscript{19} Due to both the effects of alveolar surface tension and differences in the interstitial tissue, in a normal horse the dorsal lung regions are less compliant than the ventral regions. This results in a larger portion of the inspiratory airflow filling the ventral lung, even though due to the shape of the diaphragm the pleural pressure change is greater in the dorsal region.\textsuperscript{20}

Resistance to airflow in the conducting airways influences movement of air. Resistance to flow is indirectly proportional to the cross-sectional area of the conducting tube (\(R = \left(\frac{8 \times \mu \times L}{r^4}\right)\); in which \(\mu\) is the physical properties of the gas, \(L\) is the length of the tube, and \(r\) is the radius of the tube). Therefore, individual small airways will impart more resistance to flow than larger airways.\textsuperscript{21} The total cross-sectional area of all airways increases dramatically as the airways become smaller, thus the aggregate contribution of the small airways to total airway resistance in the normal horse is small.\textsuperscript{19, 21} No regional differences in the mean diameters of airways of a similar generation have been observed,\textsuperscript{22} suggesting a homogenous distribution of lower airway resistance throughout the lung.

Rigidity of the conducting airways is imparted by cartilage, which are incomplete rings connected by trachealis muscle in the trachea, and
become less organized plates connected by bronchial smooth muscle in the bronchi. In the bronchioles, there is a lack of rigid cartilage, and the smooth muscle is arranged in a spiral fashion. Because of the lack of structural rigidity, these airways are prone to collapse during forced exhalation, particularly during periods of bronchial constriction.\textsuperscript{16}

The balance between regional tissue compliance, airway resistance, and pleural pressure gradient determine the rate of filling and emptying of the alveoli. These processes follow an exponential curve, the shape of which can be represented by a time constant. In normal horses, there appears to be uniformity of time constants throughout the lung,\textsuperscript{23, 24, 25} resulting in synchronous filling and emptying of all alveoli. Changes in regional airway resistance or tissue compliance can affect alveolar time constants, resulting in uneven alveolar filling or emptying.

C. Normal Airway Physiology in the Horse

The mechanical properties of the lung are affected by the airway smooth muscle and mucosal epithelium, which are in turn regulated by the autonomic nervous system. The airway smooth muscle has both cholinergic\textsuperscript{26} and adrenergic\textsuperscript{27} receptors, though little direct adrenergic innervation is present. The distribution of parasympathetic innervation decreases down successive generations of airways.\textsuperscript{28}
Cholinergic stimulation of airway smooth muscle results in bronchoconstriction, but little change in the airway caliber of normal horses is observed following vagotomy,\textsuperscript{29} or administration of atropine,\textsuperscript{30} implying minimal resting bronchomotor tone. Horses with airway constriction due to COPD show prompt improvement in clinical dyspnea\textsuperscript{31, 32} and a decrease in airway resistance\textsuperscript{21, 30} following administration of vagolytic agents, reflecting the activity of the parasympathetic autonomic nervous system during airway disease due to COPD.
Figure 2: Local and Systemic Control of Airway Smooth Muscle; modified from Nunn, JF:
"Applied Respiratory Physiology"

The most important type of adrenergic receptor in bronchial smooth muscle is the β-2 receptor. β-2 adrenergic receptors are primarily bronchodilators, but, due to the lack of resting bronchomotor tone, administration of β-2 adrenergic agonists and antagonists has little effect on pulmonary mechanics in normal horses.²¹, ³³, ³⁴ β-2 adrenergic receptors
also are located presynaptically on postganglionic parasympathetic nerves to augment acetylcholine release. The resulting bronchoconstriction can be observed clinically with high doses of inhaled β-2 agonists.

α-Adrenergic receptors have a minimal role in airway regulation in normal horses, but seem to play a role in the development of obstructive airway disease. α-1 adrenergic stimulation causes bronchoconstriction and signs of airway obstruction in ponies with chronic airway disease, but not in normal horses. α-2 adrenergic inhibition of cholinergic nerves has also been shown in normal horses, and is absent in horses with COPD.

The nonadrenergic-noncholinergic (NANC) branch of the autonomic nervous system may also participate in airway regulation. In other species (humans and cats), this system is believed to act in both an excitatory and inhibitory capacity, though the specific stimuli and transmitters are unclear. The existence of inhibitory NANC pathways have been confirmed in horses, but their precise role in health and disease is not clear.

The mucosa of the larger airways (trachea down to small bronchi) is lined with a dense carpet of ciliated columnar epithelium. In the small bronchi, non-ciliated mucus secreting cells become more numerous, and nonciliated epithelial cells become more prominent in the bronchioles. In contrast to other species, ciliated epithelial cells are present in the last
generation of conducting airways, and can be found adjacent to alveolar cells.\textsuperscript{40}

Ciliary activities are affected by autonomic influences. $\beta$-2 agonists have been shown to both increase ciliary beat frequency and mucociliary transport rate in horses,\textsuperscript{39} whereas anticholinergic agents slow the ciliary beat frequency.\textsuperscript{41} Increases in ciliary beat frequency resulting from stimulation by muscarinic agonists have been measured directly.\textsuperscript{41} Modification of mucociliary clearance is not seen with inhaled anticholinergic agents such as ipratropium.\textsuperscript{28} Control of mucosal secretion by autonomic pathways has been demonstrated in other species, but not in the horse.\textsuperscript{26}

D. Pulmonary Perfusion

The lung receives blood flow from both sides of the heart. The right side of the heart delivers virtually all of its output to the alveolar vascular beds through the pulmonary arteries, arterioles, and capillaries.\textsuperscript{19} Pulmonary arteries are defined as the generation of pulmonary vessels that follow the airways into the pulmonary parenchyma. Initially, the media of the vessel wall consists primarily of elastic tissue, with little smooth muscle.\textsuperscript{42} The media transitions to primarily smooth muscle as the vessels reach approximately 1 mm in diameter. The arterioles are the branches of the
arterial circulation which leave the airways and distribute blood into the alveolar capillary beds. Contrasted to their counterparts in the systemic circulation, they have virtually no smooth muscle but still account for the largest contribution to total pulmonary vascular resistance. The transition from pulmonary arteriole to capillary is marked by the disappearance of the thin elastin media of the arterioles.\textsuperscript{38, 43} The pulmonary vascular bed has lower resistance than the systemic circulation, and therefore lower mean arterial pressure (26 mm Hg vs 124 mm Hg).\textsuperscript{44}

Pulmonary blood pressure regulation is due in large part to the capacitance of the circulatory beds. At rest, there are extensive areas of unperfused and underperfused lung which, as cardiac output increases, are recruited for additional blood flow.\textsuperscript{45} As a result, cardiac output can increase 3-5 fold with only small increases in mean pulmonary arterial pressures.\textsuperscript{46} This reserve, however, is usually insufficient to completely prevent increased pulmonary vasculature pressure in the athletic horse, in which increases in pulmonary arterial pressures to 80 mm Hg or greater have been measured during exercise.\textsuperscript{47, 48}

The ability of the pulmonary vasculature to withstand the physical stress occurring during exercise has received considerable attention since the early 1970's, when it was demonstrated that the source of bleeding in horses with EIPH was the lung.\textsuperscript{2} During exercise, the mean pressures of the
pulmonary capillaries have been estimated to reach 62 mm Hg.\textsuperscript{45} West has shown in vitro that rabbit pulmonary capillaries can only withstand approximately 40 mm Hg before rupturing, and using electron microscopy he has demonstrated apparent stress failures in pulmonary capillaries in exercised horses in which the pulmonary arterial pressures reached 100 mm Hg.\textsuperscript{47} Pascoe has further supported the role of the pulmonary circulation in EIPH by demonstrating recovery of latex beads infused into the right side of the heart in BAL fluid from exercising horses.\textsuperscript{49}

The systemic circulatory contribution to lung perfusion is via the bronchial vessels. These vessels arise from the thoracic aorta and supply capillary beds of the large airways. The venous side of these capillary beds has not been demonstrated, and may, in fact, be the pulmonary veins.\textsuperscript{42} Numerous anastomotic channels are formed between the pulmonary circulation and the bronchial circulation.\textsuperscript{50} The physiologic effects of these anastomotic channels depend on their location relative to their respective capillary beds. Anastomoses from the arterial side of the bronchial capillaries potentially expose the pulmonary circulation to high systemic vascular pressures,\textsuperscript{51} whereas bronchial post-capillary anastomoses, while having more of a sparing effect with regards to intravascular pressure, result in oxygen-poor blood mixing with oxygen rich blood in the pulmonary vein.\textsuperscript{50, 52}
The pulmonary vasculature responds to autonomic influences to a lesser extent than in the systemic circulation. As a general statement, stimulation of the sympathetic system will result in vasoconstriction, whereas parasympathetic stimulation results in vasodilation.\textsuperscript{26, 44} The relaxation of pulmonary vascular smooth muscle in horses appears to be dependent on an intact endothelium, as removal of the endothelium results in constriction in response to parasympathomimetic challenge.\textsuperscript{53} Furthermore, there appears to be a difference in effect between dorsal and ventral lung regions, with the dorsal region maintaining prolonged relaxation in response to methacholine, and the ventral region demonstrating brief relaxation before subsequent contraction.\textsuperscript{53}

Reports on adrenergic activity in the pulmonary vasculature in the horse are limited. Inhaled $\beta$-2 agonists result in a transient decrease in arterial oxygen tensions in normal horses and horses with COPD,\textsuperscript{33} which is believed to be due to development of ventilation-perfusion mismatching secondary to $\beta$-2 adrenergic vasodilatation.

The pulmonary vasculature is also sensitive to a variety of circulating and locally produced vasoactive substances. Numerous prostanoids have been examined in the equine pulmonary circulation. Of them, TXA\textsubscript{2} is a potent vasoconstrictor, and PGI\textsubscript{2} a potent vasodilator. PGE\textsubscript{2}, PGF\textsubscript{2\alpha}, and the leukotrienes are mild vasoconstrictors.\textsuperscript{54} In the horse, bradykinin is a
pulmonary arterial vasodilator, and a venous vasoconstrictor. Histamine, via 
H₁ receptors, is a potent pulmonary venous vasoconstrictor. Phenylephrine 
caused vasoconstriction to a lesser degree, and carbachol had no effect.⁵⁵ 
Stimulation of H₂ receptors, on the other hand, causes relaxation of 
pulmonary vascular smooth muscle in the dog.⁵⁶ 

An important aspect of efficient pulmonary function is hypoxic 
pulmonary vasoconstriction (HPV). The existence of an endogenous 
vasoconstrictive mechanism in response to hypoxia has been established,⁴³ 
and it is a potent local control system of perfusion regulation.⁵⁶ The precise 
receptors and mediators have yet to be fully elucidated, though the hypoxic 
pulmonary vasoconstriction reflex appears to require both the intact vessel 
and the surrounding parenchyma.⁵⁶ 

Many vasoactive mediators have been considered and rejected in 
the search for the unique mediator of HPV, but in recent years a consensus 
has been growing for the inclusion of nitric oxide as an intercellular 
messenger critical to HPV. NO is synthesized by the endothelial cells, and 
is a potent smooth muscle relaxer.⁵⁷ It has an extremely short half-life, and 
is readily bound by metalloproteins such as hemoglobin. It is postulated 
that, in the absence of alveolar oxygen, the production of NO is reduced, 
resulting in reflex smooth muscle constriction and redirection of blood away 
from an unventilated alveolar unit.
Despite the magnitude of mechanical forces acting on the equine lung, there is, in the normal horse, excellent ventilation and perfusion matching. Amis and colleagues found that the gradient of perfusion which would be expected due to gravity is matched by a similar gradient in ventilation.\textsuperscript{58} Good V/Q matching has also been demonstrated in the standing horse\textsuperscript{59} and exercising horse\textsuperscript{52} using the elimination of multiple inert gases. This has been attributed to efficient regulation of pulmonary blood flow by HPV.\textsuperscript{59}

F. Gas Exchange and Transport

Gas exchange takes place in the lung at the level of the alveolus. The alveolar membrane, across which gas diffuses, is comprised of a Type I pneumocyte, a thin layer of interstitium, and an endothelial cell. The interstitium contains variable amounts of collagen, elastin, and lymphatic channels, as well as the occasional granulocyte and macrophage. Overall, this barrier, only 0.5 - 1.5 $\mu$ thick, poses little impediment to gas exchange.\textsuperscript{47} The average alveolus has a diameter of only 0.2 mm, and would be prone to collapse due to surface tension forces of the fluid lining were it not for the production of surfactant by Type II pneumocytes. Surfactant reduces the surface tension and deters alveolar collapse.\textsuperscript{43}
Equilibration of gases between the alveolus and capillary involves both the transport of respiratory gases (oxygen and carbon dioxide) across the alveolar membrane and the association or dissociation of chemical compounds which comprise the bulk of total blood gas content. Diffusion of gases across the normal alveolar membrane is quite rapid, allowing complete equilibration of the physical solution of both oxygen and carbon dioxide during rest. Both oxygen and carbon dioxide exist in circulation in chemical complexes; oxyhemoglobin in the case of oxygen, and bicarbonate and carbamino compounds in the case of carbon dioxide. The rate of the reactions which govern these compounds is a significant component of the total equilibration time for each gas.\textsuperscript{60} The coefficient for diffusion of oxygen in the normal, resting horse is 0.45 liters/min/mm Hg; the diffusion coefficient for carbon dioxide is 25 fold greater than that for oxygen.\textsuperscript{44} In a normal subject complete equilibration occurs for oxygen in 25 msec, and equilibration of carbon dioxide is believed to be virtually instantaneous.
G. Blood Oxygen Content

Oxygen is transported in the blood in 2 states: dissolved oxygen and oxygen bound to hemoglobin. The former accounts for only a small percentage of the total blood oxygen content. The solubility constant for oxygen in an aqueous solution is 0.003 ml of oxygen per mm Hg partial pressure of oxygen per 100 ml of solution. 100 ml of plasma with an oxygen partial pressure of 95 mm Hg will contain only 0.285 ml of dissolved oxygen.

The vast majority of oxygen is transported in the blood complexed with hemoglobin. Each hemoglobin molecule consists of four protein chains each possessing a heme complex and a molecule of ferric iron. A complete hemoglobin molecule can carry up to four molecules of oxygen, and a gram of hemoglobin can carry 1.39 ml of oxygen. With a normal blood hemoglobin concentration of 11 gm/dl, 100 ml of blood with fully saturated hemoglobin carries 15.29 ml of oxygen.
\[ C \times O_2 = 1.39 \times Hgb_x \times %_{o}Sat_x + 0.003 \times pxO_2 \]

Figure 4: Oxygen Content Equation (Hgb\(_x\): Hemoglobin concentration; %\(_{o}\)Sat\(_x\): Percentage oxyhemoglobin; pxO\(_2\): Oxygen tension of sample \(x\))

The saturation of hemoglobin with oxygen is dependent upon the \(pO_2\) of the blood. Equine hemoglobin has a oxygen affinity curve which is similar in appearance to human hemoglobin, but has a greater affinity for oxygen at any given \(pO_2\). (Figure 3) The oxyhemoglobin dissociation curve is sigmoid, with large changes in hemoglobin saturation (and therefore oxygen content) between a \(pO_2\) of 30 and 70 mm Hg, and smaller changes above 70 mm Hg.\(^7\) This property facilitates delivery of oxygen preferentially to tissues with oxygen deficits, yet preserves a high degree of oxygen carrying capacity even at low oxygen tensions.\(^6^3\)

Affinity for oxygen is altered in response to changes in \(pCO_2\) or pH, known as the Bohr effect. In the presence of high \(pCO_2\) or low pH, the
affinity of the hemoglobin for oxygen is decreased, and is illustrated graphically as a shift of the oxyhemoglobin affinity curve to the right. (Figure 5) In vivo, this effect allows enhanced release of oxygen from hemoglobin in tissues where increased anaerobic metabolism has resulted in local acidosis.\textsuperscript{60} Equine hemoglobin is less affected than human hemoglobin with respect to pCO\textsubscript{2} and pH.\textsuperscript{61, 64}

Temperature also affects the affinity of hemoglobin for oxygen. (Figure 6) Increased temperature has a similar effect on the oxyhemoglobin dissociation curve as does CO\textsubscript{2} and hydrogen ions, and would presumably also enhance oxygen delivery to the tissues when increased metabolic activity results in metabolic heat production.\textsuperscript{60}

The oxyhemoglobin dissociation curve is moved to the right in the presence of increased levels of 2,3-diphosphoglycerate (2,3-DPG). Increased concentrations of erythrocyte 2,3-DPG have been found in patients with chronic acidosis and anemia, and would result in improved tissue oxygen
delivery. The clinical relevance of alterations in 2,3-DPG are thought to be minor in comparison to the Bohr effect.\textsuperscript{60} Carbon dioxide is transported in numerous forms. Similar to oxygen, only a small percentage of CO$_2$ is transported as dissolved gas. The majority of CO$_2$ is transported as bicarbonate ion and carbamino compounds,\textsuperscript{19} and participates directly in acid-base homeostasis. The elimination of carbon dioxide by the lungs affects acid-base balance, and the lung serves in health as a short term effector of acid-base buffering and in disease as a cause of acid-base derangements.\textsuperscript{44} The release of CO$_2$ from the blood as the blood passes through the alveolar capillary beds results in an increase in blood pH, and respiratory insufficiency with CO$_2$ retention results in respiratory acidosis.

CO$_2$ is transported as carbamino compounds associated with blood proteins, including hemoglobin.\textsuperscript{44} It is the attachment of CO$_2$ to the NH$_3^+$ terminus of the hemoglobin subunits that is responsible for the effect of CO$_2$ on hemoglobin affinity for oxygen (the Bohr effect).\textsuperscript{63} The binding of oxygen to hemoglobin, on the other hand, decreases the affinity of hemoglobin for CO$_2$, known as the Haldane effect.\textsuperscript{60}
II: Equine Lower Respiratory Pathophysiology

A. Mechanisms of Airway Inflammation

Airway responses to noxious agents are mediated by both immune processes common to the other organs, and through pathways specific for the airways. Agents are phagocytosed by macrophages, which present these particles to lymphoid tissues, particularly bronchus-associated lymphoid tissues (BALT) for processing. The phagocytic cells release chemical mediators which result in amplification of the inflammatory response, recruitment of granulocytes, and affect vascular tone and permeability.

Airway inflammation affects mucus secretion. Normal airway mucus is a serous coating (95% water), but with inflammatory stimulus the mucus becomes more viscous, due in part to the presence of cellular DNA. Though the respiratory cilia are able to maintain a normal mucociliary clearance over a rather broad range of mucus viscosities, a decrease in mucociliary clearance is often observed due to the combination of increased mucus viscosity and impaired ciliary function. A decrease in the percentage of ciliated cells is seen with equine chronic airway disease, further impairing mucociliary clearance, whereas the numbers of mucus-secreting Goblet cells increases, resulting in more mucus production.
A prominent feature of airway inflammation is constriction of the airway smooth muscle. This reflex is mediated to a degree by the vagus nerve, as evidenced by the immediate relief of bronchoconstriction using vagolytic agents.\textsuperscript{28, 30, 31} It is believed that the presence of inflammatory mediators stimulates irritant receptors in the submucosa, resulting in neural feedback through pulmonary sensory afferent nerves and efferent tracts in the vagus nerve.\textsuperscript{16}

Histamine is an important mediator of the signs of airway inflammation. Histamine is released from a mast cell as a result of antigen binding to membrane-bound IgE. Following release from mast cells, histamine causes bronchoconstriction, increased airway permeability, and mucus secretion in the small peripheral airways.\textsuperscript{54, 67}

Aerosolized histamine causes decreased dynamic compliance in both normal and hypersensitive horses due to small airway changes,\textsuperscript{68} and causes prolongation of nitrogen washout, indicating the presence of ventilatory inefficiency.\textsuperscript{69} Histamine-induced bronchoconstriction is unrelated to the vagally mediated reflex response,\textsuperscript{30} and the vagolytic properties of atropine do not improve pulmonary mechanics in normal ponies challenged with histamine. Sodium cromoglycate inhibits the release of histamine from sensitized mast cells, and was shown to be effective in the prevention of clinical signs, including increases in ΔPpl and decreases
in paO₂, following natural inhalation challenge with environmental allergens.⁷⁰

The role of prostaglandins in airway inflammation is not completely understood. Airway smooth muscle tone is modified by prostaglandins produced by the airway epithelium and the inflammatory cells present during airway inflammation. Prostaglandin-mediated smooth muscle relaxation is probably the result of mucosal production of PGE₂, and is decreased in horses with airway inflammation.⁷¹ Cyclooxygenase inhibition with flunixin meglumine attenuated the production of thromboxane but did not change the effects of aerosolized histamine on pulmonary mechanics or airway reactivity in ponies with heaves.⁷² Another study showed that cyclooxygenase inhibition with phenylbutazone resulted in attenuation of the effects of intravenous histamine on pulmonary mechanics in ponies with heaves.⁷³ The use of different delivery routes for the histamine challenge may partially explain the differences in results from these two studies.

Furosemide can improve pulmonary mechanics in horses with airway inflammation.⁷⁴ This effect is believed to result from the release of PGE₂ from the airway epithelium. Furosemide was less effective in blocking the effects on pulmonary mechanics of intravenous histamine when compared to phenylbutazone, and attenuated the improvement in pulmonary mechanics seen with phenylbutazone.⁷³
Microscopically, equine airway inflammation is characterized by neutrophil and mononuclear cell infiltrates, often extending into the interstitium.\textsuperscript{75} In horses with COPD, the most severe signs of airway inflammation were found at the terminal bronchioles,\textsuperscript{76} and were correlated with clinical signs of disease severity. Increased numbers of eosinophils is an inconsistent finding in bronchoalveolar lavage fluid, but a common finding in pulmonary tissues.\textsuperscript{77}

B. Effects of Airway Inflammation on Lung Function

Airway inflammation has a deleterious effect on pulmonary mechanics. Resistance to airflow increases due to bronchospasm, accumulation of airway exudate,\textsuperscript{28} and dynamic collapse of non-rigid small airways during forced exhalation.\textsuperscript{21} Compliance of the lung decreases due to small airway obstruction and peribronchial edema. In long-standing cases, fibrosis of the peribronchial tissues can also decrease pulmonary compliance. These changes not only increase the basal work of breathing,\textsuperscript{16, 44, 78} but also alter the pattern of ventilation distribution.\textsuperscript{0, 16, 79, 80, 81}

Activation of phagocytic alveolar and intravascular macrophages by foreign particles can indirectly impair the regulation of perfusion distribution. Activated macrophages produce proinflammatory cytokines which, in turn,
stimulate local leukocytes and endothelial cells to produce vasoactive substances such as prostaglandins, leukotrienes, and kinins. These substances have been incriminated in the development of hypoxemia in equine endotoxemia, and may also be responsible in part for the hypoxemia seen with other types of pulmonary inflammation.

Perfusion derangement may also result from the production of excess nitric oxide. A calcium-independent form of nitric oxide synthase is produced by smooth muscle in response to the proinflammatory cytokine, tumor necrosis factor (TNF). In the horse, a potential source of TNF is the pulmonary macrophage. Production of excess nitric oxide may be a cause of altered perfusion distribution in airway inflammation.

The end result of both impaired ventilation and perfusion distribution is ventilation-perfusion mismatching. V/Q mismatching is considered the most important mechanism in the development of gas exchange impairment, and has been found in horses with COPD, bronchiolitis, and EIPH. In horses with COPD, the decrease in oxygenization is due in part to ineffective alveolar ventilation and carbon dioxide trapping, and in part due to ventilation/perfusion mismatching resulting from inefficient distribution of airflow. In horses with EIPH, V/Q mismatching can be demonstrated primarily in the dorsocaudal lung regions, coinciding with the predominant location of bronchiolitis and hemorrhage.
PART 2: RESPIRATORY EXAMINATION

I: Physical Examination

The most basic measurement of pulmonary function is the physical examination. The recording of the rate, character, and degree of distress and effort in breathing is standard for every respiratory examination.\textsuperscript{7,8} The rate and character of the ventilatory efforts of the patient are controlled by the central nervous system in response to circulating respiratory gas tensions, and are thus an indirect indicator of adequacy of pulmonary gas exchange. However, CNS regulation of ventilation is relatively insensitive to decreases in oxygen tension, and substantial decreases in $\text{paO}_2$ (<70 mm Hg) must be present before detectable changes in respiratory rate and effort are noted.\textsuperscript{9} Additionally, the rate and character of breathing can be influenced by environmental and systemic factors, such as heat, humidity, fever, acid-base derangements, and excitement.

Auscultation is a component of any respiratory examination. Auscultable noise is generated in the airways due to airflow turbulence and explosive equalization of pressure differentials across obstructed airways. The threshold of auscultable noise is dependent on the rate of airflow; therefore, the technique is more sensitive for detection of abnormalities in larger airways where mean rate of airflow is highest. Auscultation can be
enhanced through the use of CO₂ rebreathing to increase the velocity of airflow and improve the detection of airway abnormalities in airways of intermediate diameters. The earliest lesions seen histologically in airway inflammation are in the small airways,\textsuperscript{72,79} and sufficient airflow to generate auscultable noise in the small airways is probably not achieved even with rebreathing exercises.

The assessment of respiratory effort, character, and auscultatory findings is quite subjective, and scoring systems have been used in an attempt to quantitate and standardize the severity of these evaluations.\textsuperscript{88} Unfortunately, the overall subjectivity of some of these measurements and the number of physiologic determinants limits the usefulness of the physical examination. Poor correlation has been observed between clinical signs of dyspnea and the degree of inflammation in the airways.\textsuperscript{89} Poor correlation has also been found between clinical score and most measures of pulmonary mechanics.\textsuperscript{90} The exceptions were ΔPpl and peak expiratory flow rates, which were positively correlated. Clinical signs and laboratory parameters did not correlate well with prognosis for horses with COPD.\textsuperscript{91}
II: Airway Cytology

The examination of airway secretions has improved the detection of lower airway inflammation in the horse. The tracheal aspirate yields a sample representative of the larger airways. The bronchoalveolar lavage (BAL) yields a sample representative of the small airways. The bronchoalveolar lavage has been found to correlate well with histologic criteria of pulmonary disease, whereas the tracheal aspirate has been shown to have variable correlation. With both techniques, examination of the cytologic characteristics of the sample can provide valuable information regarding the nature of airway disease.
III: Thoracic Imaging

Thoracic radiography can assist in the diagnosis of pulmonary disease through the detection of changes in the radiodensity of the pulmonary tissue. Certain patterns of radiodensity have been correlated with specific tissue pathology, as in the case of pulmonary consolidation, bronchiolitis, and alveolar edema. In addition, the detection of focal lesions has been described, such as abscessation and the dorsocaudal distribution of increased radiodensity characteristic of chronic EIPH.

The specific techniques for imaging the thorax in the horse have been described. A range of 75 to 90 kilovolts with an exposure of 64 to 100 milliamphere-seconds is typically required. For ideal clarity and detail, an X-ray generator of considerable power (400-600 ma) is necessary to allow a suitably short exposure time. These machines are found in well-equipped hospitals, but are not available for the ambulatory practitioner.

Ultrasonography can be used in the diagnosis of pulmonary disease. Transthoracic ultrasonography is commonly used to identify presence of pleural effusions, and can occasionally be used to location solid masses immediately adjacent to the visceral pleura. Because sound waves transmitted to aerated tissue produce reverberation echoes, ultrasound can not be used to visualize the deeper portions of aerated lung.
IV: Pulmonary Mechanics

Among the oldest and most common research tests measuring pulmonary function are the measurement of resistance and compliance. Both calculations require the measurement of intrapleural pressure which, though possible to measure directly, is commonly measured indirectly using an intraesophageal balloon.\textsuperscript{20} In the horse, lung compliance is represented by dynamic compliance ($C_{\text{Dyn}}$), which is measured during normal breathing, rather than during breath holding as is done in humans. An indirect method of measuring resistance and compliance has recently been developed using sound wave oscillation to measure the resistance of the respiratory tract, and to mathematically derive compliance.\textsuperscript{97, 98} Changes in the cross-sectional diameter of larger airways are represented by changes in resistance, whereas changes in smaller airways and the lung parenchyma will result in changes in compliance.\textsuperscript{28}

Changes in the measured dynamic compliance due to airway inflammation can be multifactorial, as compliance measurements are directly affected by changes in airway resistance, particularly in the smaller, peripheral airways.\textsuperscript{23} Dynamic compliance decreases with airway inflammation secondary to extension of inflammation into the parenchyma surrounding the airways.\textsuperscript{99} Measured dynamic compliance will vary with increasing respiratory rate (frequency dependence) if there is
nonhomogeneity of regional time constants due to airway resistance. No frequency dependence of dynamic compliance was observed in normal ponies, arguing for uniformity of time constants in normal horses.\textsuperscript{23, 24} The uniformity of time constants in normal horses has also been demonstrated by nitrogen washout studies.\textsuperscript{25}

Measurement of dynamic compliance, total resistance, and change in pleural pressure have been the mainstay in research into the pathophysiology of equine airway disease. Due to the large number of potentially confounding variables which affect these methods, they are considered too insensitive for the detection of subclinical lung disease.\textsuperscript{92, 100, 101}
V: Blood Gas Analysis

The most direct measure of pulmonary function is the arterial blood gas analysis. Arterial blood can be obtained from many different sites on the horse; small, easily accessible arteries are located on the head and hind legs, and the carotid artery can be relocated in experimental horses to a subcutaneous site. A wide range of normal values for arterial pO₂ and pCO₂ have been published. Normal mature horses are considered by some authors to have a paO₂ as low as 84 mm Hg, while other reference texts have cited a paO₂ of 95 mm Hg as typical for an average healthy Thoroughbred. Normal values for paCO₂ are generally cited as 35 - 45 mm Hg.

Hypoxemia is considered to result from one or more of five physiologic processes: low inspired oxygen tension, diffusion impairment, right-left pulmonary shunts, hypoventilation, and ventilation-perfusion mismatching. The last, V/Q mismatching, is considered the most common cause of hypoxemia in the horse. Hypercapnia is generally considered to be caused only by hypoventilation, though severe V/Q mismatching can also lead to hypercapnea.
VI: Ventilation and Perfusion Efficiency

A. Ventilatory Equivalents

Ventilatory equivalents are indices which address the overall efficiency of ventilation with respect to metabolic rate. The oxygen ventilation equivalent is the calculated ratio between the minute ventilation vs the minute oxygen consumption (VE/VO₂), and the carbon dioxide ventilatory equivalent is the similar ratio between minute ventilation and minute CO₂ production (VE/VCO₂). In both cases, the lower the ratio, the more efficient the usage of minute ventilation with respect to gas exchange. Horses compare favorably with other species, despite having a relatively larger percentage of dead space, indicating efficient gas exchange. Ventilatory equivalents have been used to demonstrate a mild improvement in ventilatory efficiency in response to training in adult horses. Abnormalities in these variables have not been attributed to any specific pathologic process; indeed there are numerous factors which may affect these ratios.

B. Inert Gas Washout Tests

Nitrogen washout tests have been used to determine the relative efficiency of ventilation in horses. Under normal conditions breathing room air, the body tissues are at equilibrium with the atmosphere with respect to
nitrogen. In this test, pure oxygen,\textsuperscript{79, 105} or oxygen mixed with argon,\textsuperscript{25} is inspired through a non-rebreathing valve, and the expired gas is collected in a spirometer to a predetermined volume. When this volume is achieved, the concentration of nitrogen is measured in the next expiration.\textsuperscript{105} In general, the higher the concentration of nitrogen in the expired air, the worse the ventilation efficiency.\textsuperscript{79} High concentrations have been correlated in horses with increased pulmonary resistance and, presumably, disease.\textsuperscript{79} These results agree with those of another study, which also documented an improvement in nitrogen washout parameters following administration of a bronchodilator to horses with COPD.\textsuperscript{105} Good correlation between nitrogen washout data and histologic changes was found in a study comparing BAL, pulmonary mechanics, nitrogen washout, and postmortem histology of horses with degrees of disease severity ranging from clinically normal to severely compromised.\textsuperscript{106} Recently, more precise analysis of the expired nitrogen curve of a multiple breath nitrogen washout was used to demonstrate regional alveolar emptying homogeneity in healthy tranquilized horses.\textsuperscript{25} The nitrogen washout tests are noninvasive and require minimal patient cooperation.

Using the same principal as the nitrogen washout tests, multiple inert gas elimination tests have been used to calculate the relative presence of different regions of \textit{V}/\textit{Q} ratios. Six different inert gases with different
solubilities in aqueous solutions are injected intravenously into the patient, and the rate of elimination through the respiratory tract is measured using continuous expired gas sampling. These rates are then used to calculate the relative contributions of high, low, and ideal V/Q areas to overall lung function.\textsuperscript{52, 59} Multiple inert gas studies have been used to demonstrate the increases in ventilation of high V/Q areas and in dead space ventilation in horses with chronic bronchitis.\textsuperscript{80} Increased scattering of midrange V/Q values was also seen. While more precisely demonstrating the relative contributions of V/Q mismatching to overall lung efficiency, as well as defining the contribution of diffusion limitation,\textsuperscript{52} this test does not localize these areas to a specific region of the lung. Additionally, this test is more invasive than the nitrogen washout, and requires considerable amounts of sophisticated equipment. Its use has been restricted to the laboratory setting.\textsuperscript{7}

C. Scintigraphy

Scintigraphic visualization of pulmonary ventilation and perfusion patterns has been used both experimentally and clinically to examine regional derangements in V/Q matching.\textsuperscript{106} Ventilation distribution can be determined using radiolabelled, aerosolized particles,\textsuperscript{106} and radioactive inert gases.\textsuperscript{56} Perfusion scans use either intravenous radioactive gases\textsuperscript{58} or
radiolabelled, macroaggregated albumin.\textsuperscript{8} Using these techniques, typical patterns of V/Q derangement have been identified for horses suffering from EIPH and for horses suffering from COPD.\textsuperscript{8, 81} The equipment, though expensive, is becoming more widespread due to its utility in examining other areas of the horse.\textsuperscript{7} The disadvantages of this technique include the logistical restrictions placed on this technology by regulatory agencies and the cost of the test itself.
PART 3: CLINICAL METHODS OF MEASURING PULMONARY EFFICIENCY

I: Alveolar Dead Space

A. Theory

The alveolar dead space equation is used to estimate the inefficiency of alveolar ventilation. Any tidal gas which enters unperfused or under perfused alveoli does not participate in gas exchange. The measurement of alveolar dead space is an attempt to quantify the relative degree of alveolar overventilation with respect to perfusion by comparing arterial carbon dioxide with end-tidal carbon dioxide. In an ideal lung, all ventilated alveoli are properly perfused, and the mean alveolar pCO₂ is equal to the arterial pCO₂. Alveolar gas which has not participated in gas exchange will have a pCO₂ similar to that of inspired air. The difference between the actual mean alveolar pCO₂ and the ideal mean alveolar pCO₂, when expressed as a fraction of the ideal mean alveolar pCO₂, represents the fraction of alveolar air which did not participate in gas exchange.

The composition of ideal alveolar air cannot be measured directly, so certain substitutions are made to allow calculation of the alveolar dead space. In the ideal situation, the assumption is made that blood CO₂ equilibrates completely with the alveolar gas; this allows the substitution of
pulmonary end-capillary CO₂ for ideal mean alveolar CO₂. If all of the pulmonary circulation participates in gas exchange, then arterial pCO₂ can be substituted for pulmonary end-capillary pCO₂.

The composition of actual alveolar gas is equally difficult to measure directly. In the alveolar dead space equation, it is assumed that the flow of air during exhalation is orderly:

\[
\frac{V_{DB}}{V_T} = \frac{paCO_2 - E\text{t}CO_2}{paCO_2}
\]

Figure 7: Alveolar Dead Space Fraction (paCO₂: Arterial CO₂ tension; E\text{t}CO₂: End-tidal CO₂ tension)

the air moves as a column with the anatomic dead space being exhaled first, followed by the alveolar gas. The pCO₂ of the end-expiratory gas will then approximate the alveolar gas pCO₂. The result of these assumptions is the clinically applicable equation for alveolar dead space, seen in Figure 7.

These assumptions, while necessary logistically, must be examined closely before interpretation of the alveolar dead space fraction can proceed. As previously described, CO₂ exists in blood not only as free dissolved gas, but also in equilibrium with bicarbonate and as dissolved gas complexed with plasma protein. The rate of equilibration depends not only on the solubility of the gas and its ability to diffuse rapidly across the alveolar-capillary interface, but also the rate constants that control the reactions that liberate CO₂ from bicarbonate stores and plasma proteins.
The overall rate appears to be quite fast, since no evidence for the existence of a CO₂ diffusion deficit across the alveolar-capillary membrane has been found in normal patients.¹⁰⁸

The assumption that end-tidal pCO₂ is equal to mean alveolar pCO₂ is more questionable. A normal expiratory capnograph contains a pseudo-plateau, which is thought to represent alveolar air exiting the patient. This plateau is not flat, thus either the terminal air is not purely alveolar, or the alveolar pCO₂ tension is not constant, or both.¹⁰⁸ It is likely that in a patient with high expiratory flow rates or with nonhomogeneous emptying of the alveoli there is enough turbulence in airflow within the large airways that some fraction of anatomic dead space is mixed with alveolar air.¹⁰⁹ It is also conceivable that, even in ideal situations of perfect V/Q matching and homogeneous alveolar emptying, alveolar pCO₂ fluctuates with the respiratory cycle, gradually increasing even during exhalation. Therefore, end-tidal pCO₂ would not necessarily represent mean alveolar pCO₂ even in health, but would represent the gas tensions of the alveoli with the slowest time constants.¹⁰⁸,¹¹⁰

The substitution of arterial pCO₂ for pulmonary end-capillary pCO₂ is perhaps the least controversial. For arterial pCO₂ to be equivalent to alveolar pCO₂, there can be no contribution to blood CO₂ levels by the metabolic activity of the circulating cells and no venous admixture. The
contribution of circulating cells is generally felt to be minimal, and though recognized, is disregarded. Using mathematical models, it is evident that a great magnitude of venous admixture must be present in order to affect the calculation of alveolar dead space (A venous admixture of 10% only raises the \( \text{paCO}_2 \) by 0.7 mm Hg).\textsuperscript{102}

The same equation used for the alveolar dead space fraction has also been referred to as the Bohr dead space fraction. Due to the assumptions that the investigator must make in order to use the measurement, the value calculated by the alveolar dead space equation does not represent any definable lung volume; rather, it represents the sum of many anatomic and physiologic processes within the lung. For this reason, Nunn has suggested the term “Bohr dead space” to distinguish the value calculated using the equation in Figure 7 from values for alveolar dead space obtained using other methods.\textsuperscript{102}

B. Clinical Applications

The alveolar dead space fraction has been used to compare normal horses and horses with COPD.\textsuperscript{107} In this study, values were considerably higher than those previously reported for normal horses \textsuperscript{111} (6.1% vs 2.7%). An explanation for this may lie in the location of the testing. In the 1982 study, testing was conducted in Pretoria, S.A. at an altitude of 1300 meters,
whereas the earlier tests were conducted at sea level. Changes in environmental conditions may have resulted in different respiratory patterns, which may have widened the arterial-ektidtial pCO₂ difference.¹¹⁰

In the same study, normal horses occasionally had negative arterial-ektidtial differences; these values were discarded with the belief that sampling error had occurred. This may not be the case, as Piiper has demonstrated.¹¹⁰ Prolonged ventilatory cycles result in physiologic breath holding, and equilibration of alveolar air with mixed venous blood. If end-ektidal air represents pure alveolar air, the end-ektidal pCO₂ will approximate mixed venous pCO₂, and be 6-8 mm Hg higher than arterial pCO₂.

Horses with COPD had significantly higher alveolar dead space fractions than normal horses.¹⁰⁷ Nunn has suggested that changes in individual time constants of alveolar units is the chief cause of ventilation maldistribution, a factor which may also cause widening in the arterial-ektidtial pCO₂ difference.¹⁰² Thus, increases in alveolar dead space may represent ventilatory maldistribution due to airway obstruction.
II: Physiologic Shunt Fraction

A. Theory

The physiologic shunt fraction is used to estimate the amount of pulmonary venous return which has not participated in gas exchange. The pulmonary arterial blood enters the lungs with a low oxygen content, and in an ideal situation leaves the lung fully saturated with oxygen. The difference between the ideal pulmonary end-capillary oxygen content and the actual arterial oxygen content, when expressed as a fraction of the total possible oxygen transfer, is the basis of the physiologic shunt fraction (Figure 8).

The derivation of the $Q_s/Q_T$ equation, similar to the alveolar dead space equation, relies on numerous assumptions. To arrive at a value for ideal pulmonary end-capillary oxygen content, it is first assumed that, in the ideal lung, there is complete oxygen and carbon dioxide equilibration between the pulmonary vasculature and the perfused alveoli. This is generally accepted in the resting horse, but is not true in the exercising horse due to diffusion limitation. Second, it is assumed that in the ideal
\[ pAO_2 = (P_{BAR} - P_{H_2O}) \times F_{iO_2} - paCO_2 / R \]

Figure 9: Alveolar Oxygen Equation (\( P_{BAR} \): Ambient barometric pressure; \( P_{H_2O} \): Vapor pressure of water at patient temperature; \( F_{iO_2} \): Inspired oxygen fraction; \( paCO_2 \): Arterial CO\(_2\) tension; \( R \): Respiratory exchange ratio)

Lung all of the pulmonary capillary blood participates to a full extent in gas exchange. This assumption allows the substitution of alveolar oxygen tension for ideal pulmonary end-capillary oxygen tension. Similar to the difficulties encountered in the calculation of the alveolar dead space fraction, alveolar oxygen tension can not be directly measured. End-tidal oxygen tension can not be used because it represents both perfused and unperfused alveoli; therefore alveolar oxygen tension must be calculated using the alveolar oxygen equation (Figure 9).

Alveolar oxygen tension differs from inspired oxygen tension due to the effects of humidification, carbon dioxide transfer from the blood to the alveolus, and unequal mass transfer of oxygen versus carbon dioxide. Numerous versions of the alveolar oxygen equation have been used in the study of equine pulmonary gas exchange\(^{104, 112}\) with varying degrees of precision, but all rely on some basic assumptions. The first assumption is the alveolar gas is completely humidified during inhalation. It is also assumed that the alveolar concentration of CO\(_2\) is equal to the arterial
partial pressure of $\text{CO}_2$. Finally, it is assumed that the respiratory quotient of the patient is the value that the examiner assumes (or measures).

The humidification and warming of inspired air occurs as the air passes over the mucous membranes from the nares to the alveoli. Indirect evidence of the complete humidification of inspired air exists in the models of lung injury that can be produced by inadequately humidified air, but no direct evidence supports this contention in the horse. Environment plays a role, with colder, drier air being less likely to be completely warmed and humidified during inspiration; however, the logistical methods needed to accurately determine this in a clinical setting are insurmountable, and it is generally assumed for the sake of expediency that complete humidification occurs.

The carbon dioxide tension of the perfused alveolus is considered to be equal to the pulmonary end-capillary carbon dioxide tension of the alveolar-capillary unit due to the rapid equilibration of $\text{CO}_2$ across the alveolar membrane. Because in the ideal lung all blood participates in gas exchange, the systemic arterial blood $p\text{CO}_2$ is unchanged by venous admixture, and is a reliable representation of mean pulmonary end-capillary, and therefore mean alveolar, $p\text{CO}_2$. This is not necessarily the case with large magnitudes of venous admixture, but is an acceptable substitution for most clinical situations.
The determination of the patient respiratory exchange ratio (R), while feasible, often is not performed. Investigators have reported values for horses at rest of 0.85\textsuperscript{104} and 0.8.\textsuperscript{112} A value based on the patient's metabolic status is usually assumed,\textsuperscript{84} and mathematically results in little variation in the final index. In exercising horses, a steady state is often not achieved, and even measured respiratory quotients may be inaccurate.\textsuperscript{19}

B. Clinical Applications

The physiologic shunt fraction (Q_s/Q_T) is considered to be the "gold standard" in assessing pulmonary gas exchange in a clinical setting.\textsuperscript{113} By taking into consideration the effects of nonlinear oxyhemoglobin dissociation, it is considered more reliable than the alveolar-arterial oxygen gradient (A-aO_2) in the clinical monitoring of human patients, particularly those in which the FiO_2 may be changing.\textsuperscript{113} In studies of equine pulmonary gas exchange, Q_s/Q_T has been used to study the effects of recumbency, in both anesthetized and conscious horses.\textsuperscript{112} Values for Q_s/Q_T have been reported for normal resting horses. Robinson cited 5-8% Q_s/Q_T for normal horses in a review of equine respiratory physiology, but did not characterize the method of patient selection or Q_s/Q_T calculation.\textsuperscript{19} Gillespie has reported values for pure shunt (measured while the patient is breathing 100% oxygen, which technically eliminates simple V/Q matching as a contributing factor) of 5.24 % in normal horses, and 8.9% in COPD
horses.\textsuperscript{114} Littlejohn followed up on this by comparing values obtained for $Q_S/Q_T$ with horses breathing room air and horses breathing 100% oxygen, and found an additional $Q_S/Q_T$ of 10-17% could be attributed to poor V/Q matching.\textsuperscript{115} Nyman, however, found much lower values for $Q_S/Q_T$ measured in normal horses while breathing room air.\textsuperscript{80} In these studies, saturation coefficients for equine hemoglobin were not used.
III: Objectives

Because it is suspected that subclinical equine lower respiratory disease contributes to poor exercise performance, improved diagnostic tests are needed to detect subclinical disease, and improve the monitoring of therapy. In this project, the clinical methodology for collecting the data to use in the calculation of the physiologic shunt fraction and alveolar dead space fraction is reported. The values for these indices of gas exchange efficiency in horses which were rigorously screened for the absence of lower respiratory disease are also reported and differences in these indices in horses with lower respiratory disease are demonstrated. The changes observed in the dissolved blood gas tensions and indices of gas exchange efficiency following treatment of clinically significant lower airway disease with a commonly used bronchodilator (albuterol) are also reported.
MATERIALS AND METHODS

I. Pilot study

Six adult horses were selected from the research population of the Marion duPont Scott Equine Medical Center. The absence of clinical respiratory disease was determined by physical examination, including measurement of vital signs and thoracic auscultation at rest. Arterial and venous pO$_2$, pCO$_2$, and pH were measured using a clinical blood gas analyzer$^a$. Arterial blood was drawn anaerobically from the transverse facial artery using a 25 gauge, 5/8 inch needle and a heparinized 3 cc syringe. Venous blood was drawn from the jugular vein using a 20 gauge, 1 inch needle and a heparinized 3 cc syringe, respectively. Values were corrected for the subject's rectal temperature. End-tidal carbon dioxide tension was determined using an infrared capnograph$^b$ from a sample continuously aspirated from the trachea through a 20 gauge, 1.5 inch needle placed percutaneously into the trachea at midthoracic level.

Alveolar partial pressure of oxygen (pAO$_2$) was calculated using the alveolar air equation in Figure 9. The respiratory quotient was assumed to be 0.9, and the partial pressure of alveolar water vapor was assumed to be

$^a$ IL 1306 Blood Gas Analyzer, Instrumentation Laboratories

$^b$ Multinex 4100, Datascope Inc.
47 mm Hg. Inspired oxygen fraction was assumed to be 0.208. Barometric pressure was obtained from the blood gas analyzer.

Oxygen content (CxO₂) was calculated using the equation in Figure 3. Saturation of equine hemoglobin was calculated using the algorithms derived for equine oxyhemoglobin dissociation curve. 61 Hemoglobin content was estimated as one third of the horse's hematocrit.

Physiologic shunt fraction (Qś/Qₜ) was calculated using the equation in Figure 8. Ideal end-capillary oxygen content (Cc'O₂) was calculated using the alveolar partial pressure of oxygen as the end-capillary oxygen tension.

Alveolar dead space fraction (VDB/VT) was calculated using the equation in Figure 7. End-tidal CO₂ (EtCO₂) was the subjective mean as determined by observation of the reported end-tidal CO₂ of the capnograph. Inspired pCO₂ was assumed to be 0 mm Hg.

3 horses had measurements performed on three separate days each, for a total of 9 measurements. One horse had two measurements performed, and two horses were measured once each. The average for each index for horses sampled more than once was calculated, and 95% Confidence intervals for all horses were determined for Qś/Qₜ and VDB/VT using a one-sample Student's t Test.

To investigate the suitability of substituting jugular venous oxygen content for mixed venous oxygen content, in a separate study 8 adult
horses had pulmonary arterial polyethylene catheter\(^c\) placed via the jugular vein. Placement of the end of the catheter in the pulmonary artery was confirmed by the appropriate changes in pressure waveform as the catheter was advanced from the jugular vein, through the right atrium, right ventricle, and pulmonary artery. Simultaneous arterial, jugular venous, and mixed venous blood samples were collected and analyzed for dissolved gas tensions as previously described. \(Q_s/Q_T^\prime\) was calculated using the mixed venous oxygen content, and \(Q_s/Q_T\) was calculated using the oxygen content of the jugular blood sample substituted for the mixed venous oxygen content in the physiologic shunt fraction equation. The correlation between the values for \(Q_s/Q_T\) and \(Q_s/Q_T^\prime\) was performed using the Spearman's Rank Sum statistic.

\(^c\)90 French Intramedic PE tubing, Clay Adams Inc.
II. Control Horses

Six horses from the research herd and patient population of the Marion duPont Scott Equine Medical Center were included in Group 1 (Asymptomatic) based on the findings of a complete lower respiratory examination, including physical examination, auscultation while rebreathing, thoracic radiographs, and transtracheal aspiration. All horses were maintained on pasture with occasional supplementation of grain. Body weight and rectal temperature were recorded. For illustrative purposes, clinical examination findings for

<table>
<thead>
<tr>
<th>Physical Examination Score</th>
<th>0 - No abnormalities found at rest and rebreathing 1 - No abnormalities at rest, increased respiratory noise during rebreathing 2 - No abnormalities at rest, intolerance to rebreathing 3 - Increased respiratory noise at rest 4 - Dyspneic at rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal Aspirate Score</td>
<td>0 - Clear, low cellularity, low mucus content 1 - Slightly cloudy, increased mucus content, normal cellularity 2 - Slightly cloudy, increased cellularity 3 - Grossly flocculent, increased cellularity and mucus 4 - Thick mucopus</td>
</tr>
<tr>
<td>Radiographic Score</td>
<td>0 - No abnormalities 1 - Focal increases in bronchial or vascular patterns 2 - Diffuse increases in bronchial or vascular patterns</td>
</tr>
<tr>
<td>Hematology Score</td>
<td>0 - No abnormalities 1 - Leukocytosis or hyperfibrinogenemia</td>
</tr>
</tbody>
</table>

Figure 10: Disease Severity Score
each part of the lower respiratory examination were evaluated based on a severity score for each procedure (Figure 10). Due to the subjective nature of the severity score, no statistical analysis of the horses based on this scoring system was performed.

Percutaneous sampling of tracheal gas as performed in the pilot study was found to be unreliable, as the needle would frequently become obstructed due to tracheal fluid. For this reason, tidal gases were sampled from a facemask. A close-fitting low dead-space facemask\(^d\) was fitted with a heated pneumotachometer\(^e\) mounted directly in front of the nares, and the sampling line from an infrared capnograph\(^b\) was attached to the front of the facemask beneath the pneumotachometer. The capnograph sampled at a rate of 220 ml/min. The pneumotachometer was attached to a variable reluctance transducer\(^f\) and exciter/signal conditioner\(^g\). Calibration of the pneumotachometer was performed using a 3 liter syringe\(^h\).

In observing the CO\(_2\) waveform on the capnograph, it was found that the end-tidal CO\(_2\) tension was somewhat variable. In the pilot study, a value

\(^d\)Aeromask modified, Canadian Monaghan Ltd., Canada

\(^e\)Fleisch #4, Zelco Associates, Marion, SC

\(^f\)Validyne DP-45, Zelco Associates, Marion, SC

\(^g\)Validyne CD-15, Zelco Associates, Marion SC

\(^h\)Hans Rudolph, Columbus OH
for $E_t\text{CO}_2$ that was the subjective mean (as determined by operator observation) of the reported the end-tidal CO$_2$ tension of those breaths which displayed a satisfactory waveform (rapid rise to a plateau) was used. This was recognized as too arbitrary, and for the subsequent studies analysis of a digitized signal of the CO$_2$ waveform was used to acquire a value for $E_t\text{CO}_2$. The maximum end-tidal CO$_2$ tension of the sampling period, rather than the mean end-tidal CO$_2$ tension, was used to eliminate error caused by occasional incomplete exhalations. Analog signal from the capnograph and signal conditioner was sampled by an analog-to-digital sampling board$^1$ for 2 minutes at a rate of 10 Hz by data acquisition software$^2$, and stored in a personal computer. These files were imported into a spreadsheet program, and the maximum end-tidal CO$_2$ ($E_t\text{CO}_2$) during the sampling period was determined.

During the period of electronic sampling, blood samples were obtained from the transverse facial artery and jugular vein, and were analyzed for $pO_2$, $pCO_2$, and pH using a clinical blood gas analyzer.$^a$ Temperature correction and hemoglobin saturation was calculated using algorithms derived for equine hemoglobin.$^{61}$

$^1$DAS-1200, Keithley Metrabyte, Taunton, MA

$^2$Easyest-AG, Keithley Metrabyte, Taunton, MA
Physiologic shunt fraction was calculated using the equation in Figure 8. Alveolar dead space was calculated using the equation in Figure 7. The complete equations are shown in Appendix 1.

The means, standard deviations, and 95% Confidence Intervals for each measured and calculated parameter were determined using the one-sample Student's t test.
III: Clinical Horses

Twenty horses with lower respiratory disease were selected from the clinical population at the Equine Medical Center. The housing for each horse was variable, ranging from complete stall confinement to pasture turnout. Each horse had a complete respiratory examination performed similar to the control horses.

Prior to sedation for thoracic radiographs and transtracheal aspiration, tidal gas flow and composition was sampled for two minutes, and blood samples from the transverse facial artery and jugular vein were obtained anaerobically. $pO_2$, $pCO_2$, and pH were determined using a clinical blood gas analyzer. Calculation of pulmonary gas exchange indices was performed in the same manner as those in Part B.

Symptomatic horses were divided prospectively into two groups, based on the severity of the physical examination abnormalities: Group 2 - Asymptomatic at rest, symptomatic at exercise (as reported in the history obtained from the owner and/or trainer); and Group 3 - Symptomatic at rest. Comparison between groups for age, weight, and measured and calculated indices was performed using the Kruskal-Wallis test, and if the results indicated a significant difference across the groups ($p<0.05$), the Mann-Whitney U test was used to compare individual groups, with $p<0.05$ considered significant.
Following the initial physical examination and data collection, some of the horses included in Group 2 were administered albuterol\textsuperscript{1} from a metered-dose inhaler through an equine facemask at a rate of 90 μg/100 kg. Tidal gas flow and composition measurements, and blood gas analysis were repeated 15 minutes post-treatment with albuterol. Measured and calculated indices were compared to pretreatment values using the one-sample Students t-test for paired data, with \( p<0.05 \) considered significant.

\textsuperscript{1}Proventil, Schering
RESULTS

I: Pilot Study

All horses tolerated the sampling procedures well. No complications from arterial sampling (hematomas) were observed, and the repeated sampling of arteries was not difficult.

The mean ± std dev. for Q_s/Q_T was 2.519 ± 1.924 %, with a 95% confidence interval of (0.499, 4.536). The mean ± std dev. for V_{DE}/V_T was -9.47 ± 10.86 %, with a 95% confidence interval of (-20.87, 1.93).

The substitution of jugular oxygen content for mixed venous oxygen content caused a small difference in the value calculated for Q_s/Q_T (Mean Q_s/Q_T = 0.274%, mean Q_s/Q_T’ = 0.310%) The Spearman Correlation coefficient for the association between Q_s/Q_T and Q_s/Q_T’ was 0.976.
II: Control Horses

Six horses (body weight 382 - 527 kg; age 1-16 yrs) were used for Part II. No evidence of pulmonary disease was found in the examination of these horses. Rebreathing was well tolerated, and minimal mucous and granulocytes were observed in the tracheal aspirate.

The mean ± S.D. and 95% confidence intervals of the measured and calculated parameters for horses are shown in Table 1.

Table 1: Gas Exchange Values for Group 1 (Asymptomatic) Horses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± Std. Dev. (n=6)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>paO₂ (mm Hg)</td>
<td>103 ± 3</td>
<td>(100, 106)</td>
</tr>
<tr>
<td>paCO₂ (mm Hg)</td>
<td>41.3 ± 1.8</td>
<td>(39.4, 43.1)</td>
</tr>
<tr>
<td>V_{DB}/V_T (%)</td>
<td>-18.2 ± 7.5</td>
<td>(-26.0, -10.3)</td>
</tr>
<tr>
<td>Q_s/Q_T (%)</td>
<td>0.37 ± 0.94</td>
<td>(-0.62, 1.35)</td>
</tr>
</tbody>
</table>
III: Clinical Horses

Eleven horses were included in Group 2 and nine horses in Group 3, based on the results of history, physical examination and rebreathing. Disease severity scores for each horse are shown in Table 2. For four horses, thoracic radiographs were not available, and for two horses hematology results were not available. There was no significant difference found between groups with regards to body weight ($p = 0.280$), but there was a significant difference found between the groups with regards to age ($p = 0.005$). There was no significant difference between Groups 1 and 2, but Group 3 was significantly different from Group 1 ($p = 0.0392$) and Group 2 ($p = 0.0017$) with regards to age.

The range and median values of the groups for each gas exchange parameter, as well as age and weight, are shown in Table 3. There were significant differences across the groups for $Q_s/Q_T$ ($p=0.003$) and $V_{DB}/V_T$ ($p=0.025$). The results of the comparisons between Groups 1, 2, and 3 are shown in Table 4. Comparisons which were considered significant ($p < 0.05$) are noted with an asterisk. $V_{DB}/V_T$ was significantly different between Group 1 and Group 3. $Q_s/Q_T$ and $paO_2$ were significantly different between Group 1 and Group 2, and between Group 1 and Group 3, but not between Group 2 and Group 3. No other measured or calculated parameter was significantly different in more than one comparison.
Seven horses in Group 2 were tested following aerosol administration of albuterol by facemask. The pre- and post-treatment means and standard deviations for each gas exchange parameter are reported in Table 5. The differences between pre- and post-treatment measurements of paO$_2$ and Q$_{a}$/Q$_T$ were significant ($p<0.05$). No significant differences ($p > 0.05$) between pre- and post-treatment measurements were found for any other measured or calculated parameter.
Table 2: Signalment and Disease Severity Scores
(See Figure 10 for Descriptions of Scores)

<table>
<thead>
<tr>
<th>Horse</th>
<th>Group</th>
<th>Weight (kg)</th>
<th>Age (yr)</th>
<th>Breed</th>
<th>PE</th>
<th>TTA</th>
<th>Rad</th>
<th>Hem</th>
</tr>
</thead>
<tbody>
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<td>11646</td>
<td>0</td>
<td>423</td>
<td>7</td>
<td>Tb</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11991</td>
<td>0</td>
<td>382</td>
<td>4</td>
<td>Tb</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12004</td>
<td>0</td>
<td>480</td>
<td>8</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>12164</td>
<td>0</td>
<td>449</td>
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<td>Tb</td>
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<td>12259</td>
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<td>527</td>
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<td>Sdl</td>
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<td>0</td>
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<td>12275</td>
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<td>432</td>
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<td>Tb</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9523</td>
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<td>466</td>
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<td>Tb</td>
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<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
</tr>
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<td>0</td>
</tr>
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<td>1</td>
<td>599</td>
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<td>Tb</td>
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<td>1</td>
<td>1</td>
<td>0</td>
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<td>12115</td>
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<td>444</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>12385</td>
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<td>655</td>
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<td>Tb</td>
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<td>0</td>
</tr>
<tr>
<td>12585</td>
<td>1</td>
<td>473</td>
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<td>Tb</td>
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<td>12588</td>
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<td>Tb</td>
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<tr>
<td>12626</td>
<td>1</td>
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<td>3</td>
<td>Tb</td>
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<td>11693</td>
<td>2</td>
<td>348</td>
<td>29</td>
<td>Pony</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>12051</td>
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<td>618</td>
<td>17</td>
<td>Wmbl</td>
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<td>4</td>
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<td>0</td>
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<tr>
<td>12076</td>
<td>2</td>
<td>241</td>
<td>11</td>
<td>MorX</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12331</td>
<td>2</td>
<td>499</td>
<td>12</td>
<td>TnWk</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12426</td>
<td>2</td>
<td>439</td>
<td>7</td>
<td>Mor</td>
<td>3</td>
<td>3</td>
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<td>1</td>
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<td>1</td>
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<tr>
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<td>QtrX</td>
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<td>2</td>
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<td></td>
</tr>
<tr>
<td>12666</td>
<td>2</td>
<td>636</td>
<td>13</td>
<td>Tb/Cl</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Breeds: Tb: Thoroughbred; Sdl: Saddlebred; Wmbl: Warmblood; Mor: Morgan; MorX: Morgan cross; TnWk: Tennessee Walking horse; QtrX: Quarterhorse cross; Tb/Cl: Thoroughbred/Clydesdale cross.
Table 3: Group Medians and Ranges for Signalment and Gas Exchange Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 Median (Range)</th>
<th>Group 2 Median (Range)</th>
<th>Group 3 Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>441 (382 ↔ 527)</td>
<td>480 (432 ↔ 655)</td>
<td>499 (241 ↔ 636)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>6 (1 ↔ 16)</td>
<td>4 (2 ↔ 16)</td>
<td>13 (7 ↔ 29)</td>
</tr>
<tr>
<td>(\text{paO}_2) (mm Hg)</td>
<td>103 (98 ↔ 106)</td>
<td>94 (76 ↔ 102)</td>
<td>85 (61 ↔ 100)</td>
</tr>
<tr>
<td>(\text{paCO}_2) (mm Hg)</td>
<td>41.9 (38.4 ↔ 42.7)</td>
<td>43.6 (37.6 ↔ 47.4)</td>
<td>40.6 (33.7 ↔ 51.1)</td>
</tr>
<tr>
<td>(V_{DB}/V_T) (%)</td>
<td>-20.94 (-25.39 ↔ -8.84)</td>
<td>-11.40 (-26.70 ↔ 28.48)</td>
<td>-5.09 (-17.50 ↔ 57.04)</td>
</tr>
<tr>
<td>(Q_S/Q_T) (%)</td>
<td>0.40 (-0.83 ↔ 1.78)</td>
<td>1.97 (0.71 ↔ 16.99)</td>
<td>8.730 (0.60 ↔ 23.19)</td>
</tr>
</tbody>
</table>

Table 4: Group Mann Whitney Comparisons for Age and Gas Exchange Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 vs Group 2</th>
<th>Group 1 vs Group 3</th>
<th>Group 2 vs Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>p = 0.65</td>
<td>p = 0.039*</td>
<td>p = 0.0017</td>
</tr>
<tr>
<td>(\text{paO}_2)</td>
<td>p = 0.0090*</td>
<td>p = 0.0027*</td>
<td>p = 0.13</td>
</tr>
<tr>
<td>(\text{paCO}_2)</td>
<td>p = 0.097</td>
<td>p = 1.000</td>
<td>p = 0.49</td>
</tr>
<tr>
<td>(V_{DB}/V_T)</td>
<td>p = 0.21</td>
<td>p = 0.016*</td>
<td>p = 0.07</td>
</tr>
<tr>
<td>(Q_S/Q_T)</td>
<td>p = 0.0057*</td>
<td>p = 0.0056*</td>
<td>p = 0.095</td>
</tr>
</tbody>
</table>

*p-values with asterisks are considered significant
Table 5: Results of Treatment with Albuterol of Horses with Clinical Lower Respiratory Disease

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pre-treatment mean ± S.D.</th>
<th>Post-treatment mean ± S.D.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>paO₂ (mm Hg)</td>
<td>83 ± 12</td>
<td>76 ± 8</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>paCO₂ (mm Hg)</td>
<td>41.0 ± 6.2</td>
<td>43.1 ± 3.5</td>
<td>p = 0.16</td>
</tr>
<tr>
<td>Qs/Qt (%)</td>
<td>9.14 ± 6.92</td>
<td>13.39 ± 5.56</td>
<td>p = 0.003</td>
</tr>
<tr>
<td>V_{DE}/V_{T} (%)</td>
<td>-0.045 ± 0.13</td>
<td>0.014 ± 0.087</td>
<td>p = 0.12</td>
</tr>
</tbody>
</table>
DISCUSSION

In the pilot study the goal was to determine the feasibility of the clinical measurement of physiologic shunt fraction and alveolar dead space fraction in a group of horses, and to show an acceptable degree of repeatability for these parameters for horses with similar levels of pulmonary health. For these parameters to be clinically useful the methods used to obtain necessary samples from the horse must be tolerated by the horse, and have a low incidence of complications.

The respiratory health of the horses used in the pilot study was determined only through physical examination and auscultation at rest. These methods alone will not detect mild pulmonary disease, and significant subclinical disease may have been present in some or all of the subjects. If the physiologic shunt fraction and alveolar dead space fraction are sensitive to subclinical respiratory disease, the values obtained in the pilot study for these parameters may not represent the range of values expected in healthy horses. Some of the horses used in the pilot study were examined in a more thorough manner later, and were found to have subclinical respiratory disease. For this reason, they were excluded from the later stages of this study.
The horses used in the pilot study tolerated repeated sampling of the transverse facial artery, and acceptable samples for blood gas analysis were obtained without any complications. No special restraint was necessary to obtain the samples and complications of arterial sampling (hematoma formation, infection) were not observed in any of the horses.

Transtracheal sampling of tidal gases was well tolerated and easily performed. When inserted percutaneously into the trachea, the needle was prone to obstruction by intra-airway fluid or inadvertent contact with the airway mucosa, and required constant stabilization to maintain an intraluminal position. No complications from the transtracheal needle placement were observed, however, we recognized the need for a more stable, reliable sampling technique. For this reason, subsequent examinations included tidal gas sampling from a low deadspace facemask.

There was considerable variation in end-tidal CO$_2$ tension as displayed by the capnograph. In the pilot study, the subjective mean of the values for end-tidal CO$_2$ displayed by the capnograph while the horse appeared to be breathing regularly were used to represent the horse's typical end-tidal CO$_2$. This introduced the potential for operator bias in the calculation of the alveolar dead space fraction, and illustrated the need for a more objective method for determining the value of end-tidal CO$_2$. 
Two objective methods for determining a suitable end-tidal pCO₂ value were considered: The mean end-tidal CO₂ of all breaths during the sampling period, and the maximum end-tidal CO₂ during the sampling period. In trying to determine an average end-tidal pCO₂, incomplete breaths will result in an end-tidal pCO₂ which represents a mixture of anatomic dead space and alveolar gas. Incomplete breaths occurred to varying degrees in the horses examined, and added an unacceptable degree of variability to the measurement of mean end-tidal pCO₂. The calculation of maximal end-tidal pCO₂ depended on the presence of at least one complete breath during the sampling period. This occurred reliably with each horse, resulting in less variability between horses.

Despite the considerable potential for variability, a narrow confidence interval for Q₅/Q₇ was obtained, while a larger confidence interval was found for V₁/V₂. Because the techniques used to obtain these values were well tolerated by the test subjects, a more rigorous evaluation of these indices and their relationship to lower respiratory disease was pursued.

Rigorous standards for inclusion of horses in Group 1 were used. Using resources available in a clinical setting, six horses which had no abnormalities on physical examination, auscultation, transtracheal aspiration, and thoracic radiographs were selected. More sensitive techniques have the potential to improve the detection of pulmonary
disease, such as nitrogen washout testing, \textsuperscript{105} multiple inert gas elimination, \textsuperscript{59} and V/Q scintigraphy\textsuperscript{8} were not available.

The reliability of transtracheal aspirate cytology to detect the presence of airway exudate has been questioned. Though Mair has found it to be useful in the clinical evaluation of lower airway disease, \textsuperscript{93} others have found cytology of bronchoalveolar lavage fluid more representative of lower airway disease. \textsuperscript{92} With bronchoalveolar lavage, however, it is difficult to obtain uncontaminated cultures of the lower airways. We selected transtracheal aspiration based upon the desire to obtain reliable lower airway cultures, the ease of obtaining a sample, and the familiarity of our laboratory personnel in the handling and interpretation of the sample.

The absence of neutrophilic and mucoid exudate in the transtracheal aspirate as a criteria for normal lower respiratory health was more stringent than has been previously used by other investigators. Only one other study has used this criteria, and found the incidence of lower respiratory disease to be quite high (90\%).\textsuperscript{1} This coincides with our experience, in that we examined many horses without apparent respiratory disease that were subsequently excluded from Group 1 based on the transtracheal aspirate.

The values obtained for arterial pO\textsubscript{2} from the horses of Group 1 were higher than those published as normal values.\textsuperscript{38, 44, 86} This probably reflects
the exclusion in our study of animals with subclinical disease through stringent examination.

The effect of the facemask on lower respiratory function in our horses is not known. The deadspace of facemasks can increase rebreathing of CO₂ and increase inspired CO₂ tension, thus arterial pCO₂ would be expected to rise. Facemasks may also increase respiratory resistance and change breathing strategy in horses. Other investigators have described variable changes in blood gas tensions and pulmonary mechanics in horses wearing facemasks.¹¹⁶ Comparisons between horses in this study are valid due to similar testing conditions, but comparison between this study and other studies should be made carefully, due to the possibility that dissimilar testing conditions could affect the results.

The values obtained for the physiologic shunt fraction were lower than those previously reported.¹⁸,¹¹⁴,¹¹⁵ In addition to higher paO₂ values in Group 1 horses compared to previous studies, the use of equine oxyhemoglobin dissociation constants could contribute to the lower Q₅/Q₇ values we obtained. Equine hemoglobin has a greater affinity for oxygen than human hemoglobin at similar oxygen tensions.⁶¹ Therefore, at a given paO₂ with equal amounts of hemoglobin, equine blood will contain more oxygen than human blood. The effect of substituting equine oxyhemoglobin dissociation constants for human oxyhemoglobin dissociation constants will
be minimal at the high \( \text{paO}_2 \) values in the Group 1 horses, as both calculations result in an estimated oxyhemoglobin saturation >95%. This is probably not sufficient to explain the differences in the gas exchange parameters found in this study and other studies.

For the calculation of \( Q_S/Q_T \) to be an acceptable clinical tool, we felt it necessary to eliminate, if possible, the need for mixed venous blood. The placement of a pulmonary artery catheter can be difficult and has the potential for significant complications. In comparing the values obtained for \( Q_S/Q_T \) using mixed venous blood to those obtained using peripheral venous blood, no significant differences were detected. Therefore, the substitution of jugular venous oxygen content for mixed venous oxygen content in calculating \( Q_S/Q_T \) was considered acceptable.

There were no significant differences found between groups with regards to body weight (\( p = 0.280 \)), but there was significant differences found between the groups with regards to age (\( p = 0.005 \)). This is not unexpected, as the criteria established prospectively for inclusion in Group 3 would tend to select horses with chronic allergic airway disease, and these horses tend to be older. It is not possible to separate the effects of age and with regard to these indices based on this study. Future clarification of this issue would require more rigorous standardization of subjects with regard to age and nature of lower respiratory disease.
The alveolar dead space fraction was consistently less than zero in our Group 1 horses, and often less than zero in our symptomatic horses (Groups 2 and 3). Other investigators have reported obtaining negative values, which were discarded on the assumption that equipment error had occurred. Due to the long tidal cycle of the horse, it is possible for end-tidal gas to have a higher CO₂ tension than arterial blood. Alveolar CO₂ tension is not constant throughout the tidal cycle, but continues to increase until equilibrium is reached with the mixed venous blood.\textsuperscript{108} Arterial pCO₂ represents the mean of alveolar pCO₂, whereas end-tidal pCO₂ represents the alveolar gas from the regions with the longest time constants. In the ideal horse lung, which has a long tidal cycle to allow complete blood-gas equilibration and synchronous emptying of alveoli with perfect V/Q matching, end-tidal pCO₂ would closely approximate mixed venous pCO₂. Because mixed venous pCO₂ is approximately 10 mm Hg higher than arterial pCO₂, negative values for the alveolar dead space are possible.

In Part III, we compared the values obtained for pulmonary volumes and gas exchange indices from asymptomatic horses (Group 1) to those obtained from horses with varying degrees of lower respiratory disease (Groups 2 and 3). The goal of Part III was to show that horses with mild and moderate lower respiratory disease would have significant increases in physiologic shunt fraction and alveolar dead space. As Table 4 shows,
there were significant differences between the groups for $Q_S/Q_T$ and $V_{DB}/V_T$. This demonstrates that impairment of gas exchange efficiency is detectable in mild, as well as moderate, lower respiratory disease.

The mechanism by which lower respiratory disease in these horses increased $Q_S/Q_T$ is unknown. Impairment of perfusion regulation mechanisms, leading to poor V/Q matching, is possible. Locally-released inflammatory mediators could either directly affect vascular smooth muscle tone, or damage the vascular endothelium, resulting in interference of the vascular smooth muscle regulation necessary for precise V/Q matching.

Eighteen of the twenty horses in Groups 2 and 3 had airway inflammation, based upon the transtracheal aspirate. Long-standing airway inflammation results in bronchial neovascularization, the venous drainage of which may be the pulmonary veins. An increased volume of deoxygenated systemic blood mixing with oxygenated pulmonary venous blood would decrease the overall systemic arterial oxygen content, resulting in larger values for $Q_S/Q_T$. Further investigations regarding the degree of bronchial venous admixture are necessary to evaluate this as a contributing factor in resting hypoxemia.

Factors which will result in an increase in $V_{DB}/V_T$ include those which will decrease the $E TC02$. Decreases in $E TC02$ can result from mixing of alveolar gas with either anatomic dead space gas or alveolar dead space.
gas. Contamination of end-tidal gas with anatomic dead space gas may occur with rapid flow rates or asynchronous time constants, conditions often found in horses with lower respiratory disease.\textsuperscript{16, 21} Contamination of end-tidal gas with alveolar dead space gas can occur with the development of V/Q mismatching and areas of alveolar overventilation or underperfusion.\textsuperscript{110} These changes have been found in horses with lower airway disease,\textsuperscript{8} and thus may contribute to the increased $V_{DE}/V_T$ in our clinically ill horses.

In Part III, we attempted to illustrate the use of physiologic shunt fraction and alveolar dead space fraction in measuring the change in gas exchange efficiency following bronchodilator administration to horses with airway disease. Using $p < 0.05$ as significant, $paO_2$ and $Q_S/Q_T$ were different from baseline following aerosol administration of albuterol. The immediate decrease in $paO_2$ following administration of $\beta$-2 adrenergic agonists has previously been demonstrated in horses with airway disease,\textsuperscript{33} and is thought to be the result of pulmonary arterial vasodilatation and increased perfusion. This is supported by the findings of this study, in that the increase in $Q_S/Q_T$ observed could be the result of increase in areas with low V/Q ratios.

Evaluation of the horses in Part III following bronchodilator administration occurred 15 minutes following administration of the medication. Failure to detect airway smooth muscle relaxation may be due
to the short interval between medicating and testing, or lack of airway smooth muscle relaxation in response to the initial dosing of bronchodilator, rather than a lack of sensitivity of the testing methods. Measurement of pulmonary gas exchange indices over an extended period of time following medication, and corroboration of alteration in pulmonary mechanics following bronchodilators in the test subjects, is indicated to clarify this issue.
SUMMARY AND CONCLUSIONS

In order to justify investigation into the clinical usefulness of the alveolar dead space fraction \( V_{DB}/V_T \) and physiologic shunt fraction \( Q_S/Q_T \) in the evaluation of equine lower respiratory disease, we measured these indices in horses with no evidence of lower respiratory disease based on physical examination. We found the calculation of these indices to be easily performed in a clinical setting with minimal complications, and standardized the techniques to be used in future applications of these indices.

We measured \( Q_S/Q_T \) and \( V_{DB}/V_T \) in 6 horses for which the absence of lower respiratory disease was stringently determined, based on the physical examination, thoracic radiography, and analysis of aspirated tracheal secretions. Using the data obtained with this group of horses, we described 95\% confidence intervals for these indices to be used in future clinical work.

To demonstrate that changes in the degree of lower respiratory disease would be represented by changes in \( Q_S/Q_T \) and \( V_{DB}/V_T \), we examined 20 horses with lower respiratory disease. These horses were categorized based on the severity of their clinical signs of lower respiratory disease, and were compared to the control group. Significant differences
between the control horses and abnormal horses were found for $Q_S/Q_T$ and $V_{DB}/V_T$. 
BIBLIOGRAPHY


96. Warner, A.E.; "Diagnostic procedures for the respiratory system." Large Animal Internal Medicine, pp 489-505, ed. B.P. Smith, C.V. Mosby, St. Louis, MO; 1990.


Appendix 1: Equations

Physiologic dead space fraction equation:

\[ \frac{V_D}{V_T} = \frac{(paCO_2 - E_MCO_2)}{paCO_2} \]

Alveolar dead space fraction equations:

\[ \frac{V_{DB}}{V_T} = \frac{(paCO_2 - E_TCO_2)}{paCO_2} \]

Physiologic shunt fraction equation:

\[ \frac{Q_S}{Q_T} = \frac{(Cc'O_2 - CaO_2)}{(Cc'O_2 - CvO_2)} \]

Alveolar air equation:

\[ pAO_2 = (BP - pH_2O) \times FiO_2 - paCO_2/R \]

Oxygen capacity equation:

\[ CxO_2 = (Hb \times \%HBO_2 x 1.39) + (pxO_2 \times 0.0021) \]
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TEACHING ACTIVITIES:
Lecture - Equine Lower Respiratory Disease
Professional Student 4th year rotation - Equine Medical Center
Overview of clinical equine lower respiratory disease diagnosis, pathogenesis, and treatment
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Lecture - Equine Neonatology
Professional Student 4th year rotation - Equine Medical Center
Overview of clinical aspects of neonatal problem recognition and treatment
1993-1994

Lecture - Failure of Passive Transfer
Professional Student 3rd year didactic - VM 9034 Equine Pediatrics and Neonatology
Discussed pathogenesis, recognition, treatment, and prevention of failure of passive transfer of immunity in foals
1993

Lecture - Equine Neonatal Respiratory Disease
Professional Student 3rd year didactic - VM 9034 Equine Pediatrics and Neonatology
Discussed diagnosis, pathogenesis, and treatment of prematurity, pulmonary sepsis, and respiratory insufficiency in foals.
1993
RESEARCH:

The effect of nasal insufflation of oxygen on transtracheal FiO₂ in the adult horse
Davis MS, Donaldson LL
SOURCE: In-house discretionary funds
$100

Investigation into the clinical usefulness of gas exchange pulmonary function testing
Davis MS
SOURCE: In-house discretionary funds
$893

Evaluation of clinical methods of pulmonary function assessment in the standing horse
Murray MJ, Davis MS, Donaldson LL, Furr MO
SOURCE: Clinical Initiatives Grant, Virginia-Maryland Regional College of Veterinary Medicine
$1,854

Evaluation of clinical measurements of pulmonary gas exchange efficiency in the horse
Murray MJ, Davis MS, Donaldson LL, Furr MO
SOURCE: Paul Mellon Equine Research Fund
$11,661

Lower respiratory disease in Thoroughbred racehorses: Quantifying lung dysfunction and response to treatment
Murray MJ, Davis MS, Fregin GF, Donaldson LL, Furr MO
SOURCE: Chrysler Corp Triple Crown Awards
$33,000

PRESENTATIONS:

Research Data


Continuing Education

"Equine Lower Respiratory Disease" Equine Medical Center Continuing Education Forum, November 11, 1994


PUBLICATIONS:

Abstracts

"The effect of nasal insufflation of oxygen on transtracheal FiO₂"  
Davis MS, Donaldson LL  
12th Meeting of the Comparative Respiratory Society, 1993.

"A preliminary evaluation of physiologic shunt fraction, Bohr dead space and respiratory rate in horses with respiratory disease."  
Davis MS, Murray MJ, Donaldson LL, Furr MO  
