2. Literature review

2.1. Importance of neonatal respiratory disease

Respiratory disease and dysfunction in neonatal foals may present as a primary condition or occur secondary to other disease processes (Koterba 1990). Respiratory abnormalities can result from the failure of the lung to make a complete transition from a collapsed fluid filled organ in utero, to an air-filled structure serving as an effective gas exchange unit post partum (Beech 1985). The newborn foal exchanges gases inefficiently, with a higher mean ventilation per kilogram bodyweight than adults. Foals also have a higher metabolic rate but smaller and fewer alveoli per gram weight of lung and thus less air tissue interface area of lung to body surface (Gillespie 1975).

The newborn has a high chest wall / lung compliance ratio compared to adults. The flexible chest wall is an essential prerequisite for the delivery through the birth canal, and a relatively stiff lung results from the presence of fluid in the interstitium (Mortola 1983). However, studies in healthy term foals have shown that chest wall compliance, normalized to body weight, is lower in neonatal foals than in less precocious neonates. Compared to other species, mature foals therefore appear to have a sufficiently rigid chest wall and do not need to actively maintain end-expiratory lung volume (Koterba, Wozniak et al. 1994). However, the high compliance of the immature chest wall may play an important role in determining the efficiency of gas exchange. Respiratory distress in the weak, premature foal may be accompanied by pulmonary atelectasis in the dependent lung. This is influenced by combined effects of recumbency on a compliant chest wall and ineffective reflex control of breathing to maintain end-expiratory lung volume (or functional residual capacity, FRC) (Kosch, Koterba et al. 1984).

Pathologic disorders of the lung are often related to abnormal perinatal respiratory development, abnormal parturition, aspiration, perinatal asphyxia, seeding of primary infectious agents or opportunistic pathogens infecting the immunocompromised host (Paradis

1989). The clinical onset of neonatal respiratory disease may be extremely insidious and thus difficult to recognize by physical examination alone.

2.2. Conditions associated with respiratory disease or distress in the foal

Respiratory disease in neonatal foals is associated with a variety of intra and extrathoracic conditions. Airway obstruction may be related to congenital malformations (e.g. choanal atresia, pharyngeal cysts), laryngeal edema or paralysis, tracheal malformation, stenosis or collapse as well as traumatic insults. Developmental disorders, including pulmonary hypoplasia and diaphragmatic herniation occur less commonly. Infectious or non-infectious lung parenchymal diseases are most commonly associated with neonatal respiratory dysfunction (pneumonia, atelectasis, hyaline membrane disease, pulmonary edema or congestion, aspiration syndromes, air leaks [e.g. pneumothorax], pulmonary hemorrhage) (Koterba 1990).

Additionally, multiple non-pulmonary causes of respiratory dysfunction have been recognized in the equine neonate. These may include perinatal asphyxia, central nervous lesions, metabolic derangements (e.g. severe acidosis), severe anemia and hypovolemia, persistent pulmonary hypertension, pleural effusion (e.g. chylothorax, pleuritis) and congestive heart failure. Apparent signs of respiratory dysfunction may also be associated with pain, abdominal crisis, fever or high environmental temperatures, excitement and transient tachypnea syndromes (Koterba 1990).

2.2.1. Equine neonatal pneumonia

Neonatal pneumonia is generally defined as inflammation of the pulmonary parenchyma in equine neonates. The term neonatal is restricted to foals up to four weeks of age. The etiologies of pneumonia in the newborn foal include hematogenous spread of infection (sepsis), aspiration, inhalation of airborne pathogens and prematurity (Bedenice and Paradis 2002). Hematogenous (ascending) infections occur in association with sepsis, which may be

acquired in utero from a placental infection or perinatally through environmental contamination (e.g. omphalitis, omphalophlebitis). Perinatal pulmonic infections are more common in foals that are immunocompromised due to failure of absorption of maternal antibodies (failure of passive transfer or FPT). E. coli, Klebsiella spp., Actinobacillus equuli, Salmonella spp. and Streptococcus spp. are some of the more common bacteria involved (Beech 1985; Bedenice and Paradis 2002).

Equine Viral Arteritis (EVA) and Herpes viral infections (EHV 1&4) have been implicated in in utero viral infections (Vaala 1992). However, fatal adenoviral pneumonia is primarily associated with combined immunodeficiency (CID) in Arabian foals (Thompson, Spradborw et al. 1976), although involvement of adenovirus in the pathogenesis of pneumonia has also been reported in a thoroughbred foal (Webb, Knight et al. 1981). Additionally, a recent report documents the isolation of Influenza A virus from a 7-day-old foal with bronchointerstitial pneumonia (Britton and Robinson 2002).

An immature ciliary apparatus and the presence of fewer alveolar macrophages in neonates in comparison to adult horses leads to decreased bacterial clearance from the lungs (Zink and Johnson 1984). Reduced complement values in neonates may further contribute to decreased humoral defense against invading bacterial infections. If colostrum intake is insufficient and immunoglobulin G levels remain low, the foal is not only deprived of specific antibody protection, but neutrophil function is also seriously impaired (LeBlanc 1988).

Descending respiratory infections may be related to inhalation pneumonia (transmission of viral, bacterial or fungal airborne pathogens), aspiration of infected amnionic fluid due to placental infection, aspiration of gastric reflux, iatrogenic aspiration (oil, medication, oral supplements), milk and meconium aspiration. Aspiration of meconium occurs in utero in foals that experience fetal distress. Though the meconium is commonly sterile at this time it creates mechanical airway obstruction, surfactant inactivation and pulmonary inflammation, caused by vasoactive mediators, chemotactic and inflammatory cytokines (including tumor necrosis factor α, interleukin 1β and interleukin 8) (Klingner 1999). The consequent edema and

vasoconstriction may lead to hypoperfusion of the pulmonary parenchyma, with damage to type II pneumocytes and decreased production of surfactant (Ghidini and Spong 2001).

Severe meconium aspiration syndrome (MAS) may result in perinatal death despite mechanical ventilation. However, reviews of the human literature have suggested that meconium aspiration by itself does not cause severe lung damage. Severe MAS may rather be caused by other concurrent pathologic processes occurring in utero, primarily chronic asphyxia and intrauterine infection (Ghidini and Spong 2001).

Neurological dysfunction, severe inflammation or structural abnormalities of the upper airways predispose to aspiration pneumonia post partum (e.g. milk aspiration). Descending infection secondary to bacterial colonization of the mucosal surfaces of the oro- and nasopharynx rarely occurs, except if other predisposing factors are present.

2.2.2. Prematurity and respiratory distress syndrome (RDS)

Neonatal foals that lack maturity are generally classified into three different groups:

1. Premature foals are those born before 320 days of gestation

2. Dysmature foals are born full term (average: 345 days of gestation), but carry the attributes of prematurity. Placental insufficiency (e.g. placentitis) is usually identified as the cause of intrauterine growth retardation (Koterba 1990).

3. Immature foals are normal-sized, full term foals that lack maturity, without evidence of placental insufficiency.

    (Rossdale, Ousey et al. 1984)

The clinical characteristics of these three groups are similar and may not be readily differentiated by physical examination. Generally, dysmature foals may be considered small-for-gestational-age (SGA). The term "SGA" originated from human medicine and is used to describe a neonate that is too small for the gestational age. The advantage of this terminology is that the foal may be classified according to both gestational age and body size. A foal may

therefore be premature and SGA, or premature and appropriate for gestational age (Koterba 1990).

Foals that lack maturity can display a spectrum of clinical and clinicopathological abnormalities including short silky hair coat, "floppy" ears, incomplete ossification of the tarsal and carpal bones, hyperextended fetlock joints, domed forehead, abnormal temperature regulation (normal to low body temperature), depressed mentation, weakness, poor suckle reflex and respiratory dysfunction (Paradis 1989; Fenger 1998).

Respiratory failure of prematurity is related to a deficiency of mature surfactant in the neonatal lung and is commonly referred to as Respiratory Distress Syndrome (RDS) (Paradis 1989). Surfactant phospholipids are produced by type II pneumocytes. The phospholipid composition in the surfactant lipoprotein complex changes during late gestation, with saturated phosphatidylcholine (lecithin) being the principal surface active component of mature surfactant (Hallman and Gluck 1982).

Primary surfactant deficiency is uncommon in foals. An early study has shown that surfactant maturation in horses begins at or after 88% of gestational length (i.e. approximately 300 days), but remains incomplete until the foal is full term (Pattle, Rossdale et al. 1975). Release of endogenous corticosteroids and beta-adrenergic agonists (e.g. chronic stress) may stimulate maturation of the surfactant system by accelerating epithelial maturation of the lung and increasing phospholipids synthesis (Koterba 1990).

A primary quantitative surfactant deficiency and a qualitative surfactant deficiency i.e. immature or abnormal composition, may not be differentiated clinically but historical information may aid in the determination. A premature foal born by c-section may initially appear normal, but develop signs of RDS within 24 hours. Pulmonary hyaline membranes are classically seen on post mortem examination of these foals.

Secondary surfactant deficiency develops as a consequence of direct interference with surfactant or as a result of type II pneumocyte dysfunction. Secondary surfactant deficiency may develop following perinatal asphyxia, meconium aspiration syndrome, sepsis,

endotoxemia, pulmonary edema, hypothermia, viral (EHV 1 or adenovirus) or bacterial pneumonia, prolonged atelectasis or shock and hypoperfusion (Koterba and Paradis 1990).

Surfactant deficiency will lead to impaired alveolar expansion (atelectasis), diffusion impairment, ventilation / perfusion (VQ) mismatch or pulmonary shunt, increased work of ventilation and secondary respiratory failure (Fenger 1998). In severe cases of respiratory failure, the prognosis in neonatal foals is poor, despite aggressive therapy with mechanical ventilation and supplemental oxygen administration (Paradis 1989).

2.2.3. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)

The term "lung injury" is used to describe the pulmonary response to a wide range of injuries occurring either directly to the lung or as a consequence of injury or inflammation at other sites in the body (Bellingan 2002). Lung injury is a common sequel to sepsis, aspiration or trauma and may be clinically difficult to differentiate from primary pneumonia or respiratory distress syndrome (RDS) in premature foals.

The pathophysiology of acute lung injury (ALI) is driven by an aggressive inflammatory reaction. In the exudative or acute phase, injury to the alveolar epithelial barrier leads to an increase in permeability to protein, extravasation of solutes and water. Pathologically, diffuse alveolar damage with neutrophils, macrophages, erythrocytes, protein rich edema fluid in the alveolar spaces, hyaline membranes, capillary injury and disruption of the alveolar epithelium is evident (Tweardy 2003).

ALI is diagnosed in humans if acute respiratory symptoms, a P_aO_2 (arterial oxygen) / FiO_2 (inspired oxygen fraction) ratio < 300 mmHg and non-cardiogenic radiographic infiltrates occur following direct lung injury (aspiration, lung contusion, diffuse pulmonary infection etc.) or indirect lung injury (sepsis, severe non-thoracic trauma, hypertransfusion etc.). Acute respiratory distress syndrome (ARDS) represents the more severe form of lung injury and is associated with a P_aO_2 / FiO_2 ratio < 200 mmHg in humans (Desai 2002). Mortality rates from

ARDS in humans have ranged from 40-60% in the past with the majority of deaths being related to sepsis or multi-organ dysfunction rather than primary respiratory causes (Tweardy 2003). More recent studies indicate a reduced mortality of 36 and 34% (Abel, Finney et al. 1998).

2.2.4. Perinatal asphyxia syndrome (PAS)

Perinatal asphyxia is defined as a reduction in tissue oxygenation, which can result from hypoxemia (decreased oxygen content in the blood) or ischemia (decreased blood flow) in the neonate (Furr, Tinker et al. 1997). Ultimately, any condition which leads to a decrease in placental perfusion, umbilical blood flow, tissue oxygenation or perfusion in the foal may be involved in the pathogenesis of the perinatal asphyxia syndrome (PAS). The hypoxic-ischemic insult results in a variety of body wide pathophysiological abnormalities, including damage to the renal, endocrine, gastrointestinal, cardiorespiratory, and nervous systems (Furr 1996). Resulting changes in carbohydrate and energy metabolism of the brain may lead to profound central nervous system disturbances (hypoxic ischemic encephalopathy or HIE). Energy failure leads to cellular swelling, intracellular Ca^{++} accumulation and eventual cell death, while the release of free radicals, production of excitatory aminoacids and loss of cerebrovascular autoregulation further complicate hypoxic ischemic brain damage (Furr 1996). HIE in turn can result in a centrally mediated reduction of respiratory drive, further hypoxemia and respiratory acidosis. A previous study reports that 52% of foal deaths associated with respiratory disease, were directly caused by asphyxia (Drummond and Koterba 1990).

Perinatal asphyxia, severe acidosis and hypoxia are known to cause increased pulmonary vascular resistance with right to left shunting through fetal channels and depression of surfactant metabolism in humans, small ruminants and other species (Thibeault, Hall et al. 1984). It has been speculated that the anoxic insult may acutely deplete glycogen storage of the surfactant producing type II pneumocytes, resulting in defective formation of glycerol and

palmitate moieties of surfactant phospholipids (Zhukova and Hallman 1982). Furthermore, asphyxia may increase the distribution and synthesis of myoinositol in type II cells, which blocks the phosphatidylglycerol synthesis, an essential lung surfactant phospholipid (Zhukova and Hallman 1982).

Chronically asphyxiated neonates may develop lung disease compatible with persistence of the fetal circulation and persistent pulmonary hypertension (PPH). PPH is characterized by right to left shunting (through a patent ductus arteriosus or foramen ovale) as a result of pulmonary vasoconstriction (Ghidini and Spong 2001). The latter is mediated by a temporary spasm caused by pulmonary vasoactivity or permanent anatomical changes in the pulmonary arterioles. Muscle hypertrophy of pulmonary arterioles and mascularization of distal intra-acinar arteries is considered the hallmark of PPH and has been primarily related to chronic in utero asphyxia (Ghidini and Spong 2001). Once in place, PPH results in hypoxia, metabolic acidosis and further subsequent pulmonary vasoconstriction.

Some studies in humans have indicated that acute asphyxia per se does not cause a life-threatening persistent fetal circulation or PPH. However, infants with underlying lung disease in addition to asphyxia commonly develop life threatening pulmonary dysfunction (Thibeault, Hall et al. 1984).

2.2.5. Systemic inflammatory response syndrome (SIRS)

The term "systemic inflammatory response syndrome" (SIRS) is generally defined as the body's systemic inflammatory response to a variety of severe clinical insults, including, but not limited to infection, trauma, asphyxia, ischemia, endotoxemia and immune mediated organ injury. When SIRS is the result of a confirmed infectious process, it is termed "sepsis" (Committee 1992). Bacteremia commonly leads to the manifestation of sepsis, but can only be confirmed by a positive blood culture. However, negative blood culture results in the presence of systemic infection may be related to prior antibiotic treatment, transient bacteremia, poor sampling or culture techniques. Wilson et al reported a negative blood culture rate of 19% in 47 confirmed cases of equine neonatal sepsis (Wilson and Madigan 1989).

The clinical manifestations of SIRS are identical to those of sepsis, which complicates the clinical differentiation of these conditions, specifically in pretreated animals (Committee 1992). The term SIRS in our study was used to include patients with a modified sepsis score $\geq$ 11 according to Brewer et al (Brewer and Koterba 1988; Brewer, Koterba et al. 1988), a positive blood culture or a known focus of systemic infection. The sepsis score incorporates a combination of historical, clinical and laboratory variables that are used to establish the likelihood of neonatal infection (Figure 1). It is intended for use as a diagnostic aid to identify early sepsis at a treatable stage (Vaala and House 2002). The scoring system was originally developed by Brewer et al (Brewer and Koterba 1988) and was based on a relatively small number of foals. Although the sensitivity and specificity was favorable in a subsequent study (Brewer, Koterba et al. 1988), the uncritical use of the sepsis score as a definition of sepsis still remains controversial. One may propose the sepsis score to be a better indicator of severe whole body insult or multiple organ dysfunction as a complication of SIRS, than of confirmed systemic infection.

Figure 1

Modified sepsis score (Brewer and Koterba 1988)

Foal's name: _____   Date: _____   Total score: _____
Case #:   _____   Check one:
                                                                                   _____ at admission?
                                                                                   _____ # days subsequent
                                                                                             to admission

Information collected:	4	3	2	1	0	This Case
CBC Neutrophil count		< 2000 / mm^3	2000-4000 or > 12000	8000-12000	normal	
Band neutrophil count		>200/mm^3	50-200		<50	
Doehle bodies, toxic, granulation or vacuoli-zation in neutrophils	marked	moderate	slight		none	
Fibrinogen			> 600	500-600	≤ 400	
Other laboratory data hypoglycemia			<50 mg/dl	50-80	> 80	
Immunoglobulin	< 200	200-400	401-800		> 800	
Clinical examination Petechiation or scleral injection not secondary to eye trauma		marked	moderate	mild	none	
Fever			> 102 oF	< 100 oF	normal	
Hypotonia, coma, de-pression, convulsions			marked	mild	normal	
Anterior enteritis, diarrhea, respiratory distress, swollen joints, open wounds		yes			no	
Historical data Placentitis, vulvar discharge prior to delivery, dystocia		yes			no	
Prematurity (days)		< 300	300-310	310-330	>330	

Total Points _____

A score of 11 is used as the cut-off point in differentiating non-septic (<11) from septic (≥11)

foals (Brewer and Koterba 1988).

2.3. Clinical manifestation of respiratory disease in neonatal foals

The clinical manifestation of equine neonatal respiratory disease can be variable and depends on the disease severity and underlying or associated problems.

2.3.1. Historical findings

Colostrum leakage prior to parturition or colostrum deprivation of the foal may be reported (poor colostrum quality or quantity). Other historical findings may include prematurity, dystocia, placental abnormalities, insufficient vaccination of the dam, perinatal weakness, and milk discharge from nostrils after nursing (Bedenice and Paradis 2002).

2.3.2. Physical examination findings

Early in life, localizing clinical signs of respiratory infection may be absent even in the presence of extensive disease. Dyspnea may be seen in severely affected foals and manifest as an increase in respiratory rate, effort or thoraco-abdominal asynchrony (paradoxical breathing). However, signs of respiratory distress and hypoxemia are frequently vague. Even some severely hypoxemic foals may only show restlessness, considerable resistance and struggling when being handled or restraint. Abnormal respiratory sounds (crackles or wheezes) may be heard on auscultation. However, even normal foals may show crackles on the down lung after having remained in lateral recumbency for a prolonged period of time. Furthermore, foals with no auscultable abnormalities may still have extensive pulmonary disease. Cyanosis is a sign of severe hypoxemia. The arterial P_aO_2 (partial pressure of oxygen) must, however, reach very low levels (35 – 45 mmHg) before clinical cyanosis is observed. Additionally, weakness, depression, anorexia, weak or absent suckle reflex, dehydration and fever may be noted in foals with respiratory disease. Cough and nasal discharge are usually absent in the early stages of neonatal pulmonary disorders (Bedenice and Paradis 2002).

2.4. Diagnostic evaluation of neonatal respiratory disease

2.4.1. Alterations in the complete blood count (CBC)

A neutrophilic leukocytosis or neutropenia, with or without a left shift, can be found in bacterial infections as well as viral diseases. Neutropenia is commonly related to bacterial sepsis or endotoxemia. Profound lymphopenia may be found in acute viral diseases as well as sepsis and endotoxemia (Morris 2002). Lymphocyte counts are generally higher than neutrophil counts in the fetus and may reflect the degree of immaturity in premature foals. Neutropenia and N/L (neutrophil to lymphocyte) ratios < 1.5 during the first 24 hours have been reported to carry a poor prognosis for survival (Furr, Tinker et al. 1997).

Physiologically, neutrophil counts in neonatal foals increase to mean values of approximately 8×10^3 neutrophils/µl during the first 24 hrs post parturition, due to endogenous glucocorticoid steroid release. Lymphocyte counts may physiologically decrease to mean values of 1.4×10^3 lymphocytes/µl a few hours after birth, and then gradually increase to 5×10^3 lymphocytes/µl at three months of age (Koterba 1990).

2.4.2. Alterations in the serum biochemistry analysis

Neonatal respiratory disease is not consistently related to specific abnormalities in the serum biochemistry analysis. However, several age-related physiologic alterations should be considered in neonatal foals. Decreased total protein values are commonly observed prior to nursing, although these findings are not considered to serve as an accurate measure of failure of passive transfer in foals. Increased values of alkaline phosphatase (ALP) are related to a higher osteoblastic activity in neonates, ongoing hepatic maturation and intestinal pinocytosis during the first 24 hrs of life. Elevations in gamma-glutamyl-transferase (GGT) and sorbitol dehydrogenase (SDH) are observed in foals between 5 – 14 days of age due to hepatocellular maturation. Physiologic moderate neonatal hyperbilirubinemia appears to be a transient finding and is related to hepatic maturation and an accelerated breakdown of neonatal red blood cells (Koterba 1990).

Increased creatinine levels (up to 3.5 mg/dl) can occur during the first 36 hrs post partum and may reflect serum levels of the dam or the excretory function of the placenta. Significantly higher plasma creatinine concentrations have also been reported in immature compared to term infants, which persist longer than can be attributed to maternal transfer of creatinine. This phenomenon has been associated with renal tubular reabsorption of creatinine in the immature, developing kidneys of preterm human and rodent neonates (Matos, Duarte-Silva et al. 1998). Thus, elevated creatinine concentrations in the hydrated foal may potentially infer placental dysfunction (chronic asphyxia) or immaturity.

Volume depletion is a common finding in critically ill neonatal foals. A low glomerular filtration rate and enhanced proximal tubular sodium and water reabsorption in the face of volume depletion decreases distal tubular fluid delivery, thereby diminishing potassium secretion. Low systemic blood flow and hyperkalemia have further been reported in preterm infants (Kluckow and Evans 2001). Additionally, hyperkalemia is commonly associated with systemic acidosis and disorders of the cellular Na/K pump in human patients with perinatal asphyxia, SIRS, sepsis or hypovolemia.

2.4.3. Serum anion gap (AG)

The anion gap (AG) is a serum parameter that is used to evaluated patients with an acid-base disturbance. The body continually thrives to achieve an electrochemical balance of the ionic elements in the extracellular fluid (Marino 1998). Thus the number of cations must equal the number of anions in body solutions. All ions participate in this balance, but those that are routinely measured, include the major measured cations, sodium (Na) and potassium (K), as well as the major measured anions, chloride (Cl) and bicarbonate (HCO_3). The unmeasured anions (UA) and unmeasured cations (UC) have the following relationship to the commonly measured electrolytes:

unmeasured anions + [Cl + HCO$_3$] = unmeasured cations + [Na +K]

or (rearranged)

UA – UC = [Na +K] – [Cl + HCO$_3$]

The relationship of unmeasured anions to unmeasured cations (UA – UC) is a quantification of the relative abundance of unmeasured anions, referred to as the anion gap (AG) (Marino 1998). High anion gaps occur in the presence of increased serum concentrations of unmeasured anions (proteins, volatile fatty acids, lactate, pyruvate, sulfates and phosphates) or decreases in the concentration of unmeasured cations (calcium, magnesium) (Lorenz JM 1999). Relevant decreases in unmeasured cations are uncommon. Plasma proteins are the major source of unmeasured anions and a 50% reduction in plasma proteins can result in a 75% reduction in the AG (Marino 1998).

A previous study reported the normal anion gap in healthy quarterhorse foals to be 15.2 +/- 2.9 mEq/L, versus 10.4 +/- 1.2 mEq/L in two year old horses. The lower calcium and higher serum phosphate concentrations are believed to explain the increased anion gap of healthy suckling foals relative to breed matched adults (Gossett and French 1983). In the presence of acidemia, an elevated anion gap usually indicates an organic acidosis or lactic acidosis (Lorenz JM 1999).

2.4.4. Arterial blood gas analysis

Samples for arterial blood gas analysis may be obtained from the great metatarsal, brachial, femoral, facial and carotid arteries of neonatal foals. Introduction of room air into the sample (unsealed syringes or air bubble formation) will artificially increase the P$_a$O$_2$, decrease the P$_a$CO$_2$ and result in a more alkaline pH. However, sealed samples may be stored on ice for 2 hours without major changes in P$_a$O$_2$. Tables 1 and 2 may serve as a guideline for the interpretation of arterial blood gas results in equine neonates.

Table 1: Arterial blood gas values (Bedenice and Paradis 2002)

Parameter	Value	Interpretation
P_aO_2	> 80 mmHg	Within normal limits - neonatal foals will reach a P_aO_2 of 60 mmHg within 30 minutes after birth, and a P_aO_2 of 80 mmHg within 12 hours post partum (Koterba 1990)
	60 – 80 mmHg	Mild hypoxemia. Possible causes include: - respiratory compromise (see discussion below) - prolonged recumbency (especially if samples are taken from the hind limb)
	< 60 mmHg	Hypoxemia - a P_aO_2 of 60 mmHg indicates an oxygen saturation of approximately 90%
P_aCO_2	> 60 mmHg	Marked hypercapnia (inadequate CO_2 elimination relative to production), which may necessitate mechanical ventilation

In the absence of alterations in the inspired oxygen fraction (FiO_2), arterial hypoxemia may result from four basic processes, including (1) hypoventilation, (2) diffusion impairment, (3) ventilation – perfusion inequality (VQ mismatch), and (4) shunt (intra- and extrapulmonary shunt) (West 1982). Alveolar hypoventilation is defined as a reduced volume of inspired air reaching the alveoli per unit time, and is always related to an increase in P_aCO_2. Conditions associated with alveolar hypoventilation include drug induced respiratory depression (morphine, barbiturates), brainstem disease, abnormal respiratory muscle function (botulism, diaphragmatic hernia), thoracic cage abnormalities (rib fractures), increased airway resistance (upper and lower airway obstruction) and pleural space disease (Wilson and Lofstedt 2002).

Impaired diffusion results from an increase in the blood – gas barrier. Since carbon dioxide diffuses more readily than oxygen, the P_aCO_2 is usually not increased in conditions that only cause impaired diffusion. Diffusion impairments may be seen in conditions such as pulmonary edema, pneumonia, atelectasis or pulmonary contusion (Wilson and Lofstedt 2002).

The adequacy of pulmonary gas exchange is also determined by the balance between pulmonary ventilation and capillary blood flow, which is commonly expressed as the ventilation – perfusion (VQ) ratio. A VQ ratio above 1 describes conditions where ventilation is excessive relative to capillary blood flow (dead space ventilation). In normal human subjects, dead space ventilation accounts for 20-30% of the total ventilation. Dead space ventilation increases when the alveolar-capillary interface is destroyed (e.g. emphysema), when blood flow is reduced (e.g. low cardiac output, pulmonary thrombo-embolism, persistent pulmonary hypertension) or when the alveoli are overdistended by positive pressure ventilation. A VQ ratio below 1 describes the condition where capillary blood flow is excessive relative to ventilation (pulmonary edema, pneumonia, atelectasis etc.) (Marino 1998).

Shunting, on the other hand, is defined as any mechanism by which blood that has not passed through ventilated areas of the lung is added to arteries of the systemic circulation (Wilson and Lofstedt 2002). In normal human subjects, intrapulmonary shunt flow represents less than 10% of the total cardiac output (Marino 1998). Increased intrapulmonary anatomical shunting can result from pulmonary artery to venous fistulas or severe lung lobe consolidation. Cardiac shunting (right to left shunt) may be related to congenital heart disease (e.g. tetralogy of Fallot, persistent ductus arteriosus) or persistent fetal circulation (PFC). It is important to note that hypoxemia, unresponsive to oxygen therapy is suggestive of shunt (Wilson and Lofstedt 2002).

The difference in partial pressure of oxygen between alveolar gas (P_AO_2) and arterial blood (P_aO_2) is termed A-a gradient. The A-a gradient can be used as an indirect measure of VQ abnormalities and shunt, and is determined by the following alveolar gas equation:

$$P_AO_2 = [P_iO_2 - P_aCO_2 \,/\, RQ]$$

Index:

P_iO_2 = inspired oxygen pressure (150 mmHg in animals breathing room air)

P_aCO_2 = arterial pressure of carbon dioxide

RQ = respiratory quotient (ratio of CO_2 production over O_2 consumption)

= 0.8 at resting conditions

The alveolar gas equation may be used to calculate the A-a gradient as follows:

$$\text{A-a gradient} = P_AO_2 - P_aO_2$$

$$= [P_iO_2 - P_aCO_2 \,/\, RQ] - P_aO_2$$

Simplified formula of the A-a gradient in animals breathing room air:

$$\text{A-a gradient} = 150 - P_aCO_2 \,/\, 0.8 - P_aO_2$$

The normal A-a gradient is less than 15 mmHg in healthy foals and increases with VQ mismatch, shunt and severe diffusion impairments. Furthermore, it is important to note that the normal A-a gradient increases 5-7 mmHg for every 10% increase in the inspired oxygen fraction (F_iO_2 = 21% in room air). This effect is presumably caused by the loss of regional hypoxic vasoconstriction of the lungs after oxygen administration (Marino 1998). The clinical significance of the arterial blood gas analysis is summarized in Table 2 (Koterba 1990).

Table 2: Interpretation of arterial blood gases in neonatal foals (Koterba 1990)

Problem	P_aO_2	P_aCO_2	pH	A-a gradient	Response to 100% oxygen	Typical clinical conditions
VQ mismatch	↓	normal to ↓ from hypoxic drive	normal to ↓ from tissue hypoxia	↑	good	atelectasis, RDS, pneumonia, aspiration
Right to left shunt	↓	normal to ↓ from hypoxic drive	normal to ↓ from tissue hypoxia	↑	poor	atelectasis, PFC, congenital heart disease
Hypo-ventilation	↓	↑	↓ , unless full metabolic compensation	normal	very good	CNS depression, fatigue, airway obstruction
Diffusion limitation	↓	normal to ↓ from hypoxic drive	normal to ↓ from tissue hypoxia	↑	good	common in most parenchymal conditions
low inspired oxygen	↓	normal to ↓ from hypoxic drive	normal to ↓ from tissue hypoxia	normal	very good	iatrogenic

2.4.5. Other Laboratory Data

Blood cultures are helpful in identifying the etiologic agent of underlying sepsis. Other bacterial cultures and cytology (sampling of the respiratory tract) can be used to confirm the diagnosis of bacterial pneumonia and establish a pathogen specific antimicrobial therapy. Virus isolation from nasal swabs or respiratory tract sampling may also be beneficial. Viral serology, however, is undependable in the diagnosis of neonatal viral infections, as antibodies may have been acquired from the dam's colostrum. The presence of specific IgM antibodies may be useful in diagnosing selected congenital viral infections in the future (Koterba 1990). Serum immunoglobulin (IgG) levels should be measured, especially in cases of suspected sepsis.

2.4.6. Clinical pulmonary function testing in neonatal foals

Clinical pulmonary function testing (PFT) is generally divided into the assessment of respiratory mechanics (mechanical properties of the respiratory system) and gas exchange. Analysis of gas exchange investigates ventilation-perfusion (VQ) matching, shunt, diffusion capacity and dead space to tidal volume ratios (V_D/V_T). Lung mechanics determine the static and dynamic properties of the lung (resistance, compliance, functional residual capacity [FRC] and ventilatory parameters) (Hoffman 2002). The anatomy, size, behavior, cooperation, technological and economical constraints have limited the development and application of pulmonary function testing in neonatal foals. Most information on neonatal pulmonary function has been obtained in the research setting (Hoffman, Couetil et al. 1999) or in anesthetized foals (Stewart, Young et al. 1987; Koterba, Wozniak et al. 1994) and has not yet found broad application to veterinary patients in most referral centers. In order to address this shortfall, the development of non-invasive pulmonary function testing for veterinary patients is a major focus of research at the Lung Function Testing Laboratory at Tufts University School of Veterinary Medicine (North Grafton, USA) (Hoffman 2002).

Potential applications of non-invasive PFT in neonatal foals include measurements of FRC and the diffusion capacity of carbon monoxide (D_lCO), via helium and carbon monoxide dilution (Amis and Jones 1984). FRC is a measure of the amount of gas that remains in the lung at end-expiration (FRC = residual volume + expiratory reserve volume). FRC is lower in patients with increased "lung stiffness" (reduced elastic recoil of the lung) as well as airway inflammation. Additionally, pulmonary vascular resistance depends on FRC. Overdistension of the lung with an increased positive end-expiratory pressure (PEEP) during mechanical ventilation may increase pulmonary vascular resistance and decrease cardiac output, resulting in reduced delivery of oxygen to peripheral tissues (Schibler and Frey 2002). D_lCO is related to the surface area available for gas exchange. Measurement of pulmonary diffusion capacity and VQ mismatch via nuclear scintigraphy may be used to monitor pulmonary disease progression in neonatal foals. A lower diffusion capacity may be found in foals with pulmonary parenchymal disease.

Arterial blood gas analysis is commonly used to determine oxygenation and ventilation in neonates (see section 2.4.3). The calculation of the alveolar-arterial oxygen gradient (A-a gradient) may provide further insight into ventilation-perfusion (VQ) matching, but requires FiO_2 (fraction of inspired oxygen) measurements in animals on supplemental oxygen (Marino 1998). Pulse oximetry provides a continuous measure of arterial oxygen saturation. However, this technique has not been rigorously tested in the foal and accurate readings are difficult to obtain.

Capnography (assessment of end-tidal CO_2 [P_ECO_2] via face mask) may serve as another non-invasive means to measure pulmonary gas exchange in neonatal foals. It serves as a non-invasive approximation of P_aCO_2 in animals without significant VQ mismatch, pulmonary hypoperfusion or shunt. Additionally, the following formula for measuring the dead space to tidal volume ratio (V_D/V_T) estimates the percentage of ventilated lung where no gas exchange occurs:

$V_D/V_T = [P_aCO_2 - P_ECO_2] / P_aCO_2$ (Marino 1998)

In summary, better clinical monitoring will improve the clinician's assessment with regards to (1) aggressive management of lung disease, (2) decisions for ventilation, (3) intensity of monitoring, (4) ventilator weaning, (5) cost analyses and (6) prognosis of foals with neonatal respiratory disease.

2.4.7. Upper airway endoscopy

Upper airway endoscopy is an invaluable diagnostic tool for the assessment of upper airway dysfunction in neonatal foals (e.g. dynamic pharyngeal collapse, laryngeal paralysis, cysts). Indications for upper airway endoscopy include inspiratory stridor, milk reflux from the nares and unexplained external swelling. A 7 mm outer diameter endoscope is usually small enough to pass through the ventral nasal meatus of most foals over 15 kg (Koterba 1990).

2.4.8. Thoracic ultrasound examination

Thoracic ultrasonography has become a popular diagnostic tool for the assessment of equine lung and pleural disease. Consolidation, pleural effusion, abscesses, penetrating thoracic wounds, rib fractures and diaphragmatic hernias are some of the diseases that have been detected ultrasonographically in the lung and pleural cavity of adult horses and foals. The side(s) of the thorax affected, as well as the precise location of the lesion, can be determined in most cases. Aspirates or biopsies of small masses (0.5 cm) or small fluid collections can therefore be safely performed using ultrasonographic guidance. However, deep pulmonary lesions and interstitial disease may only result in non-specific subtle abnormalities of the visceral pleural surface or normal lung appearance. Lesions that do not involve the periphery of the lung cannot be imaged ultrasonographically (Reef 1998).

2.5. Thoracic Radiography

Thoracic radiography is often helpful in establishing the presence of respiratory disease and determining the type and extent of pulmonary involvement (Koterba 1990). In contrast to non-invasive and invasive pulmonary function testing (PFT, section 2.4.6), thoracic radiography is used extensively in the clinical setting. The ability to obtain thoracic radiographs is usually available for foals with suspected respiratory disease admitted to referral centers. However, the correlation between radiographic pattern and disease condition in not as advanced as in human neonatology (Koterba 1990). An early study in humans reviewed chest radiographs of 337 newborn babies, who were referred for the examination of respiratory distress (Tudor, Young et al. 1976). A radiographic scoring system assessed the severity of hyaline membrane disease based on the degree of radiographic changes. An 82% correlation between radiological and pathological findings post mortem, was observed in this study. A descriptive radiographic – pathologic correlation in foals with respiratory disease revealed that thoracic radiographs of neonatal foals were helpful in identifying the presence of pulmonary dysfunction and in formulating a preferential diagnosis. However, radiographs were not always considered sufficiently specific to obtain a definitive diagnosis (Toal and Cudd 1987).

Correlations of radiographic and microscopic finding in normal and abnormal lungs have been reported for humans (Heitzman 1984) and certain domestic animal species, including adult horses (Wisner, O'Brien et al.; Farrow 1981; Heitzman 1984; Lakritz, Wisner et al. 1995). Lakritz et al determined a strong correlation of estimated volume density of alveolar tissue, measured volumetrically at post mortem, and the apparent prominence of bronchial and bronchointerstitial patterns observed radiographically in normal adult thoroughbred race horses (Lakritz, Wisner et al. 1995). A strong negative correlation between measured estimates of alveolar size and the prominence of the radiographic bronchial and vascular pattern further suggested that these radiographic patterns are inversely related to the mean size of air spaces. Thus, as the mean size of airspaces increases, the ability to perceive

vascular and bronchial patterns decreases. Studies in racehorses with exercise induced pulmonary hemorrhage (EIPH) have further shown that increased radiographic interstitial opacities in the caudodorsal lung field in affected horses are associated with 4 major types of histopathological changes: bronchiolitis, increased number of hemosiderophages, increased connective tissue and areas of marked eosinophilic infiltration (Wisner, O'Brien et al.).

2.5.1. Radiographic pattern recognition

Neonatal radiographic evaluation of the chest is facilitated by classifying the image appearance into radiographic patterns of pulmonary disease. Three major lung patterns (interstitial, alveolar and bronchial patterns) are commonly used to define evidence of radiographic respiratory disease in neonatal foals. Although the vascular pattern may be helpful in assessing pulmonary perfusion, venous congestion and cardiac anomalies, it plays a lesser role in the assessment of foal pneumonia.

2.5.1.1. Interstitial Lung Pattern

The pulmonary interstitium is composed of a connective tissue framework, containing the pulmonary vasculature, lymphatics, nerves and bronchi. Inflammatory infiltrate that affects these structures may produce radiographic changes that are referred to as the interstitial pattern. The hallmark of the interstitial pattern is an increase in background opacity, which results in the loss of visualization of the fine vascular structure of the normal, well aerated lung (Butler 1995).

The interstitial pattern is the most common radiographic abnormality in foals with pulmonary disease. It may be associated with bacterial or viral pneumonia, pulmonary edema, meconium aspiration, lung immaturity, acute lung injury, and prolonged recumbency due to dependent atelectasis (Lester and Lester 2001). Compression of the lung by distended abdominal viscera may also result in an apparent increase in the interstitial pattern due to decreased lung inflation.

2.5.1.2. Bronchial Lung Pattern

Although the bronchi are a component of the interstitial complex, diseases of this system are often considered separately. Radiographic signs of bronchial disease include increased thickness and density of the bronchial walls, or changes in bronchial lumen diameter (e.g. bronchiectasis). A bronchial lung pattern is more suggestive of chronic disease. However, a peribronchial pattern indicates infiltrates surrounding the bronchi and may be seen in acute and chronic inflammatory conditions (Butler 1995).

2.5.1.3. Alveolar Lung Pattern

The alveolar or air-space pattern is characterized by displacement of air from the alveoli by transudate, exudate or blood, leading to a relatively homogenous increase in soft tissue opacity. Air bronchograms may appear as lucent branching structures within an opaque lung field. The finding of air bronchograms is an indication of nearly complete alveolar filling (Butler 1995). Edema, aspiration, infection, hemorrhage and pulmonary consolidation commonly produce an alveolar pattern in neonatal foals (Lamb and O'Callaghan 1989). Additionally, a diffuse air-space pattern can result from RDS, ALI and ARDS in the equine neonate (Lamb, O'Callaghan et al. 1990).

2.5.2. Radiographic Scoring Systems

A wide variety of Chest Radiograph Scoring Systems have been developed in humans in an attempt to quantify the type and severity of pulmonary disease (Wood, Sinkin et al. 1987; Maconochie, Greenough et al. 1991; Weinstein, Peters et al. 1994). The ideal criteria for a useful scoring system include reproducibility and a reasonable correlation with pulmonary function data (O'Laoide, Fahy et al. 1991). Additionally, an objective score allows easy comparison between radiographs and decreases the degree of subjective variability of interpretation. Wood et al evaluated the frequency and severity of RDS using a simple radiographic scoring system in which pulmonary parenchymal densities and the severity of

air-bronchograms were used as an indicator of widespread atelectasis. The radiographic assessment of lung disease consistently correlated with data on oxygen and mean airway pressure requirements for infants (Wood, Sinkin et al. 1987). The Chrispin-Norman, Wisconsin, Brasfield and Northern chest radiograph scores were developed for the assessment of cystic fibrosis in people (Weatherly, Palmer et al. 1993; Conway SP 1994). The Northern Score was designed for rapid assessment by a single physician and evaluates each lung quadrant according to increasing disease severity. Furthermore, Maconochie et al developed a chest radiograph scoring system to predict chronic oxygen dependency in low birth weight infants. The scoring system assessed the preterm infant's chest radiograph at one month of age and graded the images according to volume, presence of opacification, cystic elements and interstitial changes. Infants who were chronically oxygen dependent had significantly higher radiographic scores (Maconochie, Greenough et al. 1991).

Similar radiographic scoring systems have not been developed for equine patients although various authors have proposed the use of pulmonary pattern scoring for the assessment of radiographic disease severity (Lakritz, Wisner et al. 1995). Lakritz et al based their radiographic assessment on the numeric scoring of the four major lung patterns (vascular, bronchial, interstitial and alveolar patterns). A score of 0-3 was assigned for normal, mild increase, moderate increase and marked increase of the specified pulmonary pattern. Radiographic scores for each pattern were summoned to obtain a total pulmonary score. Additionally, a mixed broncho-interstitial pulmonary pattern score was calculated in this study (Lakritz, Wisner et al. 1995).

2.6. Risk factors and prognostic variables of equine neonatal disease

Intensive care for the critically ill newborn foal can be a long financial, emotional and labor intensive investment. Several studies in foals have looked at different variables to aid prediction of patient survival (Hoffman, Staempfli et al. 1992; Furr, Tinker et al. 1997; Barton, Morris et al. 1998; Gayle, Cohen et al. 1998). Gayle et al determined that the ability to stand at admission, a respiratory rate $\geq$ 60 breaths per minute (bpm) and normal-appearing mucous membranes were significantly associated with survival of septic foals (Gayle, Cohen et al. 1998). Foals with a history of induced parturition were significantly less likely to live. Additionally, the following hematological and serum biochemical variables were correlated with survival ($p<0.05$): leukocyte count $\geq$6000 cells/μL, neutrophil count >4000 cells/μL, serum albumin concentration >2.2 g/dL, serum glucose concentration >120 mg/dL, blood pH $\geq$ 7.35 and a positive base excess (Gayle, Cohen et al. 1998). Hoffman et al studied prognostic factors in critically ill neonates with a variety of primary problems and reported that decreased P_vO_2 and increased anion gap were indicators of poor prognosis (Hoffman, Staempfli et al. 1992). Furr et al found a low neutrophil count, decreased neutrophil to lymphocyte ratio, hypothermia and the presence of maternal disease during pregnancy to be associated with decreased survival rates in neonatal foals (Furr, Tinker et al. 1997). In contrast to all previous reports, the objective of our study was to explore the association between selected clinical variables, the manifestation of neonatal thoracic radiographic abnormalities and the prognosis of foals with respiratory disease admitted to a referral center.

.