3. Study design

3.1. Objectives

3.1.1. Section one

The clinical and prognostic significance of radiographic pattern, distribution and severity of thoracic radiographic changes in neonatal foals

1. Explore the association between selected clinical parameters and the type of pulmonary radiographic disease manifestation in neonatal foals.
2. Investigated the impact of pattern recognition, distribution, and severity of pulmonary changes on short-term survival.

3.1.2. Section two

Risk factors and prognostic variables for survival of foals with radiographic evidence of pulmonary disease

1. Identify clinical variables in neonatal foal, which may predispose neonates or indicate respiratory disease
2. Identify clinical variables, which may predict survival of foals with radiographic evidence of pulmonary infiltrates.

3.2. Material and Methods

3.2.1. Section one: The clinical and prognostic significance of radiographic pattern, distribution and severity of thoracic radiographic changes in neonatal foals

The first section of our study was designed as a descriptive, retrospective cohort analysis of neonatal thoracic radiographs obtained from 128 hospitalized foals, less than 4 weeks of age, at Tufts University School of Veterinary Medicine [TUSVM], between 1990-1998. Three independent and blinded interpreters each reviewed a total of 207 thoracic radiographs. A maximum of three serial images was evaluated in patients that underwent repeated diagnostic imaging throughout their hospital stay. The initial radiograph was taken within 48 hours of presentation (rad1), the second radiograph (rad2) half way through the assessment period, and the last thoracic radiograph (rad3) prior to discharge or death. The reason for the radiographic evaluation was not always defined, but most commonly related to a suspicion of respiratory disease based on physical examination and blood gas analysis. Final outcome was defined as survival (discharged alive) or non-survival (not discharged alive), with a sub-categorization into foals that had died and those that were euthanized. A summary of the study design is outlined in Table 3.

Table 3: Study summary

Item	**Section one**		**Section two**	
	Part one	**Part two**	**Part one**	**Part two**
Total number of scored radiographs	207	207	163	163
Total number of included foals	128	128	163 [a]	163
Foals with radiographic changes	75 [a] (upon presentation)	75 [a] (upon presentation)	121	121 [a]
Fraction of surviving foals with respiratory disease	49 / 75 (65%)	49/75 (65%)	83/121 (69%)	83/121 (69%)
Type of study	**Assessment of clinical data** (effect on radiographic pattern)	**Outcome analysis** (effect of radiographic pattern on outcome)	**Assessment of clinical data** (effect on the presence of respiratory disease)	**Outcome analysis** (effect of clinical data on outcome)
Number of tested clinical variables	12	not applicable	27	27

[a] Primary group of foals that was statistically analyzed

3.2.1.1. Image quality criteria

The radiographic evaluation was based on a single right-lateral view of the recumbent animal, using a modified ceiling-mounted x-ray machine [a]. Prior to the diagnostic assessment, a series of image quality criteria were evaluated to eliminate studies of poor diagnostic quality (Table 4).

Table 4: Image quality criteria (Vano 1995)

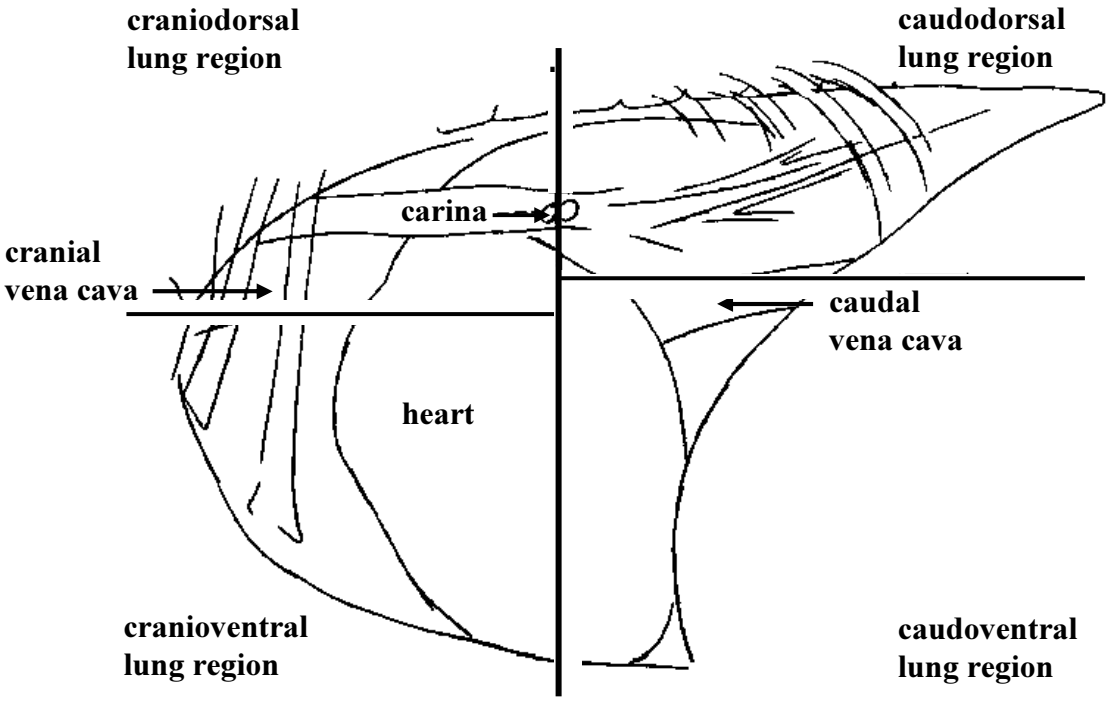
Evaluation of correct patient positioning
1. The entire lung field is visible from the first thoracic vertebra to the caudodorsal lung recess.
2. Both forelimbs are extended in the laterally recumbent patient. The extremities do not obscure any area of the lung field located below the ventral border of the trachea.
3. No significant rotation of the thorax is evident. The curved proximal third of the ribs is visualized ventral to the costo-vertebral joints.
Evaluation of correct radiographic exposure
1. The 3rd rib can be differentiated as it superimposes over the 3rd thoracic vertebral body
2. Vessels can be visualized overlying the cardiac contour
3. The caudal three sternebrae are distinctly visualized
4. The carina appears black and shows good tissue contrast resolution to the surrounding tracheal lumen

[a] modified ceiling-mounted Philips ® x-ray machine; Grand Praire, Texas 75050

3.2.1.2. Scoring System for equine neonatal thoracic radiographs

Radiographically, the thorax was studied in four separate anatomical locations: craniodorsal (crd), cranioventral (crv), caudodorsal (cd) and caudoventral (cv) regions, which are defined in Figure 2. Radiographs were only included in the study if at least three lung regions could be evaluated.

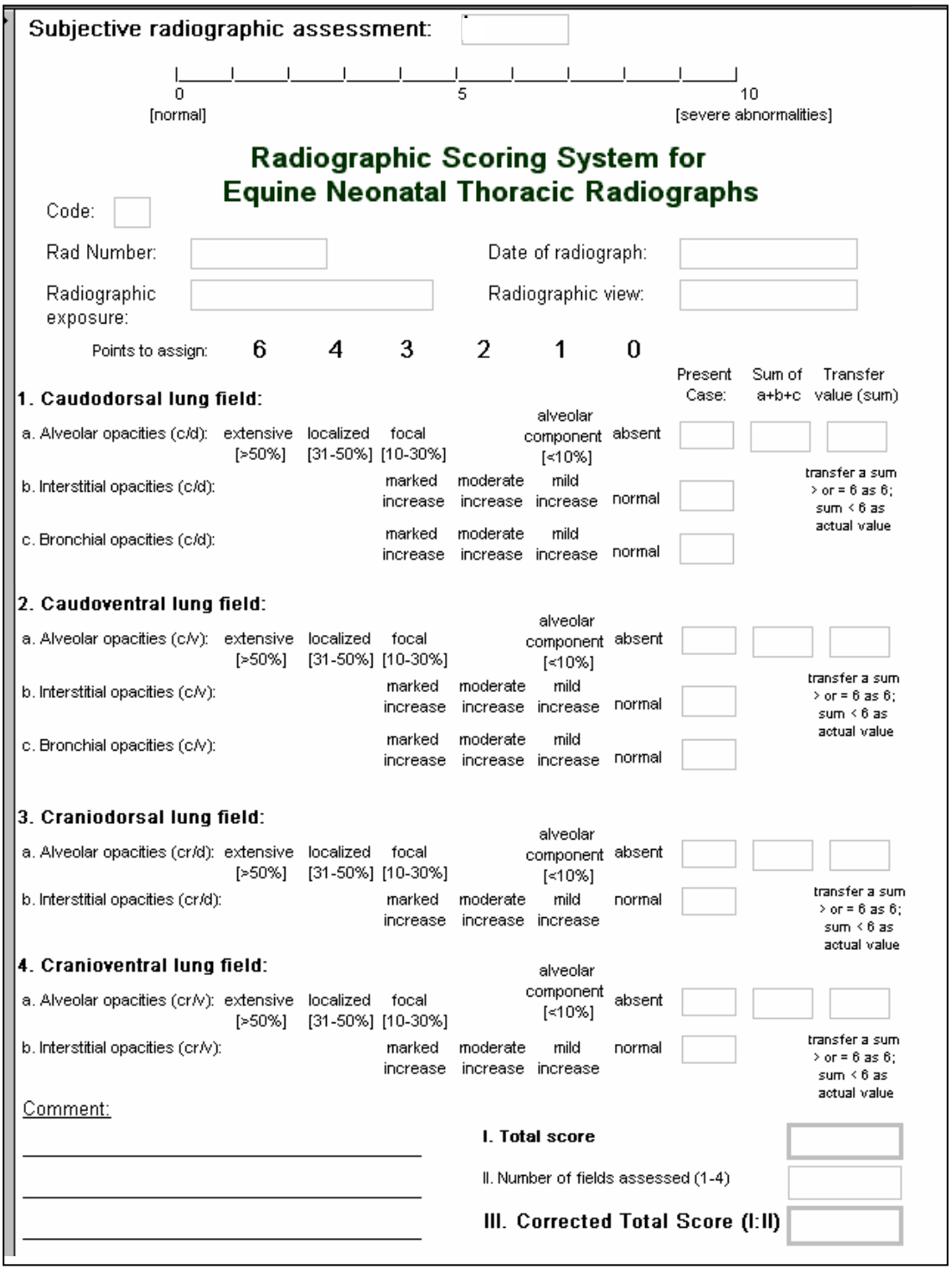
Figure 2: Partition of the caudodorsal (cd), caudoventral (cv), craniodorsal (crd) and cranioventral (crv) quadrants in neonatal thoracic radiographs



1. Separation of the cranial and caudal lung regions: Vertical line drawn through the center of the carina.
2. Separation of the craniodorsal and cranioventral regions: Horizontal line overlying the ventral margin of the cranial vena cava
3. Separation of the caudodorsal and caudoventral regions: Horizontal line overlying the dorsal margin of the caudal vena cava

In order to achieve a more objective and comparative assessment, a scoring system was developed for the radiographic evaluation (Figure 3).

Figure 3: Equine Neonatal Thoracic Scoring System



Subjective radiographic assessment:

0 [normal] 5 10 [severe abnormalities]

Radiographic Scoring System for Equine Neonatal Thoracic Radiographs

Code:

Rad Number: Date of radiograph:

Radiographic exposure: Radiographic view:

Points to assign: 6 4 3 2 1 0

							Present Case:	Sum of a+b+c	Transfer value (sum)
1. Caudodorsal lung field:									
a. Alveolar opacities (c/d):	extensive [>50%]	localized [31-50%]	focal [10-30%]		alveolar component [<10%]	absent			
b. Interstitial opacities (c/d):			marked increase	moderate increase	mild increase	normal			transfer a sum > or = 6 as 6; sum < 6 as actual value
c. Bronchial opacities (c/d):			marked increase	moderate increase	mild increase	normal			
2. Caudoventral lung field:									
a. Alveolar opacities (c/v):	extensive [>50%]	localized [31-50%]	focal [10-30%]		alveolar component [<10%]	absent			
b. Interstitial opacities (c/v):			marked increase	moderate increase	mild increase	normal			transfer a sum > or = 6 as 6; sum < 6 as actual value
c. Bronchial opacities (c/v):			marked increase	moderate increase	mild increase	normal			
3. Craniodorsal lung field:									
a. Alveolar opacities (cr/d):	extensive [>50%]	localized [31-50%]	focal [10-30%]		alveolar component [<10%]	absent			
b. Interstitial opacities (cr/d):			marked increase	moderate increase	mild increase	normal			transfer a sum > or = 6 as 6; sum < 6 as actual value
4. Cranioventral lung field:									
a. Alveolar opacities (cr/v):	extensive [>50%]	localized [31-50%]	focal [10-30%]		alveolar component [<10%]	absent			
b. Interstitial opacities (cr/v):			marked increase	moderate increase	mild increase	normal			transfer a sum > or = 6 as 6; sum < 6 as actual value

Comment:

I. Total score

II. Number of fields assessed (1-4)

III. Corrected Total Score (I:II)

Table 5: Guidelines for pulmonary pattern recognition: [loosely adapted from (Myer 1980; Myer 1980; Suter 1981; Thrall 1994; Butler 1995)]

Alveolar lung pattern:

Vessels are not visualized. There is displacement of air from the distal air spaces of the lung leading to a relatively homogenous increase in soft tissue opacity. Formation of air bronchograms is usually associated with the pattern, but not always present.

Assessment	**Description**
Absent	The pulmonary vessels are well visualized
Minimal alveolar component [<10%]	No visualization of vessels in < 10% of the lung field. Usually occurs in conjunction with a moderate or severe interstitial lung pattern.
Focal [11-30%]	No visualization of vessels in 11 - 30% of the lung field. Air bronchograms may or may not be present within < 30% of the lung field.
Localized [31 - 50%]	No visualization of vessels in 31 - 50% of the lung field. Air bronchograms may or may not be present within < 50% of the lung field.
Extensive [>50%]	No visualization of vessels in ≥ 50% of the lung field. Air bronchograms may or may not be present throughout the entire quadrant.

Table 5: Guidelines for pulmonary pattern recognition (continued):

Interstitial lung pattern:

Characterization of the non-air-containing elements of the lungs, excluding the macroscopic blood vessels and bronchi

Assessment	**Description**
Normal	Clear visualization of vessels, with well-defined borders.
Mild increase	The pulmonary vessels appear slightly ill defined (hazy borders with loss of visualization of the fine vascular structures). Mildly lacy appearance of the lung field.
Moderate increase	The vessels are ill defined resulting in a moderately lacy appearance and increased opacity of the lung field.
Marked increase	Significantly increased opacity; vessel borders are barely recognizable.

Table 5: Guidelines for pulmonary pattern recognition (continued):

Bronchial lung pattern:	
Characterized by alterations in bronchial wall thickness and density, or in bronchial lumen diameter. Please note: Peri-brochial cuffing is used as a feature of the interstitial, not bronchial pattern.	
Assessment	**Description**
Normal	Bronchial structures (seen in cross section) appear as small, thin-walled hollow rings between paired vessels. The bronchial walls are barely distinguishable when viewed side-on and are not clearly visualized at the periphery of the lung field. [no thickening]
Mild increase	A mild thickening of the smaller bronchi is noted in the hilar region, but cannot be followed far into the periphery.
Moderate increase	A few thickened bronchial walls (thick rings, commonly known as "doughnuts", when seen in cross section) can be observed in the periphery. Longitudinal views can appear as paired branching "tramlines" extending up to 2/3 into the periphery. *Note:* bronchial walls will converge as the bronchi become progressively smaller, while two adjacent vessels will diverge as they extend into the periphery.
Marked increase	Extensive bronchial thickening may be observed, extending far into the periphery of the visible lung field.

Each lung region (crd, crv, cd and cv) was assessed for the presence or absence of pulmonary abnormalities following the guidelines of pattern recognition (Table 5). The bronchial and interstitial lung patterns were graded as normal, mild, moderate or severe, obtaining a score of 0, 1, 2 or 3 respectively. The alveolar pattern was assessed for extent of the lesion and graded as absent, minimal, focal, localized and extensive, with a score of 0, 1,

3, 4 and 6 respectively. The alveolar, interstitial and bronchial scores of each lung region were combined to form a summarized regional score (Figure 3, Sum a+b+c). A maximum value of 6 could be obtained for any one region (crd, crv, cd, cv). If a specific lung pattern could not be assessed (e.g. an overwhelming alveolar pattern hampered the assessment of bronchial structures) this pattern was not included in the evaluation. The affected lung region would automatically obtain a maximum value of 6 due to the presence of an extensive alveolar pattern, which limited further assessment. If a certain lung region could not be evaluated (e.g. the scapula was overlying the craniodorsal lung), the assessment of this quadrant was omitted.

The total score of a thoracic radiograph was obtained by adding the regional scores. In order to correct for the number of lung regions that were evaluated, a corrected radiographic score was obtained by dividing the total score by the number of lung regions assessed. Therefore, the maximum obtainable value of the total score was 24 (sum of regional scores) and the maximum obtainable value of the corrected total score was 6 (total score : number of lung regions). For comparative reasons, an “assessment” score between 0 and 10 was also established for each thoracic radiograph. The “assessment” score graded the general severity of pulmonary infiltrates as viewed by each individual interpreter. A value of zero was considered normal, while a score of 10 indicated the most severe radiographic changes.

The presence of radiographic abnormalities was defined as a corrected radiographic score ≥ 1 out of 6. Foals with a corrected total score < 1 were determined to be radiographically normal and served as controls. In summary, the following radiographic variables were collected for each film: “assessment” score, total score, corrected total score, regional score of the cd, cv, crd and crv region (cd, cv, crd and crv regional sum), score of the bronchial lung pattern in the cd and cv region (cd and cv bronchial score; cranial assessment was inconclusive and therefore omitted), score of the interstitial lung pattern in the cd, cv, crd and crv region (cd, cv, crd and crv interstitial score), score of the alveolar lung pattern in the cd, cv, crd and crv region (cd, cv, crd and crv alveolar score) and the added sum of the alveolar and interstitial lung pattern scores in the cd, cv, crd and crv region (cd, cv, crd and crv alveolar-interstitial sum).

3.2.1.3. Part one: The association between selected clinical parameters and the type of pulmonary radiographic disease manifestation

The first part of section one of our study explored the potential impact of 12 selected clinical parameters on pattern recognition, distribution, and severity of pulmonary changes in 75 foals with radiographic abnormalities at the time of admission. All clinical variables selected for analysis are listed and defined in Table 6.

Table 6: Clinical and laboratory variables of neonatal foals, collected at the time of admission [categorical data]

I. Physical exam findings upon presentation
- Suckle reflex present / absent
- Abnormal respiratory sounds (crackles or wheezes)
- Dyspnea (significantly increased respiratory effort)
- Milk reflux from nares
- Tachypnea (RR>50 bpm)
- Fever (T≥102^{o}F)
II. Clinical Diagnoses
- systemic inflammatory response syndrome (SIRS)
- Upper airway pathology
- Immaturity
- Serum IgG ≤ 400 mg/dL

Table 6: Clinical and laboratory variables of neonatal foals, collected at the time of admission (continued)

III. Laboratory evaluation (CBC, chemistry) within 24 hr of presentation
- Neutropenia (Neutrophils < 4 x10^3/μL)
- Hyperfibrinogenemia (Fibrinogen concentration > 400 mg/dL)
IV. Arterial blood gas analysis upon presentation
- pH (pH < 7.3)
- Bicarbonate (bicarbonate < 15 mEq/L)
- P_aCO_2 ($P_aCO_2 \geq 50$ mmHg)
- P_aO_2 ($P_aO_2 \leq 60$ mmHg)
- Anion Gap (AG ≥ 20 mEq/L)
V. Outcome
- Survival to discharge

In this study, the definition of systemic inflammatory response syndrome (SIRS) included patients with a modified sepsis score ≥ 11 (Brewer and Koterba 1988; Brewer, Koterba et al. 1988), a positive blood culture or a known focus of systemic infection. The classification of immature foals included patients with traditional signs of immaturity, dysmaturity or prematurity (gestational age < 320 days). Endoscopy, fluoroscopy or radiography was used to determine upper airway disease. All clinical and laboratory variables were reported as categorical parameters for purposes of modeling and to provide results considered clinically useful (Table 6). The plasma anion gap (AG, mEq/L) was calculated by the formula: [Na + K] – [bicarbonate + Cl].

3.2.1.4. Part two: The impact of pattern recognition, distribution, and severity of pulmonary changes on short-term survival

In the second part of section one of our study, we investigated the impact of pattern recognition, distribution, and severity of pulmonary changes on short-term survival. In order to determine the prognostic significance of changes in radiographic appearance over time, the difference in the corrected total score between rad2 and rad1, rad3 and rad1 as well as rad3 and rad2 (corrected score difference 2-1, 3-1 and 3-2, respectively) were also determined.

3.2.2. Section two: Risk factors and prognostic variables for survival of foals with radiographic evidence of pulmonary disease

Section two of this study was also designed as a descriptive, retrospective cohort analysis of hospitalized foals which presented to Tufts University School of Veterinary Medicine [TUSVM] between 1990-1998. An exploratory analysis of clinical parameters was performed to identify potentially predictive variables of survival and risk factors or indicators of respiratory disease. The medical records of 163 neonatal foals, less than 4 weeks of age, for which thoracic radiographs were obtained within 48 hours of admission, were reviewed. Three independent interpreters with no previous knowledge of the animals' clinical history, assessed thoracic radiographs based on the single right-lateral view of the standing or recumbent animal [a]. Assessment of radiographic abnormalities was largely based on the presence or absence of the three major recognized lung patterns [bronchial, interstitial and alveolar pattern]. The radiographs were evaluated by following a predetermined scoring system as described in section one.

All clinical data were obtained from an institutional software database [b], which is based on the medical records of neonatal foals. It records diagnoses, initial laboratory results and clinical findings documented within 24 hours of admission. A pre-selection of potentially predictive clinical variables of neonatal thoracic radiographic disease was based on previous

[a] modified ceiling-mounted Philips ® x-ray machine; Grand Praire, Texas 75050
[b] Microsoft Access: Microsoft Office 97, Seattle, Washington

studies (Hoffman, Staempfli et al. 1992; Furr, Tinker et al. 1997; Barton, Morris et al. 1998; Gayle, Cohen et al. 1998) as well as a panel of experts. A total of 27 clinical variables were selected for further analysis (Table 7). All clinical and laboratory variables were reported as categorical parameters for purposes of modeling and to provide results considered clinically useful. The cut off points for conversion of continuous data into categorical variables are listed in Table 7. The plasma anion gap (mEq/L) was again calculated by the formula: [Na + K] – [bicarbonate + Cl].

Table 7: Historical, clinical and laboratory variables of neonatal foals, collected at the time of admission [categorical data]

I. Historical findings
- Admission age (≤ 7 day)
- History of dystocia or prepartum complications
II. Physical exam findings upon presentation
- Depression (reduced response to stimulation)
- Suckle reflex present / absent
- Abnormal respiratory sounds (crackles or wheezes)
- Dyspnea (significantly increased respiratory effort)
- Milk reflux from nares
- Tachypnea (RR>50 bpm)
- Fever (T≥102^{o}F)
III. Clinical Diagnoses
- SIRS
- Septic arthritis
- Omphalitis
- Diarrhea
- Patent urachus
- Hypoxic ischemic encephalopathy (HIE)
- Upper airway pathology
- Immaturity
- Serum IgG ≤ 400 mg/dL

Table 7: Historical, clinical and laboratory variables of neonatal foals, collected at the time of admission (continued)

IV. Laboratory Evaluation within 24 hr of presentation

i) Chemistry analysis:

- Hypoglycemia (Glc < 95 mg/dL)
- Decreased globulin (Globulin < 1.5 g/dL)
- Hyperkalemia (K > 4.7 mEq/L)
- Increased blood urea nitrogen (BUN > 30 mg/dL)
- Increased creatinine (Creatinine > 1.7 mg/dL)

ii) Complete blood count:

- Neutropenia (Neutrophils < 4 x103/μL)
- Neutrophilia (Neutrophils > 9 x103/μL)
- Hyperfibrinogenemia (Fibrinogen > 400 mg/dL)
- Increased hematocrit (Hct > 44%)

iii) Arterial blood gas analysis:

- pH (pH < 7.3)
- Bicarbonate (bicarbonate < 15 mEq/L)
- P_aCO_2 ($P_aCO_2 \geq 50$ mmHg)
- P_aO_2 ($P_aO_2 \leq 60$ mmHg)
- Anion Gap (AG ≥ 20 mEq/L)

V. Outcome

- Survival to discharge

Similar to section one, the definition of SIRS included patients with a modified sepsis score ≥ 11 (Brewer and Koterba 1988; Brewer, Koterba et al. 1988), a positive blood culture or a known focus of systemic infection. Final outcome was characterized by survival (discharged alive) or non-survival (not discharged alive), with a sub-categorization into foals

that had died and those that were euthanized. The classification of immature foals included patients with traditional signs of immaturity, dysmaturity or prematurity (gestational age < 320 days). Endoscopy, fluoroscopy or radiography was again used to determine upper airway disease.

3.3. Statistical Analysis

3.3.1. Section one

Three independent and blinded interpreters, scoring all 207 neonatal radiographs, established the reproducibility of the scoring system. The interviewer correlation was determined using the intra-class correlation coefficient (ICC) of a commercial statistical program [c]. The reliability analysis used a two-way random effects model with a confidence interval of 95%. Only the three investigators' mean total scores, regional and pattern scores with an ICC ≥ 0.90 were utilized for further analysis (Table 8).

The pattern and region of disease distribution was analyzed descriptively in 75 abnormal neonatal radiographs, obtained upon presentation. The regional distribution of radiographic disease was divided into the following categories: cd only, cv only, crv only, cd+cv, cd+crv, cv+crv and cd+cv+crv. Subsequently, the number of survivors and non-survivors was compared in all categories that contained more than 10 foals, using Contingency Table Chi Square analysis (Pearson Chi-Square or Fischer's Exact Test).

The distribution of radiographic scores was analyzed graphically to determine the appropriateness of parametric statistics. Subsequently, the association between 12 selected clinical parameters (Table 6, I-III) and four radiographic variables with an ICC ≥ 0.90 (corrected total score, regional cd, cv and crv scores) was examined in a multiple stepwise linear regression, using standard criteria of 0.05 for entry and 0.1 for removal of variables. Similarly, a multiple stepwise linear regression analysis was performed to relate five arterial blood gas parameters (Table 6, IV) to the aforementioned 4 radiographic variables in a subset of 50 foals, for which arterial blood gas results were available.

In the second part of section one of our study, four radiographic variables with an ICC ≥ 0.90 (corrected total score, regional cd, cv and crv sores) were compared between survivors and non-survivors, by independent-samples t-test. All radiographic variables that were possibly different between survivors (S) and non-survivors (NS, $p \leq 0.1$) were subsequently

[c] SPSS 10.00, SPSS Inc, 33 South Woctier Drive, Chicago, Ill 6060

entered into a stepwise forward logistic regression. In order to reduce bias in the analysis, all radiographic variables were also compared between foals that were euthanized and those which died naturally, by independent samples t-test. A similar evaluation compared foals with and without blood gas results.

It should be noted that this study involves extensive examination of data extracted from radiographs and records collected in the past. The study was designed to find and measure relationships and to generate hypotheses rather than test a hypothesis. In this process, multiple statistical procedures were performed, certainly more than could be strictly justified without extreme corrections for multiple comparisons. We therefore, wherever practical, present point estimates of these relationships with their confidence intervals. Additionally, we present p-values as guides to the relative importance of the relationships found, and not as indications of absolute statistical significance. In the discussion section we use the results of these data analyses to shape our exploration of biological and clinical implications. These results are further used to initiate a discussion and to suggest plausible hypotheses rather than as a sole basis of firm conclusions and clinical recommendations.

3.3.2. Section two

Twenty-seven clinical and laboratory variables (Table 7) were explored to identify their potential role as risk factors or indicators of radiographic pulmonary disease in 163 neonatal foals. The predictive value of five arterial blood gas parameters (Table 7; IV iii) was further evaluated in a subset of 86 foals, for which an initial arterial blood gas was available.

All categorical variables were compared between foals with radiographic evidence of pulmonary disease (PD) and patients without radiographic abnormalities (no disease, ND), using contingency table Chi-Square analysis (Pearson Chi-Square or Fischer's Exact Test). A statistical significance in the univariate analysis was defined as $p \leq 0.05$. All variables that were found to be possibly different between groups with $p \leq 0.1$ (PD vs. ND), were subsequently entered into a stepwise forward logistic regression.

A Chi-Square analysis and subsequent stepwise forward logistic regression was similarly performed in 121 foals with radiographic pulmonary disease, to compare categorical variables between survivors (S) and non-survivors (NS). The predictive significance of arterial blood parameters on outcome was analyzed separately in a subset of 67/121 foals with arterial blood gas results. In order to reduce bias in the analysis, all clinical parameters, including outcome and prevalence of respiratory disease, were compared between foals which did or did not have arterial blood gas results (contingency table Chi-Square analysis). A similar evaluation was performed to compare dead foals that were euthanized to those which died naturally.

Results of the arterial blood analysis were also available as continuous data and a linear regression analysis was performed for selected clinical variables. All data were analyzed using commercially available statistical software [c].

[c] SPSS 10.00, SPSS Inc, 33 South Woctier Drive, Chicago, Ill 6060