RETROSPECTIVE CLINICAL AND PATHOMORPHOLOGICAL EVALUATION OF PNEUMONIAS IN FOALS WITH SELECTED IMMUNOHISTOCHEMICAL INVESTIGATIONS

THESIS
Submitted in partial fulfilment of the requirements for the degree
-Doctor of Veterinary Medicine-
Doctor medicinae veterinariae
(Dr. med. vet.)

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Hannover 2010
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Day of the oral examination: 31.08.10
To my parents and brother for their support and love
Many times the malice or stupidity place impediments in the new idea, hence it is necessary to fight hard to achieve mutual and unconditional tolerance. Only then science flourishes and advances, because its foundation is self-experimentation and research.

Max Nettlau
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Abbreviations

“A”
round hypo- to hyperechoic area of different sizes near pleura surface at ultrasonographic examination

ABC
Avidin-Biotin-Peroxidase-Complex

A. equuli
*Actinobacillus equuli*

A. fumigatus
*Aspergillus fumigatus*

B. bronchiseptica
*Bordetella bronchiseptica*

BAL
bronchoalveolar lavage

BALT
bronchus-associated lymphoid tissue

BSA
bovine serum albumin

BW
body weight

CBC
complete blood count

CD4+
cluster of differentiation 4

dimethyl sulfoxide

CEV
cresyl violet

Ch. spp.
*Chlamydia* spp.

CF test
complement fixation test

centimeters

CO₂
carbon dioxide

diffuse

DAB
3, 3'-diaminobenzidine-tetrahydrochloride

DDS
4, 4-diaminodiphenylsulfone

desoxyribonucleic acid

D. arnfieldii
*Dictyocaulus arnfieldii*

EAV
Equine Arteritis Virus

EBE
acetic-n-butylester

EHV
Equine Herpes Virus

EIV
Equine Influenza Virus

ELISA
enzyme-linked immunosorbent assay

EMPF
Equine Multinodular Pulmonary Fibrosis

*E. coli*
*Escherichia coli*
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>EvG</td>
<td>Elastica van Gieson’s stain</td>
</tr>
<tr>
<td>F</td>
<td>focal</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>g/L</td>
<td>gram per liter</td>
</tr>
<tr>
<td>GMS</td>
<td>Grocott’s methenamine silver stain</td>
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<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HE</td>
<td>Hematoxylin and Eosin stain</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>ICS</td>
<td>intercostal space</td>
</tr>
<tr>
<td>IF</td>
<td>immunofluorescence</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>i. m.</td>
<td>intramuscular</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>Kb</td>
<td>kilobases</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>L/min</td>
<td>liter per minute</td>
</tr>
<tr>
<td>M</td>
<td>mol</td>
</tr>
<tr>
<td>M</td>
<td>multifocal</td>
</tr>
<tr>
<td>Mab</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>M. hemolytica</td>
<td><em>Mannheimia hemolytica</em></td>
</tr>
<tr>
<td>mm Hg</td>
<td>millimeter of mercury</td>
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<tr>
<td>mg/kg</td>
<td>milligram per kilogram</td>
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<tr>
<td>µg/kg</td>
<td>microgram per kilogram</td>
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<tr>
<td>µl</td>
<td>microliter</td>
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<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>min.</td>
<td>minutes</td>
</tr>
<tr>
<td>no.</td>
<td>number</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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</tbody>
</table>
O₂  oxygen
PaCO₂  arterial partial pressure of carbon dioxide
PaO₂  arterial partial pressure of oxygen
PAS  Periodic acid-Schiff
PBS  phosphate-buffered saline
pH  pondus Hydrogenii
P. equorum  Parascaris equorum
PCR  Polymerase chain reaction
P. carinii  Pneumocystis carinii
rr  reference range
R. equi  Rhodococcus equi
RNA  Ribonucleic acid
Ssp.  subspecies
Sc. equi ssp. equi  Streptococcus equi ssp. equi
Sc. equi ssp. zooepidemicus  Streptococcus equi ssp. zooepidemicus
S. pneumoniae  Streptococcus pneumoniae
TBA  tracheobronchial aspiration
TBS  Tris-buffered saline
TMS  trimethoprim-sulfamethoxazole
Vap  virulence-associated protein
W  watt
ZN  Ziehl Neelsen’s stain
1 Introduction

Pneumonia is a very frequent disease and a major cause of death in foals. Up to 14% of all foals develop pneumonia, and death rate due to respiratory disorders may be as high as 45 to 50%. A North American survey indicated that respiratory disorders are the third-leading cause of disease in thoroughbred foals and rank second as a cause of death, following injury and wounds (NEUMANN, 2006).

Pneumonia in foals comprises a heterogeneous group of pulmonary disorders. Etiologic classification distinguishes between infectious and non-infectious causes, but from all Rhodococcus (R.) equi is considered the most common pathogen of pneumonia in foals between 1 and 6 months of age (GIGUÉRE, 2001). Bacterial pneumonia, being the most common type of pneumonia in foals, can be also acquired secondary to parasitic pulmonary migration, viral respiratory infection, and stress due to transportation, overcrowding, and/or weaning.

R. equi pneumonia of foals occurs characteristically in individuals at one to six months of age and is characterized by a granulomatous to pyogranulomatous pneumonia. In the past the mortality of foals on farms with endemic rhodococcosis was as high as 80%. Early recognition of affected foals and appropriate treatment has reduced the losses dramatically.

Much less frequent are interstitial pneumonias in foals. These are challenging for the veterinarian because of the sudden onset of clinical signs and the very poor prognosis of the disorder. The cause of this disease is often unclear even at post mortem, but often Pneumocystis (P.) carinii and some virus like EHV 2 and Influenza seem to be involved in the pathogenesis. P. carinii is a common opportunistic pathogen causing a peracute and severe interstitial pneumonia. P. carinii infection is rarely described in horses; most reports are related to Arabian foals that are suffering from severe combined immunodeficiency or to foals treated with immunosuppressive drugs. As clinical findings of P. carinii pneumonia are non-specific and the identification of the pathogen is only possible in the bronchoalveolar lavage (BAL) and lung biopsy, the diagnosis is seldom made in vivo. Further diagnostic methods as ultrasonography of the thorax have not yet been evaluated in these patients and comparison of ultrasonographical findings with morphological changes is missing.

The aim of the present study was to evaluate retrospectively different types of pneumonias in foals based in morphological, clinical and ultrasonographical findings with respect to R. equi
and *P. carinii*. Based on the ultrasonographical findings, foals were organized in two different groups. Another group resulted from those foals without clinical and ultrasonographical data. The last group resulted from two foals used as control, without clinical and ultrasonographical findings of respiratory disease. Furthermore, from this organization, the first two groups with ultrasonographical findings were compared with the pathomorphological findings. Additionally microbiological results regarding *R. equi* were compared with immunohistochemical findings and the classical identification of *P. carinii* with the Grocott’s methenamine silver (GMS) stain was compared to immunohistochemistry methods.
2 Literature

2.1 Pneumonia in foals

2.1.1 General aspects

Pneumonia is the leading cause of morbidity and mortality in foals up to 6 months, and constitutes a major cause of economic loss to the equine industry due to mortality, cost of treatment and prophylaxis, growth and performance retardation and loss of value (WILSON, 1992, 1997). Although non-infectious noxes have been described as causes of pneumonia, infectious pathogens represent the main cause of respiratory tract lesions in foals (WILSON, 1997; COLAHAN et al., 1999; DUNKEL et al., 2005; DUNKEL, 2006). Viruses and bacteria are the leading agents and viral infections can be the primary cause of bacterial pneumonia.

There are several predisposing factors leading to infectious pneumonia including high environmental temperature, dry dusty conditions, environmental irritants, overcrowding, parasitism, and other factors that impair the effectiveness of respiratory tract defense mechanisms (LAKRITZ et al., 1993; DUNKEL et al., 2005). Furthermore, in hot climates the demand for heat dissipation may stress the respiratory system of foals which appear to be less able than adults to tolerate extreme temperatures (WILSON, 1997).

Most infections that cause foal pneumonia are thought to be acquired by inhalation of aerosolized or dustborn pathogens. Infectious agents suspended in aerosols or on dust particles tend to be deposited on the mucosa of the respiratory tract at the bronchiolar-alveolar junction (WILSON, 1997). Further hematogenous spread starting from the lung with subsequent septicemia also occurs, especially in neonates (DUNKEL et al., 2005; DUNKEL, 2006).

2.2 Clinical classification and findings

2.2.1 Non-infectious pneumonia

2.2.1.1 Meconium aspiration

In utero asphyxia or prolonged compression of the umbilical cord could result in evacuation of meconium into the amniotic fluid. Fetal meconium staining is generally recognized as a marker of fetal stress and asphyxia (KOTERBA et al., 1990; PARADIS, 2006). SCHOON and KIKOVIC (1987) suggested that meconium aspiration can also occur in the course of a
bacterial placentitis, because of intrauterine hypoxia, resulting in a decrease of placental perfusion.

Foals with meconium aspiration may have brown-tinged nasal secretions. Meconium may obstruct airways, resulting in air trapping (emphysema) and resorption atelectasis. Additionally, it also causes chemical irritation, bronchopneumonia and death. Lung sounds may be normal or increased in intensity, such as respiratory rate (PARADIS, 1999; PARADIS, 2006). Thoracic radiography reveals a caudo-ventral granular infiltrate (BEECH, 1985), consolidation and hyperinflation (KNOTTENBELT et al., 2005). Blood gas abnormalities vary from normal to those of hypoxia or respiratory acidosis (PARADIS, 1999; PARADIS, 2006). In the most severe cases, focal atelectasis can be observed grossly in the lung, indicating failure of the lung to be fully inflated because of the mechanical obstruction and the chemical effect of meconium on pulmonary surfactant (PARADIS, 2006).

In animals surviving more than 48 hours, microscopically, meconium and keratin lamellae are present in bronchi, bronchioles and alveoli, accompanied by mild alveolitis characterized by infiltration of neutrophils followed by macrophages and occasional giant cells as well as hyaline membranes (SCHOON and KIKOVIC, 1987; MCGAVIN et al., 2007).

2.2.1.2 Milk aspiration

Milk aspiration is usually secondary to dystocia, to a soft palate defect or displacement. The defect is a failure of the soft palate to separate tightly the oro- and the nasopharynx. Cleft palate is a malformation of the soft and sometimes hard palate, where the right and left side fail to unite, forming a cleft. Fusion occurs in a rostral to caudal direction, and therefore defects in the caudal soft palate occur more commonly than the combined soft and hard palate clefts (ALMAIER and MORRIS, 1993; PARADIS, 2006). Displacement appears to be due to a flaccidity of the soft palate and an inability of the foal to replace it in the normal position during swallowing (ALMAIER and MORRIS, 1993). Both problems result in aspiration of milk during nursing. As a first clinical sign milk appears at the nostrils following suckling and a gurgling sound can be heard in the throat region. On chest radiographs a consolidation of the accessory lobe of the lung is usually seen (PARADIS, 1999; PARADIS, 2006).

Grossly, consolidation of the accessory lobe of the lung is usually observed (PARADIS, 1999; PARADIS, 2006).
2.2.1.3 Neonatal Respiratory Distress syndrome

Neonatal respiratory distress syndrome (NRDS) is a condition characterized by failure of pulmonary gas exchange usually seen in premature foals (PARADIS, 1999; PARADIS, 2006). It is characterized by increased permeability of blood vessels and pulmonary edema, and is clinically recognised by increased respiratory effort, tachypnea, cyanosis refractory to oxygen therapy, and decreased lung compliance. In the exudative phase, injury to the alveolar epithelial barrier leads to an alveolar edema and can manifest as an alveolar-interstitial radiographic pattern (PITTET et al., 1995). Foals with neonatal respiratory distress syndrome also show hyperfibrinogenemia (fibrinogen > 4.0 g/L; reference range [rr]: 2 – 4 g/L), and leucocytosis (leucocytes above 12 x 10^3 per µl blood; rr: 6 – 12 x 10^3 per µl blood) (DUNKEL et al., 2005; DUNKEL, 2006; PARADIS, 2006).

Post mortem examination shows diffusely enlarged lungs that fail to collapse after opening the thorax. Histological findings include congestion and edema of the interstitium, hyaline membrane formation, interstitial fibrosis, and type II pneumocyte proliferation. Diffuse or patchy damage to the alveolar septae are essential features of this syndrome. This leads in the production of intra-alveolar and interstitial exudates consisting of fluid, proteins, and cellular debris (LAKRITZ et al., 1993; DUNKEL et al., 2005).

2.2.2 Infectious pneumonias

2.2.2.1 Bacterial pneumonias

There is evidence that pneumonia in older foals (more than one month of age) is caused by bacteria normally resident in the upper respiratory tract, whereas pneumonia in neonatal foals most often occurs in association with septicemia caused by gram-negative aerobic bacteria (KOTERBA et al., 1984; KOTERBA et al., 1990). General routes of infection include placental infection, aspiration of vaginal bacteria during delivery, omphalogenic, oral or nasal entrance (BEECH, 1985; DUNKEL et al., 2006; RUSH and MAIR, 2006). Especially in foals with complete or partial failure of maternal antibody transfer this post natal infection will lead to a clinical disorder (KOTERBA et al., 1984; KOTERBA et al., 1990; RUSH and MAIR, 2006).
2.2.2.1 Gram-negative bacteria

Escherichia coli

Escherichia (E.) coli is a Gram-negative, non-sporulating facultative anaerobic bacillus with both a fermentative and respiratory type of metabolism. E. coli is commonly found in the intestine of warm-blooded animals. Foals may get infected in utero or in the first 3 weeks of life. E. coli pneumonia may result from microaspiration of upper airway secretions that have previously colonized with this organism. Aspiration of infected amniotic fluid is the most common route of infection in cases of dystocia (WILKINS, 1997; SANCHEZ, 2005; MAPES et al., 2007). Additionally E. coli sepsis in neonatal foals results in hematogenous pneumonia (SANCHEZ, 2005).

Actinobacillus equuli

Actinobacillus (A.) spp. are pleomorphic Gram-negative rods. Actinobacilli are commensal organism of mucus membranes of the upper respiratory and lower genital tract of animals and are spread by aerosol and by passage through the birth canal (BIBERSTEIN, 1990). Several species of Actinobacillus have been associated with infections in horses. A. equuli, being the most common leads to an acute, highly fatal septicemia, pleuropneumonia or peritonitis of newborn foals (MARTENS et al., 1986; BREWER and KOTERBA, 1990; ASHHURST-SMITH et al., 1998; DONAHUE et al., 2006; PATTERSON-KANE et al., 2008). Foals are infected in utero or shortly after birth. The disease commonly results in death of affected foals (BREWER and KOTERBA, 1990; ASHHURST-SMITH et al., 1998).

Mannheimia haemolytica

Mannheimia (M.) spp. are Gram-negative, non-motile, short rods or coccobacilli. The organism is a normal inhabitant of mucus membranes of the respiratory and genital tract and capable to cause primary as well as opportunistic secondary infections (SCHLATER, 1989; PUSTERLA et al, 2009). Outbreaks of M. haemolytica have been described in horses with neonatal septicemia, pneumonia, abortion, and ulcerative lymphangitis (PUSTERLA et al, 2009). One of the most common signs is respiratory infection resulting in a nasal mucoid discharge (PUSTERLA et al., 2009).
**Klebsiella pneumonia**

*Klebsiella (K.) pneumonia* is a Gram-negative, facultative anaerobic, non-motile rod-shaped bacterium that possesses a prominent polysaccharide capsule (ROBERTS et al., 2000). *Klebsiella* spp. are commonly isolated from lung tissue at post mortem examination. *K. pneumonia* is commonly isolated from septicemic foals (PUSTERLA et al., 2009). The organism is a normal inhabitant of the respiratory, intestinal and genital tract of animals and humans. It may be also associated with opportunistic infections (LAKRITZ et al., 1993).

**Bordetella bronchiseptica**

*Bordetella (B.) bronchiseptica* is a Gram-negative, respiratory pathogen in several species, but its role in equine respiratory diseases has yet to be defined (COLAHAN et al., 1999). The organism appears to be overlooked as a significant respiratory pathogen in foals. However, the organism is frequently isolated in clinically sick foals from TBA or BAL and may be associated with herd outbreaks of pneumonia in foals. The primary role of *B. bronchiseptica* in bronchopneumonia of foals is not clearly established (PRESCOTT, 1997; RUSH and MAIR, 2006). In species with recognized pathogenicity, it attaches to airway surfaces, causing acute inflammation, mucus secretion, and altered mucociliary clearance (BEECH, 1999).

**Chlamydophila spp.**

*Chlamydophila (Ch.)* spp. is a Gram-negative, typically coccoid- or rod-shaped, aerobic, intracellular pathogen that requires growing cells to remain viable. *Chlamydophila* spp. resemble bacteria in the composition of the cell wall and in multiplication by binary fission, which occurs only within the host cells (GEENS et al., 2005). *Chlamydophila* spp. have been rarely detected in horses with acute respiratory infections (MOORTHY and SPRADDBROW, 1978; MCCHESNEY et al., 1982; THEEGARTEN et al., 2008). MAIR and WILLS (1992) found culturable chlamydiae in 5% of equine nasal and conjunctival swabs in a prevalence study, but no association between isolation and clinical disease was seen. *Chlamydophila* spp. can cause systemic infections in many species. In horses, it has been associated with keratoconjunctivitis, rhinitis, bronchopneumonia, abortion, polyarthritis, diarrhea, and hepatitis. In foals, it has not been described yet (THEEGARTEN et al., 2008). The most efficient methods
of diagnosis in horses are immunohistochemistry (IHC) and polymerase chain reaction (PCR) (SZEREDI et al., 2005).

2.2.2.1.2 Clinical, pathological findings and diagnosis of Gram-negative bacterial pneumonia

As in most neonatal infections, the clinical presentation of pneumonia can be nonspecific. Signs of respiratory distress may or may not be present (WILKINS, 1997). Clinical signs are more like septicemia including weakness, diarrhea, hypothermia, respiratory distress, elevated respiratory rate, and sometimes nasal discharge (KOTERBA et al., 1990; WILKINS, 1997; DUNKEL et al., 2005; DUNKEL, 2006; RUSH and MAIR, 2006; PARADIS, 2006; MCGORUM et al., 2007). The most common radiographic finding in the antero-ventral view is a prominent diffuse pulmonary alveolar opacity, in addition to a diffuse broncho-interstitial and alveolar, pulmonary pattern (LAKRITZ et al., 1993; RUSH and MAIR, 2006). Bacteria can be isolated from BAL and TBA. In these cases blood analysis, as an auxiliary tool for diagnosis, show hyperfibrinogenemia with leucocytosis. At necropsy a heavy, enlarged, non-collapsed lung with an interstitial pneumonia or abscess formation is noted (LAKRITZ et al., 1993). Histologically, a broncho-interstitial pneumonia is seen characterized by epithelial necrosis, hyaline membrane formation, and hyperplasia of type II pneumocytes. In addition, accumulation of bizarre epithelioid-like cells, macrophages, lymphocytes, and neutrophils in alveoli and interstitium, along with increased amounts of collagen is observed (LAKRITZ et al., 1993).

2.2.2.1.3 Gram-positive bacteria

Streptococcus equi ssp. zooepidemicus

Streptococcus (Sc.) equi ssp. zooepidemicus is a Gram-positive beta-hemolytic pathogen, belonging to the Lancefield group C. It is a commensal organism of the equine tonsil and nasopharyngeal mucosa that may opportunistically invade the respiratory tract, causing purulent rhinitis and bronchitis in weaning foals and pneumonia in horses of all ages (HOFFMAN et al., 1993; TIMONEY, 2004). Because S. equi ssp. zooepidemicus cannot invade mucous membranes, it is most important as a secondary invader in foals with compromised respiratory tract resulting from viral infection or stress from transport
(COLAHAN et al., 1999; TIMONEY, 2004). Inside the lung, resistance of the pathogen to phagocytosis is mediated by a complex polysaccharide capsule that forms a hydrophilic gel on the surface of the organism. Inhalation of the organism can result in infection of the upper respiratory tract including sinusitis and abscessation of lymph nodes. Subsequently, the lower respiratory tract can become infected resulting in pneumonia (COLAHAN et al., 1999; TIMONEY, 2004).

Common clinical signs consist of fever, anorexia, depression, and mucopurulent nasal discharge. A complete physical examination can reveal indicative signs, e.g. lymphadenopathy. Sinuses that are dull on percussion indicate fluid accumulation, and lung sounds are suggestive of pneumonia with consolidation or pleural fluid (BEECH, 1999). Diagnosis is based on isolation of the organism from the site of infection (BLUNDEN et al., 1994).

Histological findings comprise suppurative bronchopneumonia, but also include acute, purulent, necrotizing pneumonia with vasculitis, secondary thrombosis, and scattered foci of pulmonary infarction (ROONEY and ROBERTSON, 1999).

**Streptococcus equi ssp equi**

*Streptococcus (S.) equi ssp. equi* causes strangles, a highly contagious infection of the upper respiratory tract and associated lymph nodes of solipeds (TIMONEY, 2004).

Fever, depression, anorexia, a serous nasal discharge, that becomes mucopurulent, are characteristic findings. Pneumonia is rare but is the most fatal complication. Nodular consolidations extending to the surface of the lung can be diagnosed on ultrasonography in foals. The nodular consolidations appear as thick-walled pockets that contain material of variable echogenicity in foals. Thoracic radiographs are usually required to confirm the diagnosis of lung abscess appearing as multiple nodular opacities or large cavitary lesions in which a gas-fluid interface may be visible (LAVOIE, 1997). Culture of nasal swabs, nasal washes or pus from abscesses is essential for confirming the presence of *S. equi ssp. equi* in foals, although polymerase chain reaction (PCR) is more sensitive than culture. At necropsy, a consolidation and non-collapsed lung can be observed. Histologically, abscesses contain numerous neutrophils and necrotic debris (TIMONEY, 2004).
*Streptococcus pneumoniae*

*Streptococcus (S.) pneumoniae*, also called pneumococcus, is an alpha-hemolytic, Gram-positive diplococcus. *S. pneumoniae* is a normal resident of the upper respiratory tract of foals and other animals (AUSTRIAN and THORN, 1979; VARVIO et al., 2009). BENSON and SWEENEY (1984) speculated that the pneumococcal capsular polysaccharide may accumulate in the alveolar spaces and further compromise lung capacity, as well as hinder the normal first-line non-specific host defence mechanisms. The low incidence of recovery of *S. pneumoniae* from foals, compared with the human carrier rate suggests that these foals acquire the infecting agent from a human source (AUSTRIAN and THRON, 1979; TIMONEY, 2004). *Post mortem* examination reveals local pneumonia, especially in the cardiac area and accessory lobes (TIMONEY, 2004).

*Rhodococcus equi*

*Rhodococcus (R.) equi* was first isolated in 1923 from Swedish foals and described initially as *Corynebacterium equi* (MAGNUSSON, 1923). *R. equi* is a Gram-positive, pleomorphic coccobacillus, non motile without flagella, non-sporulating, facultative intracellular, that may also be weakly acid fast (YANAGAWA and HONDA, 1976; HONDALUS and MOSSER, 1994). It causes granulomatous to pyogranulomatous pneumonia in foals one to five months of age. The discovery of virulence-factors has permitted the classification of the strains to virulent and avirulent *R. equi* (TAKAI et al., 1991a; TAKAI et al., 1991b; TAKAI, 1997). Virulent *R. equi* is characterized by the presence of virulence-associated 15-17-kilodalton (kDa) antigens (VapA), and virulence plasmid DNA of 85-90 kilobases (kb) (TAKAI et al., 1991c). The virulent plasmid encodes a family of eight closely related virulence-associated proteins designated VapA and VapC through VapI (SEKIZAKI et al., 1995; TAKAI et al., 2000; POLIDORI and HAAS, 2006). This plasmid is critical for intracellular replication within macrophages and for the development of disease in foals (HONDALUS and MOSSER, 1994; GIGUÈRE et al., 1999; JAIN et al., 2003). Isolates that lacked the virulence plasmid and consequently the 15-17 kDa antigens, are unable for both, replication and survival in macrophages and do not cause disease (TAKAI et al., 1991b; GIGUÈRE et al., 1999; JAIN et al., 2003).
R. equi has been isolated on every continent except Antarctica (ELLENBERGER and GENETZKY, 1986). R. equi pneumonia is virtually nonexistent in horses over six months of age, suggesting age-dependent maturation of protective mechanisms (ELLENBERGER et al., 1984; NERREN et al., 2009). Epidemiological evidence indicates that foals with R. equi pneumonia are most commonly infected during the first few days of life (GIGUÈRE and PRESCOTT, 1997; HOROWITZ et al., 2001; CHAFFIN et al., 2003), but clinical signs typically do not develop until foals are 30 to 60 days old and may not be apparent for several months (HIGUCHI et al., 1997; HOROWITZ et al., 2001; CHAFFIN et al., 2003; ALTHAUS 2004). This infection is related to up to 10% of mortality and up to 45% of pneumonia in foals on endemic farms. In about half of the cases, pneumonia is accompanied by intestinal lesions including enterocolitis and typhlitis with multifocal granulomatous or suppurative mesenteric lymphadenitis (ZINK et al., 1986; CHAFFIN and MARTENS, 1997). Other clinical signs may include nonseptic polysynovialitis, septic arthritis, osteomyelitis, uveitis, and subcutaneous abscesses (CHAFFIN and MARTENS, 1997; CHAFFIN et al., 2003).

High-ambient summer temperatures, sandy soil, and dusty conditions favor multiplication and dissemination of the organism in the environment. R. equi is a common inhabitant of the soil, and inhalation of dust is believed to be the primary route of exposure (CHAFFIN et al., 2003; MCGAVIN et al., 2007). Swallowing infectious sputum and feces can lead to enteric disease (MEIJER and PRESCOTT, 2004). Once in the lung, R. equi is phagocytized by alveolar macrophages and the agent is able to evade destruction (FERNANDEZ-MORA et al., 2005). In macrophages, it is located exclusively within membrane-enclosed vacuoles, and intracellular persistence appears to correlate with failure of phagosome-lysosome fusion (ZINK et al., 1987; FERNANDEZ-MORA et al., 2005). Inhibition of the acidification blocks vesicular delivery to lysosomes, pH of the phagosome controls fusion events (MEIJER and PRESCOTT, 2004). The basis of pathogenicity of R. equi is its ability to multiply in and eventually to destroy alveolar macrophages. Released lysosomal enzymes are responsible for extensive caseous necrosis of the lung and the intense recruitment of neutrophils, macrophages, and giant cells. These changes lead to pyogranulomatous pneumonia with abscesses and tracheo-bronchial pyogranulomatous lymphadenitis (GIGUÈRE and PRESCOTT, 1997; MCGAVIN et al., 2007).
R. equi causes chronic suppurative bronchopneumonia which is clinically unapparent until pulmonary lesions are extensive and well advanced. (COLAHAN et al., 1990; CHAFFIN et al., 2003; PRESCOTT and GIGUÈRE, 2005; MCGORUM et al., 2007). The clinical signs of the disease may include decreased appetite, mild lethargy, fever (38.9 to 40.5°C), tachypnea, dehydration, and increased effort of breathing characterized by nostrils flaring and increased abdominal effort. On auscultation, moist, loud breath sounds with crackles and wheezes may be heard (PRESCOTT and GIGUÈRE, 2005; MCGORUM et al., 2007). A muco-purulent nasal discharge and cough are common, but not always present (ELLENBERGER and GENETZKY, 1986; RUSH and MAIR, 2006).

On chest radiographs of affected foals a prominent alveolar pattern with regional consolidation can be observed (HINES, 2007). Lung radiographic findings progress from a prominent interstitial pattern to dense patchy alveolar opacities, lung consolidation and abscesses (ELLENBERGER and GENETZKY, 1986; KOTERBA et al., 1990; MCGORUM et al., 2007). At ultrasonography, pulmonary abscessation at the periphery of the lung can be observed as multifocal nodular hypoechoic consolidation (GIGUÈRE and PRESCOTT, 1997; RUSH and MAIR, 2006; MCGORUM et al., 2007). Pathologic blood values commonly seen at endemic farms for R. equi are hyperfibrinogenemia and leucocytosis (PRESCOTT, 1991; GUIGÈRE et al., 2003; MCGORUM et al., 2007). TBA and BAL cytology and/or culture are required for a positive diagnosis. In a recent study, it was shown that the diagnostic potential of the culture is slightly higher than that of PCR. Furthermore it was stated that for the diagnosis of R. equi pneumonia in foals the combination of both microbiological culture with PCR should be used for examination of samples of the airways of foals to enhance the rate of diagnosis (VENNER et al., 2007). TAKAI and coworkers (1993) developed a monoclonal antibody specific for the 15-17 kDa antigen (Mab 10G5) of R. equi that is supposed to be highly sensitive and specific in an immunoassay for the identification of virulent R. equi.

At necropsy, in chronic disease the lungs are diffusely consolidated with miliary to large confluent abscesses and partly atelectatic areas (MAGNUSSON, 1923; HINES, 2007). In more slowly progressing disease there are frank abscesses ranging from 1 to 6 cm in diameter. Although the distribution of lesions may be variable, lesions are bilateral in most cases and are more severe in the cranio-ventral region. They consist of areas of caseous necrosis, and in most cases there is no distinct fibrous capsule around the necrotic tissue (ROONEY and...
ROBERTSON, 1999; RETTEG et al., 2009). The pulmonary exudates vary from a thick yellow to crumbly and caseous material. In all forms bronchial and mediastinal lymph nodes have similar lesions (AINSWORTH, 1999). Histologically, there is a pyogranulomatous bronchopneumonia. The peracute, disseminated disease is characterized by diffuse inflammatory cell infiltration of alveoli and distal airways with striking accumulations of basophilic bacteria in the cytoplasm of macrophages and giant cells. Central areas of necrotic debris are surrounded by congestion, edema and a mixed population of inflammatory cells (BARTON and HUGHES, 1980; 1984). The bronchioles may contain macrophages, neutrophils, necrotic and mucous substances (ISHINO et al., 1992; WEIMAR, 2006).

2.2.2.2 Viral pneumonias

2.2.2.2.1 DNA Viruses

Adenovirus

Adenoviruses are widely distributed DNA-containing viruses (ARDANS et al., 1973; KOTERBA et al., 1990; POWELL, 1991; MCGORUM et al., 2007). Infection commonly involves the respiratory tract and the conjunctiva (KOTERBA et al., 1990; POWELL, 1991). Infection is acquired by foals from their dams during nursing, or as droplet of respiratory or ocular secretions (POWELL, 1991; STUDDERT, 2003). Fatal adenovirus pneumonia has been associated with combined immunodeficiency syndrome seen in Arabian foals (KOTERBA et al., 1990; MCGORUM et al., 2007). The virus replicates in epithelial cells throughout the respiratory tract producing lysis and sloughing of cells with subsequent hyperplasia of underlying uninfected cells (STUDDERT, 2003). Experimental infection of normal and colostrum-deprived neonatal foals, 24 to 48 hours of age, resulted in signs of pneumonia, like fever, cough and nasal discharge, within seven days post inoculation, but no foals died of the infection (KOTERBA et al., 1990; MCGORUM et al., 2007). Foals may develop bronchopneumonia and interstitial pneumonia, which usually resolves spontaneously if it is not complicated by secondary bacterial infection (STUDDERT, 2003). Concurrent infections with R. equi and Pneumocystis (P.) carinii have been observed. Cell smears prepared from nasal and conjunctival epithelium reveal basophilic intranuclear
inclusion bodies. Serologic methods to confirm infection include neutralizing, hemagglutination inhibition, complement fixation and precipitin antibody tests (POWELL, 1991). In most cases of adenovirus infection it can be observed an interstitial pneumonia combined with a bronchopneumonia. At necropsy, up to 25% of the total lung volume, particularly in the cranio-ventral portion is consolidated and purple in color. Additionally small purple depressed areas are scattered in the pulmonary tissue near the line of demarcation. Upon incision the dark depressed areas are consolidated and yellow mucus exudate is found in bronchi and bronchioles lumina (CHESNEY et al., 1970; JONES et al., 1997). Throughout the lungs, there are also some atelectatic areas. The remaining lung tissue is normal in color, markedly emphysematous and distended, and fails to collapse (MCGORUM et al., 2007). Histologically, bronchiolar thickening that results from proliferation of the lining epithelial cells and accumulation of leucocytes around the bronchioles is observed. Common finding is hyperplastic epithelial lining of small bronchi and bronchioles ranging from 3 to 12 cells in depth with consequent luminal narrowing. The nuclei of many of these cells contain large, basophilic inclusion bodies. Necrosis of cells results in cellular debris partially filling the lumen (CHESNEY et al., 1970; JONES et al., 1997; MCGORUM et al., 2007).

**Equine Herpes Virus 1 and 4**

Equine herpesvirus 1 (EHV-1) and EHV-4, are alpha herpesviruses. They are DNA-containing viruses, and occur ubiquitously in horse populations worldwide. Foals may acquire EHV-1 in utero, during birth or postnatally. Newborn foals are most commonly infected at or before birth, and as the virus induces abortion in mares, many are born dead. Those born alive are weak (with features of prematurity/dysmaturity), and may show icterus, pneumonia, neurological signs, microulcerative keratitis, and death is frequent within three days (KNOTTENBELT et al., 2005). There is a high mortality in foals within the first 48 hours of life and survivors are often left with secondary bacterial bronchopneumonia (KNOTTENBELT et al., 2005).

Transmission occurs horizontally by direct or indirect contact with infectious nasal secretions, aborted fetuses, placenta, or placental fluids. The upper respiratory tract is the natural portal of entry for EHV, and the respiratory mucosal epithelium is the primary target tissue for infection (ALLEN, 2002; MCGORUM et al., 2007). Quickly following replication in the
upper respiratory tract epithelium, the virus is carried by migratory dendritic cells and macrophages into the draining lymph nodes (KYDD et al., 1994).

Diagnostic confirmation is predicated on demonstrating the presence of EHV in either nasopharyngeal secretions or venous blood leukocytes (ALLEN, 2002). In culture, EHV-1 and EHV-4 have a characteristic cytopathic effect, forming acidophilic intranuclear inclusions (WANG et al., 2007).

Grossly EHV-1 and EHV-4 in aborted fetus causes pleuritis and pneumonia, it is also seen in infected newborn foals but they die few days after birth (KLEIBOEKER et al., 2002; KNOTTENBELT et al., 2005).

Histological findings in an EHV 4 infection range from mild, lympho-histiocytic, and suppurative bronchiolitis with peribronchial interstitial inflammatory cell infiltrates to lungs that contain marked, diffuse accumulations of histiocytes and neutrophils with areas of interstitial fibrosis and necrotizing bronchiolitis. Syncytial cells that contain up to 20 visible nuclei and intranuclear eosinophilic inclusion bodies are commonly seen (KLEIBOEKER et al., 2002; MCGORUM et al., 2007).

In EHV-1 pneumonia, microscopic examination of the lungs reveals extensive subpleural and intralobular edema and presence of foamy macrophages in alveolar spaces. Multifocal areas of acute hemorrhages and necrosis within the parenchyma can also be observed. Small aggregates of mononuclear inflammatory cells are observed around the bronchioles. Occasional intranuclear eosinophilic inclusion bodies in alveolar cells are observed (HAMIR et al., 1994).

**Equine Herpes Virus 2 and 5**

EHV-2 and EHV-5 are gamma herpesviruses and are also called equine cytomegaloviruses. EHV-2 is known to be widely spread in horse populations, frequently establishing persistent and/or latent infections. In contrast, EHV-5 has so far only been isolated in a few countries, like Australia, Switzerland, Germany and New Zealand, and very little is known about the pathogenic role of this virus. In Australia, EHV-5 was originally isolated from horses suffering from upper respiratory disease (NORDENGRHAN et al., 2002). Until now EHV-5 has only been isolated from adult horses (WILLIAMS et al., 2007). EHV-2 induces a common infection in foals at early life time (MURRAY et al., 1996). Latency certainly plays a key role
in the epidemiology of EHV-2, since the majority of adult horses (89–93%) and almost all foals harbor latent infection in circulating lymphocytes (SLATER, 2007).

EHV-2 seemed to cause keratoconjunctivitis in adult horses and might induce respiratory disease in foals, although it has also been associated with upper respiratory disease, pneumonia, pharyngitis, fever, enlarged lymph nodes, inappetence, general malasia, and poor performance (AGIUS and STUDDERT, 1994; MCGORUM et al., 2007; KAPPE et al., 2009), but its role in clinical disease is uncertain (SLATER, 2007). Some have suggested that EHV-2 may act as an immunosuppressive agent in foals predisposing for other infections, including other respiratory viruses and R. equi (PÁLFI et al., 1978; BELÁK et al., 1980, WANG et al., 2007). EHV-2 has a “slowly” cytopathic effect, and does not form inclusion bodies (STUDDERT, 1993; WANG et al., 2007).

Grossly EHV-2 presents a suppurative to necrotizing bronchopneumonia and an interstitial pneumonia. Histologically, alveolar damage of different stages can be observed, characterized by hyaline mebranes formation attached to alveolar walls, desquamation and proliferation of type II pneumocytes (KAPPE et al., 2009), and giant cells formation (SCHLOKER et al., 1995).

EHV-5 casues the equine multinodular pulmonary fibrosis (EMPF), and is macroscopically observed as multifocal to coalescing nodules of fibrosis throughout the whole lung. The individual nodules range from <1 to 5 cm in diameter. The nodules are pale tan-white and moderately firm. Little of unaffected lung is usually present when this form of disease is recognized. Histologically, the lesions are largely confined to the alveolar parenchyma. The nodules are sharply demarcated from adjacent less affected lung. The nodules consist of marked interstitial expansion by well-organized mature collagen. Intersitium is infiltrated by variable numbers of mixed inflammatory cells, consisting primarily of lymphocytes, with smaller numbers of macrophages, neutrophils, and occasional eosinophils (WILLIAMS et al., 2007).
2.2.2.2 RNA Virus

Equine Influenza Virus

Equine Influenza virus (EIV) is an Orthomyxovirus and contains RNA as its genetic material. EIV constitutes one of the major epizootic diseases of the horse. These viruses are endemic in Europe and North America. EIV is contracted by inhalation and is extremely contagious. The persistent dry cough releases large quantities of virus particles into the atmosphere, and horses sharing the same air space can be infected (CULLINANE, 2005). The virus infects the ciliated epithelial cells of the upper and lower airways and can cause deciliation of large areas of the respiratory tract within four to six days. As a result, the mucociliary clearance mechanism is compromised and tracheal clearance rates may be reduced allowing less pathogenic bacteria to gain access to the respiratory epithelium (BRITTON and ROBINSON, 2002; KNOTTENBELT et al., 2005). Bronchitis and bronchiolitis develop followed by interstitial pneumonia accompanied by congestion, edema and neutrophilic infiltration. Disease is limited to the upper respiratory tract in normal foals but can cause pneumonia in foals that are very young or immunocompromised. Foals can also present cough, nasal discharge, being serous at first becoming catarrhal and then purulent as secondary infection develop and limb edema. Secondary bacterial infections are common and represent the most serious aspect of the disease (KNOTTENBELT et al., 2005).

Laboratory confirmation is achieved by isolation of virus from nasopharyngeal swabs or retrospective serology (ANESTAD and MAAGAARD, 1990). EIV infection has been reported in association with severe, acute, fatal interstitial pneumonia in two foals. Post mortem findings in these two foals were consistent with a viral pneumonia including a diffuse bronchitis, alveolar necrosis, hemorrhage and fibrin exudation (BRITTON and ROBINSON, 2002; PEEK et al., 2004).

Equine Viral Arteritis

Equine Viral Arteritis (EVA) is a Togavirus and contains RNA as its genetic material. The natural reservoir of EVA that ensures its persistence in various horse populations throughout the world is the carrier stallion (TIMONEY and MCCOLLUM, 1993). EVA can cause sporadic respiratory disease and sudden death in foals, and abortion in mares (DEL PIERO, 2000). Acutely infected horses shed EVA for a limited period of time in various body
secretions and excretions. The greatest concentration of virus is usually shed via the respiratory tract with aerosol transmission as the most important mode of spread (MCCOLLUM et al., 1971; MCGORUM et al., 2007). Additionally virus is disseminated on breeding farms venereally by acutely infected stallions (TIMONEY and MCCOLLUM, 1993). Transmission of EVA may also occur in utero resulting in abortion or at birth as congenitally infected live but diseased foal (DOLL et al., 1957; DEL PIERO et al., 1997; SZEREDI et al., 2003).

In foals clinical signs include, profound depression, weakness, hind limb and facial edema, conjunctivitis, tachypnea, leucopenia, thrombocytopenia and terminally respiratory distress like polypnea and dyspnea with sudden death (DEL PIERO et al., 1997; SZEREDI et al., 2003). Affected foals with EVA present severe respiratory distress of acute onset. Radiographic findings are consistent with an interstitial pneumonia. The disease is almost uniformly fatal (MCGORUM et al., 2007). The severity of EVA tends to be greater in very young or aged horses, in debilitated individuals, and in horses that are being physically stressed (TIMONEY and MCCOLLUM, 1993).

In foals, EVA infection is frequently confirmed by virus isolation, PCR and/or immunohistochemistry using an N protein-specific Mab (DEL PIERO et al., 1997; SZEREDI et al., 2003). Several ELISA tests have been developed for the diagnosis (CHO et al., 2000).

In foals, at necropsy, the lungs appear wet, heavy, increased in volume with rib impression present on the parietal surface of the lung and contain multifocal hemorrhages (DEL PIERO et al., 1997; SZEREDI et al., 2003).

Microscopical examination reveals a mild interstitial pneumonia accompanied by severe edema and hemorrhages. The pneumonia is characterized by hypertrophy and hyperplasia of type II pneumoncytes and eosinophilic laminar to granular material scattered in the alveolar lumen, free or attached to the alveolar wall, sometimes entrapping a few degenerated cells (DEL PIERO et al., 1997; MCGORUM et al., 2007). The inflammatory cells are mostly macrophages and rarely neutrophils. In large areas of the lung, the alveoli, bronchioles and bronchi are partly or completely filled with hyaline membranes. In the blood vessels, especially in the small muscular arteries, edema, swollen endothelial cells, vasculitis, perivasculitis, and fibrinoid necroses are observed (DEL PIERO et al., 1997; SZEREDI et al., 2003; MCGORUM et al., 2007).
2.2.2.3 Parasitic pneumonias

*Parascaris equorum*

*Parascaris (P.) equorum* is a large nematode of the small intestine in horses. The larval stages migrate through the lungs of foals less than six months of age. Foals of this age are more susceptible to the infection and are the source of the greatest egg production (CLAYTON, 1986; RUSH and MAIR, 2006). Clinical respiratory disease has been reported in foals at two to four weeks of age (CLAYTON and DUNCAN, 1978; RUSH and MAIR, 2006; LYONS et al., 2008). The life cycle of *P. equorum* starts with swallowing eggs containing third-stage larvae. These eggs hatch at the gastrointestinal tract and the resulting larvae burrow into the wall of the small intestine, where they pass through portal blood veins, and migrate to the liver, heart and subsequently to the lungs. After migration into airways, the larvae are coughed up, swallowed, return to the small intestine and mature in the duodenum and proximal jejunum. After completion of their migration, the parasites grow in size, and first eggs appear in the feces between 72 and 110 days after infection (CLAYTON, 1986; RUSH and MAIR, 2006; LYONS et al., 2008). When Parascarid eggs are ingested, larvae can be found seven to 14 days later in the lungs, where they incite eosinophilic inflammation of airways and alveoli, mucus exudation, and focal hemorrhage (NICHOLS et al., 1978; LYONS et al., 2008).

Foals infected at young age cough, have a mucoid or muco-purulent nasal discharge, and may have an increased respiratory rate. The presence of eosinophils in tracheal aspirates and lack of evidence of sepsis support the diagnosis (COLAHAN et al., 1999). Small necrotic foci and petechiae occur in the liver, lungs and hepatic and tracheobronchial lymph nodes. These changes are also apparent microscopically, but eosinophils are also prominent as part of granulomatous pneumonia associated with larvae. Eosinophilic alveolitis, perivasculitis, bronchitis and bronchiolitis are present (ROONEY and ROBERTSON, 1999; MCGAVIN et al., 2007). The chronic nodules consist of aggregates of lymphoid tissue with fibrosis and mineralization (DUNGWORTH, 2007).
2.2.2.4 **Fungal pneumonia**

*Aspergillus fumigatus*

*Aspergillus (A.) fumigatus* affects immunosuppressed animals, those on prolonged antibiotic or corticosteroid therapy or enteric mucosal compromise. Pulmonary aspergillosis, although extremely rare in horses, has been associated with enterocolitis, often due to *Salmonella* spp. infection (HATTEL et al., 1991; GUIDA et al., 2005; HILTON et al., 2009). Some studies suggest that damaged digestive mucosa serves as a portal of entry with subsequent spread to the lungs (SLOCOMBE and SLAUSON, 1988; CARRASCO et al., 1997; HILTON et al., 2009).

Reports of pulmonary aspergillosis are rare in neonatal foals. Although respiratory disease resulting from opportunistic pathogens such as adenovirus and *Pneumocystis carinii* is a common finding in foals with immunodeficiency, pulmonary aspergillosis has not been reported (PERRYMAN et al., 1978, HILTON et al., 2009).

HILTON and coworkers (2009) comment a case of cavitary lung disease resulting from aspergillosis that is the first time seen in a foal.

*Pneumocystis carinii*

As a pathogen, *Pneumocystis (P.) carinii* was likely described in humans and guinea pigs for the first time by CHARGAS in 1909. But in 1957 the organism was primarily associated with several epidemics of interstitial plasma cell pneumonia among premature or malnourished infants (GEORGIEV, 1998; ALIOUAT-DENIS et al., 2008). Until recently, *P. carinii* was considered to be a unicellular parasite and classified as protozoon. Subsequently, it was demonstrated that it was more closely related to fungi, and was classified as Ascomycota (GEORGIEV, 1998). *P. carinii* pneumonia is associated with immunodeficiency syndromes in foals from six to twelve weeks of age (RUSH and MAIR, 2006).

*P. carinii* is the most common pulmonary infection related to human immunodeficiency virus, and has been associated with combined immunodeficiency syndrome, corticosteroid administration, *R. equi* infection in foals and CD+4 lymphopenia in humans (HUI and KWOK, 2006; RUSH and MAIR, 2006; JIANCHENG et al., 2009). Healthy hosts are usually able to eliminate the parasites from their lungs. However, as long as they remain infected, they are able to transmit *Pneumocystis* by the airborne route to naive immunocompetent hosts,
like young mammals (CHABÉ et al., 2004). After the foal inhales the organism, small, uninucleated trophic forms establish themselves in the alveoli (Fig. 2.1). The thin-walled trophic forms are pleomorphic as they adapt to the contours of neighbouring organisms or cells. They may either divide by a process described as being binary fission-like to produce two daughter cells resembling the trophic forms, or by sexual reproduction resulting in highly pleomorphic cysts (VAN RENSBURG, 2005). These forms have a multinucleated appearance, as each cyst contains two to eight intracystic bodies. The trophozoites could likely leave the cyst, presumably by a preformed pore located at the thickest part of the mature cyst’s wall (ITATANI, 1994), releasing the intracystic bodies into the alveoli which attach to type I pneumocytes and give rise to a new generation of small, mobile, thin-walled trophic forms (DEI-CAS et al., 2004). Empty mother cysts collapse to form crescent-shaped bodies (VAN RENSBURG, 2005), and are frequently encountered in alveoli during microscopical examination of infected lungs (CAMPBELL, 1972; DEI-CAS et al., 2004).

**Fig. 2.1 A:** Hypothetical life-cycle of *Pneumocystis* species. Parasites are represented as observed in the lung using transmission electron microscopy. Pleomorphic, thin-walled mononuclear trophic forms are shown attached to type I pneumocytes close to an capillary vessel (star). Trophic forms (small arrows) evolve into thick-walled sporocytic stages, in which multiple nuclear division leads to the formation of eight spores. These forms are able to leave the cyst and to attach specifically to type I pneumocytes (ALIOUAT-DENIS et al., 2008).
After inhalation and attachment to type I pneumocytes, an interstitial inflammatory response consisting mainly of an infiltration with histiocytes, lymphocytes and plasma cells is evoked. Degeneration of type I pneumocytes is accompanied by increased permeability of the alveolar capillary membrane resulting in exudation of fibrinogen and serum proteins. This helps to constitute the foamy eosinophilic matrix. These changes together with the continual multiplication of the organisms lead to filling of the alveoli, disturbances in blood-gas exchange, and ultimately to death of the foal (VAN RENSBURG, 2005).

Clinical signs include non-productive cough, tachypnea, dyspnea and tachycardia. There may be a slight elevation of body temperature. At auscultation loud wheezes, crackles and whistling sounds over the entire lung field on both sides may be audible. Complete blood count (CBC) may show slight leucocytosis, mild eosinophilia and normally also hyperfibrinogenemia (PERRON LEPAGE et al., 1999; VAN RENSBURG, 2005). In severe cases the mucous membranes may be cyanotic. Blood-gas analysis may indicate hypoxemia and hypercapnia (PERRON LEPAGE et al., 1999).

The clinical specimens used for conventional cytochemical staining include sputum, BAL, bronchial brushing and lung tissue biopsy (MCGAVIN et al., 2007; JIANCHENG et al., 2009). Lavage specimens and lung tissue slides can be stained for *P. carinii* in a variety of ways, including GMS, toluidine blue, Gram-Weigert, cresyl echt violet (CEV), and Giemsa stain (GILL et al., 1987; JIANCHENG et al., 2009). GMS stain, toluidine blue, CEV and Gram-Weigert stain cyst walls of *P. carinii* leaving the trophozoites and internal sporozoites unstained. Giemsa stains the internal sporozoites as well as the trophozoites, but leave the cyst walls unstained. A disadvantage of both, Gram-Weigert and Giemsa stains, is the non-specific staining of tissue cells and debris (GILL et al., 1987). Immunohistology using commercially available antibodies specific for *P. carinii* may also be applied on lung tissue or BAL (RUSH and MAIR, 2006).

Mainly, foals with fatal pneumocystosis have uniformly meaty, non-collapsed, enlarged and consolidated lungs. The cut surface of affected lungs is fairly dry and sometimes diffuse pink and yellow-brown mottled (VAN RENSBURG, 2005; RUSH and MAIR, 2006). Histopathological evaluation reveals diffuse distention of alveoli and terminal bronchioles flooding with foamy macrophages and proteinaceous, finely vacuolated material giving them a “honeycomb” appearance, which contains small, faintly staining, basophilic particles
representing the nuclei of the organisms (VAN RENSBURG, 2005; MCGAVIN et al., 2007). Interstitial spaces are infiltrated with lymphocytes and plasma cells. Hyaline membrane formation may be prominent with mild to marked type II pneumocyte hyperplasia (RUSH and MAIR, 2006).

### 2.3 Lung ultrasonography

Ultrasonographic examination of the lung can be performed using a linear or a sector transducer with either a frequency of 5.0- or 7.5-MHz. With 7.5-MHz a depth of four to twelve centimetres can be penetrated, which is ideal for the thoracic examination of a foal (SLOVIS et al., 2005). The area examined should include both sides of the thorax from the 16th to the 3rd intercostal space (ICS) and from the dorsal to the ventral portion of the thorax (REEF, 2004a).

Ultrasonographic waves are completely reflected at the normal aerated lung interface, allowing only the pleural surface to be evaluated (REIMER, 1990). Therefore, the normal visceral pleural edge of the lung appears as a straight hyperechoic line and characteristic equidistant reverberation air artifacts deeper in the image (Fig. 2.2) (REEF, 2004a). Only when fluid or cellular accumulation occurs immediately beneath the visceral pleural surface will an acoustic window be created, allowing visualization of pulmonary pathology. The affected area of the lung is hypoechoic and lacks the normal air echo at the surface (SLOVIS et al., 2005). Comet tail artifacts are created by the presence of a small area of fluid or cellular infiltrate in the pulmonary periphery. The ultrasonographical beam is transmitted through this small area of fluid or cellular infiltrate and then encounters air which is highly reflective, creating an air artifact that appears like a comet tail (REEF, 2004a). Nodular focal or multifocal consolidations are variable in size and are located anywhere in the lung, being most frequent cranially and ventrally. They are identified by cavitated appearance and the absence of normal pulmonary structures. The center may appear hypoechoic, isoechoic, or septate, depending on the type of fluid present. Pulmonary nodular consolidations in horses are rarely encapsulated, with R. equi abscesses in foals being the most notable exception. In these foals, more echoic capsules can be imaged surrounding the hypoechoic to echoic fluid within the pulmonary abscess (REEF, 2004b). Pulmonary consolidation is hypoechoic, and occurs due to
the replacement of alveolar air with fluid or cells, which produces an acoustic window (RAMIREZ et al., 2004).

Fig. 2.2 Image of an ultrasonographical healthy lung of a foal showing at the top of the image the skin, then the hypoechoic parallel lines are the intercostal muscles, and beneath the muscle is the visceral plaura as a hyperchoic line and beneath the pleura is the normal aerated lung.

2.4 Morphological classification of pneumonias and pathogenesis

2.4.1 Bronchopneumonia

Bronchopneumonia refers to a particular type of pneumonia in which injury and the inflammatory process take place primarily in the bronchial, bronchiolar, and alveolar lumen (MCGAVIN and ZACHARY, 2006). Bronchopneumonia occurring as suppurative or fibrinous form is the most common type of pulmonary inflammation seen in domestic animals. The lesions are located more often at the cranio-ventral regions of the lungs with neutrophils and sometimes fibrin or macrophages filling the bronchioles and alveoli. Bronchopneumonia is often caused by bacteria and less frequently by aspiration of feed or gastric contents, or anecdotally by improper tubing or fungal infections (MCGAVIN and
ZACHARY, 2006; DUNGWORTH, 2007). Bronchopneumonia is in most cases caused by opportunistic bacterial pathogens (DUNGWORTH, 2007). The pathogens arrive in the lungs via inspired air, either from contaminated aerosols or from the nasal flora. Before causing lesions, pathogens overwhelm or evade the pulmonary defense mechanisms. The initial injury is centred on the mucosa of bronchi; from there, the inflammatory process can spread downward to the bronchioles. Typically, the inflammatory exudate accumulates in bronchial, bronchiolar, and alveolar lumina leaving the alveolar interstitium unchanged (MCGAVIN and ZACHARY, 2006). If the inflammatory process cannot control the inciting cause of injury, the lesion spreads rapidly from lobe to lobe through alveolar pores and destroys alveolar walls, until the entire lobe or a large portion of the lung is involved (MCGAVIN and ZACHARY, 2006). At the early stages, the pulmonary vessels are engorged with blood and the bronchi, bronchioles, and alveoli contain some exudate. In cases in which the injury is mild to moderate, cytokines locally released in the lung cause rapid recruitment of neutrophils and alveolar macrophages into bronchioles and alveoli. When pulmonary injury is much more severe, proinflammatory cytokines induce more pronounced vascular changes by further opening endothelial gaps, thus increasing vascular permeability resulting in fibrinous exudates and sometimes haemorrhages in the alveoli. Alterations in permeability can be further exacerbated by structural damage to pulmonary capillaries and vessels directly caused by microbial toxins (MCGAVIN and ZACHARY, 2006). The final result is that blood vessels become permeable and allow substantial leakage of plasma including proteins into the alveoli. The typical gross appearance of bronchopneumonia is irregular consolidation of the cranioventral regions of the lung. The cut surface is usually edematous, and catarrhal or purulent material can be pressed out of the small airways (DUNGWORTH, 2007).

2.4.1.1 Suppurative bronchopneumonia

During the first twelve hours of suppurative bronchopneumonia, when bacteria are rapidly multiplying, the lungs become hyperemic and edematous. Soon after neutrophils start filling the airways and by 48 hours the parenchyma starts to consolidate and becomes firm in texture. Three to five days later, hyperemic changes are less obvious, but the bronchial, bronchiolar, and alveolar spaces continue to fill with neutrophils and macrophages. At this stage, the affected lung has a grey-pink color, and on cut surface purulent exudate can be expressed
from bronchi. In favorable conditions where the infection is under control of the host defense mechanisms, the inflammatory process begins to regress, known as resolution. Complete resolution in favorable conditions could take one to two weeks. Around seven to ten days after infection, the lungs become pale gray and take a “fish flesh” appearance. This appearance is the result of purulent and catarrhal inflammation, obstructive atelectasis, mononuclear cell infiltration, lymphoid hyperplasia, and early fibrosis. Complete resolution is unusual in chronic bronchopneumonia, and scars, bronchiectasis, adhesions, and abscesses may remain unresolved for a long time (MCGAVIN and ZACHARY, 2006).

Microscopically, suppurative bronchopneumonia is characterized by abundant neutrophils, macrophages, and cellular debris within the lumina of bronchi, bronchioles and alveoli (MCGAVIN and ZACHARY, 2006). Hyperplasia of bronchus-associated lymphoid tissue (BALT) is commonly seen.

### 2.4.1.2 Fibrinous bronchopneumonia

Fibrinous bronchopneumonia is similar to suppurative bronchopneumonia except that the predominant exudate is fibrinous rather than neutrophilic, and has also a cranio-ventral distribution (MCGAVIN and ZACHARY, 2006). The inflammatory process involves numerous contiguous lobes, and the exudate moves quickly through pulmonary tissue until the entire pulmonary lobe is affected. Externally, early stages are characterized by severe congestion and haemorrhage giving the affected lung a characteristically intense red discoloration. A few hours later, fibrin starts to accumulate on the pleural surface, giving the pleura a ground glass appearance and eventually forming plaques of fibrinous exudate over a red, dark lung. At this stage, a yellow fluid accumulates in the thoracic cavity (MCGAVIN and ZACHARY, 2006). The color of fibrin deposited over pleural surface is also variable depending on the proportion of red blood cells, hemoglobin, and leucocytes. On the early stages, the cut surface appears as simple red consolidation (MCGAVIN and ZACHARY, 2006).

Microscopically, in the initial stage there is massive exudation of plasma proteins into the bronchioles and alveoli, and as a result most of the airspaces become obliterated by fluid and fibrin. Leakage of fibrin and fluid into alveolar lumina is due to extensive disruption of the integrity and increased permeability of the air-blood barrier. Leucocytes are present after the
onset of fibrinous inflammation. As inflammation progresses, fluid exudate is gradually replaced by exudates composed of fibrin, neutrophils, macrophages, and necrotic debris (MCGAVIN and ZACHARY, 2006).

2.4.2 Interstitial pneumonia

Interstitial lung disease primarily affects foals between three days and six months of age, although the disease can also occur in adult horses (BUERGELT et al., 1986; LAKRITZ et al., 1993; PERRON LEPAGE et al., 1999; NOUT et al., 2002; PATTERSON-KANE et al., 2008). Etiologic classification of interstitial pneumonia in veterinary medicine includes infectious, chemical, or allergic factors (BUERGELT et al., 1986; NOUT et al., 2002). Infectious agents are the most common known cause of interstitial pneumonia in domestic animals (TURK et al., 1981; BUERGELT et al., 1986; LAKRITZ et al., 1993; PERRON LEPAGE et al., 1999; PATTERSON-KANE et al., 2008).

Acute interstitial pneumonia begins with injury to either type I pneumocytes or alveolar capillary endothelium, which induces a disruption to the blood-air barrier with subsequent exudation of plasma proteins into the alveolar space, constituting the exudative phase (HALL, 1995; DUNGWORTH, 2007; MCGAVIN et al., 2007). In some cases of diffuse alveolar damage, exudated plasma proteins mix with lipids and other components of pulmonary surfactant. These components form elongated membranes that become partially attached to the alveolar and bronchial walls and are referred to as hyaline membranes. In addition to intraalveolar exudation of fluid, inflammatory edema and neutrophils accumulate in the alveolar interstitium and cause thickening of the alveolar walls (DUNGWORTH, 2007; MCGAVIN et al., 2007). This phase is followed by the proliferative phase characterized by hyperplasia of type II pneumocytes to replace the lost type I alveolar cells. As a consequence, the alveolar walls become increasingly thickened (HALL, 1995; DUNGWORTH, 2007; MCGAVIN et al., 2007). When the source of alveolar injury persists, the proliferative and infiltrative lesions can progress into a different morphologic stage referred to a chronic interstitial pneumonia. The hallmarks are fibrosis of the alveolar walls with presence of collagen bands, accumulation of mononuclear inflammatory cells in the interstitium, and persistence of hyperplastic type II pneumocytes (MCGAVIN et al., 2007). The fibrosis ultimately destroys the gas-exchange units (HALL, 1995) resulting in reduction of the number
of functional alveoli, thus altering pulmonary function (DERKSEN, 1993). Pulmonary gas exchange is impaired due to maldistribution of air and blood. Total and vital lung capacities decrease due to a loss in gas exchange units and change in the elastic properties of the lung. Stiff, fibrotic lungs increase the effort of breathing resulting in dramatic exercise intolerance and dyspnea (DERKSEN, 1993).

Important gross findings are the failure of lungs to collapse when the thoracic cavity is opened, the occasional presence of rib impressions on the lung’s pleural surface, indicating poor deflation, and the lack of visible exudates in airways unless complicated with secondary bacterial pneumonia (MCGAVIN et al., 2007). The color of affected lungs varies from diffusely red to diffusely pale grey and to a mottle red, pale appearance. Pale lungs are caused by severe obliteration of alveolar capillaries, particularly due to fibrosis. Lungs with uncomplicated interstitial pneumonia have an elastic or rubbery consistency and on a cut surface, there is no evidence of exudate (MCGAVIN et al., 2007).

2.4.3 Embolic pneumonia

Embolic pneumonia refers to a particular type of pneumonia in which injury is hematogenous, and the inflammatory response is typically centred in pulmonary arterioles and alveolar capillaries (MCGAVIN and ZACHARY, 2006). To cause pulmonary infection, circulating bacteria must first attach to the vascular endothelium and then evade phagocytosis by intravascular macrophages. Infected thrombi facilitate entrapment of bacteria in the pulmonary vessels and provide a favorable environment to escape phagocytosis. Once trapped in the pulmonary vasculature, usually in alveolar capillaries, offending bacteria spread from the vessels to the interstitium and then to the surrounding lung, forming finally a nidus of infection. Early lesions are characterized mainly by the presence of very small, white foci in the lung surrounded by a discreet, red, hemorrhagic halo. Unless emboli arrive in massive numbers causing severe pulmonary edema, embolic pneumonia is seldom fatal. In most instances, acute lesions if unresolved rapidly progress into abscesses that are randomly distributed in all pulmonary lobes (MCGAVIN and ZACHARY, 2006; MCGAVIN et al., 2007). The early inflammatory lesions are always focal. As a differential diagnosis in embolic pneumonia with abscess formation pulmonary abscesses arising from chronic bronchopneumonia have to be considered (DUNGWORTH, 2007). Multiple, widely
distributed abscesses usually indicate hematogenous origin and are often associated with an obvious source of septic emboli elsewhere in the body (DUNGWORTH, 2007).

2.4.4 Granulomatous pneumonia
Granulomatous pneumonia refers to a particular type of pneumonia in which aerogenous or hematogenous injury is caused by organisms or particles that cannot be normally eliminated by phagocytosis and that evoke a local inflammatory reaction with numerous alveolar and interstitial macrophages, lymphocytes, a few neutrophils, and sometimes giant cells (MCGAVIN and ZACHARY, 2006; MCGAVIN et al., 2007). The unique pattern of inflammatory response makes this pneumonia a distinctive type, which may result in the formation of granulomas. As a rule, agents causing granulomatous pneumonia are resistant to intracellular killing by phagocytic cells and to the acute inflammatory response and persist in affected tissue for a long time (MCGAVIN and ZACHARY, 2006). Granulomatous pneumonia is characterized by the presence of variable number of caseous or noncaseous granulomas randomly distributed in the lungs. On palpation, lungs have a typical nodular character given by well-circumscribed, variably sized nodules that, generally, have a firm texture (MCGAVIN and ZACHARY, 2006). Microscopically pulmonary granulomas are composed of a centre of necrotic tissue, surrounded by a rim of macrophages and giant cells and an outer delineated layer of connective tissue infiltrated by lymphocytes and plasma cells (MCGAVIN et al., 2007).

2.5 Treatment
2.5.1 General consideration
Regardless of the etiologic agent causing pulmonary disease, stall rest and supportive care are essential in the treatment of pulmonary disease in foals. Foals with pneumonia should be kept in a controlled environment that is, avoiding extreme conditions of temperature and humidity (LEGUILLETTE et al., 2002). Good-quality hay and clean water should be provided at all times (VAN MAANEN and CULLINANE, 2002). Wetting hay and providing clean bedding, including dust-free wood shavings or chopped paper seem to be beneficial (POWELL, 1991). In severe recumbent cases, simple therapies for respiratory support include maintenance of sternal position or alternating lateral recumbency every two to four hours, gentle chest
coupage to help loosen lower airways exudates, and maintenance of adequate hydration (BENTZ, 2005). When hypothermia is present gradual warming could be used, and can be achieved using blankets, hot-water bottles, and warm fluids (BENTZ, 2005). In case of fever, techniques for external thermoregulatory control, such as an alcohol bath, an air-conditioner stall and/or a fan can be used (RUSH and MAIR, 2006). Important is also the provision of caloric intake, administered by voluntary nursing, bottle feeding or nasogastric intubation (BENTZ, 2005).

In animals with severe hypoxemia (PaO$_2$ below 65 mm Hg), the primary objective is to support these foals providing an increased percentage of inspired O$_2$ and decreased CO$_2$ in the blood. Mechanical ventilation with supplementary oxygen is the most effective way; at 10 L/min and 100% oxygen (KOSCH et al., 1984; KOTERBA et al., 1990; COLAHAN et al., 1999; PARADIS, 2006; MCGORUM et al., 2007). Intranasal oxygen therapy is always a good supportive measure with persistent need of intranasal therapy. Humidification of the oxygen is necessary using an attachable in-line oxygen bubbler (KOTERBA et al., 1990; BENTZ, 2005; MCGORUM et al., 2007).

2.5.2 Medical therapy

2.5.2.1 Non-infectious pneumonias

Antimicrobial coverage is required for treatment or prevention of generalized septicemia (KOTERBA et al., 1990; BENTZ, 2005; KNOTTENBELT, 2006; PARADIS, 2006; MCGORUM et al., 2007). Amikacin is generally considered to be the aminoglycoside of choice for neonatal and pediatric foals, and the combination with penicillin is frequently used for critical foals (BENTZ, 2005; MCGORUM et al., 2007). Third generation of cephalosporines is commonly used alone or in combination with amikacin (KOTERBA et al., 1990; BENTZ, 2005).

Other medications that are worthy of consideration include those associated with anti-endotoxic and/or anti-inflammatory properties including meloxicam, flunixine meglumine or ketoprofen. Corticosteroids like prednisolone or dexamethazone are also recommended (DUNKEL, 2006). Neonatal foals may benefit from the administration of a respiratory stimulant. Caffeine is a respiratory stimulant that may help increase respiratory drive and lower PaCO$_2$. By linear extrapolation from clinical experience in human infants for caffeine, a
loading dose of 10 mg/kg administered orally followed by a maintenance dose of 2.5 to 3.0 mg/kg, per os, every 24 hours has been recommended for foals (GIGUÈRE et al., 2007; GIGUÈRE et al., 2008). Doxapram as a respiratory stimulant is used, only being effective for the primary apnea at a dose of 0.5 mg/kg intravenously as a bolus (BENTZ, 2005; KNOTTENBELT et al., 2005; GIGUÈRE et al., 2007; GIGUÈRE et al., 2008). It is also helpful to administer a bronchodilator like terbutaline through β-adrenergic effects at 0.03-0.07 mg/kg orally every eight hours, subcutaneously 3.6 μg/kg every four hours or by nebulizer (KNOTTENBELT et al., 2005). An intravenous loading dose of aminophylline of 4-7 mg/kg can be followed by 2-4 mg/kg intravenously, or 4-7 mg/kg orally every twelve hours, for maintenance and this may help considerably (KNOTTENBELT et al., 2005). There is some evidence that alveolar epithelial fluid clearance is stimulated by systemic or inhaled β-adrenergic agonists, offering an alternative rationale for their usage (PIANTADOSI and SCHWARTZ, 2004). Inhaled furosemid attenuates or prevents bronchospasm and exerts an anti-inflammatory and immunomodulatory effect by decreasing cytokine production (DUNKEL, 2006).

It is often beneficial to administer at least 1 L of hyperimmune plasma, regardless of the IgG concentration. Because many ill foals are in a negative energy balance, they will use this protein as a source of calories or will loose IgG levels in response to overwhelming sepsis or localized infections (BENTZ, 2005).

For the neonatal respiratory distress syndrome in some cases it is recommended to administer exogenous surfactant. There are multiple types of exogenous surfactant; however, natural surfactants, which contain most of the phospholipids and surfactant proteins, have been shown to be the best treatment of surfactant deficiency (SURESH and SOLL, 2003). Unfortunately natural surfactants are not licensed for animals yet and synthetic forms are still unaffordable. Multiple studies in foals have shown that surfactant is most effective when it is given prophylactically, within 30 minutes of birth, rather than as a rescue treatment. Even so, it may be necessary for the neonate to receive multiple doses (up to four to six) in a 48-hour period (SURESH and SOLL, 2003; PARADIS, 2006).
2.5.2.2  Infectious pneumonias

2.5.2.2.1  Bacterial pneumonias

Bacterial pneumonia may require long-term therapy with antibiotics. Antibiotic sensitivity testing of the isolates should be performed to choose the appropriate drug (COLAHAN et al., 1999; PARADIS, 2006; MCGORUM et al., 2007). Gram-negative bacteria like Pasteurellaceae sp. are sensitive to gentamicin, cephalothin, chloramphenicol, tetracycline, amikacin, kanamycin and neomycin, and like E. coli are sensitive to gentamicine (SWEENEY et al., 1991). For Sc. equi ssp. zooepidemicus, the best therapy are β-lactam antibiotics, that include penicillin G, trimethoprim-sulfadiazine (TMS) and ceftiofur that are usually effective against most pulmonary bacterial isolates of foals. A combination of erythromycin and rifampin, administered orally, was considered as an effective therapy for R. equi infection in the past. Nowadays other macrolids as azithromycin or clarithromycin are antiinfectives of choice for the treatment of R. equi pneumonia (DAVIS et al., 2002; VENNER et al., 2009). Combinations of penicillin G, ampicillin or ceftiofur with an aminoglycoside most likely give additive or synergistic effects against mixed aerobic bacterial pathogens (PRESCOTT, 1997). Resolution of clinical signs, normalisation of plasma fibrinogen and radiographic or ultrasonographic resolution of lung lesions are commonly used to guide the duration of therapy which generally ranges between four and nine weeks (GIGUÈRE, 2001). Potent antiinflammatories are essential in treatment of foals with pneumonia (LAKRITZ et al., 1993). Oral steroids like prednisolone at 1 mg/kg per os every 8 hours may be clinically beneficial in some cases of foal pneumonia in terms of reducing the severity of pulmonary dysfunction and shortening the course of illness (DUNKEL et al., 2005; DUNKEL, 2006). Dexamethasone at 0.1mg/kg intravenously once is also recommended (PARADIS, 2006). Supportive therapy including bronchodilators and antipyretic agents may be necessary in foals with impaired pulmonary function. Commonly used bronchodilators are methylxantine derivates, such as theophylline and aminophylline, and the α2-adrenergic agonists clenbuterol and terbutaline (LEGUILLETTE et al., 2002).
2.5.2.2.2 Viral pneumonia

In cases with suspected viral infection the animal has to be isolated to avoid spreading of the agent to other foals. Antipyretics are recommended for foals with fever > 40°C or a depressive state. Antibiotic therapy is started upon suspicion of secondary bacterial infection. In congenitally infected neonatal foals or in young horses in which clinical signs of bacterial infection are severe, progressive or involve the lower airways, optional antimicrobials for treatment include amikacin, procaine penicillin G, ceftiofur, tircacillin, and ceftazidine (ALLEN, 2002). For foals, the administration of dimethyl sulphoxide has not proven any clear positive effect. On the opposite parenteral corticosteroid dexamethazone and prednisolone are standard (ALLEN, 2002). Non-steroidal anti-inflammatory agents, like phenylbutazone or flunixin meglumine, are also used for reduction of respiratory tract inflammation (ALLEN, 2002). The use of the virostatic “acyclovir” to treat interstitial pneumonia of suspected viral origin in young foals is worth of consideration but has not been confirmed as effective yet (POWELL, 1991). Antiviral agents have been used for the prevention and treatment of influenza such as amantadine and rimantadine that inhibit the M2 protein of the EIV, but have not been used on foals yet (HAYDEN, 1996).

2.5.2.2.3 Parasitic pneumonia

The only treatment and prevention for lungworms in foals is a correct deworming program. Successful treatment of both horses and donkeys has been described using macrocyclic lactones (moxidectin, ivermectin) and benzimidazoles at elevated dosage (MATTHEWS, 2002; VERONESI et al., 2009). LYONS and coworkers (2008) proved that oxibendazole was the most effective antiparasitic for ascardis. Other treatments include fenbendazole (60 mg/kg) once per os (COLAHAN et al., 1999); or moxidectin (400 µg/kg) once per os (MATTHEWS, 2002).

2.5.2.2.4 Fungal pneumonia

Treatment of fungal infection is difficult. Treatment is to be managed with antifungal agents as amphotericin B, kenazoles, and iodides (KNOTTENBELT, 2002). The antimicrobial choice of treatment and prophylaxis of P. carinii infection in foals as in infants is TMS (FISHMAN, 1998; RUSSIAN and KOVACS, 1998; COLAHAN et al., 1999; WALKER and
MESHNICK, 1998; RODRIGUEZ and FISHMAN, 2004). In humans, aerosolized pentamidine has been effective and could be used in affected foals (COLAHAN et al., 1999). CLARK-PRICE and coworkers (2004) suggested the use of a sulfone like dapsone in foals (4, 4’-diaminodiphenylsulfone, DDS) with *P. carinii* pneumonia. Dapsone may be of use as an adjunctive treatment to administration of TMS or as a single agent treatment for foals that cannot tolerate TMS (CLARK-PRICE et al., 2004). Pulmonary aspergillosis is probably best regarded as untreatable in the horse although in theory at least amphotericin B may be effective (KNOTTENBELT, 2002).
3 Materials and methods

3.1 Study animals

39 foals sent to post mortem examination in the years 2003 to 2007 were included in this study. These foals originated from a large breeding farm in the north of Germany with endemic pulmonary rhodococcosis.

Retrospective analysis of necropsy reports of foals with morphological lung alterations was performed. Furthermore lung sections of the foals were histologically evaluated to confirm pneumonia. Then clinical and ultrasonographical records of 20 out of these 37 foals were interpreted in order to assess the respiratory disorder. Clinical and ultrasonographical data of 17 animals were not available. The clinical and ultrasonographical findings of 20 foals were then compared with the pathomorphological findings. Two foals originating from the same horse breeding farm and showing neither clinical symptoms nor pulmonary ultrasonographical findings of pneumonia where used as control (no. 38, 39).

Based on the ultrasonographical evaluation 22 of the 39 foals (no. 1 – 20 and 38, 39) were organized in three different groups. 17 foals (21 - 37) were evaluated pathomorphologically but clinical and ultrasonographical data were not available.

3.1.1 Archive material

A standardised health monitoring of the foals sets weekly clinical findings of all foals and of the sick foals prior to euthanasia or death. All together 37 foals of both gender aged between 1 and 218 days of age were evaluated (Tables 9.5.1 and 9.5.2). The animals were selected considering the presence of lesions of pneumonia at the histological evaluation. Parhomorphological and immunohistochemical investigations were performed in the 39 foals. Out of the 39 foals included in this study, 16 (no. 1, 2, 3, 6, 13 - 16, 25, 27, 28, 30, 31, 35, 36, 38) were previously used in an ultrasonographical study (WEIMAR, 2006).

3.1.2 Clinical data

Clinical data of the foals were gathered from the individual foal records including day of birth, gender, age at first clinical signs of pneumonia, approximate duration of clinical signs and treatment, age at euthanasia or death, days from the last clinical
examination to the day of death or euthanasia. Individual clinical signs of each foal are summarised in the annex (Table 9.5.2).

The longest interval between last clinical and ultrasonographical evaluation and necropsy was five days. In five cases (no. 23 - 26, 29) the interval was longer than five days. These foals were grouped together with other 12 foals (no. 21, 22, 27, 28, 30 - 37) that were not examined ultrasonographically because they were clinically healthy and no ultrasonographical evaluation was performed, were gathered in the 3\textsuperscript{rd} group of this study.

Clinical examination of each foal had been performed on the farm once weekly. A scoring system regarding respiratory clinical signs was adapted from the scoring of OHNESORGE, (1998) (Table 3.1). Each clinical finding got a grade of severity. All grades were added resulting in the “clinical score” indicating the severity of the disease. The following ranges were chosen: 0-1 = healthy; 2-3 = mild disease; 4-6 = moderate disease; and more than 6 = severe respiratory disease.

Clinical score and temperature were mathematically evaluated with media, standard deviation and quartiles.

*Table 3.1 Clinical score for the assessment of the severity of respiratory disease of foals (adapted from OHNESORGE, 1998)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Findings</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal discharge</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Serous, sero-mucoid</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Purulent</td>
<td>2</td>
</tr>
<tr>
<td>Cough</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>2</td>
</tr>
<tr>
<td>Mandibular</td>
<td>No abnormality</td>
<td>0</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Enlarged</td>
<td>1</td>
</tr>
<tr>
<td>Dyspnea at rest</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Increased intercostal effort</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Or/and nostril flaring</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Vesicular</td>
<td>0</td>
</tr>
<tr>
<td>Auscultation</td>
<td>rattle, crackles, wheezes</td>
<td>2</td>
</tr>
<tr>
<td>Trachea</td>
<td>No abnormality</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rattle</td>
<td>2</td>
</tr>
<tr>
<td>Clinical Score</td>
<td></td>
<td>0 – 16</td>
</tr>
</tbody>
</table>
3.1.3 Ultrasonographical evaluation of the thorax

When foals showed clinical signs of respiratory disorders or when blood leucocytes were elevated above $13 \times 10^3 \text{ per } \mu l$ blood, an ultrasonographical examination of the thorax was accomplished. The ultrasonographical examination of the thorax was performed with two portable battery-powered ultrasound units: “Sonovet 2000” (Kretztechnik AG) or “My sono™ 201” (Willi Pütz Medizintechnik, Massing), both with a 7.5 MHz linear scanner. Both sides of the thorax were thoroughly scanned in a dorsal to ventral plane from the 16th to the 3rd intercostal space. Alcohol at a concentration of 99% was copiously applied to the hair coat to provide surface contact between the transducer and the foal and to reduce intervening trapped air.

Findings of the ultrasonographical examination of each foal were interpreted according to ALTHAUS (2004) (Table 3.2). The findings had been documented in the foal’s records. Ultrasonographical signals include diffuse “hyperechoic comet tail artifacts” interpreted as cellular diffuse consolidation of the lung parenchyma, “multifocal nodular hypo- to hyperechoic areas” interpreted as nodular consolidated pulmonary alteration or abscesses and “mixed, nodular hypo- to hyperechoic areas with diffuse comet tail artifacts”.

The comet tail artifacts (vertical hyperechoic lines originating from the lung surface) were evaluated, and counted. Their number corresponds to the grade of consolidation of the lung parenchyma. Score “0” was given when intercostal muscles, pleura and normal lung surface were observed. Score “1” was given, when one or two comet tail artifacts were observed in one picture frame. (Fig. 3.1) If more than two comet tail artifacts were observed, it was scored as “2” (Fig 3.2), and score “3” was noted when five or more pulmonary comet tail artifacts were present in one picture frame (Table 3.3).

“A” was defined as a nodular hypo- to hyperechoic area of different sizes near the pleural surface (Fig. 3.3). The diameter of all pulmonary nodular hypo- to hyperechoic areas of both sides of each foal was added up to give the “nodular hypo- to hyperechoic area diameter”(Fig. 3.6). These diameters where taken into account for choosing the appropriate treatment protocol.
Table 3.2 Lung ultrasonographic score by ALTHAUS (2004)

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Muscle sheets, pleura and normal air artifacts, normal anatomic</td>
</tr>
<tr>
<td>1</td>
<td>One to two lung comet tail artifacts</td>
</tr>
<tr>
<td>2</td>
<td>More than two lung comet tail artifacts</td>
</tr>
<tr>
<td>3</td>
<td>More than five lung comet tail artifacts</td>
</tr>
<tr>
<td>A</td>
<td>Nodular hypo- to hyperechoic area</td>
</tr>
</tbody>
</table>

Fig 3.1 Ultrasonograhical image of a comet tail artifact of “1” (WEIMAR, 2006)
The ultrasonographic findings were analyzed also according to the “comet tail artifact score” (Table 3.3) for the assessment of the severity of the pulmonary alterations, evaluating the quantity and distribution of comet tail artifacts in the whole lung parenchyma. Score “0” was given if there were neither “3” nor “2” observed in the whole lung; score “1” was given if there were no “3” observed and half of the lung on both sides were “2” observed; if more than half of the lung on both sides was full of “2”
and less than \( \frac{3}{4} \) of the lung had “3” became a score of “2” (Fig. 3.4); and a score of “3” was given if there were “3” observed in more than \( \frac{3}{4} \) of the lung on both sides (Fig. 3.5) (Table 3.3).

According to the ultrasonographical findings two different groups of foals with respiratory disease were determined.

**Table 3.3 Comet tail artifact score for the whole lung parenchyma of the foal**

<table>
<thead>
<tr>
<th>Score</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Only “0” and “1” in both sides</td>
</tr>
<tr>
<td>1</td>
<td>No “3” and till half of the lung in both sides with ”2”</td>
</tr>
<tr>
<td>2</td>
<td>More than half of the lung in both sides with ”2” and less than ( \frac{3}{4} ) of the lung with ”3”</td>
</tr>
<tr>
<td>3</td>
<td>More than ( \frac{3}{4} ) of the lung in both sides with ”3”</td>
</tr>
</tbody>
</table>

*Fig. 3.4 Ultrasonographical findings of the right lung of a comet tail artifact (CTA) score of 2 in foal no. 1. The small numbers above indicate the intercostal spaces, and the letters at the left side indicate the region of the thorax. “A” for the dorsal region, “B” for the middle region, and “C” for the ventral region of the thorax.*
Material and Methods

3.1.4 Treatment evaluation

The treatment was adapted considering clinical and ultrasonographical findings. The therapy consisted of antibiotics like TMS, or rifampin in association with azithromycin, clarithromycin, or with tulathromycin, depending on the severity of the findings. If the lung was severely and diffusely affected with numerous comet tail artifacts and a clinical score of “3”, glucocorticoids e.g. dexamethazone, or prednisolone, or
cyclosporine were also administered. In cases with fever and depression non-steroidal anti-inflammatory drugs as flunixin meglumine were given. After starting the treatment sick foals were reevaluated by clinical and ultrasonographical examination of the lung every day in severe cases and once a week in milder cases, during the first two weeks of treatment. If response to treatment was satisfying, treatment was prolonged for four more weeks. If findings were showing a poor response to treatment, antibiotic regime was changed and the foal was reevaluated in the same manner.

Drugs, dosages, application intervals and route of administration used for the foals in this study are presented in table 3.4.

**Table 3.4** Drugs, dosage, application intervals and route of administration given to the foals.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Application Interval</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim-sulfadiazine</td>
<td>30 mg/kg BW</td>
<td>Every 12 hours</td>
<td>p. o.</td>
</tr>
<tr>
<td>Rifampin</td>
<td>10 mg/kg BW</td>
<td>Every 12 hours</td>
<td>p. o.</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>10 mg/kg BW</td>
<td>Every 24 hours</td>
<td>p. o.</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>7.5 mg/kg BW</td>
<td>Every 12 hours</td>
<td>p. o.</td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>2.5 mg/kg BW</td>
<td>Once a week</td>
<td>i.m.</td>
</tr>
<tr>
<td>Dexamethazone</td>
<td>0.1 mg/kg BW</td>
<td>Once</td>
<td>i. v.</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>2mg/kg BW</td>
<td>Every 8 or 12 hours</td>
<td>i. v. or p. o.</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>1 mg/kg BW</td>
<td>Every 12 hours</td>
<td>p. o.</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>0.5 mg/kg BW</td>
<td>Every 12 to 24 hours</td>
<td>i. v.</td>
</tr>
</tbody>
</table>

*BW: body weight; i. m.: intra-muscular; i. v.: intra-venous; p. o.: per os*

**3.1.5 Archive pathology material**

The necropsy reports of 37 foals and two control foals (no. 1 – 37, and 38, 39; tables 9.5.13, 9.5.14, 9.5.15, and 9.5.16) were evaluated regarding gross findings with particular focus on the respiratory tract. Types of lesions were recorded for comparison with histological findings.
Material and Methods

Paraffin wax tissue blocks and histological sections of the lungs stained with hematoxylin and eosin (HE) were obtained from the block and slide archive. Two foals (no. 38, 39) from the necropsy material showing neither anamnestic nor morphological evidence of respiratory affections were added as controls.

3.1.6 Microbiology and virology

_Intra vitam_ and _post mortem_ microbiological aerobic and anaerobic investigations of TBA samples and lung tissue (Tables 9.5.13, 9.5.14, 9.5.15, and 9.5.16) were performed in 10 and 23 out of 39 foals, respectively, at the Department of Microbiology, University of Veterinary Medicine Hannover. Additionally virological examination was performed in six out of 39 foals for the detection of EHV-1 by means of polymerase chain reaction (PCR), EHV-4 antibody titer by means of ELISA, and EIV by means of cell culture. These investigations were performed by the Department of Virology, University of Veterinary Medicine Hannover and a private laboratory (Innovative Veterinärdiagnostik, Hannover, Germany).

3.2 Histological methods

Additional paraffin sections of 3 µm thickness were cut using a rotation microtome (2030, Leica Instruments GmbH, Nussloch). The sections were placed in cold distilled water and then transferred into 55°C warm water for stretching. The sections were placed on glass slides (Menzel-GLäSER Super® Frost, Thermo Scientific, Braunschweig, Germany). For drying and removing excess paraffin wax the slides were incubated 20 minutes in an oven at 60°C (Medite, Burgdorf, Germany).

Selected sections were stained with HE to complete the archival series, if some slides were missing. In addition, sections were stained with special stains including Periodic acid-Schiff’s (PAS) reaction, Grocott’s methenamine silver stain (GMS), Elastica van Gieson’s (EvG) stain, and Gram’s stain. In one case (no. 3) von Kossa’s stain for calcium deposition was performed. For immunohistochemistry four unstained sections were cut additionally.

3.2.1 Hematoxylin and Eosin-Stain

This staining method involves the application of the basic dye hematoxylin, which colors acid structures blue-purple and alcohol, based acidic eosin, which colors base
structures bright pink. The staining was achieved with an automatic staining machine (Varistain 24-3, Shandon, Frankfurt, Germany).

1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 2 min. and descending series alcohols (96%, 70%, and 50% ethanol), each for one min.
2. Rinsing in distilled water for 5 min.
3. Differentiation in Mayer’s hemalaun solution (Roth, Karlsruhe, Germany) (annex 9.1.1) for 15 min.
4. Rinsing in tap water.
5. Counter staining in 1% eosin solution (annex 9.1.1) for 1 min.
6. Rinsing in distilled water.
7. Dehydration twice for 2 min. each time through ascending series of alcohol (50%, 70%, and 96% ethanol), once in isopropanol, clearing in EBE® “acetic acid-n-butylester”, (Roth, Karlsruhe, Germany), and finally automatic mounting (promountes® RCM2000, Medite, Burgdorf, Germany).

3.2.2 Periodic Acid-Schiff Reaction

Periodic Acid-Schiff (PAS) reaction is used for the demonstration of polysaccharides, neutral mucusubstances and basement membranes in tissue sections. Polysaccharides appear in a pink color (BANCROFT and STEVENS, 1996).

1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 2 min. and descending series alcohols (96%, 70%, and 50% ethanol), each for 1 min.
2. Rinsing in distilled water for 5 min.
3. Immersion in 1% periodic acid solution (Roth, Karlsruhe, Germany) (annex 9.1.1) for 10 min.
4. Rinsing in tap water for 10 min.
5. Rinsing two times, each for 2 min. with distilled water.
6. Differentiation in Schiff’s reagent (Roth, Karlsruhe, Germany) for 10 to 25 min. at room temperature.
7. Rinsing in warm tap water for 5 min.
8. Rinsing in distilled water.
9. Counter staining in Mayer’s hemalaun solution (Roth, Karlsruhe, Germany) for 5 min.
10. Rinse in tap water for 5 to 10 min.
11. Rinse in distilled water.
12. Dehydration twice for 2 min. each time through ascending series of alcohol (50%, 70%, and 96% ethanol), once in isopropanol, clearing in EBE® “acetic acid-n-butylester” (Roth, Karlsruhe, Germany), and finally automatic mounting (promountes® RCM2000, Medite, Burgdorf, Germany).

3.2.3 Elastica van Gieson’s Stain
Elastica van Gieson’s (EvG)-stain is a combination of Verhoeff’s elastic stain. This stain is a hematoxylin stain containing ferric chloride, Wright’s iodine solution, van Gieson stain. Elastic fibres are stained blue-black to black, collagen pale red, other tissue elements yellow, and nuclei blue to black.
1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 2 min. and descending series alcohol (96%, 70%, and 50% ethanol), each for 1 min.
2. Rinsing in distilled water for 5 min.
3. Immersion in resorcin fuchsin solution (Waldeck, Münster, Germany) for 30 min.
4. Clearing in 96% alcohol.
5. Rinsing with distilled water.
6. Differentiation in Weigert’s iron A+B (1+1) (annex 9.1.1) for 5 min.
7. Differentiation in hydrogen chloride.
8. Rinsing with distilled water.
9. Rinsing in tap water for 15 min.
10. Counter staining in van Gieson’s solution (Waldeck, Münster, Germany) for 5 min.
11. Dehydration twice for 2 min. each time through ascending series of alcohol (50%, 70%, and 96% ethanol), once in isopropanol, clearing in “acetic acid-n-butylester” EBE® (Roth, Karlsruhe, Germany), and finally automatic mounting (promountes® RCM2000, Medite, Burgdorf, Germany).

3.2.4 Grocott’s methenamine silver stain
Grocott’s methenamine silver stain (GMS) is used for the detection of fungi in tissue sections by staining the cell wall of fungi in a brown to black color. The staining solution reacts with polysaccharides of the fungal cell wall (BANCROFT and STEVENS, 1996).
1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 2 min. and descending series alcohols (96%, 70%, and 50% ethanol), each for 1 min.
2. Rinsing in distilled water for 5 min.
3. Oxidation in 5% chromic acid (Riedel de Haën, Seelze, Germany) solution for 1 hour.
4. Rinsing in running tap water for 10 min.
5. Immersion in aqueous sodium bisulfate (Riedel de Haën, Seelze, Germany) for 1 min. to remove any residual chromic acid.
6. Rinsing in tap water for 5 to 10 min.
7. Rinsing in distilled water two times for 3 min. each.
8. Immersion in working methenamine silver solution (Merck, Darmstadt, Germany) (annex 9.1.1) at 60°C in a water bath for 15 to 30 min. or until the section have a yellow-brown color.
9. Rinsing in distilled water two times for 2 min. each.
10. Immersion in 0.1% gold chloride for 5 min.
11. Rinsing in distilled water.
12. Removing unreduced silver by treating with 2% sodium thiosulfate (Merck, Darmstadt, Germany) for 2 min.
13. Rinsing thoroughly in tap water for 10 to 15 min.
14. Counterstaining with methanol yellow (Riedel de Haën, Seelze, Germany) (annex 9.1.1) for 5 to 10 min.
15. Dehydration twice for 2 min. each time through ascending series of alcohol (50%, 70%, and 96% ethanol), once in isopropanol, clearing in EBE® “acetic acid-n-butylester” (Roth, Karlsruhe, Germany), and finally automatic mounting (promountes® RCM2000, Medite, Burgdorf, Germany).

3.2.5 Gram’s Stain
The Gram’s stain is used for the differentiation of Gram positive (+) and Gram negative (−) bacteria staining Gram + bacteria in a blue-purple color. (MADIGEN et al., 2004).
1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once in isopropanol for 2 min. and descending series alcohol (96%, 70%, and 50% ethanol) each for 1 min.
2. Rinsing in distilled water for 5 min.
3. Immersion in 0.1% nuclear fast red solution (Merck, Darmstadt, Germany) for 10 min.
4. Rinsing in distilled water.
5. Immersion in Crystal Violet solution (Roth, Karlsruhe, Germany) for 5 min.
6. Blot sections dry with filter paper.
7. Differentiation in Lugol’s iodine solution as a mordant for 5 min.
8. Rinsing shortly in distilled water.
9. Blot sections dry with filter paper.
10. Decolorisation of sections with aniline-xylene (Glukachemie, Stuenheim, Germany) (2:1) solution, until the sections are again red.
11. Rinsing some times with xylene.

3.2.6 **Von Kossa’s stain**

The von Kossa’s stain represents a method for demonstrating calcium deposits in tissue sections. The stain is based on the substitution of tissue calcium, bound to phosphates, by silver ions and the subsequent visualization of silver ions by hydroquinone reduction to metallic silver. Deposits of calcium are stained dark brown to black (RUNGBY et al., 1993).

1. Deparaffination and rehydration by immersion twice for 2 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 2 min. and descending series alcohol (96%, 70%, and 50% ethanol) each for 1 min.
2. Rinsing in running tap water.
3. Rinsing in distilled water
4. Immersion in 3-5% silver nitrate solution (Merck, Darmstadt, Germany) for 2 hours with exposure to a 100 watt incandescent desk lamp.
5. Rinsing with distilled water.
6. Differentiation in 5% aqueous sodium thiosulfate (Merck, Darmstadt, Germany) for 5 min.
7. Rinsing in distilled water.
8. Counter staining in nuclear fast red solution (Merck, Darmstadt, Germany) for 5 to 10 min.
9. Rinsing briefly in distilled water
10. Dehydration twice for 2 min. each time through ascending series alcohols (50%, 70%, and 96% ethanol), once in isopropanol, clearing in EBE® “acetic acid-n-butylester”, (Roth, Karlsruhe, Germany), and finally automatic mounting (promountes® RCM2000, Medite, Burgdorf, Germany).

11.  

3.3 Immunohistochemistry

Immunohistochemistry (IHC) was used for the identification of *R. equi* and *P. carinii* in tissue sections.

3.3.1 Reagents

Primary antibodies were diluted using phosphate-buffered saline (PBS) (annex 9.1.1), pH 7.1, containing 1% bovine serum albumin (BSA) (annex 9.2.1). Secondary antibodies and the detection system were diluted in PBS. For *R. equi* a murine monoclonal antibody directed against the 15 - 17 kDa VapA (Mab 10G5) was used kindly provided by Prof. S. Takai (Department of Animal Hygiene, School of Veterinary Medicine and Animal Science, Kitasato University, Japan). For *P. carinii* a commercially available murine fluorescein (FITC)-labelled monoclonal antibody was used (Mab 092; Abcam, Hamburg, Germany). The optimal dilution of the antibodies were evaluated by chess board titration using varying antibody dilutions ranging from 1:500 to 1:6000 for Mab 10G5 (RETTEG et al., 2009) and 1:10, 1:50, 1:100, 1:500 and 1:1000 for Mab 092.

3.3.1.1 Blocking serum

Non-specific binding was blocked with inactivated (normal) goat serum diluted 1:5 in PBS.

3.3.1.2 Secondary antibodies

A biotin-labelled goat-anti mouse IgG (Vector Laboratories, Burlingame, CA, USA) for Mab 10G5 and a biotin-labelled goat-anti fluorescein (Vector Laboratories, Burlingame, CA, USA) for the Mab 092 were used (working dilution: 1:200 in PBS).
3.3.1.3 Detection system

Avidin-Biotin-Peroxidase-Complex (ABC; “Vectastain Elite ABC Kit” PK 6100, Vector Laboratories, Burlingame, CA, USA) (annex 9.1.1) was applied by adding 15 µl reagent A to 1 ml PBS (pH 7.1). After vigorous shaking 15 µl reagent B is added followed again by shaking. It has to be prepared 30 min. before use.

3.3.2 Protocol for immunohistochemical staining (ABC method)

1. Deparaffination and rehydration by immersion twice for 5 min. each time into Roticlear® (Roth, Karlsruhe, Germany), once into isopropanol for 5 min. and descending series alcohols (96%, 70%, and 50% ethanol) each for 5 min.
2. Rinsing in distilled water.
3. Immersion in 3 ml H₂O₂ 30% in 197 ml methanol to inhibit endogenous peroxidase activities for 20 min.
4. Rinsing in 3 changes of distilled water for 3 to 5 min. each time, then once in phosphate-buffered saline (PBS; pH 7.1).
5. Antigen retrieval: Incubation in acitric acid monohydrate solution (pH 6.2; 0.01M) for 25 min. in a microwave oven (800W) (annex 9.1.1).
6. After cooling, rinsing in 3 changes of distilled water for 3 to 5 min. each time, then once in PBS.
7. Placing slides in Shandon Racks (Coverplates™ Sequenza®, Pittsburg, USA), in each Shandon Rack 100 to 200 µl of the primary antibody dilution with PBS.
8. Incubation with blocking serum at room temperature for 30 min.
9. Incubation with primary antibody (Mab 10G5 or Mab 092) for 1.5 hours at room temperature.
10. Rinsing in 3 changes of distilled water for 3 to 5 min. each time, then once in PBS.
11. Incubation with secondary antibody for 30 min. at room temperature, in each Shandon Rack 100 to 200 µl of the 1 : 200 dilution of the second antibody in PBS.
12. Rinsing in 3 changes of distilled water for 3 to 5 min. each time, then once in PBS.
13. Incubation with ABC for 30 min. at room temperature.
14. Rinsing in 3 changes of distilled water for 3 to 5 min. each time, then once in PBS.
15. Incubation in 0.05% (w/v) fresh and filtrated 3', 3'-diaminobenzidine-tetrahydrochloride (DAB; Fluka, Buchs, Switzerland) for 10 min. at room temperature and under constant stirring with H₂O₂ 0.03% (v/v) as substrate in 0.1 M
Tris-buffered saline (TBS) (Tris-hydroxymethyl-aminomethane; Merck, Darmstadt, Germany), pH 7.6.

16. Rinsing in 3 changes of distilled water for 5 min. each time, then once in TBS.
17. Rinsing in 3 changes of distilled water for 5 min. each time.
18. Counter staining with Mayer’s hematoxylin (Roth, Karlsruhe, Germany), duration according to desired color intensity.
19. Rinsing in 3 changes of distilled water for 5 min. each time.
20. Dehydration twice for 2 min. each time through ascending series of alcohols (50%, 70%, and 96% ethanol), once in isopropanol, clearing in EBE® “acetic acid-n-butylester” (Roth, Karlsruhe, Germany).

3.3.2.1 **Negative control**

For negative control purposes, the primary antibody was replaced by ascitic fluid from non-immunized Balb/cJ mice (Biologo, Kronshagen, Germany). Histologically normal lung tissue of a foal, which was bacteriologically free of *R. equi*, and another lung tissue of a foal free of *P. carinii* originating from the necropsy cases of the Department of Pathology, University of Veterinary Medicine, Hannover, were used as negative tissue control. In addition, the *R. equi* primary antibody was applied also on lung tissue from a horse and a pig infected with *Streptococcus* sp. Also one slide selected randomised was tested with a canine distemper antibody (SeroTec MCA 1893, Germany) as negative control to prove the absence of cross-reaction.

3.3.2.2 **Antigen retrieval**

For the identification of *R. equi*- (RETTEG et al., 2009) and for *P. carinii*-antigens microwave treatment has turned out to be the most suitable method for antigen retrieval (annex 9.1.1). Tissue sections were immersed in citrate buffer (pH 6.0-6.5) and incubated in a microwave oven (800 watt) for 20 min. Subsequently sections were left at room temperature for 10 min. for cooling.

3.3.3 **Evaluation of histological and immunohistochemical findings**

The slides were evaluated using a standard binocular light microscope with different objectives (Zeiss, Oberkochen, Germany). The evaluation of the histological and
immunostained tissue sections was performed independently by the author and an experienced pathologist and findings were discussed. Examination of tissue sections was performed semiquantitatively and the severity of lesions was assessed viewing high power field (40x objective). Histological lesions were recorded qualitatively as mild, moderate and severe. The distribution of lesions was described as focal (F) for single lesions being localized centrally at a focus of the section; multifocal (M), for lesions being localized in two or more foci of the section; or diffuse (D), for lesions distributed throughout the whole section. For the immunohistological evaluation the quantity and localization of immunolabelled cells the following score was used by evaluating the whole slide: minimal (0-10 positive cells), few (11 - 30 positive cells), moderate (31-50 positive cells), or severe (> 50 positive cells). Locations without lesions were designated as normal (= 0).
4 Results

4.1 Arrangement of Study animals

The selection criteria of the foals was morphological lung lesions of pneumonia. Based on the recorded ultrasonographical findings the 37 selected study animals from the necropsy reports were divided into four groups:

**Group 1:** Consisted of 6 animals (no. 1 – 6) presenting numerous comet tail artifacts at ultrasonographical evaluation.

**Group 2:** Consisted of 14 foals (no. 7 – 20), which presented nodular hypo – to hyperechoic areas and some combined with comet tail artifacts.

**Group 3:** Consisted of 17 foals (no. 21 – 37), in which ultrasonographical examination had not been performed and clinical data were lacking.

**Group 4:** As control, two foals that had neither clinical signs nor pulmonary ultrasonographical findings of pneumonia originating from the same horse breeding farm where used (no. 38, 39).

4.2 Clinical findings

4.2.1 Group 1

*Breed and gender:* All foals (n = 6) were of the Warmblood breed, including 4 (67%) colts and 2 (33%) fillies.

*Age at date of diagnosis:* Respiratory findings were initially observed between 41 to 63 days of age (median 59). However, in this group all except for one foal (no. 3) showed clinical signs after 40 days of age.

*Duration of respiratory signs:* The time from diagnosis to euthanasia or death ranged from 1 to 120 days (median: 11 days), with a duration of sickness of less than 20 days in all foals except for foal no. 6 (120 days).

*Dyspnea:* Three out of six foals (no. 1, 4, 5) presented dyspnea with a duration of 3 to 5 days (median: 4 days).

*Duration of medical treatment:* This period ranged from 6 to 18 days (median: 10.5 days). Treatment duration was assessed from the onset of severe or moderate clinical respiratory signs to the day of euthanasia or death.
Days from the last clinical examination to the day of euthanasia or death: In this six foals (no. 1 - 6) this time period lasted from 0 to 5 days (median: 2 days).

Mode of death: Four out of six foals (no. 2 – 4, 6) were euthanized and two (no. 1, 5) died spontaneously.

Age at death: Affected foals of this group died at an age between 25 to 161 days (median: 70.5 days), with most foals (five out of six; 83%) being older than 30 days of age.

Details of clinical data of group 1 are included in tables 9.5.1, 9.5.5, and 9.5.9.

4.2.2 Group 2

Breed and gender: All foals (n = 14, no. 7 - 20) were of the Warmblood breed, including 7 (50%) colts and 7 (50%) fillies.

Age at date of diagnosis: Respiratory findings were initially observed between 6 to 82 days (median 47). However, ten out of 14 foals (no. 8 -10, 14 - 20) showed first clinical signs of respiratory disorders after 30 days of age.

Duration of respiratory signs: The time from diagnosis to euthanasia or death ranged from 5 to 75 days (median: 22.5 days), with a duration of sickness of more than 13 days in all foals except for three (no. 7, 12, 16).

Dyspnea: Eight out of 14 foals (no. 9 – 11, 15 - 19) presented dyspnea with a duration of 1 to 13 days (median: 2 days). All foals in this group, except for one (no. 18) presented dyspnea for less than one week.

Duration of medical treatment: This period in 13 out of 14 foals ranged from 3 to 75 days (median: 13.5 days). Foal no. 20 did not receive any treatment, because it had nodular hypo-to hypechoic areas less than 5cm. Treatment duration was assessed from the onset of severe or moderate clinical respiratory signs to the day of euthanasia or death. Eight out of 14 foals had treatment duration longer than 10 days.

Days from the last clinical examination to the day of euthanasia or death: In all 14 foals this time period lasted from 0 to 5 days (median: 1.5 days).

Mode of death: Eight out of 14 foals (no. 8, 13 – 18, 20) were euthanized and six died spontaneously (no. 7, 9 – 12, 19).

Age at death: Affected foals of this group died at an age between 12 to 191 days (median: 67 days), with most foals (no. 8 – 11, 13 - 20) being older than 30 days of age.
Details of clinical data of group 2 are included in tables 9.5.2, 9.5.6, and 9.5.9.

4.2.3 Group 3

*Breed and gender:* All foals (n = 17) were of the Warmblood breed, including 10 (59%) colts and 7 (41%) fillies.

*Age at date of diagnosis:* Respiratory findings from five out of 17 foals (no. 23, 25, 26, 29, 34) were initially observed between 40 to 174 days (median 59).

*Duration of respiratory signs:* The time from diagnosis to euthanasia or death of five out of 17 foals (no. 23, 25, 26, 29, 34) ranged from 12 to 109 days (median: 31 days).

*Duration of medical treatment:* This period for five out of 17 foals (no. 23, 25, 26, 29, 34) ranged from 10 to 109 days (median: 10.5 days). Treatment duration was assessed from the onset of severe or moderate clinical respiratory signs to the day of euthanasia or death. Four out of these five foals had treatment duration of less than 45 days (no. 23, 25, 26, 29).

*Dyspnea:* Two out of 17 foals (no. 26, 34) presented dyspnea with a duration of 5 or 59 days respectively. Foal no. 34 with dyspnea for 59 days was recurrently and not continuously ill.

*Days from the last clinical examination to the day of euthanasia or death:* In five out of 17 foals (no. 23, 25, 26, 29, 34) this time period lasted from 8 to 59 days (median: 12 days). The other 12 animals had more than 5 days in between the last clinical examination and euthanasia or death.

*Mode of death:* Nine out of 17 foals were euthanized (no. 21, 23, 25, 27, 29, 31, 32, 36, 37) and eight foals (no. 22, 24, 26, 28, 30, 33 - 35) died spontaneously.

*Age at death:* Affected foals of this group died between 1 and 218 days of age (median: 45 days), with more than the half of the foals in this group (9/17) living less than 60 days of age. Seven (no. 21, 22, 24, 27, 30, 33, 36) of these nine foals were neonatal (less than one week old).

Details of clinical data of group 3 are included in tables 9.5.3, and 9.5.7.

4.2.4 Group 4

*Breed and gender:* The two foals were of the Warmblood breed, including one colt and one filly.

*Mode of death:* Both foals were euthanized because of orthopedical disorders.
**Age at death:** Affected foals of this group died at the age of 1 and 40 days.

Details of data of group 4 are presented in tables 9.5.4, 9.5.8 and 9.5.12. Clinical data of the “Foal records” were evaluated with respect to the last data recorded of the foal. Clinical findings of each of group no. 1 and 2 are presented in table 4.1, not taking into consideration the foals of the control group and of group 3 because of missing clinical data close enough to the time of death.

*Table 4.1 Clinical signs of pneumonia of foals with comet tail artifacts (group 1) and foals with nodular hypo- to hyperechoic areas (group 2) at ultrasonography of the lung.*

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>No. of foals in group 1</th>
<th>No. of foals in group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean nostrils</td>
<td>6/6 (no. 1 – 6)</td>
<td>8/14 (no. 7, 8, 12 – 14, 16, 17, 19)</td>
</tr>
<tr>
<td>Serous to seromucoid nasal secretion</td>
<td>0/6</td>
<td>4/14 (no. 9, 15, 18, 20)</td>
</tr>
<tr>
<td>Purulent nasal discharge</td>
<td>0/6</td>
<td>2/14 (no. 10, 11)</td>
</tr>
<tr>
<td>Normal lung sounds</td>
<td>4/6 (no. 2, 3, 5, 6)</td>
<td>6/14 (no. 12 – 14, 16, 17, 20)</td>
</tr>
<tr>
<td>Crackles, wheezes or rattle at lung</td>
<td>2/6 (no. 1, 4)</td>
<td>8/14 (no. 7 – 11, 15, 18, 19)</td>
</tr>
<tr>
<td>Normal tracheal auscultation</td>
<td>6/6 (no. 1 – 6)</td>
<td>10/14 (no. 7, 8, 10, 12 – 14, 16, 17, 19, 20)</td>
</tr>
<tr>
<td>Rattle at trachea</td>
<td>0/6</td>
<td>4/14 (no. 9, 11, 15, 18)</td>
</tr>
<tr>
<td>Small submandibular LN</td>
<td>4/6 (no. 1 – 3, 6)</td>
<td>5/14 (no. 12 – 14, 16, 17)</td>
</tr>
<tr>
<td>Enlarged painful LN</td>
<td>2/6 (no. 4, 5)</td>
<td>9/14 (no. 7 – 11, 15, 18 – 20)</td>
</tr>
<tr>
<td>Cough</td>
<td>0/6</td>
<td>4/14 (no. 8, 9, 11, 18)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3/6 (no. 1, 4, 5)</td>
<td>8/14 (9 – 11, 15 – 19)</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>2/6 (no. 1, 5)</td>
<td>7/14 (no. 9, 12, 13, 15, 16, 18, 19)</td>
</tr>
</tbody>
</table>

LN: lymph nodes
The clinical findings were evaluated according to OHNESORGE (1998) resulting in a clinical assessment of the severity of disease based on a scoring system with a range from 0 to 16 (Table 3.1).

Clinical score and temperature of each of the two groups with clinical findings are presented in Table 4.2.

Table 4.2 Clinical score and temperature of each of the two groups with clinical findings.

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantity of foals</strong></td>
<td>n = 6</td>
<td>n = 14</td>
</tr>
<tr>
<td><strong>Clinical score</strong></td>
<td>4 ± 4.96</td>
<td>7 ± 5.99</td>
</tr>
<tr>
<td>(x, SD)</td>
<td>[0, 8.75]</td>
<td>[2.25, 12.75]</td>
</tr>
<tr>
<td>[Q25%, Q75%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Temperature °C</strong></td>
<td>38.5 ± 1.02</td>
<td>39.05 ± 0.95</td>
</tr>
<tr>
<td>(x, SD)</td>
<td>[38.175, 39.575]</td>
<td>[38.325, 39.05]</td>
</tr>
<tr>
<td>[Q25%, Q75%]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

x: median; Q: quartiles; SD: standard deviation

4.3  Ultrasonographical findings of the lung

Ultrasonographical examination of the lung of 20 foals allowed identification of pulmonary nodular hypo- to hyperechoic and/or comet tail artifacts. According to the comet tail artifact score the severity of the lung lesions was assessed (Table 3.3) (Fig. 4.2). Diameter of nodular hypo- to hyperechoic areas of both pulmonary lobes, were added together to get a “nodular hypo-to hyperechoic area diameter”.

4.3.1  Group 1

This group included six foals (no. 1-6) with multifocal to coalescing diffuse hyperechoic areas, roughly vertical narrow-based artifacts spreading from the pulmonary surface away from the scanner, named “comet tail artifacts”, and interpreted as interstitial lung lesions.

Table 4.3 presents the severity of the lesions described as the “comet tail artifacts score”, the distribution and pattern of comet tail artifacts at the ultrasonographical evaluation of foals in group 1 (no. 1-6).

The detailed ultrasonographical findings of this group are included in Table 9.12.
Table 4.3 Score, distribution and pattern of comet tail artifacts of ultrasonographical evaluation of foals in group 1 (no. 1 - 6).

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>CTA score</th>
<th>Distribution</th>
<th>Pattern CTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Right: Cr.</td>
<td>M to D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left: Cr-V,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and V to C.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Right: Cr-V,</td>
<td>M to D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V to C.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left: C card</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>iac notch.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Cr cardiac</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>notch</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Whole lung</td>
<td>D</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Whole lung</td>
<td>D</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Cr</td>
<td>M to D</td>
</tr>
</tbody>
</table>

CTA: comet tail artifacts; Cr: cranial; Cr-V: cranio-ventral; V: ventral; C: caudal; D: diffuse; M: multifocal; F: focal.

4.3.2 Group 2

This group included 14 foals (no. 7 –20), which showed focal to multifocal nodular hypo- to hyperechoic areas in three foals (no. 7, 12, 14) and hypo- to hyperechoic areas combined with comet tail artifacts in 11 foals (no. 8 – 11, 13, 15 – 20). Most nodular lesions were surrounded by an area rich in comet tail artifacts. Nodular focal to multifocal lung consolidation in all foals was mostly found in the cranio-ventral part of the lung of right and left lung lobes, but found throughout the whole lung, and varied in depth and size from 0.3 up to 6 cm into the lung parenchyma.

The diameter of all nodular lesions of a foal was added resulting in a nodular diameter. The summarized nodular diameter of the foals from this group measured from 1 to 18.8 cm, with a median of 4.8 ± 4.6 cm.

Eleven out of 14 foals (no. 8 – 11, 13, 15 - 20) presented comet tail artifacts throughout the whole lung, being more severe in most of the foals in the cranio-ventral part of the thorax.

From these eleven foals, one foal (no. 13) had a comet tail artifact score of 1, six foals (no. 8, 10, 16 – 18, 20) presented a comet tail artifact score of 2 and four foals (no. 9, 11, 15, 19) presented a comet tail score of 3 (Table 4.4 and Figure 4.1).

The detailed ultrasonographical findings of this group are included in table 9.13.
Table 4.4 Nodular hypo- to hyperechoic areas measures and comet tail artifact score at pulmonary ultrasonography of foals in group 2 (no. 7 - 20).

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Diameter NA (cm)</th>
<th>Distribution NA</th>
<th>Pattern NA</th>
<th>CTA score</th>
<th>Distribution CTA</th>
<th>Pattern CTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2</td>
<td>Right: C</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>4.5</td>
<td>Cr-V</td>
<td>M</td>
<td>2</td>
<td>Extending from C-V to Cr-V, Cr-Do</td>
<td>M to D</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>Cr-V</td>
<td>M</td>
<td>3</td>
<td>Cr-Do</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>Cr-V</td>
<td>M</td>
<td>2</td>
<td>Extending from Mi to Cr.</td>
<td>D</td>
</tr>
<tr>
<td>11</td>
<td>5.2</td>
<td>Cr-V</td>
<td>M</td>
<td>3</td>
<td>Cr-Do</td>
<td>D</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>Right: C</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>3.3</td>
<td>Cr-V</td>
<td>M</td>
<td>1</td>
<td>Whole lung</td>
<td>D</td>
</tr>
<tr>
<td>14</td>
<td>3.6</td>
<td>Cr-Do</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>10.5</td>
<td>Do, Cr-V</td>
<td>M to D</td>
<td>3</td>
<td>Cr-Do</td>
<td>D</td>
</tr>
<tr>
<td>16</td>
<td>8.5</td>
<td>Cr-V</td>
<td>M</td>
<td>2</td>
<td>Cr-V, right lobe Cr-Do</td>
<td>M to D</td>
</tr>
<tr>
<td>17</td>
<td>1.5</td>
<td>Cr-V</td>
<td>M</td>
<td>2</td>
<td>Whole lung</td>
<td>D</td>
</tr>
<tr>
<td>18</td>
<td>15.8</td>
<td>Cr-V</td>
<td>M to D</td>
<td>2</td>
<td>Cr, V in Mi</td>
<td>D</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>V 11th ICS</td>
<td>F</td>
<td>3</td>
<td>Cr-Do</td>
<td>D</td>
</tr>
<tr>
<td>20</td>
<td>5.4</td>
<td>Cr-V</td>
<td>M</td>
<td>2</td>
<td>Cr-V</td>
<td>D</td>
</tr>
</tbody>
</table>

CTA: comet tail artifacts; NA: nodular hypo- to hyperechoic areas; D: diffuse; M: multifocal; F: focal; Cr-Do: cranio-dorsal; Cr-V: cranio-ventral; C: caudal; Do: dorsal; V: ventral; Mi: middle of the lung; ICS: intercostal space; -: no comet tail artifacts.
Fig. 4.1 Comet tail artifact (CTA) score (left, black) and nodular hypo- to hyperechoic areas (NA) diameter in cm (right, white) in the 14 foals of group 2.

4.3.3 Comparison of ultrasonographic findings in the two groups with ultrasonographic evaluation

Group 1 consisted of foals showing only comet tail artifacts at the ultrasonographical evaluation and resulted in a mean of 2 ± 0.8 (quartiles: 2, 3).

Group 2 consisted of foals showing nodular hypo- to hyperechoic areas at lung ultrasonography with a mean of 4.8 ± 4.7 (quartiles: 2.3, 6.6). Eleven of these 14 foals additionally presented comet tail artifacts at the ultrasonographical evaluation and resulted in a mean of 2 ± 1.2 (quartiles: 1.2, 2.7).

The values were evaluated and compared with each group.
4.4 Evaluation of treatment

Medication data was obtained from the foals records of each foal. The medication given to the foals in the horse breeding farm was mostly focused against an endemic *R. equi* infection. Therefore the antibiotics given were rifampin, tulathromycin, azithromycin, clarithromycin or trimetoprim-sulphamethoxazole (TMS). Corticosteroids (prednisolone or dexamethazone) were given shortly in patients presenting a severe dyspnea. Cyclosporine was administered in severe cases of dyspnea not responding to antibiotics and corticosteroids. The non-steroidal anti-inflammatory drugs (NSAID) used included flunixine meglumine, and phenylbutazone. The individual treatment of each foal of the first three groups is summarized in table 9.10.

4.4.1 Group 1

All foals of this group (n = 6; no. 1-6) were treated with antibiotics. Three of them (no. 1, 4, 5) were treated additionally with corticosteroids over a period of one to seven days.

---

**Fig. 4.2** Comet tail artifact (CTA) score in foals 1 to 20: 3 = more than ¾ of the lung in both sides with “3”; 2 = more than half of the lung in both sides with “2” and less than ¾ of the lung with “3”; 1 = no “3” and till half of both sides of the lung with “2”; 0 = only “0” and “1” in both sides of the lung. Pink = group 1 (No. 1 – 6), blue = group 2 (No. 7-20)
4.4.2 Group 2
Except for foal no 20 all foals were treated primarily with antibiotics. Nine out of these 13 foals (no.8, 9, 11, 12, 15 – 19) were additionally provided with dexamethazone, prednisolone and/or cyclosporine until improvement. Foals presenting fever for more than one occasion (no. 9, 11, 15 – 17, 19) were treated additionally with an NSAID as flunixine meglumine.

4.4.3 Group 3
Data about the treatment of ten of the 17 animals of this group (n = 17; no. 21 - 37) are not available except for seven foals (no. 21, 23, 25, 26, 29, 34, 36), that received rifampin combined with tulathomycin for a range from 13 to 109 days and glucocorticoids like dexamethazone, prednisolone and/or cyclosporine until improvement in two foals (no. 34, 36). However, 13 foals (no. 21 – 24, 27 – 32, 35 - 37) of this group did not need treatment, because they were clinically healthy (“Clinical Score” of 1 or 0).

4.4.4 Group 4
These two foals where clinically healthy, so they did not get any treatment.

4.5 Pathologic-anatomic findings

4.5.1 Group 1
The morphologic findings in the six foals (no. 1 - 6) of this group were indicative for interstitial pneumonia. The pulmonary parenchyma was poorly retracted and diffusely consolidated with a meaty consistency. Except for one foal (no. 6) additional nodular lesions in the pulmonary parenchyma were reported in all animals of this group. The extension of these foci ranged from 0.5 to 4 cm located mostly in the caudal lobe deep in the lung parenchyma. An overview of distribution, quantity, diameter and morphologic character of nodular changes in the pulmonary parenchyma of five foals (no. 1 - 5) in this group is shown in table 4.5. Furthermore, in two foals (no. 1, 3) moderate multifocal alveolar emphysema and in four foals (no. 2, 3, 4, 5) mild multifocal alveolar edema was observed. The necropsy findings of this group are included in table 9.12.
Table 4.5 Distribution, pattern, diameter and morphologic character of nodular lesions found at necropsy of 5 foals (no. 1 - 5) in group 1.

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Distribution</th>
<th>Pattern of NA</th>
<th>Diameter in cm</th>
<th>Morphologic character</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cr</td>
<td>D</td>
<td>1.5 x 1</td>
<td>Suppurative to necrotizing and fibrosing</td>
</tr>
<tr>
<td></td>
<td>C-Do</td>
<td>M</td>
<td>3 x 2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5th ICS</td>
<td>F</td>
<td>0.5</td>
<td>Suppurative to fibrosing</td>
</tr>
<tr>
<td>3</td>
<td>Whole lung</td>
<td>M</td>
<td>1</td>
<td>Fibrosing</td>
</tr>
<tr>
<td>4</td>
<td>Left lung:</td>
<td></td>
<td>Left lung:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11th ICS, Ce, Su</td>
<td>F</td>
<td>0.5</td>
<td>Left lung:</td>
</tr>
<tr>
<td></td>
<td>9th ICS, Ce, Pr</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>9th ICS, Do, Pr</td>
<td>F</td>
<td>2 x 05</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>9th ICS, V, Pr</td>
<td>F</td>
<td>1 x 0.2</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>9th ICS, Ce, Pr</td>
<td>F</td>
<td>1 x 0.6</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>7th ICS, Do, Pr</td>
<td>F</td>
<td>1 x 0.9</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>7th ICS, Do, Pr</td>
<td>F</td>
<td>2 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>7th ICS, Ce, Pr</td>
<td>F</td>
<td>1 x 1</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>6th ICS, Do, Pr</td>
<td>F</td>
<td>1 x 0.3</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>4th ICS, Do, Pr</td>
<td>F</td>
<td>1 x 1</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>Cr, Su</td>
<td>F</td>
<td>2 x 08</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>Right Lung:</td>
<td></td>
<td>Right Lung:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11th ICS, Ce, Su</td>
<td>F</td>
<td>1 x 1.3</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>11th ICS, Do, Pr</td>
<td>F</td>
<td>1 x 0.6</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>10th ICS, Do, Pr</td>
<td>F</td>
<td>3 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>10th ICS, Ce, Pr</td>
<td>F</td>
<td>3 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>9th ICS, V, Pr</td>
<td>F</td>
<td>1 x 0.3</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>9th ICS, V, Pr</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>8th ICS, Ce, Pr</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>7th ICS, Do, Su</td>
<td>F</td>
<td>1 x 0.6</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>6th ICS, Do, Su</td>
<td>F</td>
<td>1 x 0.3</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>5th ICS, V, Su</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>4th ICS, V, Pr</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>4th ICS, V, Pr</td>
<td>F</td>
<td>1 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
<td>F</td>
<td>2 x 0.5</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>5</td>
<td>Right, Cr, Su</td>
<td>M to D</td>
<td>4 x 4</td>
<td>Suppurative</td>
</tr>
<tr>
<td></td>
<td>Left, Pr</td>
<td>M to D</td>
<td>4 x 4</td>
<td>Suppurative</td>
</tr>
</tbody>
</table>

Cr: cranial; C-Do: caudo-dorsal; Ce: In the center of the lung; V: ventral; Do: dorsal; D: diffuse; M: multifocal; F: focal; ICS: intercostal space; Su: superficial location in the pulmonary parenchyma; Pr: profound location in the pulmonary parenchyma.
4.5.2  Group 2

In this group (n = 14, no. 7 - 20) focal to multifocal firm nodular lesions characterized by abscess formation, white to yellowish or red firm foci up to 2 cm in diameter or nodular necroses were located mostly bilaterally in the cranial lobes and were the common finding in ten foals (no. 7, 9, 12 - 16, 18 - 20). These nodular lesions were interpreted as pyogranulomatous pneumonia (no. 9, 12, 14, 18, 19), suppurative to necrotizing pneumonia (no. 13 - 16, 20), as well as fibrosing and necrotizing pneumonia (no. 7).

In addition eight out of 14 foals (no. 9 - 11, 15 - 19) presented consolidated, heavy, rubber-like and non-collapsed lungs with a dry cut surface interpreted as interstitial pneumonia.

In nine of these foals additional lesions were found:

• Foals no. 8, 13, 15 and 20: mild multifocal alveolar edema.
• Foals 9 – 11: acute diffuse alveolar edema.
• Foal no. 20: mild diffuse interstitial edema.
• Foals no. 8, 10, 13, 20: mild diffuse alveolar emphysema.
• Foal no. 9: severe acute diffuse alveolar emphysema.
• Foal no. 7: multifocal fibrinous to necrotizing pleuropneumonia with 3.5 litres of exudate in the thoracic cavity.
• Foal no. 11: severe multifocal fibrinous pleuritis.
• Foal 14: atelectatic areas at the cranial part of the lung in both lung lobes.
• Foal 20: mild diffuse interstitial edema.

The necropsy findings of this group are included in table 9.13.

A summary of the distribution, pattern, diameter and morphological character of lung lesions observed at necropsy in all 14 foals in group 2 is shown in table 4.6.
**Table 4.6** Distribution, pattern, diameter and morphological character of lung lesions in eleven foals (7 – 9, 12 - 16, 18 - 20) with nodular lesion in group 2.

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Distribution</th>
<th>Pattern</th>
<th>Diameter (cm)</th>
<th>Morphologic character</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Cr-V</td>
<td>M</td>
<td>1</td>
<td>Fibrosing and necrotizing</td>
</tr>
<tr>
<td>8</td>
<td>Whole lung</td>
<td>D</td>
<td>-</td>
<td>Alveolar edema and emphysema</td>
</tr>
<tr>
<td>9</td>
<td>Cr</td>
<td>F</td>
<td>0.5 – 1</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>12</td>
<td>7th ICS V left</td>
<td>F</td>
<td>0.3 x 0.3</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>13</td>
<td>4th ICS left 6th ICS left 4th ICS right</td>
<td>F</td>
<td>0.1 0.3 0.1</td>
<td>Suppurative to necrotizing Pyogranulomatous</td>
</tr>
<tr>
<td>14</td>
<td>5th ICS right 6th ICS left</td>
<td>F</td>
<td>1.5 x 1.7 2 x 2 x 1</td>
<td>Suppurative to necrotizing Pyogranulomatous</td>
</tr>
<tr>
<td>15</td>
<td>Whole lung</td>
<td>M</td>
<td>1 x 0.2</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>16</td>
<td>Whole lung</td>
<td>M</td>
<td>1 – 2</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>18</td>
<td>Cr</td>
<td>M</td>
<td>1-2</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>19</td>
<td>Whole lung</td>
<td>M</td>
<td>1</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>20</td>
<td>Whole lung</td>
<td>M</td>
<td>1 – 2</td>
<td>Suppurative to necrotizing</td>
</tr>
</tbody>
</table>

Cr: cranial; Cr-V: cranio-ventral; D: diffuse; M: multifocal; F: focal; ICS: intercostal space; -: not presented

4.5.3 **Group 3**

Multifocal nodular pulmonary changes characterized by firm whitish to dark grey nodules with variable sizes were observed in ten out of 17 foals (no. 21 – 23, 25, 27, 31, 32, 34 - 36). These lesions were interpreted as suppurative to necrotizing pneumonia in six foals (no. 21 - 23, 27, 34, 36), as suppurative pneumonia in two foals (no. 31, 35), as granulomatous pneumonia in one foal (no. 32), and as pyogranulomatous pneumonia in one foal (no. 25). Additionally seven out of 17 foals (no. 26, 27, 31 - 33, 35, 36) presented a multifocal to coalescing firm, non-collapsed lung indicative for interstitial pneumonia.

Distribution, pattern, diameter and morphological character of the nodular lesions found at necropsy of ten foals (no. 21 - 23, 25, 27, 31, 32, 34 - 36) with nodular pulmonary changes in group 3 are shown in table 4.7.
Table 4.7 Distribution, pattern and diameter and morphological character of nodular lesions in ten foals (no. 21 - 23, 25, 27, 31, 32, 34 - 36) of group 3.

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Distribution</th>
<th>Pattern</th>
<th>Diameter (cm)</th>
<th>Morphologic character</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Cr left more than right</td>
<td>M to D</td>
<td>1</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>22</td>
<td>Cr-Do</td>
<td>M</td>
<td>1</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>23</td>
<td>Cr-V, C</td>
<td>M to D</td>
<td>1</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>25</td>
<td>7th ICS V left</td>
<td>M</td>
<td>0.3 x 0.3</td>
<td>Pyogranulomatous</td>
</tr>
<tr>
<td>27</td>
<td>C</td>
<td>M</td>
<td>0.5</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>31</td>
<td>4th ICS</td>
<td>M</td>
<td>0.5 – 1</td>
<td>Suppurative</td>
</tr>
<tr>
<td></td>
<td>5th ICS</td>
<td>M</td>
<td>0.3 – 1</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>9th ICS</td>
<td>F</td>
<td>1 x 1 x 1.2</td>
<td>Granulomatous</td>
</tr>
<tr>
<td></td>
<td>Do right</td>
<td>F</td>
<td>2 x 1</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Cr-V</td>
<td>F</td>
<td>1</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td>35</td>
<td>Cr</td>
<td>F</td>
<td>0.3</td>
<td>Suppurative</td>
</tr>
<tr>
<td>36</td>
<td>4th ICS</td>
<td>F</td>
<td>0.5 x 0.8</td>
<td>Suppurative to necrotizing</td>
</tr>
<tr>
<td></td>
<td>4th ICS</td>
<td>F</td>
<td>0.3 x 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4th ICS</td>
<td>F</td>
<td>0.3 x 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4th ICS</td>
<td>F</td>
<td>0.1 x 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Cr: cranial; C: caudal; Cr-Do: cranio-dorsal; Cr-V: cranio-ventral; Do: dorsal; D: diffuse; M: multifocal; F: focal; ICS: intercostal space.

Foal no. 21 presented additionally atelectatic areas in the right cranial lobe, and a fibrinous pleuritis at the left cranial lobe.

In two foals (no. 30, 35) whitish mucoid aspirated material in the bronchial lumina was observed.

As additional findings in nine foals (no. 25, 26, 28 - 30, 32, 35 - 37) alveolar edema was observed, in six out of these nine foals (no. 25, 26, 29, 32, 36, 37) of a moderate, and in three foals (no. 28, 30, 35) of a severe degree.

Additionally one foal (no. 24) presented a moderate multifocal interstitial edema.

In seven foals (no. 24 - 26, 28, 30, 36, 37) moderate acute diffuse alveolar emphysema was observed.

The necropsy findings of this group are included in table 9.14.
4.5.4 Group 4

In both foals (no. 38, 39) mild acute diffuse alveolar edema and hyperemia were observed. Additionally, foal no. 39 had mild multifocal alveolar and moderate multifocal interstitial emphysema.

The necropsy findings of this group are included in table 9.15.

4.6 Pathologic-histological and immunohistological findings

4.6.1 Group 1

All foals of this group (no. 1 - 6) presented a moderate to severe multifocal to coalescing or diffuse interstitial pneumonia characterized by interstitial infiltration of lymphocytes, macrophages, and plasma cells (Fig. 4.6.1), multifocal alveolar PAS-positive, hyaline membranes (no. 1, 4, 5) (PAS-reaction: positive), and proliferation and desquamation of type II pneumocytes (no. 1 - 6) (Fig. 4.6.2 and 4.6.3). In some foals few multinucleated giant cells were seen (no. 1 – 3, 5). Additionally some interstitial (no. 1, 5) and alveolar (no. 1 - 5) neutrophils were observed.

Additional findings consisted of:

- mild focal to multifocal interstitial fibrosis (EvG-stain: positive) in all foals;
- foamy or finely honeycombed eosinophilic intra-alveolar proteinaceous fluid in four foals (no. 1, 2, 4, 5) (Fig. 4.6.3);
- mucus accumulation in bronchial and bronchiolar lumina in four foals (no. 1, 2, 4, 5).
- focal pyogranulomatous or granulomatous pneumonia composed of focal necrosis surrounded by neutrophils, macrophages, lymphocytes, and few multinucleated giant cells in three foals (no. 2, 4, 5) (Fig. 4.6.4 and 4.6.5);
- suppurative bronchopneumonia with accumulation of high amount of neutrophils and macrophages in bronchial, bronchiolar and alveolar lumina in foals no. 1 and 4;
- aspirated keratin lamellae with infiltration of mainly macrophages in bronchi, bronchioles and alveoli in two foals (no. 2, 6);
- hyaline thrombi in interalveolar capillaries in foal no. 1;
- diffuse interstitial fibrosis with severe calcification (von Kossa's stain: positive) in foal no. 3 (Fig. 4.6.6);
• multifocal mild granulocytic alveolitis in foal no. 4 (Fig 4.6.1 and 4.6.2).

Using *Gram's stain* in two foals (no. 2, 4) intralesional intracellular cocci (Fig. 4.6.7) were observed.

Using *GMS stain*, in four animals (no. 1, 2, 4, 5) small mycotic cysts with a double membrane and dark brown to black stained cyst walls ranging from 5 to 7 µm in diameter were observed (Fig. 4.6.8). These organisms appeared sometimes attached to the alveolar wall and were interpreted as cysts of *P. carinii*.

*Immunohistochemistry* revealed a moderate to marked focal to multifocal cytoplasmic staining of *R. equi*-antigen as pale to dark brown finely granular deposits in macrophages, multinucleated giant cells and some neutrophils of three foals (no. 2, 4, 5) localized mostly in pyogranulomatous lesions (Fig. 4.6.9).

*Immunohistochemically*, high amounts of *P. carinii* cysts and trophozoites were demonstrated in the alveolar and bronchiolar lumina in four foals (no. 1, 2, 4, 5) (Fig. 4.6.10).

The histological and immunohistochemical findings of this group are included in table 9.12.

### 4.6.2 Group 2

13 out of 14 foals (no. 7, 9 - 20) of this group presented focal to multifocal nodular inflammatory lesions of the pulmonary parenchyma consisting of suppurative bronchompneumonia in seven foals (no. 11, 13, 15 - 17, 19, 20). The lesions were characterized by infiltration of high number of neutrophils and macrophages. Suppurative to necrotizing pneumonia characterized by infiltration of numerous of in part degenerated neutrophils and macrophages were observed in three foals (no. 13 – 15). Focal interstitial pneumonia characterized by septal thickening with cell infiltration predominantly of macrophages and lymphocytes surrounded by fibrotic tissue (*EvG stain*) was shown in three foals (no. 10, 14, 16; Fig. 4.6.11). Granulomatous to pyogranulomatous pneumonia characterized by occasional central necrosis and/or surrounded by neutrophils. In the periphery infiltration of macrophages, lymphocytes, plasma cells and fibrosis was observed in five foals (no. 9, 12, 14, 15, 18) (Fig. 4.6.12; 4.6.13 and 4.6.14). Fibrosing and necrotizing pneumonia characterized by severe focal necrosis with neutrophils and macrophages infiltration and collagenous fibers surrounding elastic fibers was observed in one foal (no. 7).

Additional findings consisted of:
interstitial pneumonia characterized by multifocal to diffuse mild peribronchial, peribronchiolar, interstitial and perivascular infiltration of mainly macrophages, lymphocytes and plasma cells in all foals (no. 7 - 20), occasionally admixed with some neutrophils (no. 7, 15, 16, 19) and single multinucleated giant cells (no. 9, 15 - 18); proliferation of type II pneumocytes (no. 9, 11, 12 – 19; Fig. 4.6.15) and PAS-positive hyaline membranes attached to alveolar walls (no. 9, 11, 15 – 19; Fig. 4.6.16);

alveolar lumina containing varying amounts of neutrophils (no. 7 - 20) and multinucleated giant cells (no. 11, 13 - 20).

mild to moderate focal to multifocal non-suppurative pleuritis in seven foals (no. 8, 11, 15, 16, 18 - 20) characterized by inflammatory cell infiltration and additionally edema (no. 7, 11, 12, 14, 15, 18 - 20), fibrosis (no. 11, 16, 18, 20) and emphysema (no. 15) in the pleura;

hyaline thrombi in interalveolar capillaries of foals no. 7, 10, 19 and 20;

moderate to severe multifocal to coalescing interstitial edema in two foals (no. 15, 19)
intralesional bacterial colonies in two foals (no. 16, 18);

moderate multifocal subpleural atelectatic areas in one foal (no. 8);

severe multifocal alveolar emphysema in one foal (no. 10),

moderate multifocal alveolar edema in one foal (no. 14),

severe multifocal proliferation of the intima layer with obliteration of arterial lumina in foal no. 12;

moderate multifocal keratin lamellae interpreted as amniotic fluid in the bronchi and bronchioles of one foal (no. 20).

Using EvG stain an increase of elastic and collagenous fibers was found in the interstitium and adjacent to the inflammatory foci in two foals (no. 12, 14).

Gram’s stain was made in three foals (no. 7, 12, 19). In foals no. 7 and 19 cytoplasmic Gram-positive coccoid bacteria were found. Extracellular Gram-positive cocci forming clumps were located in necrotic pulmonary areas of foal no. 12.

Using GMS stain, in foals no. 11, 14, 16 and 17 cysts of P. carinii appeared as almost round, cup- or crescent-shaped, occasionally with a thick dark capsular dot, mostly attached to the alveolar wall, but also intraalveolar. Additionally, some parenthesis-like bodies were observed.
**Immunohistochemistry** revealed a focally mild pale brown cytoplasmic granular staining of *R. equi*-antigen predominantly in macrophages and some single multinucleated giant cells adjacent to necrotic tissue of the lung of six foals (no. 9, 15 - 19; Fig. 4.6.17).

**Immunohistochemical** demonstration of *P. carinii*-antigen characterized by few round cysts stained dark to light brown with a thick rim and some amorphous mass (corresponding to the extracellular matrix and all stages of the organism) were observed in alveolar lumina and alveolar walls in foals no.11, 14, 16, 17, and 19 (Fig. 4.6.18).

The histological and immunohistochemical findings of this group are included in table 9.13.

### 4.6.3 Group 3

15 out of 17 foals (no. 21, 23, 24, 26 - 37) of this group (no. 20 - 37) presented a moderate to severe multifocal to coalescing interstitial pneumonia characterized by interstitial, peribronchial, peribronchiolar and perivascular infiltration of macrophages, lymphocytes, plasma cells, with some intra-alveolar multinucleated giant cells (no. 32), foamy macrophages (no. 26), and interstitial neutrophils (no. 25, 26, 30, 34), proliferation of type II pneumocytes (no. 21, 26, 27, 29, 31 - 34), and PAS-positive, hyaline membranes attached to alveolar walls (Fig. 4.6.19) (no. 30, 31, 34). In addition, alveolar lumina contained varying amounts of neutrophils (no. 21, 22, 24, 27, 29 - 34, 37) and multinucleated giant cells (no. 22, 27, 29, 31, 32, 34) were observed.

Additional findings consisted of:

- focal to multifocal nodular inflammatory lesions of the pulmonary parenchyma in 15 out of 17 animals (no. 21, 23, 24, 26 - 37) consisting of suppurative bronchopneumonia in seven animals (no. 27, 30 - 32, 34, 35, 37). The lesions were characterized by infiltration of huge amounts of neutrophils and macrophages (Fig. 4.6.20).

- suppurative to necrotizing pneumonia characterized by infiltration of huge amounts of in part degenerated neutrophils and macrophages observed in six foals (no. 21 - 24, 29, 36).

- pyogranulomatous or granulomatous pneumonia characterized by a necrotic area at the centre surrounded by neutrophils, macrophages, lymphocytes, plasma cells, and few
multinucleated giant cells and peripherally surrounded by fibrosis in four foals (no. 23, 25, 33, 36; Fig. 4.6.21).

- suppurative bronchitis characterized by bronchi infiltrated with neutrophils and some macrophages in one foal (no. 24).

- fibrosing and necrotizing pneumonia characterized by severe focal necrosis with neutrophils and macrophages infiltration and collagenous fibres surrounding elastic fibres in foal no. 25.

- focal interstitial pneumonia characterized by septal thickening with cell infiltration predominantly of macrophages and lymphocytes surrounded by fibrotic tissue (EvG stain; Fig. 4.6.22) in one foal (no. 29).

- severe diffuse alveolar edema in nine foals (no. 23, 27, 28 – 32, 35, 37);

- mild to moderate focal to multifocal non-suppurative pleuritis in seven foals (no. 21, 22, 27, 28, 30, 31, 35) characterized by inflammatory cell infiltration and additionally edema (no. 21, 27, 28, 30, 35), and fibrosis (no. 21, 35);

- mild to moderate focal to multifocal interstitial fibrosis (EvG stain: positive) in seven foals (no. 21, 24, 26, 30 - 32, 36);

- aspirated keratin lamellae in bronchial or alveolar lumina in seven foals (no. 21, 24, 27, 30, 33, 35, 36) accompanied by inflammatory infiltrates mostly neutrophils and macrophages (Fig. 4.6.23);

- moderate to severe multifocal to coalescing alveolar emphysema in six foals (no. 28, 29, 31, 35 - 37);

- bacterial colonies in alveoli, bronchi and/or bronchioli in five foals (no. 21, 22, 24, 27, 33);

- severe diffuse interstitial edema in two animals (no.24, 30);

- focally severe granulomatous to necrotizing pneumonia with intralesional fungi in foal no. 23 (Fig. 4.6.24) characterized by branching septate hyphae up to 6 µm in width and associated with cellular debris and exudate. The inflammatory infiltrate consisted of numerous neutrophils, macrophages, few lymphocytes, and plasma cells. In many lesions macrophages and epitheloid cells predominated; occasionally multinucleated giant cells were present. Using GMS stain mycotic hyphae stained dark brown to black (Fig. 4.6.24 inset) were observed.
• severe fibrotic focus (*EvG stain*: positive) in foal no. 25.
• moderate multifocal mucus accumulation in bronchial lumina in one foal (no. 26).
• severe multifocal proliferation of the intima layer of an artery with obliteration of the lumen in foal no. 29;

Using *Gram’s stain* in two foals (no. 25, 35), in foal no. 25 some Gram-positive intralesional cytoplasmic cocci mainly in macrophages were observed.
Using *GMS stain* mycotic cysts of *P. carinii* attached to the alveolar wall were observed in three foals (no. 26, 28, 29). Cysts were empty and surrounded by a thick dark-brown to black double-layered membrane.

*Immunohistochemistry* revealed moderate to severe focal to multifocal cytoplasmic staining of *R. equi*-antigen as fine to coarse granular deposits in macrophages mostly adjacent to necrotic areas of three foals (no. 29, 32, 34; Fig. 4.6.25).

*Immunohistochemical* demonstration of numerous *P. carinii* cysts and trophozoites was noted in the alveolar lumina or attached to the alveolar walls, and occasionally in the lumina of bronchioles of four foals (no. 26, 28, 29, 32; Fig. 4.6.26).

The histological and immunohistochemical findings of this group are included in table 9.14.

### 4.6.4 Group 4

Histological findings in these animals comprised mild alveolar edema and emphysema. In foal no. 38 additionally focally mild interstitial emphysema and aspirated material, most likely meconium, were observed. Foal no. 39 presented a mild multifocal peribronchial and bronchiolar infiltration of lymphocytes.

Histological special stains (*PAS-reaction, EvG-stain, and GMS stain*) as well as immunohistochemistry for *R equi*- and *P. carinii*-antigens were negative.

The histological and immunohistochemical findings of this group are included in table 9.15.
Histological documentation

4.6.5 Lesions in animals of group 1

*Fig. 4.6.1* Foal no. 4: interstitial pneumonia characterized by infiltration of lymphocytes, macrophages and plasma cells with granulocytic alveolitis (open arrows) and hyaline membranes formation (arrows). HE, objective magnification 20 x, bar = 140 µm

*Fig. 4.6.2* Foal no. 4: same as *fig. 4.6.1*, interstitial pneumonia characterized by thickened interalveolar septae (asterisk), intraalveolar hyaline membranes (arrow) and granulocytic alveolitis (open arrows). HE, objective magnification 40 x, bar = 70 µm

*Fig. 4.6.3* Foal no. 4: interstitial pneumonia with diffuse distention of alveoli flooding with foamy macrophages (arrow) and proteinaceous material (open arrows) giving a “honey comb” appearance, and proliferation of type II pneumocytes (arrow head). HE, objective magnification 20 x, bar = 140 µm
Fig. 4.6.4 Foal no. 2: pyogranulomatous pneumonia composed of a center of necrotic tissue with neutrophilic granulocytes (asterisks), surrounded by a rim of macrophages (open arrow), single giant cells, and an outer delineated layer of connective tissue (arrows) infiltrated by lymphocytes and plasma cells. HE, objective magnification 20 x, bar = 140 µm

Fig. 4.6.5 Foal no. 5: granulomatous pneumonia with focal necrosis (arrows) and thick fibrous demarcation (asterisks) with inflammatory cell infiltration HE, objective magnification 5 x, bar = 560 µm

Fig. 4.6.6 Foal no. 3: diffuse interstitial fibrosis with calcification (arrows). HE, objective magnification 20 x, bar = 140 µm. Inset: calcium deposits stained by von Kossa’s stain (arrow), objective magnification 10 x, bar = 70 µm
Fig. 4.6.7 Foal no. 4: interstitial pneumonia with focal granulomatous to pyogranulomatous pneumonia and intralesional cytoplasmic Gram-positive cocci in macrophages (arrows). Gram’s stain, objective magnification 40 x, bar = 70 µm

Fig. 4.6.8 Foal no. 4: interstitial pneumonia with numerous dark brown intraalveolar cysts of *P. carinii* (arrows); Grocott’s methenamine silver stain, objective magnification 40 x, bar = 70 µm

Fig. 4.6.9 Foal no. 4: interstitial pneumonia with focal pyogranulomatous to necrotizing pneumonia, immunolabelling of *R. equi* antigen in macrophages (arrows). Immunohistochemistry, Nomarsky differential interference contrast filter, objective magnification 40 x, bar = 70 µm
Results

4.6.6 Lesions in animals of group 2

Fig. 4.6.10 Foal no. 4: interstitial pneumonia, immunolabelling of *P. carinii* antigen in alveoli (arrows). Immunohistochemistry, Nomarsky differential interference contrast filter, objective magnification 40 x, bar = 70 µm

Fig. 4.6.11 Foal no. 13: focally severe interstitial pneumonia with septal thickening and cell infiltration demarcated by fibrotic tissue (arrows) and adjacent hyperemic pulmonary tissue without inflammation (asterisks), EvG stain, objective magnification 1.25 x, bar = 2240 µm

Fig. 4.6.12 Foal no. 9: focally severe pyogranulomatous pneumonia with central cellular debris (A) surrounded by inflammatory cells with a fibrous demarcation (asterisks). HE, objective magnification 10 x, bar = 280 µm
Results

**Fig. 4.6.13** Foal no. 14: pyogranulomatous to necrotizing pneumonia with necrosis (A) at the centre of the lesion surrounded by inflammatory cell infiltration. HE, objective magnification 5 x, bar = 560 µm. Inset: Infiltration of predominantly macrophages (open arrow), lymphocytes (arrowhead), plasma cells and few giant cells (arrow) HE, objective magnification 40 x.

**Fig. 4.6.14** Foal no. 12: focal pyogranulomatous to necrotizing pneumonia characterized by necrosis (A), infiltration of in part degenerated neutrophils (asterisk) and peripherally surrounded by fibrosis (arrows). HE, objective magnification 10 x, bar = 280 µm.

**Fig. 4.6.15** Foal no. 13: interstitial pneumonia characterized by infiltration of lymphocytes (arrow head), macrophages (open arrows), plasma cells, proliferation of type II pneumocytes (arrow). HE, objective magnification 40 x, bar = 70 µm.
**Fig. 4.6.16** Foal no. 9: interstitial pneumonia characterized by infiltration of inflammatory cells, hyaline membranes (arrows), and proliferation of type II pneumocytes, HE, objective magnification 10 x, bar = 280 µm. Inset: Infiltration of lymphocytes, macrophages (arrow head), plasma cells and some giant cells and proliferation of type II pneumocytes (open arrow). HE, objective magnification 40 x

**Fig. 4.6.17** Foal no. 16: suppurative to necrotizing pneumonia, immunolabelling of *R. equi* antigen in the cytoplasm of macrophages (arrows), some neutrophils (arrow head) and extracellularly in cellular debris (open arrow). Immunohistochemistry, Nomarsky differential interference contrast filter, objective magnification 40 x, bar = 70 µm

**Fig. 4.6.18** Foal no. 17: interstitial pneumonia, immunolabelling of *P. carinii* antigen in cysts of *P. carinii* along the alveolar wall (arrows) Immunohistochemistry Nomarsky differential interference contrast filter, objective magnification 40 x, bar = 70 µm
4.6.7 Lesions in animals of group 3

*Fig. 4.6.19* Foal no 26: interstitial pneumonia characterized by infiltration of lymphocytes, macrophages (arrow head) and plasma cells with proliferation of type II pneumocytes (open arrows), hyaline membrane formation (arrow) and septal thickening (asterisks). HE, objective magnification 20 x, bar = 140 µm

*Fig. 4.6.20* Foal no. 30: suppurative pneumonia with severe alveolar infiltration of neutrophils (arrows) and few macrophages. HE, objective magnification 20 x, bar = 140 µm. Inset: infiltration of neutrophils (arrow), macrophages (open arrow) and bacterial colonies (arrow head), HE objective magnification 40 x

*Fig. 4.6.21* Foal no. 25: focal chronic granulomatous pneumonia with necrotic center (A) surrounded by extensive fibrosis (arrows) and collagenous fibers; EvG, objective magnification 5 x, bar = 560 µm
Fig. 4.6.22 Foal no. 29: focally severe interstitial pneumonia with septal thickening, surrounded by fibrotic tissue (A), hyperemic pulmonary tissue without inflammation (asterisk). HE, objective magnification 2.5 x, bar = 1120 µm
Inset: infiltration of macrophages (arrow) and lymphocytes (open arrow). HE, objective magnification 40 x

Fig. 4.6.23. Foal no. 33, two days old: suppurative pneumonia with aspirated keratin lamellae (arrows), and neutrophils (open arrows) and macrophages (arrow head) infiltration, HE, objective magnification 40 x, bar = 70 µm

Fig. 4.6.24 Foal no. 23: focal granulomatous to necrotizing pneumonia with intraleisonal fungi (arrows), HE objective magnification 10 x, bar = 280 µm.
Inset: mycotic hyphae stained with Grocott’s methenamine silver stain; objective magnification 40 x
Fig. 4.6.25 Foal no. 29: focal interstitial pneumonia, immunolabelling of *P. carinii* antigen in alveoli. Immunohistochemistry, Nomarsky differential interference contrast filter, objective magnification 10 x, bar = 280 µm

Fig. 4.6.26 Foal no. 28: interstitial pneumonia, immunolabelling of *P. carinii* antigen in cysts of *P. carinii* located in the bronchiolar lumen (arrows). Immunohistochemistry, Nomarsky differential interference contrast filter, objective magnification 40 x, bar = 70 µm
4.7 Microbiological and virological findings

*Intra vitam* and *post mortem* microbiological aerobic and anaerobic investigations of TBA samples and lung tissue were performed in 23 (59%) and 10 (26%) out of 39 foals, respectively. By means of anaerobic methods no bacteria were isolated.

Viral detection methods revealed on samples of six out of 39 foals (15%; investigation on EHV-1 in foal no. 5, 11, 15, 22, EHV-4 in foal no. 22, Equine Rotavirus (ERV) in foals no. 12 and 24, and EIV in foal no. 11).

4.7.1 Group 1

TBA samples from three out of six foals (no. 2 - 4) resulted in isolation of *R. equi* (no. 3), *Sc. equi* ssp. *zooepidemicus* (no. 2), *B. bronchiseptica* (no. 2), *E. coli* (no. 2, 3) and non-specific bacteria (no. 2 - 4).

Microbiology of lung tissue obtained *post mortem* from three out of six foals (no. 2 - 4) resulted in isolation of *R. equi* (no. 2, 4), *Sc. equi* ssp. *zooepidemicus* (no. 2, 3), *E. coli* (no. 2, 4), *K. pneumoniae* (no. 4), and non-specific bacteria (no. 2 – 4).

The investigation of one out of six foals (no. 5) on EHV-1 was negative.

The microbiological results of this group are integrated in table 9.12.

4.7.2 Group 2

TBA samples from four out of foals (no. 9, 14, 16, 20) resulted in isolation of *R. equi* (no. 9), *Sc. equi* ssp. *zooepidemicus* (no. 9, 20), *E. coli* (no. 14), *B. bronchiseptica* (no. 14), *K. pneumoniae* (no. 14), and non-specific bacteria (no. 14, 16, 20).

Microbiological *post mortem* investigation of lung tissue from ten out of 14 foals (no. 7, 9 - 11, 14 – 16, 18 - 20) resulted in isolation of *R. equi* (no. 10, 14 – 16, 18), *Sc. equi* ssp. *zooepidemicus* (no. 7, 11, 15, 18, 20), *E. coli* (no. 7, 10, 11, 14, 16, 18, 20), *K. pneumoniae* (no. 11, 14, 15), *B. bronchiseptica* (no. 14) and some non-specific bacteria (no. 9 – 11, 14 – 16, 18 - 20).

The investigation on EHV-1 in two out of 14 foals (no. 11, 15) was negative. Additionally in foal no. 11 cell culture for EIV was negative for virus isolation.

TBA and lung tissue microbiological investigation of this group is included in table 9.13.
4.7.3 Group 3
TBA samples from three out of 17 foals (no. 27, 28, 36) resulted in isolation of *Sc. equi* ssp. *zooepidemicus* (no. 36), *E. coli* (no. 27, 28) and non-specific bacteria (no. 27, 28).

Microbiological *post mortem* investigation of lung tissue from ten out of 17 foals (no. 21 – 24, 30 – 33, 36, 37) resulted in isolation of *R. equi* (no. 21, 31, 32), *Sc. equi* ssp. *zooepidemicus* (no. 31, 32), *E. coli* (no. 21, 23, 24, 30, 32, 36, 37), *K. pneumoniae* (no. 24, 30, 32, 36), *P. haemolytica* (no. 24), *A. equuli* (no. 24), and non-specific bacteria (no. 21 - 23, 30, 31, 33, 36, 37).

Foal no. 22 investigated for EHV-1 was negative and for EHV-4 resulted in 2,341 (ELISA/Obtical density values) which could not be differentiated from vaccination antibodies.

TBA and lung tissue microbiological investigation of this group is included in table 9.14.

4.7.4 Group 4
Microbiology of TBA samples taken *intra vitam* (no. 38, 39) and lung tissue (no. 39) obtained *post mortem* resulted in isolation of non-specific bacteria.

TBA and lung tissue microbiological investigation of this group is included in table 9.15.

4.8 Comparison of clinical, ultrasonographical, pathological and etiological findings

4.8.1 Clinical and ultrasonographical correlation
In the following chapter the clinical and ultrasonographical findings of groups 1 and 2 are compared. For the other foals (groups 3 and 4) this was not possible because clinical and ultrasonographical evaluations of the lung were not performed close to the time of death.

4.8.1.1 Group 1
In this group multifocal to coalescing comet tail artifacts were diagnosed at ultrasonography throughout the whole lung surface. These findings did mostly match with the clinical findings taking into consideration the clinical score (Table 3.1). Three foals (no. 2, 3, 6) were found clinically healthy and three of the foals were severely sick (no. 1, 4, 5).
From these three severe cases, two foals (no. 4, 5) had a comet tail artifact score of 3 corresponding to the severe clinical score, and foal no. 2 presented a comet tail score of 2 and was severely sick.

From the three foals with a clinical score of 0 or 1, in one foal (no. 3) comet tail artifact score of 1 and a healthy clinical score were matching. Foal no. 2 showed a clinical score of < 1, not being parallel with the comet tail artifact score of 2. In foal no. 6 there was an imbalance between clinical and ultrasononographical findings, showing a comet tail artifact score of 3 and a healthy clinical score (Figure 4.3).

![Fig 4.3 Correspondence between comet tail artifact (CTA) score (left side, dark pink) and clinical score (right side, red) of the six foals in group 1 (comet tail artifacts at ultrasonography). Red: severe diseased cases; healthy: foals no. 2, 3 and 6 with a clinical score of zero.](image)

Clinical findings of foals in this group are included in tables 9.1, 9.5 and 9.9. Ultrasononographical findings are included in table 9.12 and figures 4.1 and 4.2.

4.8.1.2 Group 2

Ultrasononographical images showed multifocal to coalescing nodular hypo- to hyperechoic areas combined in eleven foals with comet tail artifacts, where three foals (no. 12 – 14) were clinically healthy, two foals (no. 7, 20) were mildly sick, one foal (no. 8) had a moderate disease and eight foals (no. 9- 11, 15 - 19) presented a severe disease (Fig 4.4 and 4.5).
Ultrasonographical findings and the clinical score of the three clinically healthy foals did not correspond.

From the two mild sick foals, one (no. 7) corresponded well with the clinical score, showing at the ultrasonographical evaluation only nodular hypo- to hyperechoic areas with a diameter of 2 cm. The other mild case (no. 20) was a disassociation between the mild sickness and the comet tail artifact score.

In the moderate diseased foal (no. 8) the clinical score of 5 corresponded well with the ultrasonographical findings, showing a comet tail artifact score of 2 and a nodular diameter of 4.5 cm.

From the eight severe cases, there was a good matching between the clinical score and the ultrasonographical findings in six foals. Foals no 16 and 18, although they presented a comet tail artifact score of 2, they had nodular hypo- to hyperechoic areas of 8.5 and 18.8 cm in diameter, respectively. In the last two foals (no. 10, 17) clinical score and ultrasonographical findings were not matching.

![Fig. 4.4 Correspondence between comet tail artifact (CTA) score (left side, purple) and clinical score (right side, red) of the 14 foals in group 2; healthy (clinical score of zero): foals no. 12 - 14; yellow: mild; green: moderate; red: severe.](image-url)
Results

Fig. 4.5 Correspondence of nodular hypo- to hyperechoic area (NA) diameter (left, purple) and clinical score (right, red) of the 14 foals in group 2. Healthy (clinical score of zero): foals no. 12 - 14; yellow: mild; green: moderate; red: severe.

Clinical findings of foals in this group are presented in tables 9.2, 9.6 and 9.9. Ultrasonographical findings are included in tables 9.13 and figures 4.1 and 4.2.

4.8.2 Comparison of ultrasonographical and pathological findings

4.8.2.1 Group 1

Multifocal to coalescing comet tail artifacts throughout the whole lung observed at ultrasonographical evaluation were associated to diffuse consolidated, firm, rubber-like and non-collapsed lung typical for diffuse interstitial pneumonia that matched to the morphological and histological evaluation in all animals of this group (Fig 4.6 and 4.7).

Foal no. 3 showed a comet tail artifact score of 1, however macroscopically a diffuse firm, consolidated, non-collapsed lung and histologically an interstitial pneumonia with calcification and fibrosis was observed.

In five of six foals (no. 1 - 5), additional multiple nodular lesions characterized by suppurative to necrotizing, suppurative to fibrosing, granulomatous or suppurative pneumonia were observed at pathological examination. The extension of these foci ranged from 0.5 to 4 cm located mostly in the caudal lobe deep in lung parenchyma. In these foals no ultrasonographical finding indicating nodular lesions had been seen.

Ultrasonographical and necropsy findings of group 1 are included in table 9.12.
Fig 4.6 A: Comet tail artifact at lung ultrasonography. B: Diffuse lung consolidation of the pulmonary parenchyma interpreted as interstitial pneumonia of a foal (WEIMAR, 2006)

Fig 4.7 A: Comet tail artifact at lung ultrasonography. B: Diffuse lung consolidation of the pulmonary parenchyma interpreted as interstitial pneumonia of a foal (WEIMAR, 2006)
4.8.2.2 Group 2

In eleven out of 14 foals (no. 8 - 11, 13, 15 - 20) diffuse comet tail artifacts throughout the whole lung combined with nodular hypo- to hyperechoic areas were observed at lung ultrasonography. Comet tail artifacts were associated with diffuse interstitial pneumonia in these foals, macroscopically showing a diffuse firm, rubber-like, non-collapsed lung in eight foals, and histologically all 14 foals showed an interstitial pneumonia. Although at ultrasonographical evaluation all 14 foals showed nodular hypo- to hyperechoic areas, at necropsy nodular pulmonary changes were observed only in ten foals (no. 8, 9, 12 - 16, 18 - 20) (Fig. 4.8 and 4.9). The nodular lesions macroscopically and histologically corresponded to multifocal suppurative or fibrosing to necrotizing, granulomatous, pyogranulomatous, or focal interstitial pneumonia, and/or suppurative bronchopneumonia.

Ultrasonographical and necropsy findings of foals of group 2 are included in table 9.13.

Fig. 4.8 A: Ultrasonographical nodular hypo- to hyperechoic area with 17 mm in diameter of the pulmonary parenchyma of a foal. B: Gross appearance at necropsy of the same hypo- to hypoechoic area (abscess)
Results

Fig. 4.9 A: Ultrasonographical nodular hypo- to hyperechoic area with 17 mm in diameter of the pulmonary parenchyma of a foal. B: Gross appearance at necropsy of the same hypo- to hypoechoic area (abscess)

4.8.3 Comparison of microbiological with immunohistochemical detection of *R. equi*

An overview of the isolation and immunohistochemistry in the different types of pneumonias of the 23 foals with microbiological investigation is presented in table 4.8.

4.8.3.1 Group 1

TBA and lung tissue samples of two out of six foals (no. 2, 4) were investigated *intra vitam* and *post mortem*, respectively. *R. equi* was isolated in foal no. 2 from TBA samples and lung tissue and in foal no. 4 only from lung tissue similar to the immunohistochemical results for the evaluation for *R. equi*.

An overview of the correspondence of microbiological and immunohistochemical findings of this group is included in table 9.12.

4.8.3.2 Group 2

Lung tissue of ten out of 14 foals (no. 7, 9 - 11, 14 - 16, 18 - 20) was investigated *post mortem* and in five foals (no. 10, 14 - 16, 18) *R. equi* was isolated in lung lesions. Two foals of these five foals (no. 14, 16) were investigated *intra vitam* by TBA and in none of the samples *R. equi* was isolated. In two out of these five foals (no. 16, 18) a specific immunolabelling of *R. equi* was observed.
equi was observed. In foal no. 9 R. equi was isolated from TBA samples, and the immunohistochemical reaction was positive (table 9.13).

4.8.3.3  Group 3

Lung tissue of ten out of 17 foals (no. 21 - 24, 30 - 33, 36, 37) was investigated post mortem and in three foals (no. 21, 31, 32) R. equi was isolated in pulmonary lesions. Immunohistochemistry's positive reaction could be observed in one foal (no. 32) (table 9.14).

4.8.3.4  Group 4

TBA samples from both foals (no. 38, 39) taken for intra vitam, lung tissue of one foal (no. 39) taken post mortem were bacteriologically negative for R. equi similar to the immunohistochemical results (table 9.15).

*Table 4.8 Microbiological isolation and immunohistochemical positivity to R. equi in 23 foals with microbiological investigation in different types of pneumonia.*

<table>
<thead>
<tr>
<th>Type of pneumonia</th>
<th>Microbiology</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial</td>
<td>1/16</td>
<td>0/16</td>
</tr>
<tr>
<td>Pyogranulomatous</td>
<td>4/6</td>
<td>5/6</td>
</tr>
<tr>
<td>Granulomatous</td>
<td>1/2</td>
<td>½</td>
</tr>
<tr>
<td>Suppurative to necrotizing</td>
<td>4/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Suppurative bronchopneumonia</td>
<td>3/9</td>
<td>4/9</td>
</tr>
</tbody>
</table>

IHC: Immunohistochemistry

4.8.4  Correlation of immunohistochemistry and Grocott’s methenamine silver stain for *P. carinii*

The comparison of immunohistochemistry for *P. carinii* with GMS stain of positive foals is included in table 9.16.
4.8.4.1 **Group 1**

Four (no. 1, 2, 4, 5) out of six foals (no. 1 - 6) were positive using both methods.

4.8.4.2 **Group 2**

Four (no. 11, 14, 16, 17) out of 14 foals (no. 7 – 20) were positive using both methods. Only a positive immunohistochemical reaction was observed in foal no. 19.

4.8.4.3 **Group 3**

In three (no. 26, 28, 29) out of 17 foals (no. 21 - 37) a positive reaction was observed using both methods. In addition, in foal no. 32 *P. carinii* was identified immunohistochemically.

4.8.4.4 **Group 4**

GMS stain and IHC investigation was negative in both foals (no. 38, 39).
5 Discussion

The aim of the present study was to describe the different types of pneumonia and correlate retrospectively the pathomorphological changes with the clinical and ultrasonographical findings of the lung in foals, and further to attempt to selectively identify the contribution of *R. equi* and *P. carinii* to the different types of pneumonia. Until now, characterization of the morphological changes in specific types of pneumonias has been described only in case studies (SHIVELY et al., 1973; TANAKA et al., 1994; MURRAY et al., 1996; DEL PIERO et al., 1997; PERRON-LEPAGE et al., 1999; GIGUÈRE, 2001; PEEK et al., 2004), but no precise characterization of the different types of pneumonias in foals is available.

5.1 Clinical and ultrasonographical findings

There is consensus in the literature that clinical signs of different types of pneumonias in foals are nonspecific (AINSWORTH et al., 1993; GIGUÈRE, 2001). Presently the common thought is that no symptoms indicate the difference between a diffuse lung consolidation interpreted as interstitial pneumonia and a focal or multifocal lung consolidation. One goal of the present study was to determine whether clinical parameters would help to distinguish between diffuse and focal or multifocal lung consolidation in foals with respiratory signs. The observations made in the present study showed that the course of the disease might differ in various types of pneumonias. Foals with diffuse lung consolidation showed an acute onset of clinical symptoms. Without announcing symptoms, the foals developed clinical signs especially dyspnea within 6 to 12 hours. The clinical signs of foals with a diffuse lung consolidation interpreted as interstitial pneumonia were fever and dramatic dyspnea in 50% of the cases. The majority of the foals affected showed no further symptoms indicative of a respiratory disorder. This can be explained by the fact that in foals with diffuse consolidation of the pulmonary interstitium, the alveolar-capillary distance is increased. This results in a compromised diffusion of oxygen and CO$_2$ through the blood-air barrier. Additionally, histology confirmed that the interstitial pulmonary lesions were widespread in the lung of the foals. This leads to a generalised disturbed gas exchange in the lung and results in a dramatic hypoxemia (SHIVELY et al., 1973; AINSWORTH et al., 1993; TANAKA et al., 1993; PERRON LEPAGE et al., 1999; VAN RENSBURG, 2005). In these foals total and vital lung
capacities decrease rapidly due to a reduced gas exchange and change in the elastic properties of the lung (DERKSEN, 1993). The result is a dramatic dyspnea. This differed to the course of disease and symptoms observed in foals presenting focal or multifocal lung consolidations. In these foals a progressive onset of respiratory symptoms as nasal discharge, cough, crackles and wheezes at lung auscultation, rattle at tracheal auscultation and in some cases enlarged mandibular lymph nodes were observed. Furthermore 42% of these foals also presented dyspnea but this was characterized by a progressive onset. In cases of focal or multifocal lung consolidation the late and slow onset of respiratory symptoms can be explained by the remarkable ability of foals to compensate the progressive loss of functional lung and the slow spread of focal pulmonary lesions due to pathogens such as R. equi (GIGUÈRE, 2001). In most of the foals with R. equi pneumonia the disease progresses slowly and sub-clinically over several weeks without being clinically apparent. In cases with focal or multifocal lung consolidation, the non affected and healthy lung areas can compensate the deficit of the damaged pulmonary tissue at rest (PRESCOTT et al., 1991; PRESCOTT and GIGUÈRE, 2005). This could explain why foals presenting focal or multifocal lung consolidation show a progressive onset of the disease in contrast to foals with diffuse lung consolidation which present a more acute onset of respiratory signs. In the present study, five clinically healthy foals with typical early stage R. equi lung lesions were observed at post mortem examination, and R. equi was confirmed by immunolabelling or by microbiological culture of affected lung tissue. This supports the consensus, that in early course of the rhodococcosis in foals, clinical findings do not match with the pathological changes (ALTHAUS 2004). In contrast to this, in cases of diffuse lung consolidation, clinical findings seem to correlate better with the severity of the pulmonary lesions. The results of the present study show that the course of respiratory disorder can help differentiate between interstitial and focal/multifocal pneumopathy and that clinical signs are not 100% specific for different types of pneumonia. The evaluation of ultrasonographical findings showed that ultrasonographical evaluation of the thorax can help to differentiate a diffuse from a focal or multifocal lung consolidation. Typical ultrasonographical findings of foals with an interstitial pneumonia were numerous comet tail artifacts. Similar observations were made in horses with induced interstitial pneumopathy (VENNER, 2003). In most cases of nodular pneumonia of the foals examined in this study, thorax ultrasonography revealed
focal to multifocal consolidations as more or less clearly circumscribed areas of dense lung tissue. These ultrasonographical findings correspond to areas of the lung next to the pleura visceralis that are densified by granulomatous, pyogranulomatous, suppurative, necrotizing or fibrinous pneumonia as was shown previously (WEIMAR, 2006).

Although a final diagnosis is made by taking into account results of microbiological examination of airway samples and histological evaluation of lung biopsy or post mortem lung tissue samples, the present study shows that the information collected at clinical and ultrasonographical evaluation allows approaching the diagnosis very closely.

5.2 Pathomorphological, microbiological and immunohistochemical findings

Different types of pneumonias, such as suppurative and fibrinous bronchopneumonia, interstitial, embolic and granulomatous pneumonia, have been described in foals (MCGAVIN et al., 2007). Of these, suppurative bronchopneumonia, suppurative to necrotizing, granulomatous, pyogranulomatous and finally interstitial pneumonia were the most frequent types of pneumonia found in the present study.

5.2.1 Interstitial pneumonia

5.2.1.1 Pathomorphological findings

In the present study at necropsy 59% of the foals presented consolidated, heavy, rubber-like and non-collapsed lungs when thorax cavity was opened. The cut surface was dry. When these lesions where observed, it was interpreted as interstitial pneumonia. Lesions were diffusely distributed and involved all pulmonary lobes, or in some cases the lesions appeared to be more pronounced in the dorso-caudal aspects of the lungs. In some foals, rib impressions were observed on the lung’s pleural surface indicating poor deflation. Similar findings were made in other investigations of interstitial pneumonia in foals (SHIVELY et al. 1973; AINSWORTH et al. 1993; TANAKA et al., 1993; PERRON LEPAGE et al., 1999; VAN RENSBURG, 2005; KAPPE et al., 2009).

Interstitial lung disease includes a heterogeneous group of pulmonary disorders characterized by damage of the alveolar walls and loss of function of the alveolocapillary unit (PERRON-LEPAGE et al., 1999). Alveolar changes with variable thickening of alveolar wall and
interstitial infiltration of lymphocytes, macrophages and plasma cells in foals as reported in a case of interstitial pneumonia by PERRON-LEPAGE and coworkers (1999) was observed in 78% of the foals at histological evaluation in this study.

Interstitial pneumonia is also characterized by hyaline membranes attached to alveolar walls (SHIVELY et al., 1973; AINSWORTH et al., 1993; TANAKA et al., 1993; PERRON-LEPAGE et al., 1999; VAN RENSBURG, 2005; KAPPE et al., 2009) as observed in 45% of the foals with interstitial pneumonia in the present study. Another common finding in interstitial pneumonia is proliferation and desquamation of type II pneumocytes observed in 79% of the foals presenting interstitial pneumonia. Additionally, foamy macrophages were observed in alveolar spaces in one foal. Furthermore, in 51% of the foals with interstitial pneumonia, giant cells were observed in alveolar lumina. Finally in 21% of the foals with interstitial pneumonia finely honeycombed eosinophilic intra-alveolar fluid was observed in agreement with other interstitial pneumonia studies (SHIVELY et al., 1973; AINSWORTH et al., 1993; TANAKA et al., 1993; PERRON-LEPAGE et al., 1999; VAN RENSBURG, 2005). Interstitial fibrosis was also observed in 55% of the foals with interstitial pneumonia. Typically, collagenous fibers can be detected histologically after 5 – 7 days, and fibrosis may be well developed by 14 days (MCGAVIN et al., 2007). This demonstrates that some of the interstitial lesions were chronic, being more than a week old. However, the other 45% of foals showed interstitial changes assigned to acute lesions.

A further rare type of interstitial pneumonia was observed in one foal and was characterized by pulmonary calcification (MCGAVIN et al., 2007). Pulmonary nodular lesions composed of interstitial fibrosis associated with small firm white foci were observed throughout the whole lung. The most likely cause of pulmonary calcification in foals is hypercalcemia (COLAHAN et al., 1999; MCGAVIN and ZACHARY, 2006); however in this foal the cause could not be determined.

5.2.1.2 Staining for fungi detection

The cause of interstitial pneumopathy in foals is multiple. Fungi have been associated with interstitial pneumonia in horses and were evaluated in this study. Bacteria could not be isolated in lesions of interstitial pneumonia of our study. Viruses such as EHV-1, EHV-2,
EHV-4, EHV-5, EVA, EIV and adenovirus can cause interstitial pneumonia in foals (KAPPE et al., 2009), but were not investigated here.

In the present study 11 out of the 29 foals (38%) presenting interstitial pneumonia showed a positive reaction to the Grocott’s methenamine silver stain (GMS) of pulmonary lesions. Typically spherical to crescent-shaped small round cysts, some of which had thickened cyst walls and parenthesis-like bodies were observed and are a criterion for the diagnosis of *P. carinii* infection (SHIVELY et al., 1973; TANAKA et al., 1994). These cysts were located in the alveolar lumina and/or attached to the alveolar wall. *P. carinii* has been described as an important fungus causing interstitial pneumonia in immunocompromised foals and other mammals including humans (SHIVELY et al., 1973; AINSWORTH et al., 1993; TANAKA et al., 1993; PERRON LEPAGE et al., 1999; VAN RENSBURG, 2005). Since *P. carinii* does not grow on cell cultures, and *P. carinii* culture with fungal media formulation has not been successful, routine diagnosis of this fungus is based on direct demonstration of cysts in different samples types. Appropriate samples are BAL or TBA (FRANKLIN et al., 20002), lung biopsy or *post mortem* collected lung tissue (SHIVELY et al., 1973; PERRON LEPAGE et al., 1999). Smears of nasal swabs were not reported as suitable for *P. carinii* identification (SHIVELY et al., 1973). In foals *P. carinii* cysts are routinely identified by microscopic observation of stained organisms (SHIVELY et al., 1973; AINSWORTH et al., 1993; TANAKA et al., 1993), and occasionally by immunofluorescense (ELVIN and LINDER, 1993), immunohistochemistry (TANAKA et al., 1993; PERRON LEPAGE et al., 1999) or electron microscopy (TANAKA et al., 1993).

5.2.1.3 Immunohistological findings

In this study nearly 45% of foals (13/29) with interstitial pneumonia reacted positive to immunohistochemistry for *P. carinii*. A positive reaction of *P. carinii* showed a dark to light brown staining attached to the alveolar walls, in alveolar, and occasionally in bronchiolar lumina. Both cysts and trophozoites are stained but cannot be differentiated. Similar results were obtained in other studies of foals using the same method but different antibody (TANAKA et al., 1994) and different detection reagents (PERRON-LEPAGE et al., 1999). Similar findings were also described in humans (RADIO et al., 1990), and in pigs with the monoclonal Mab 0921 anti-*P. carinii* antibody (KONDO et al., 2000).
Although immunofluorescence is commonly used in BAL samples in humans, rats and mice with *P. carinii* pneumonia (GILL et al., 1987; ELVIN and LINDER, 1993; HASSAN et al., 1996; KONDO et al., 2000), immunohistochemistry is technically easier to perform as only a light microscope is needed. Still, immunohistochemistry has to be evaluated in BAL and TBA samples for a *pre mortem* diagnosis and detection of *P. carinii*.

### 5.2.2 Granulomatous and pyogranulomatous to necrotizing pneumonia

#### 5.2.2.1 Pathomorphological findings

Macroscopic evaluation showed that most nodules observed in the lung parenchyma were focal to multifocal with a diameter of 0.1 to 4 cm. Some nodules showed a caseous or suppurative material at the cut surface (MARIOTTI et al., 2000; ÖZSOY and HAZIROGLU, 2009). In eight foals the nodular lesions were located in the cranial lung area and in the other 25 foals in the caudal area. Previous reports on *R. equi* pneumonia in foals describe nodules located mostly in the cranio-ventral part of the lungs, and multifocal to coalescing in appearance (MCGAVIN et al., 2007). In the present study, from the 27 foals (73%) with nodular focal to multifocal lung consolidation, 55% of foals the nodules were located deep in the lung parenchyma, 11% presented superficial nodules and 33% presented both superficial and deep nodules at the lung parenchyma. In opposition to earlier observations, stating that lesions were more extensive in the right lung than the left (HILLIDGE, 1986), the lesions found in the present study were distributed equally on both sides of the lung.

At histological evaluation, eleven out of 37 foals (30%) showed pyogranulomatous or granulomatous pneumonia. Granulomatous pneumonia characterized by a center of necrotic tissue, surrounded by a rim of macrophages, epithelioid cells, giant cells and an outer delineated layer of necrotic tissue commonly infiltrated by lymphocytes and plasma cells was observed in 18% of these foals. These lesions were similar to those described in case reports of *R. equi* pneumonia in foals (TAKAI et al., 1994; MADARAME et al., 1996; MARIOTTI et al., 2000; GIGUÉRE, 2001; SZEREDI et al., 2001 and 2006; COLAHAN et al., 2007; ÖZSOY and HARIROGLU, 2009). Pyogranulomatous pneumonia observed in nine foals of our study was characterized by the same cellular exudate as the granulomatous pneumonia but additionally contained multifocal infiltrates of neutrophils and fibrin. In some granulomatous
and pyogranulomatous lesions bacteria laden macrophages, neutrophils and giant cells were observed. In some cases bacteria were also observed in necrotic areas. It is suggested that granulomatous lesions develop to pyogranulomatous pneumonia if the causative agent is not controlled by therapy (COLAHAN et al., 2007). Both types of pneumonia are associated to a cell rich infiltration of lung tissue and local reaction to it. This develops in over a period of several days and could explain why foals with nodular focal to multifocal lung consolidation such as pyogranulomatous pneumonia presented a more chronic onset of disease, than those presenting a diffuse lung consolidation.

5.2.2.2 Microbiological and mycological findings

Granulomatous or pyogranulomatous pneumonia is the lesion commonly observed in foals with R. equi infection (TAKAI et al., 1994; MADARAME et al., 1996; MARIOTTI et al., 2000; GIGUÉRE, 2001; SZEREDI et al., 2001 and 2006; ÖZSOY and HARIROGLU, 2009). Lysosomal enzymes are released by R. equi which induce cell destruction causing extensive caseous necrosis followed by pyogranulomatous pneumonia and intense recruitment of neutrophils, macrophages and formation of multinucleated giant cells (JONES et al., 1997). In the present study R. equi was isolated intra vitam from TBA samples or post mortem from lung tissue samples from 45% of the foals presenting granulomatous or pyogranulomatous lesions. These results might be influenced by the antibiotic treatment given to the foals ante mortem. In none of these foals R. equi was isolated alone. In four of the foals Sc equi ssp. zooepidemicus and in one E. coli was isolated as well. Commonly, Sc. equi ssp. zooepidemicus is associated with suppurative bronchopneumonia in foals (LAVOIE et al., 1994) and not granulomatous lesions. Furthermore Sc. equi ssp. zooepidemicus is a commensal inhabitant of foal’s airways (ANZAI et al., 2000). Because Sc. equi ssp. zooepidemicus cannot invade mucous membranes, it is most important as a secondary invader in foals with compromised respiratory tract resulting from viral or bacterial infection or stress from transport (COLAHAN et al., 1999; TIMONEY, 2004). This could explain why, in the present study, Sc equi ssp. zooepidemicus was never isolated alone.

One foal showed a focally severe granulomatous to necrotizing pneumonia with intralesional fungi. An Aspergillus spp. infection was suspected in this case because of the histological characteristics and the hyphae structures (HILTON et al., 2009) seen at Grocott’s
methenamine silver stain. As observed in this case, *Aspergillus* spp. infections are associated with multiple, septate, branching, 2 – 5 µm hyphae in pulmonary lesions (COLAHAN et al., 1999). In horses, the pathogenicity of *Aspergillus* spp. is very low and immune incompetence is assumed to be an important risk factor for invasive pulmonary aspergillosis which remains an uncommon disease. Related to this, pulmonary aspergillosis has been reported in an immunocompromised horse treated with corticosteroids (HILTON et al., 2009). However, in our case the foal had not received a corticosteroid therapy and no other cause for aspergillosis could be determined.

5.2.2.3 **Immunohistochemical findings**

In the present study, immunohistochemistry revealed a moderate to marked, focal to multifocal, cytoplasmic staining of *R. equi*-antigen. Pale to dark brown granular deposits in macrophages, in multinucleated giant cells and in some neutrophils localized in granulomatous or pyogranulomatous lesions. This positive reaction was observed in six out of nine foals with pyogranulomatous lesions and in one out of two foals with granulomatous pneumonia. These are the first results available on immunohistochemistry for *R. equi* in a population of foals with pneumonia.

Because immunohistochemistry detects intra- and extracellular antigen, this method is recommended for its high sensitivity and specificity (MADARAME et al., 1996). Compared to this method, histological stains (Gram and Ziel-Neelsen) are much less appropriate for identification of *R. equi* in tissue samples (MADARAME et al., 1996).

In the present study a mouse monoclonal antibody against 15-17 kd antigen of virulent *R. equi* was used for the detection of *R. equi* in paraffin-embedded sections of post mortem lung tissue samples of foals performed as previously described (ISHINO et al., 1992; MADARAME et al., 1996; MARIOTTI et al., 2000). ISHINO (1992), MADARAME (1996), MARIOTTI (2000) and SZEREDI with coworkers (2006) used the same antibody (Mab10G5) as in the present study and results obtained had the same staining intensity and the same distribution pattern in the lung lesions. Although SZEREDI (2001 and 2006), and ÖZSOY and HAZIROGLU (2009) used another antibody (specific anti-*R. equi* IgG), results showed the same intralesional intracytoplasmic staining of macrophages, some neutrophils and multinucleated giant cells and few extracellular in cellular debris as in the present study.
5.2.3  **Suppurative bronchopneumonia, suppurative to necrotizing pneumonia and other types of pneumonias**

5.2.3.1  **Pathomorphological findings**

In the present study macroscopic evaluation of the lungs showed multifocal nodular pulmonary changes characterized by firm whitish to dark grey nodules and white to yellowish or red firm foci up to 2 cm in diameter or nodular necroses located mostly bilaterally in the cranial lobes of 19 foals (51%) with suppurative or fibrosing to necrotizing pneumonia or suppurative bronchopneumonia. Previous reports of suppurative pneumonia and bronchopneumonia in foals describe the damage area of the lung located mostly in the cranio-ventral part of the lungs, and multifocal to coalescing in appearance (MCGAVIN et al., 2007; COLAHAN et al., 1999). In the present study, from these 19 foals (51%) with suppurative or fibrosing pneumonia or suppurative bronchopneumonia, in 53% of the foals the nodules were located deep in the lung parenchyma, 16% presented superficial nodules and 31% presented both superficial and deep nodules at the lung parenchyma.

In the present study, at the histological evaluation suppurative bronchopneumonia characterized by infiltration of high number of neutrophils and macrophages in bronchial, bronchiolar and alveolar lumina was observed in 43% (16/37) of the foals. These lesions were the same as previously described by COLAHAN et al. (1999) and LAVOIE et al. (1997) in thoroughbred foals with aspiration or Sc. equi ssp. zooepidemicus pneumonia. Furthermore, suppurative to necrotizing pneumonia characterized by lesions restricted to alveolar tissue infiltrated by numerous partly degenerated neutrophils and macrophages were observed in 27% (10/37) of the foals in the present study.

5.2.3.2  **Microbiological findings**

Microbiological investigation of *post mortem* lung tissue of foals in the present study was performed in 10 out of 16 foals with suppurative bronchopneumonia. *R. equi* was isolated in four foals and *Sc. equi* ssp. *zooepidemicus* in three foals. These bacteria are commonly isolated from lung abscesses of foals with pneumonia (LAVOIE et al., 1994). Furthermore *E. coli* and *K. pneumonia* were commonly isolated in combination with *R. equi* and *Sc. equi* ssp. *zooepidemicus*. 
In seven out of 10 foals with suppurative to necrotizing pneumonia *R. equi* was isolated in three foals in combination with *E. coli, B. bronchiseptica, K. pneumonia* and/or non-specific bacteria. *Sc. equi* ssp. *zooepidemicus* was isolated in one foal in combination with *R. equi, K. pneumonia* and non-specific bacteria. Furthermore, as *Sc. equi* ssp. *zooepidemicus* and *R. equi* were both isolated in lung tissue from 2 foals with suppurative bronchopneumonia and in one foal with suppurative to necrotizing pneumonia in the present study, both bacteria might act as a combined infection and induce pulmonary lesions (ROONEY and ROBERTSON, 1999). Our results show that in pulmonary lesions of foals with suppurative to necrotizing pneumonia *R. equi* was never isolated alone. Though no other authors have strictly evaluated bacterial growth from suppurative to necrotizing pneumonia before, bacterial isolation from airways secretions of clinical cases of pneumonia often reveal *R. equi* in combination with other bacteria. (LECLERE et al., 2009). It has also to be considered that the foals were treated with antibiotics before death, so culture results may be altered by this.

5.2.3.3 **Immunohistochemical findings**

This is the first study on immunohistochemistry for *R. equi* in suppurative bronchopneumonia and suppurative to necrotizing pneumonia in foals. From 26 cases with suppurative bronchopneumonia or suppurative to necrotizing pneumonia, *R. equi* was positive in seven animals (27%) by means of immunohistochemistry in post mortem lung tissue. Immunohistochemically, 15-17 kd antigens were detected as well-developed intra cellular granular clumps. These antigens were observed in the suppurative lesions in alveoli and to a lesser extent in the purulent debris of the bronchi. The immunolabelling was observed as dot- or rod-shaped areas in the cytoplasm of swollen macrophages, multinucleated giant cells, and neutrophils and very rarely extracellular.

These findings were similar to those in granulomatous or pyogranulomatous lesions, where the antigen is mostly distributed intracellularly (RETTEG et al., 2009).

5.3 **Comparison of microbiological with immunohistochemical detection of *R. equi* in foals with different types of pneumonia**

The present study is the first report on the comparison between the results of *R. equi* identification by microbiology and immunohistochemistry from lung tissue in foals with
Discussion

pneumonia. Although TAKAI (1993), PRESCOTT (1991), GIGUÈRE with coworkers (2001) have reported microbiological and immunohistochemical investigation separately, the results were not directly compared. In 23 foals with different types of pneumonia, *R. equi* was isolated from lung tissue samples in 10 foals and identified by immunohistochemistry in 8 foals. In only 6 foals *R. equi* was identified by both methods. In cases of negative *R. equi* culture and positive immunolabelling, antimicrobial treatment might have killed the bacteria that consequently were no longer able to grow but remained reactive to the antibody. This is one advantage of immunohistochemistry that can also be used to detect dead or altered bacteria that can no longer be cultured (ISHINO et al., 1992; MADARAME et al., 1996; MARIOTTI et al., 2000). In four foals *R. equi* was isolated at culture but resulted negative at immunohistochemistry. This discrepancy may be due to foals carrying the avirulent strain of *R. equi* which lacks the virulence-associated 15-17 kDa antigen (VapA), resulting in a viable *R. equi* culture which does not react to the antibody (TAKAI et al., 1993).

5.4 Correlation of immunohistochemistry and Grocott’s methenamine silver stain for *P. carinii*

In the present study *P. carinii* was detected by immunohistochemistry in 13 foals (35%) and by GMS stain in eleven foals (30%). All foals positive with GMS stain were also positive by immunohistochemistry. The higher identification of *P. carinii* by immunohistochemistry might be explained by the ability of the monoclonal antibody to reveal both *P. carinii* cysts and trophozoites. However cysts have been estimated to represent less than 1% of all *P. carinii* parasites in lung tissue of humans (CHATTERON et al., 1990). Immunohistochemistry for *P. carinii* is not yet commonly used for the diagnosis of *P. carinii* in foals (TANAKA et al., 1993; PERRON LEPAGE et al., 1999), though it can be highly recommended for an early diagnosis. The main obstacle for the in vivo diagnosis of *P. carinii* pneumonia is the invasiveness of sample collection (lung tissue biopsy, TBA or BAL) in patients already distressed by the dyspnea (TANAKA et al., 1993; PERRON LEPAGE et al., 1999). Since the in vivo identification of *P. carinii* is difficult and the course of the disease is very rapid, the diagnosis of *P. carinii* pneumonia is commonly not confirmed until necropsy (AINSWORTH et al., 1993; PERRON LEPAGE et al., 1999).
Comparison of ultrasonographical and pathological findings

All foals examined in the present study and presenting comet tail artifacts had an interstitial pneumonia at the pathomorphological evaluation. As normal lung contains much air and little water, the comet tail artifacts described here have the following characteristics: they are related to cell- or water-rich structures, below the resolution of the ultrasonographical beam, surrounded by air (REEF, 2004a). Comet tail artifacts are absent in a normal lung and present in alveolar damage, and can be found throughout the whole lung.

Our observations show that hypo- to hyperechoic areas seen at lung ultrasonography correlate with granulomatous, pyogranulomatous or suppurative or fibrosing to necrotizing pneumonia and suppurative bronchopneumonia. In nodular lesions, the sensitivity of ultrasonography is 80 - 90% because of the inability of lung ultrasonography to detect lesions located deep in normal aerated lung (RAMIREZ et al., 2004; WEIMAR, 2006). This was also observed in the present study, where five foals presented only comet tail artifacts at ultrasonography, and at the pathomorphological evaluation the lung revealed additional deep nodular lesions. However, as nodular lesions often also affect the lung periphery, these changes will be identified ultrasonographically (RAMIREZ et al., 2004).

When interstitial pneumonia progresses to a chronic course, interstitial fibrosis develops, and because of type II pneumocyte proliferation, it can be observed that fibrotic alveolar septa are lined with cuboidal epithelial cells. These alterations can give the appearance of an interstitial fibrotic nodule (MCGAVIN et al., 2007). Small (1 – 2 cm or less) hypo- to hyperechoic areas scattered throughout the lung field are consistent with soft tissue masses or granulomas with a widespread multifocal distribution. This ultrasonographical finding can be observed in horses or foals with pulmonary fibrosis, fibrosing interstitial pneumonia or granulomatous pneumonia (REEF et al., 2004b). This was noticed in the present study in three foals in which nodular hypo- to hyperechoic areas were observed at ultrasonographical evaluation, and at necropsy no nodular changes were found in the pulmonary parenchyma, but at histological evaluation, some focal interstitial pneumonia characterized by septal thickening with cell infiltration predominantly of macrophages and lymphocytes and proliferation of type II pneumocytes surrounded by fibrotic tissue was observed.

Three other foals did not show comet tail artifacts at ultrasonographical evaluation, though an interstitial pneumonia was observed histologically. In these cases the inflammatory...
infiltration might have been mild and consequently did not disturb the beam and failed to create a difference in acoustic impedance.

Generally it can be concluded that comet tail artifacts are indicative of interstitial changes and that focal or multifocal nodular hypo- to hyperechoic areas are associated with suppurative bronchopneumonia, granulomatous, pyogranulomatous or suppurative or fibrosing to necrotizing or focal interstitial pulmonary lesions. Finally, as seen in the present study, ultrasonographical findings often help to establish an early diagnosis of diffuse or focal to multifocal lung consolidation, although they do not always match with the pathomorphological lesions. In rare cases, ultrasonographical findings typical of one type of lesions are found in a different type of pneumonia, so care should be taken and diagnosis should be made by taking into consideration clinical, microbiological and if present cytological/histological findings.
Conclusion

Interstitial pneumonia, characterized by interstitial infiltration of lymphocytes, macrophages and plasma cells, multifocal alveolar hyaline membrane formation, and proliferation and desquamation of type II pneumocytes, was the pneumonia observed in most of the foals in the present study. *P. carinii* infection was observed in 45% of foals with interstitial pneumonia. As most of these foals presented additionally a bacterial infection and viruses were not evaluated here, it is not possible to determine if *P. carinii* is the causative agent of pneumonia in these foals, or if *P. carinii* forms a secondary, opportunistic infection.

The second most observed pneumonia in the present study was a suppurative bronchopneumonia, characterized by accumulation of partly degenerated neutrophils in bronchial, bronchiolar and alveolar lumina. This pneumonia was associated in most of the cases with *R. equi* and *Sc. equi* ssp. *zooepidemicus*. Additionally suppurative to necrotizing and granulomatous or pyogranulomatous pneumonia were also observed in foals with an *R. equi* infection.

The study demonstrated ultrasonography to be an appropriate diagnostic tool for the diagnosis of interstitial pneumonia in foals. The results further suggest that in cases with nodular focal or multifocal lung consolidation without findings at ultrasonography, radiography should be considered to enable the detection of deep nodular pulmonary lesions.

The etiology of the different types of pneumonia described here seems to be multifactorial. The present study showed that 50% of foals with *R. equi* pneumonia were additionally infected with *P. carinii*. In these foals a granulomatous, pyogranulomatous or suppurative to necrotizing pneumonia or suppurative bronchopneumonia combined with interstitial pneumonia was observed. Histopathological examination of the present study did not allow distinguishing whether *R. equi* lesions or *P. carinii* alveolar damage occurred first. As there is a lack of evidence that *P. carinii* is a pathogen in foals, we suggest that *P. carinii* acts as an opportunistic microorganism, taking advantage of an immunosuppression resulting from a viral or a bacterial infection.

Given the current data, the primary cause of the interstitial and granulomatous, pyogranulomatous or suppurative to necrotizing pneumonias or suppurative bronchopneumonia can not be determined.
6 Summary

Retteg Pauls, Stephanie: Retrospective clinical and pathomorphological evaluation of pneumonias in foals with selected immunohistochemical investigations

In the present study pathomorphological characterization of pneumonias was performed in 37 foals 1 to 218 days of age from a German horse breeding farm. Post mortem examination was performed at the Department of Pathology from the University of Veterinary Medicine Hannover. In addition to the pathomorphological evaluation and microbiological investigations, immunohistochemistry for the detection of Pneumocystis (P.) carinii and Rhodococcus (R.) equi was performed. Furthermore clinical symptoms in foals with diffuse and focal to multifocal lung consolidation were described. Finally, ultrasonographical findings of the lung of the foals were compared with pathomorphological findings. Archive material from 37 foals with pneumonia was evaluated and the type of pneumonia was characterized. Lung tissue sections were evaluated histologically and were immunostained. The evaluation of the histological and immunostained tissue sections was performed independently by the author and an experienced pathologist (Diplomate of the European College of Veterinary Pathologists) and findings were discussed. Furthermore, the pathomorphological findings of 20 of these foals were additionally compared with the ultrasonographical and clinical findings. From the other 17 foals clinical and ultrasonographical data were not available. Paraffin embedded lung sections were stained with Periodic acid-Schiff’s (PAS) stain, Gram stain, Elastica van Gieson’s (EvG) stain and Grocott’s methenamine silver (GMS) stain. Results of GMS stain were compared with the immunolabelling for P. carinii. Furthermore an immunohistochemical method using a monoclonal antibody against a 15-17 kDa antigen of virulent R. equi and a fluorescein (FITC)-labelled monoclonal antibody for P. carinii was used on formalin-fixed and paraffin-embedded lung tissue sections. The findings were later compared to the clinical and ultrasonographical findings of each of the 20 foals for which clinical and ultrasonographical findings were available.
Interstitial, suppurative, granulomatous, pyogranulomatous pneumonia and suppurative bronchopneumonia were the most common pneumonias affecting the foals in this study. Most of the foals presented more than one type of pneumonia.

From the 37 foals, 29 foals presented an interstitial pneumonia. In 24 out of these 29 foals, interstitial lesions were combined with other types of pneumonias. From these 24 foals with different types of pneumonia, *P. carinii* was detected in interstitial lesions and *R. equi*, *Sc. equi* ssp. *zooepidemicus* and some gram negative bacteria were isolated from suppurative, granulomatous or pyogranulomatous lesions. Eleven foals presented a granulomatous or pyogranulomatous pneumonia. 26 foals presented a suppurative bronchopneumonia.

*P. carinii* was identified as dark to light brown staining of cysts and trophozoites attached to the alveolar walls, in alveolar, and occasionally in bronchiolar lumina by immunohistochemistry in 13 foals and as typical spherical to crescent-shaped small round cysts, some of which had thickened cyst walls and parenthesis-like bodies by GMS in eleven foals.

*R. equi* was isolated *intra vitam* from two out of ten TBA samples and *post mortem* from ten out of 23 lung tissue samples. At immunohistochemistry for *R. equi*, twelve out of 37 foals (32%) showed pale to dark brown granular deposits in macrophages, multinucleated giant cells and some neutrophils localized in granulomatous, pyogranulomatous and suppurative to necrotizing lesions.

By means of immunohistochemistry for *P. carinii*, 13 of the 29 (45%) foals with interstitial pneumonia were associated to a *P. carinii* infection. From these 13 foals with interstitial pneumonia, eight cases had a combined infection with *R. equi* by immunolabelling and five by microbiological isolation.

From the eleven foals presenting granulomatous and/or pyogranulomatous pneumonia, in five foals *R. equi* was isolated and in seven foals a positive immunolabelling was observed.

From the 26 foals presenting suppurative pneumonia and/or suppurative bronchopneumonia, *R. equi* was isolated and detected by immunohistochemistry in seven foals. In six foals the granulomatous, pyogranulomatous or suppurative to necrotizing pneumonia and/or suppurative bronchopneumonia was observed with no other kind of pneumonia and in 20 cases, these types of pneumonia were combined with a diffuse interstitial pneumonia. *Sc. equi* ssp. *zooepidemicus* was also commonly isolated from tracheobronchial aspirate (TBA) (in 4
out of 10 samples taken) and lung tissue samples (in 9 out of 23 samples taken), and was also associated in 11 foals with suppurative to necrotizing pneumonia or suppurative bronchopneumonia. In six foals *Sc. equi* ssp. *zooepidemicus* and *R. equi* were identified in lung tissue samples. In the foals of the present study it could not be discarded a viral infection, because virus were not investigated.

An additional goal in this study was to determine whether clinical findings are useful to differentiate a diffuse lung consolidation interpreted as an interstitial pneumonia from a focal or multifocal lung consolidation interpreted as a granulomatous, pyogranulomatous, suppurative to necrotizing pneumonia or suppurative bronchopneumonia. Foals with interstitial pneumonia showed an acute onset of respiratory disorders and were often febrile with few clinical findings except for a frequent severe dyspnea. Most of the foals with nodular pneumonia presented nasal discharge, crackles and wheezes at lung auscultation, rattle at trachea auscultation, enlarged lymph nodes, cough and 42% also presented an acute dyspnoea. Comparison of ultrasonographical and histological findings showed that all foals presenting comet tails artifacts presented an interstitial pneumonia. All foals presenting nodular hypo- to hyperechoic areas at ultrasonography presented granulomatous, pyogranulomatous or suppurative to necrotizing pneumonia and/or suppurative bronchopneumonia at the pathomorphological evaluation and in most of them, *R. equi* was observed. Though ultrasonographical findings did not always match the pathomorphological findings, they can facilitate an early diagnosis of diffuse or of focal to multifocal lung consolidation in foals with respiratory disorders.
Zusammenfassung

Stephanie Retteg Pauls: Retrospektive Klinische und Pathomorphologische Charakterisierung von Fohlen-Pneumonien und ausgewählte immunohistologische Untersuchungen.


Zusammenfassung

Klinischen und sonographischen Daten von 20 Fohlen (ultrasonographische und klinische Daten waren nur von 20 Tiere verfügbar) verglichen.


Von den elf Fohlen, die eine granulomatöse und/oder pyogranulomatöse Pneumonie präsentierten, wurde R. equi in fünf Fohlen isoliert und eine positive Immungoldmarkierung in sieben Fohlen beobachtet.

Von den 26 Fohlen mit eitriger Lungenentzündung und / oder eitriger Bronchopneumonie wurde R. equi isoliert und immunhistochemisch erkannt in sieben Fohlen. Die in sechs Fohlen festgestellten granulomatösen, pyogranulomatösen oder eitrig bis nekrotisierenden Pneumonien und / oder eitrigen Bronchopneumonien wurden nicht in Kombination mit


Die Auswertung der sonographischen Untersuchung zeigte, dass Fohlen, bei denen Kometenschweife-Artefakte beobachtet wurden, eine interstitielle Pneumonie zeigen, in denen meistens *P. carinii* identifiziert wurde. Fohlen mit nodulären, hypo- bis hyperechoreichen Lungen-Areale bei der Ultraschalluntersuchung der Lunge wiesen pathomorphologisch eine eitrige, granulomatöse und/oder pyogranulomatöse bis nekrotisierende Lungenentzündung oder eine eitrige Bronchopneumonie auf, in denen meistens *R. equi* nachgewiesen werden konnte. Obwohl die Ultraschall-Ergebnisse nicht immer dem pathomorphologischen Befund entsprechen, stellt diese Untersuchung eine sehr gute Methode zur frühzeitigen Diagnose interstitieller Pneumonien und nodulärer Veränderungen der Atemwege des Fohlens dar.
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und mikrobiologische Befunde.
Stiftung Tierärztliche Hochschule Hannover, Diss.

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429

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CASWELL, C. JACKSON, N. E. ROBINSON, F. DERKSEN, M. A. SCOTT, D. B. UHAL,
Equine multinodular pulmonary fibrosis: A newly recognized Herpesvirus-associated fibrotic
lung disease.
Vet. Pathol. 44, 849-862
Foal pneumonia: an overview.  

Foal pneumonia  
In: ROBINSON N. E., Current therapy in Equine Medicine, Volume 5. Saunders, USA. 666 – 673

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Infect. Immun. **13**, 1293-1295

ZINK, M. C., J. A. YAGER and N. L. SMART (1986):  
*Corynebacterium equi* infection in horses, 1985-84: a review of 131 cases.  

Electron microscopic investigation of intracellular events after ingestion of *Rhodococcus equi* by foal alveolar macrophages.  
8 Annex

8.1 Histological stainings

8.1.1 Hematoxylin eosin stain

Hemalaun according to P. Meyer:
1 g hematoxylin (Roth, Karlsruhe, Germany) diluted in 1000 ml distilled water,
0.2 g sodiumjodate (Merck, Darmstadt, Germany),
50 g pure potassium aluminium sulfate (Roth, Karlsruhe, Germany) added and heating,
After cooling add 50 g chlora hydrate (Roth, Karlsruhe, Germany) and 1g crystalline citric acid (Roth, Karlsruhe, Germany) dissolve, cool and filtrate.

Eosin:
Dilute 10 g of eosin yellow (Merck, Darmstadt, Germany) in 1000 ml distilled water to get a 1% eosin solution.
Heat until all is dissolved.
After cooling filtrate.
For staining, in 100 ml 1% Eosin (Roth, Karlsruhe, Germany), add 2 drops of pure acetic acid (Roth, Karlsruhe, Germany).

8.1.2 PAS-reaction

1% periodic acid solution:
Dilute 10 g of periodic acid (Roth, Karlsruhe, Germany) in 1000 ml distilled water.

8.1.3 Elastica-van Gieson’s stain

Weigert’s Iron Lake:

Solution A:
- To 100 ml alcohol, add 1 g hematoxylin (Roth, Karlsruhe, Germany)

Solution B:
- To 4 ml 29% Iron lake solution (Merck, Darmstadt, Germany), add 1 ml 25% hydrochloric acid (HCl) (Roth, Karlsruhe, Germany) and 95 ml distilled water.
8.1.4 Grocott’s methenamine silver stain

Stock Methenamine Silver solution:
- To 100 ml 3% Methenamine solution (Roth, Karlsruhe, Germany), add 5 ml 5% silver nitrate solution (Roth, Karlsruhe, Germany).

Working Methenamine Iron lake solution:
For 60 ml solution:
To 33 ml stock Methenamine silver nitrate solution (Roth, Karlsruhe, Germany) add 33 ml distilled water and 2.7 ml 5% aqueous Borax (Roth, Karlsruhe, Germany).

Methanile yellow:
- To 0.25 g Methanile yellow (Merck, Darmstadt, Germany), add 100 ml distilled water and 0.25 g glacial acetic acid (Roth, Karlsruhe, Germany).

8.2 Immunohistochemistry

8.2.1 Reagents for immunohistochemistry

ABC-reagent:
Make a solution of 1 ml PBS (pH 7.1), 15 ml Reagent A, agitate the solution and add 15 ml Reagent B and agitate the solution. Prepare the solution 30 minutes before using.

Citrate buffer:
Dissolve 2.1 g citric acid monohydrate (Roth, Karlsruhe, Germany) in 1 liter distilled water. With caustic soda solution (Merck, Darmstadt, Germany) adjust to a 6.0 pH.

DAB-reagent:
Dilute 0.1 g DAB in 200 ml PBS and 2 ml 30% H₂O₂ solution.

Methanol in 30% H₂O₂ solution:
Dilute 3 ml Perhydrol (Merck, Darmstadt, Germany) in 197 ml 85% Methanol (Roth, Karlsruhe, Germany).
Phosphate buffered sodium chloride solution (PBS)
Dissolve 40 g sodium chloride (Merck, Darmstadt, Germany) and 8.97 g sodium monobasic phosphate (NaH₂PO₄) (Merck, Darmstadt, Germany) in 5 litre distilled water; with caustic soda solution (Merck, Darmstadt, Germany) adjust to a 7.1 pH.

8.2.1.1 Antigen Retrieval
Microwave:
Citric acid monohydrate solution:
Dilute 2.1 g citric acid monohydrate (Roth, Karlsruhe, Germany) in 1 liter distilled water.
Adjust pH to 6.0 – 6.5 with caustic soda solution (Merck, Darmstadt, Germany).
Cook 25 minutes in microwave (800 W) (Bauknecht, Stuttgart, Germany)
Wait for 10 minutes to get cold, or rinse with cold water slowly.

8.3 Staining and immunohistochemical resources
Abcam, Hamburg
Murine fluoresceine (FITC)-labelled monoclonal antibody against *P. carinii*, ab24263

Biologo, Kronshagen
Ascites fluid of non immunised Balb c/J mice CL 8100

BIO CYC Gesellschaft für Biotechnologie und Recyclingverfahren mbH & Co. Luckenwalde (Vertrieb quartett Immundiagnostika und Biotechnologie GmbH, Berlin)
Pro Taqs Citrate-buffered concentrate, 400300692

Carl Roth GmbH + Co. KG, Kalsruhe
Chloral hydrate, K318.2
Citric acid monohydrate, 3958.1
Cover plates agent, Roti®-Histokit, 6638.1
Cresyl violet, 1A396
Crystal Violet solution, 1.01408.
EBE® “acetic acid-n-butylester”, 4600.4
Eosin, 7089.2
Hydrogen Chloride, K025.1
Hydrogen Phosphate Monohydrate, K300.2
Isopropanol, 9866.4
Karbol fuchsin, A130.2
Methanol, 4627.6
Magnesium potassium iodide, N052.2
Potassium Aluminium sulfate, P724.1
Roticlear, A538.3
Rotihistol®, 5429.3
Silver nitrate, 9370.3
Soft fuchsin solution,

**Fluka, Buchs**
3, 3´-diaminobenzidine-tetrahydrochloride (DAB), 32750

**Leica Instruments GmbH, Nussloch**
Rotation Microtom, 2030
Automatic staining machine, Leica ST 4040

**Medite, Burgdorf**
Automatic mounting machine, promountes® RCM 2000

**Menzel-GLäser, Braunschweig**
Superfrost Plus® Glass slides, 041300

**Merck, Darmstadt**
Caustic soda, 9137
Eosin yellow, 1345
Hematoxylin, 4305
Hydrogen chloride, 1N, 9010
Iron chloride solution, 1.05512.0250
Methenamine silver solution, 4339
Nuclear fast red solution, 5189
Sodium chloride, P029.2
Sodium monobasic phosphate, 1.06580.1000
Sodium thiosulfate, St320/8102
Sodium jodate, 1.06525.100

Riedel de Haën, Seelze
Aqueous sodium sulfite, 31454
Borax, 31457
Chromic acid, 12235
Methanol yellow, 32607
Ortho-Periodic acid, 03304

Serva Feinbiochemica GmbH und CoKg., Niedelberg
Bovine serum albumin (BSA), 11930
Citric acid, 38642

Vector Laboratories, USA
Biotin-labelled goat-anti mouse IgG antiserum, GAM-b IgG (H+L), BA-9200
Biotin-labelled goat-anti fluorescein antiserum, GAM-b FITC-b, BA-0601
Vectastain Elite ABC Kit, PK 6100

Waldeck, Münster
Resorcin fuchsin solution, 2E030
Van Gieson’s solution, 2E050


8.4 Equipment resources

**Bauknecht, Stuttgart**
Microwave Excellence MWS 2924

**Gehard Menzel Glasbearbeitungswerk GmbH & Co. KG, Braunschweig**
Super Frost® Plus Slides, 041300

**Kendro Laboratory Products GmbH, Langenselbold**
Heraeus warming cupboard UTG

**Knittel W. Glasbearbeitungs GmbH, Braunschweig**
Coverslips (24 x 50mm)

**Leica Microsystems Nussloch GmbH, Nussloch**
Rotation microtome, 2030

**Medite Medizintechnik, Burgdorf**
Automatic mounting machine, promounter RCM 2000
Warming cupboard UTG

**Memmert GmbH + Co. KG, Schwabach**
Waterbath, WB 22
Waterbath, WB 45

**Mettler-Toledo GmbH, Giesen**
Weighing machine, LabStyle 204
Shandon, Frankfurt
Automatic staining machine, ST4040
Coverplates™, 72110013
Sequenza® Slideracks, 7331017
Shandon Racks

Zeiss, Oberkochen
Standard binocular light microscope
### Results tables

**Table 9.** Gender, age, age at diagnosis of pneumonia, duration of illness, duration of therapy, duration of dyspnea, days from the last clinical examination to death or euthanasia and mode of death of group 1 (no. 1 – 6)

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender</th>
<th>Age (Days)</th>
<th>Age at Diagnosis of Pneumonia</th>
<th>Duration of Therapy*</th>
<th>Duration of Illness*</th>
<th>Days from Last Exam. to Death*</th>
<th>Duration of Dyspnea*</th>
<th>Mode of Death</th>
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<td>120</td>
<td>15</td>
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</tbody>
</table>

*all data given in days

no = data no required, because foals were healthy; ♂ = colt; ♀ = filly; D = spontaneous death; E = euthanasia.

Table 9.1. Gender, age at diagnosis of pneumonia, duration of therapy, duration of illness, duration of dyspnea, days from the last clinical examination to death or euthanasia and mode of death of group 1 (no. 1 – 6)
Table 9. Gender, age, age at diagnosis of pneumonia, duration of illness, duration of therapy, duration of dyspnea, days from the last clinical examination to death or euthanasia and mode of death of group 2 (no. 7 - 20).

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<th>Duration of illness (Days)</th>
<th>Duration of dyspnea (Days)</th>
<th>Days from last exam. to death or euthanasia*</th>
<th>Mode of death*</th>
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</table>

*all data given in days
no data no required, because foals were healthy; ♀ = filly; ♂ = colt; D = spontaneous death; E = euthanasia.

Note: Table 9.2 Gender, age at diagnosis of pneumonitis, duration of illness, duration of therapy, duration of dyspnea, days from the last clinical examination to death or euthanasia and mode of death of group 2 (no. 7 - 20).
<table>
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<th>No.</th>
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<th>AGE</th>
<th>DAYS FROM LAST CLINICAL EXAMINATION</th>
<th>AGE AT DIAGNOSIS OF PNEUMONIA</th>
<th>DURATION OF ILLNESS</th>
<th>DURATION OF THERAPY</th>
<th>DURATION OF DYSPNEA</th>
<th>DAYS FROM LAST CLINICAL EXAMINATION TO DEATH OR EUTHANASIA</th>
<th>MODE OF DEATH</th>
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</table>

All data given in days.

No data no required, because foals were healthy; ♂ = colt; ♀ = filly; D = spontaneous death; E = euthanasia.

Table 9.3 Gender, age at diagnosis of pneumonia, duration of illness, duration of therapy, duration of dyspnea, days from last clinical examination to death or euthanasia and mode of death of group 3 (no. 21 - 37)
Table 9. Gender, age, age at diagnosis of pneumonia, duration of illness, duration of therapy, duration of dyspnea, days from the last clinical examination to death or euthanasia, and mode of death of group 4 (no. 38 - 39).

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Gender</th>
<th>Age</th>
<th>Days from last exam. to death</th>
<th>Dyspnea</th>
<th>Therapy</th>
<th>Illness</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Mode of death</th>
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</table>

* all data given in days

no = data no required, because foals were healthy; ♂ = colt; ♀ = filly; D = spontaneous death; E = euthanasia.
Table 9. Findings of the last clinical evaluation of foals in group 1 (no. 1–6). Findings were counted together to get a clinical score representing the interpretation of the severity of illness.

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<th>Foal no.</th>
<th>ND</th>
<th>Lu</th>
<th>Tra</th>
<th>Ln</th>
<th>Cough</th>
<th>Dyspnea</th>
<th>Clinical score</th>
<th>Result clinical</th>
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</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>Healthy</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Foal no. = number of foal; ND = nasal discharge; Lu = lung; Tra = trachea; Ln = lymph nodes. (Clinical score table 3.1)
Table 9. Findings of the last clinical evaluation of foals in group 2 (no. 7 – 20). Findings were counted together to get a clinical score representing the interpretation of the severity of illness.

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>ND</th>
<th>Lu</th>
<th>Tra</th>
<th>Ln</th>
<th>Cough</th>
<th>Dyspnoea</th>
<th>Result clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Mild</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Moderate</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Severe</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>Mild</td>
</tr>
</tbody>
</table>

Result clinical score represents the interpretation of the severity of illness.

Table 9.6 Findings of the last clinical evaluation of foals in group 2 (no. 7 – 20). Findings were counted together to get a clinical score representing the interpretation of the severity of illness.
Table 9. Findings of the last clinical evaluation of foals in group 3 (no. 21 – 37). Findings were counted together to get a clinical score representing the interpretation of the severity of illness. (This information could not be used, because the intermission between last clinical evaluation and the day of death or euthanasia was more than 5 days in some foals).

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>ND</th>
<th>Lu</th>
<th>Tra</th>
<th>Ln</th>
<th>Cough</th>
<th>Dyspnea</th>
<th>Clinical score</th>
<th>Result clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>Severe</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>Severe</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>33</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>Severe</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Healthy</td>
</tr>
<tr>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Healthy</td>
</tr>
</tbody>
</table>
Table 9. Findings of the last clinical evaluation of foals in group 4 (no. 38 – 39). Findings were counted together to get a clinical score representing the severity of illness.

Foal no. = number of foal; ND = nasal discharge; Lu = lung; Tra = trachea; Ln = lymph nodes. (Clinical score table 3.1)

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>Healthy</th>
<th>ND</th>
<th>Lu</th>
<th>Tra</th>
<th>Ln</th>
<th>Cough</th>
<th>Dyspnoea</th>
<th>Clinical score</th>
<th>Result clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>Healthy</td>
</tr>
<tr>
<td>39</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>Healthy</td>
</tr>
</tbody>
</table>
Fig. 9.9 Temperature of foals in group 1 (no. 1 - 6) and group 2 (no. 7 - 20).

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>Group 1</th>
<th>Foal no.</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.4*</td>
<td>7</td>
<td>38.3</td>
</tr>
<tr>
<td>2</td>
<td>38.6</td>
<td>8</td>
<td>38.1</td>
</tr>
<tr>
<td>3</td>
<td>38.1</td>
<td>9</td>
<td>40.5</td>
</tr>
<tr>
<td>4</td>
<td>37.9</td>
<td>10</td>
<td>38.1</td>
</tr>
<tr>
<td>5</td>
<td>39.9</td>
<td>11</td>
<td>38.3</td>
</tr>
<tr>
<td>6</td>
<td>38.4</td>
<td>12</td>
<td>39.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>38.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>39.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>38.4</td>
</tr>
</tbody>
</table>

* all data given in ° C.

Fig. 9.10 Treatment given to foals in group 1 (no. 1 - 6), group 2 (no. 7 - 20) and group 3 (no. 21 - 37).

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>Group 1</th>
<th>Foal no.</th>
<th>Group 2</th>
<th>Foal no.</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A, C</td>
<td>7</td>
<td>A</td>
<td>21</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>8</td>
<td>A, C</td>
<td>22</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>9</td>
<td>A, C, N</td>
<td>23</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>A, C</td>
<td>10</td>
<td>A</td>
<td>24</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>A, C</td>
<td>11</td>
<td>A, C, N</td>
<td>25</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>12</td>
<td>A, C</td>
<td>26</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>A</td>
<td>27</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>A</td>
<td>28</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>A, C, N</td>
<td>29</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>A, C, N</td>
<td>30</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>A, C, N</td>
<td>31</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>A, C</td>
<td>32</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>A, C, N</td>
<td>33</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>no</td>
<td>34</td>
<td>A, C, N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td>A, C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td>no</td>
</tr>
</tbody>
</table>

A = antibiotic (rifampin, azithromycin, tulathromycine, clarithromycin, trimethoprim-sulphametoxazole); C = corticosteroids (prednisolone, cyclosporine, dexamethazone); N = NSAID (non-steroidal anti-inflammatory drugs) (flunixine meglumine, phenylbutazone) no = no required treatment.
**Table 9.11** Temperature, leucocytes levels and treatment of foals in group 4 (no. 38 – 39)

<table>
<thead>
<tr>
<th>Foal no</th>
<th>Temperature °C</th>
<th>Leucocytes x 10^3 per µl blood</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>38.7</td>
<td>11.7</td>
<td>no</td>
</tr>
<tr>
<td>39</td>
<td>38.4</td>
<td>9.4</td>
<td>no</td>
</tr>
</tbody>
</table>
Table 9. Main ultrasonographic, pathomorphologic, histologic, microbiologic and immunohistochemical findings of foals in group 1

<table>
<thead>
<tr>
<th>No.</th>
<th>CT1A</th>
<th>Ultrasonographic findings</th>
<th>Macroscopic findings</th>
<th>Histologic diagnosis</th>
<th>Microbiology Lung tissue</th>
<th>Microbiology TBA</th>
<th>Immunohistochemistry (IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>++</td>
<td>Interstitial, non-specific</td>
<td>Interstitial, non-specific</td>
<td>Interstitial, non-specific</td>
<td>R. equi, Sc. equi</td>
<td>n. d.</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>R. equi, Sc. equi</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
<tr>
<td>5</td>
<td>+++</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
<tr>
<td>6</td>
<td>+++</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>Interstitial and suppurative</td>
<td>R. equi, Sc. equi</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

CT1A: Comet tail artifacts; -: negative result; n.d.: not done; Ultrasonographic findings: Ultrasonographic and immunohistochemical findings.
Table 9.1: Main ultrasonographic, pathomorphologic, histologic, microbiological and immunohistochemical findings of foals in group 2

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>Ultrasound findings</th>
<th>Macroscopic findings</th>
<th>Histologic findings</th>
<th>Microbiology</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>CTA and NA</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>++++*</td>
</tr>
<tr>
<td>8</td>
<td>CTA and NA</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>n. d.</td>
</tr>
<tr>
<td>9</td>
<td>CTA and NA</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>n. d.</td>
</tr>
<tr>
<td>10</td>
<td>CTA and NA</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>n. d.</td>
</tr>
<tr>
<td>11</td>
<td>CTA and NA</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>n. d.</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>Interstitial pneumonia</td>
<td>Multifocal interstitial pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

**Ultrasonographic findings:** CTA: comet tail artifacts; NA: nodular hypo- to hyperechoic areas; **Histologic findings:** n. d.: not done; **Immunohistochemistry:** Estimated number of immunolabelled cells: ++ = minimal (11 - 30 positive cells), +++ = mild (31 - 50 positive cells), ++++ = severe (> 50 positive cells).

<table>
<thead>
<tr>
<th>Estimated number of immunolabelled cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++ = minimal (11 - 30 positive cells)</td>
</tr>
<tr>
<td>+++ = mild (31 - 50 positive cells)</td>
</tr>
<tr>
<td>++++ = severe (&gt; 50 positive cells)</td>
</tr>
</tbody>
</table>

* Estimated number of immunolabelled cells: + = minimal (0 - 10 positive cells), ++ = mild (11 - 30 positive cells), +++ = moderate (31 - 50 positive cells), ++++ = severe (> 50 positive cells).
Continuation on Table 9.1 (foals no. 13 - 17)

<table>
<thead>
<tr>
<th>Foal no.</th>
<th>Ultrasound Findings</th>
<th>Macroscopic Findings</th>
<th>Histologic Findings</th>
<th>Diagnosis</th>
<th>Microbiology</th>
<th>TBA</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>CTA and NA</td>
<td>n. d.</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>suppurative to necrotizing pneumonia</td>
<td>R. equi, E. coli, B. bronchiseptica, K. pneumoniae, non-specific</td>
<td>+*</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>n. d.</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>suppurative pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>++</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>n. d.</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>suppurative pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>+++</td>
</tr>
<tr>
<td>16</td>
<td>CT A and NA</td>
<td>n. d.</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>suppurative pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>++++</td>
</tr>
<tr>
<td>17</td>
<td>CT A and NA</td>
<td>n. d.</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>suppurative pneumonia</td>
<td>R. equi, E. coli, non-specific</td>
<td>++</td>
</tr>
</tbody>
</table>

*estimated number of immunolabeled cells: + = minimal (0 - 10 positive cells), ++ = mild (11 - 30 positive cells), +++ = moderate (31-50 positive cells), ++++ = severe (> 50 positive cells).

- CTA: comet tail artifacts; NA: Nodular hypo- to hyperechoic areas; - negative result; n. d.: not done; Ultrasonographic findings; TBA: tracheobroncheal aspirate; IHC: Immunohistochemistry.
<table>
<thead>
<tr>
<th>Foal No.</th>
<th>CTA and NA</th>
<th>Ultrasonographic findings</th>
<th>TBA: tracheobronchial aspirate</th>
<th>IHC: Immunohistochemistry</th>
<th>Microbiology</th>
<th>Lung tissue</th>
<th>Histologic diagnosis</th>
<th>Microscopic findings</th>
<th>Field diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>+</td>
<td>Interstitial and pyogranulomatous pneumonia</td>
<td>Interstitial and suppurative bronchopneumonia</td>
<td>R. equi, Sc. equi ssp. zooepidemicus, E. coli, non-specific</td>
<td>R. equi, Sc. equi ssp. zooepidemicus, E. coli, non-specific</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>No findings</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>Interstitial and pyogranulomatous pneumonia</td>
<td>Interstitial and suppurative bronchopneumonia</td>
<td>Non-specific</td>
<td>Non-specific</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>No findings</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>Suppurative to necrotizing pneumonia</td>
<td>Interstitial and suppurative bronchopneumonia</td>
<td>Sc. equi ssp. zooepidemicus, E. coli, non-specific</td>
<td>Sc. equi ssp. zooepidemicus, E. coli, non-specific</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>No findings</td>
</tr>
</tbody>
</table>

CTA: comet tail artifacts; NA: Nodule, hypo-to hyperechoic areas; - : negative result; n. d.: not done; Ultrasound, findings: 

Estimated number of immunolabelled cells: + = minimal (0-10 positive cells), ++ = mild (11-30 positive cells), +++ = moderate (31-50 positive cells), ++++ = severe (> 50 positive cells). The estimated number of immunolabelled cells: + = minimal (0-10 positive cells), ++ = mild (11-30 positive cells), +++ = moderate (31-50 positive cells), ++++ = severe (> 50 positive cells).
Table 9.1: Main ultrasonographic, pathomorphological, histological, microbiological and immunohistochemical findings of foals in group 3.

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Ultrasonographic findings</th>
<th>Pathomorphological findings</th>
<th>Histological findings</th>
<th>Microbiology Lung tissue</th>
<th>Immunohistochemistry R. equi</th>
<th>Immunohistochemistry P. carinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Interstitial pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>E. coli, A. equuli, E. coli, K. pneumoniae</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Interstitial pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>E. coli, A. equuli, E. coli, K. pneumoniae</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Interstitial edema</td>
<td>Subacute to acute pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>E. coli, A. equuli, E. coli, K. pneumoniae</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Interstitial pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>E. coli, A. equuli, E. coli, K. pneumoniae</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Interstitial edema</td>
<td>Subacute to acute pneumonia</td>
<td>Subacute to acute pneumonia</td>
<td>E. coli, A. equuli, E. coli, K. pneumoniae</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

* Estimated number of immunolabelled cells: + = minimal (0 - 10 positive cells), ++ = mild (11 - 30 positive cells), +++ = moderate (31-50 positive cells), ++++ = severe (> 50 positive cells).

CTA: comet tail artifacts; NA: Not done; "": negative result; n. d.: not done; Ultrasonographic findings: TBA: tracheobronchial aspirates; IHC: immunohistochemistry.
### Table 9. Group 3 (foals no. 28 – 33)

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Ultrasound findings</th>
<th>Macroscopic findings</th>
<th>Histologic findings</th>
<th>Microbiology of Lung tissue</th>
<th>Microbiology of TBA</th>
<th>IHC</th>
<th>R. equi</th>
<th>P. carinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>n. d.</td>
<td>Alveolar edema and</td>
<td>Interstitial</td>
<td>E. coli, non-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>NA</td>
<td>Alveolar edema and</td>
<td>Multifocal</td>
<td>E. coli, non-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>Multifocal</td>
<td>E. coli, K. pneumoniae, non-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>n. d.</td>
<td>Interstitial and</td>
<td>N/A</td>
<td>R. equi, Sc. equi ssp. zooepidemicus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>n. d.</td>
<td>Granulomatous</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>n. d.</td>
<td>Interstitial pneumonia</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Estimated number of immunolabelled cells: = minimal (0-10 positive cells); ++ = mild (11-30 positive cells); +++ = moderate (31-50 positive cells); ++++ = severe (> 50 positive cells).*

NA: Nodular hypo- to hyperechoic areas; -: negative result; n. d.: not done; Ultrasono. findings: Ultrasonographic findings; TBA: Tracheobronchial aspirate; IHC: Immunohistochemistry.
Continuation Table 9.14 Group 3 (foals no. 34 - 37)

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Ultraso. findings</th>
<th>Macroscopic findings</th>
<th>Histologic findings</th>
<th>Microbiology Lung tissue</th>
<th>TBA</th>
<th>IHC</th>
<th>R. equi</th>
<th>P. carinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>P. carinii</td>
<td>Alveolar edema</td>
<td>Interstitial and suppurrative pneumonia</td>
<td>E. coli, K. pneumonia, non-specific</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td>E. coli, K. pneumonia, non-specific</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td>E. coli, K. pneumonia, non-specific</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CTA: comet tail artifacts; NA: Nodular hypo- to hyperechoic areas; : negative result; n. d.: not done; Ultrasound: Ultrasonographic findings; TBA: Tracheobronchial aspirate; IHC: Immunohistochemistry

*Estimated number of immunolabelled cells: + = minimal (0-10 positive cells), ++ = mild (11-30 positive cells), +++ = moderate (31-50 positive cells), ++++ = severe (> 50 positive cells).
Table 9.15: Main ultrasonographic, pathomorphologic, histologic, microbiologic and immunohistochemical findings of foals in group 4: negative result; n. d.: not done; Ultrasono. findings: Ultrasonographic findings; TBA: Tracheobronchial aspirate; IHC: Immunohistochemistry.

<table>
<thead>
<tr>
<th>No.</th>
<th>Ultrasono. findings</th>
<th>Macroscopic findings</th>
<th>Histologic findings</th>
<th>Microbiology</th>
<th>Lung tissue microbiology</th>
<th>TBA</th>
<th>IHC</th>
<th>n. d.</th>
<th>n. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>-</td>
<td>-</td>
<td>Non-specific</td>
<td>Non-specific</td>
<td>Alveolar edema and emphysema, + bronchitis</td>
<td>R. equi</td>
<td>P. carinii</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>-</td>
<td>-</td>
<td>Alveolar edema, emphysema, + bronchitis</td>
<td>Non-specific</td>
<td>Alveolar edema and interstitial emphysema</td>
<td>n. d.</td>
<td>IHC</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 9.16 Correlation of immunohistochemical labelling of *P. carinii* and Grocott’s methenamine silver stain in 13 positive foals at IHC for *P. carinii* (no. 1, 2, 4, 5, 11, 14, 16, 17, 19, 26, 28, 29, 32) out of 37 foals.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of foal</th>
<th>IHC <em>P. carinii</em></th>
<th>Grocott</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
<td>19</td>
<td>+</td>
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<td>3</td>
<td>26</td>
<td>+</td>
<td>+</td>
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<td>3</td>
<td>28</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+: positive reaction; -: negative reaction; No. of foal: number of foal
IHC: immunohistochemistry for *P. carinii* antigen
Acknowledgement

I express my gratitude to Prof. Dr. Wolfgang Baumgärtner, Ph.D., for his support and for letting me do the laboratory part of my work at the Pathology Institute and spending a nice time at the institute.

I wish to express my special thanks to Dr. Peter Wohlsein for his outstanding support, gentleness continuous motivation during the completion of my Doctor-project, and always taking the best worths to cheer me up. You had always the correct answer to my questions. It was very nice working with you, and will remember these days happily.

Frau PhD. Dr. M. Venner danke ich für die kompetente Betreuung meiner Arbeit.

I express my gratitude to Dr. S. Takai that kindly provided the specific anti-R. equi antibody for the Immunohistochemistry. I also want to thank the kindly support and always being there to answer any question.

Herrn P. Schockemöhle danke ich für die Erlaubnis zur Durchführung meiner Untersuchungen auf seinem Gestüt und die finanzielle Unterstützung.


Bei den „guten Feen“ des Labors - Bettina Buck, Petra Grünig, Claudia Herrmann, - bedanke ich mich für ihre Geduld und Hilfsbereitschaft.

I am grateful to my colleagues and friends Maria, Gorn, Visar, Anna, André, Tim, Eva Maria, Jelena, Vanessa, Florian, Sven, Kathrin, Frauke, Suliman, and the others for the best friendly work atmosphere.

I thank all past and present colleagues and employees from the Department of Pathology, University of Veterinary Medicine Hannover, who make this workplace so friendly.

Thanks to my office companion: Gorn and Doro for spending some time together, always having an answer, the good advices and laughs. Special thanks to Gron for his outstanding support, gentleness, continuous motivation and always having smart words to encourage me.

I thank Mrs. Ledwoch of the office of international academic affairs, for their dedicated service of international students and postgraduates and the constant readiness to solve our problems.

I want to thank Chris Holly for all his truthfully support and help by correcting and reading my whole work. Thankyou for spending lots of hours reading my thesis.

Sobretodo agradecer a mi panzon por su apoyo incondicional, moral y económico. Agadecida estoy que me dejaste ir a realizar este sueño. Siempre pensando en el futuro para poder apoyarme, es algo que nunca olividare. Gracias por haber estado ahí siempre cuando te
necesité y por seguir este sueño conmigo. Aunque ya no estés a mi lado, se que siempre seguiremos este sueño juntos.

Mil gracias a mi ma que siempre estuvo ahí apoyándome y sacándome adelante cuando ya no podía más, con esas tardes de niñas y lindas palabras que siempre me levantan el ánimo. También que estuvo ayudando con algunas traducciones.

Muchas gracias mi querido hermano Daniel que aunque nos dejo hace tiempo se que me hubiera estado apoyando con sabias palabras de un gran filósofo.

Mil gracias a mi familia en México por haber estado con mis papás y así yo poder estar en Alemania realizando mi sueño.

Ein besonderer Dank gilt jedoch meine Oma una mein Opa, tanten und onkel und cousienen für Ihre liebevolle Unterstützung.

Hartas gracias a mis compañeros latinos en Hannover que fueron de gran apoyo tanto en el instituto como afuera. Gracias por haber adoptado a la mexicanita huérfana.

Bei der „alte Tante“ – Britta Richter - bedanke ich mich für ihre unterstützung und die schöne und lustige Nächte und Tage während unser Aufenthaltes auf dem Gestüt und später auch.

Muchísimas gracias a Dra Myriam Boeta por dejar que cumpliera mi sueño, ese apoyo incondicional, buenos consejos, y por su gran amistad.

Millones de gracias a mis compañeros de la UNAM por haberme apoyado aunque sea de larga distancia, por haber estado ahí siempre cuando los necesité.