Szent István University
Postgraduate School of Veterinary Science

Some new aspects of equine pulmonary diagnostics

PhD dissertation

Written by:
Orsolya Kutasi

2011.

Szent István University

Postgraduate School of Veterinary Science

Supervisor:

....

Prof. Szenci Otto, PhD, MSc, DSc

Head of Clinic for Large Animals

Szent István University, Faculty of Veterinary Sciences

Advisors:

Prof. Vörös Károly, PhD, MSc, DSc

Head of Internal Medicine Department

Szent István University, Faculty of Veterinary Sciences

Prof. Gálfi Péter, PhD, MSc, DSc

Head of Pharmacology and Toxicology

Szent István University, Faculty of Veterinary Sciences

Published in 8 copies.

This is the ….. copy.

....

Orsolya Kutasi DVM

Contents

Abbreviations

Recurrent airway obstruction (RAO)
Summer pasture associated recurrent airway obstruction (SPA-RAO)
Inflammatory airway disease (IAD)
Exercise-induced pulmonary hemorrhage (EIPH)
Bronchoalveolar lavage (BAL)
Bronchoalveolar lavage fluid (BALF)
Polymerase chain reaction (PCR)
Enzyme-linked immunosorbent assay (ELISA)
Equine Herpesvirus 5 (EHV-5)
Functional residual capacity (FRC)
Respiratory tract (RT)
Respiratory secretion (RS)
Infectious disorders (ID)
Upper respiratory tract (URT)
Upper respiratory tract functional disorders (URTFD)
Small airway inflammation (SAI)
Ultrasonography (US)

Summary

Even though the respiratory system is one of the most accessible organs for diagnostic testing, it is not always easy to define respiratory diseases in horses. In mature horses results of physical examination can be difficult to interpret accurately and although pertinent ancillary diagnostic modalities can help further characterize and localize causes for respiratory dysfunction, in most cases, however the findings are not specific. Early recognition of respiratory abnormalities during the postnatal period is of special importance for successful management of critically ill foals. In most foals, physical examination is not adequate for precise identification of the cause or severity of respiratory dysfunction, even when clinical signs are present. Clinical signs have to be interpreted in conjunction with clinicopathologic and diagnostic imaging findings.

Diagnostic procedures performed by first opinion veterinarians in the field are often restricted to taking the history and performing clinical examination. Respiratory tract endoscopy, tracheal or bronchoalveolar lavage and blood sampling are sometimes used but other specific ancillary examinations are seldom performed in stable settings. Therefore, our objectives were to evaluate the diagnostic value of different techniques and examination types routinely used in the diagnostic workup of chronic equine lower airway cases in both stable and clinical circumstances. Another aim of this study was to estimate the prevalence of different chronic pulmonary disorders among horses admitted to a Hungarian referral clinic. According to the conditional inference tree method, age of the horse, history, clinical examination, respiratory tract endoscopy and bronchoalveolar lavage cytology proved to be the most valuable tools to define pathology. It was also concluded that in 22% of cases more specific ancillary diagnostic modalities, unavailable for the field veterinarian, were needed to establish the final diagnosis. According to our study, the most frequently diagnosed chronic pulmonary disorders in Hungary are of non-infectious origin, principally recurrent airway obstruction (RAO). Regardless of the cause, and interestingly including RAO as well, these diseases occur primary during the warm months.

Thoracic radiographs of foals made immediately after birth are characterized by a pronounced interstitial-alveolar opacity with blurring of small vessels. This opacity is the result of incomplete lung inflation, the presence of residual fluid in the small airways, and uptake of fetal alveolar fluid

into the lung interstitium. Foals with respiratory disease may have a similar radiographic pattern, but it typically persists beyond the normal absorption time. In the second part of my thesis the kinetics of postnatal equine lung using sequential thoracic radiography was characterized. The aim was to establish the earliest time when normal foals have clear, radiolucent lung fields, and to characterize the pattern of this clearance. Both right-to-left and left-to-right thoracic radiographs were acquired in lateral recumbency at peak inspiration within the first 30 min after birth and thereafter at 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h. Radiographs were interpreted by three observers. The overall assessment of radiographic lung clearance was followed by the evaluation of individual lung quadrants to document changes in pulmonary radiographic patterns over time. It was concluded that thoracic images in a healthy foal older than 4 h should be characterized by clear lungfields and after this period distinctions between physiologic and pathologic conditions can be made. The ventral lung cleared first, presumably due to the greater flexibility of the thoracic wall in this anatomic region.

Chapter 1. General introduction

My studies do not focus on a single equine disease or a specialized experiment series, but I have examined different aspects of equine pulmonary diagnostics. I have focused on two main fields of lower airway examinations: in the first part I studied the diagnostic value of different techniques and examination types used routinely in the diagnostic work up of chronic equine lower airway cases and in the second part I used thoracic radiography to assess the pulmonary fluid clearance in healthy neonatal foals. The primary aim of both parts of my studies was to present useful data and recommendations to first opinion veterinary surgeons working in the field.

Evaluation of the respiratory system starts with taking the history. The history, probably one of the most important aspects of a general physical examination, can be tailored to the problem for which the horse is presented (Derksen and Paradis, 1999, Laumen et al., 2010). A detailed physical examination of the horse with respiratory disease should start with simple observation of the general condition and the pattern of breathing. Examination starts at the head and then follows the route of air in the airways through pharynx, larynx and trachea and finally ends at the lungs (Vörös, 1998). Evaluation of the lung is the most challenging part of examination of the respiratory system. Auscultation and percussion have some limitations that a large portion of the cranioventral lungfields cannot be evaluated using these techniques because of the shoulder and its associated musculature. In spite of their limitations auscultation and percussion are still useful methods of evaluation (Savage, 1997; Derksen and Paradis, 1999). Pulmonary disease can result in increased or decreased intensity of breath sounds, abnormal lung sound or adventitious sounds, and auscultation should be considered a qualitative test only. Auscultation should not be used to specifically diagnose diseases or to quantitate the severity of the disease. Percussion allows delineation of the boundaries of the aerated lung and detection of pneumothorax or large space-occupying lesions, such as abscesses and pleuritis. In some cases, respiratory disease can be diagnosed after detailed physical examination of the respiratory system but in most cases ancillary aids are required.

Respiratory disorders of the newborn foal are some of the most underdiagnosed pathologies in the field (Derksen and Paradis, 1999). Clinical signs of pulmonary involvement may be subtle, and it takes experience in observing foals to recognise the problem (Kosch et al., 1984). The skills of history taking and physical examination must be combined with diagnostic tests.

1.1 Ancillary diagnostic procedures used to collect data about physiologic and pathologic conditions of the equine lung

1.1.1 **Laboratory diagnostics**

1.1.1.1 **Clinicopathology**

A routine hemogram should always be obtained in the presence of acute respiratory signs and also in chronic cases whenever the involvement of an infectious disease is considered possible (Roy and Lavoie, 2003; Pusterla et al., 2006). In some chronic diseases such as RAO, inflammatory airway disease (IAD) or exercise induced pulmonary hemorrhage (EIPH), the hemogram does not show specific changes (Lavoie, 1997). When an infectious disease is suspected, interpretation of the hemogram will depend on the phase of the disease when the blood sample was collected, as the white blood cell response varies with the phase of the disease. The hemogram may be negative also in these cases, in any phase of the disease. In the case of acute virus infections, the hemogram may show lymphopenia, mild anemia and possibly thrombocytopenia in the first febrile phase and then in the next phase it may return to normal, with the occasional development of lymphocytosis. In chronic, primary bacterial, infections neutrophilia and mild anemia are common (Lavoie et al., 1994; Ainsworth et al., 1998; Pustrela et al., 2006). Foals can present with a marked leukocytosis in the acute stage of the disease (Hoffman et al., 1993). Mild to moderate anemia developing in inflammatory diseases is the commonest type of anemia, which is often misinterpreted. The organism blocks the release of iron, necessary for bacterial growth, from the reticuloendothelial system, the response of the bone marrow to erythropoietin is depressed, and the lifespan of erythrocytes is shortened (Pusterla et al., 2006). The pronounced increase of the platelet count should also direct attention to the possibility of a chronic inflammation of bacterial origin (Sellon et al., 1997). The presence of thrombocytopenia may play an important role in the diagnosis of *Rhodococcus equi* infection in foals as well (Leadon et al., 1988).

Of the biochemical parameters, total protein concentration and, within it, first the fibrinogen level may increase in the presence of inflammation, then in chronic cases the globulin concentration also rises (Pusterla et al., 2006), while the albumin level may often decrease (Sellon et al.,

1997). Other biochemical parameters are not of major help in diagnosis of infectious conditions, except for identifying secondary processes such as pre-renal azotemia due to dehydration, electrolyte imbalance, or secondary foci of infection (Léguillette et al., 2002).
Measurement of arterial blood gas tension is the simplest quantitative test of pulmonary function available to equine practitioners. The test evaluates the most important aspect of pulmonary function such as gas exchange (Derksen and Paradis, 1999). It is also used to monitor clinical therapeutic response in critical cases. The arterial blood gas values of normal term foals vary considerably and depend on the position and excitement of the patient (Derksen and Paradis, 1999). Foals usually tolerate hypoxemia better than adult horses and may not show clinical signs of respiratory insufficiency before being severely hypoxemic (Léguillette et al., 2002).

1.1.1.2 **Collection of airway secretions**

Nasopharyngeal and pharyngeal swabs

Nasopharyngeal and pharyngeal swabs can be taken easily without any sedation of foals using a guarded culturette or a guarded uterine swab, respectively. Samples should be processed rapidly and have to use a commercial transport media. Complete bacteriologic examination of a nasal or pharyngeal swab is often not warranted because of the presence of normal bacterial flora, but it can be used to detect the presence of specific pathogens such as *Streptococcus equi* (Léguillette et al., 2002). Swab samples are also appropriate for virus isolation or other molecular diagnostic tests (Mumford et al., 1998).

Tracheal wash

The sample taken from the lower third of the trachea originates from both the peripheral and the central airways (Lavoie, 1997). The neutrophil cell count of tracheal samples shows large variation even in healthy horses, it is poorly correlated with the cell count of samples taken from the bronchoalveolar area and, therefore, it is unsuitable for the diagnosis of IAD or RAO (Derksen et al., 1989; Traub-Dagratz et al., 1992, Richard et al., 2010). Also, normal values seem to be different in foals as they tend to have a higher percentage of neutrophils than in adult horses (Hoffamn et al. 1993). In foals total cell count and the percentage of neutrophils tend to increase in cases of airway infection (Mansmann and Knight, 1972; Hoffmann et al. 1993). However, the results can be significantly affected by the sampling technique (Mair et al., 1987).

When infectious diseases are suspected, tracheal samples are routinely used for bacterial or fungal culture, cytological examination or Gram staining (Pusterla et al., 2006), or for the detection of developmental stages of parasites. In order to obtain reliable culture results, tracheal samples should be collected in a sterile manner by transtracheal aspiration or by the use of a special sheathed catheter introduced through the working channel of the endoscope (**Figure 1.**). Bacterial culture may bring a false negative result in *Rhodococcus equi* infection of young foals, but such false negative results may also occur in adult horses with other bacterial diseases.

Bronchoalveolaris lavage (BAL)

The diagnosis of chronic respiratory diseases is often based on the cytological examination results of bronchoalveolar lavage fluid (BALF) samples. After appropriate sedation, such samples may be obtained with a special bronchoalveolar lavage (BAL) catheter (BIVONA) (**Figure 2.**) or by lavage through the working channel of the endoscope (Hewson and Viel, 2002) and the lavage fluid is gained back with a foamy layer on the top (**Figure 3**).

Figure 1. Tracheal wash through endoscope chanel.

Figure 2. BAL with BIVONA catheter

Figure 3. The BAL fluid with the foamy layer as a result of surfactant content of the distal airways

Under physiological conditions, macrophages and lymphocytes are dominant in the BALF sample (Lavoie, 1997, Richard et al., 2010). In horses with RAO always a high, 15–25% neutrophil granulocyte count can be found (Grünig et al., 1989). High neutrophil granulocyte counts may also be encountered in horses with bacterial and viral infections, equine multinodular pulmonary fibrosis, tumors or idiopathic granulomatous pneumonia (Hart et al., 2008). In the presence of an infection, degenerative changes of the neutrophil cells are typical like karyolysis or karyorrhexis or the loss of segmentation. In the case of IAD, a more varied picture is seen: the elevated inflammatory cell count may be accompanied by mild neutrophilia, lymphocytosis, monocytosis (Couëtil et al., 2001; Couëtil et al., 2007), eosinophilia or an increased mast cell count (Hare et al., 1998; Hoffman et al., 1999). Hemosiderophages or, in the case of a recent hemorrhage, red blood cells are found in EIPH, but these may occur in many inflammatory diseases of the lower airways as well (Fogarty 1990; Hewson and Viel, 2002, Ferruci et al., 2009). Bacterial culture may also be done from a BAL sample, but both false negative (as the sampling cannot be accomplished in a sterile manner) and false positive results may occur.

Bronchoalveolar lavage is less frequently used as a diagnostic tool of pneumonia in foals because of the frequent contamination by the upper airway bacterial flora, but may be useful for the diagnosis of conditions such as *Pneumocystis carnii* pneumonia.

In critically ill neonates procedures like bronchoalveolar lavage or endoscopy with tracheal sampling are not always advisable because of the stress of the procedure may further compromise the patient (Derksen and Paradis, 1999). In neonates, blood culture results may be indicative of bacterial isolate from the lungs (Léguillette et al., 2002), although it may be necessary to sample the respiratory tract directly when *in utero*-acquired pneumonia is suspected (Koterba, 1990).

1.1.1.3 **Thoracocentesis and lung biopsy**

The indication for thoracocentesis is to evaluate the bacteriological and cytological content of fluid accumulating within the pleural space. The presence of pleural effusion can be suspected based on the findings of thoracic auscultation and percussion. Today with the more frequent use of ultrasonography in the field, pleural effusion can be confirmed by visualization prior to performing thoracocentesis (Norman et al., 1981). The information obtained from thoracocentesis has several benefits largely pertaining to case management. First, it will guide the clinician in the selection of an appropriate antibiotic therapy. Second, it will dictate the decision to initiate tube drainage of the pleural space.

Percutaneous lung biopsy is useful in evaluating pulmonary pathology for conditions that are diffuse within the lung, but offers limited value for evaluating focal or localized conditions unless ultrasound guidance is used to select the site of biopsy. Most frequently, lung biopsy is utilized as a complementary diagnostic aid to bronchoalveolar lavage and pulmonary function testing in order to determine the degree of airway remodeling such as inflammatory cell infiltrate, smooth muscle hypertrophy, as well as collagen formation and deposition.

Complications associated with lung biopsies are minimal, and are generally restricted to self-limiting hemoptysis not requiring medical intervention (Raphel and Gunson, 1981). However, rare complications such as tachycardia, tachypnea, pneumothorax, respiratory distress, epistaxis, pulmonary hemorrhage, pale mucous membranes, great vessel hemorrhage, collapse, and peritonitis from inadvertent intestinal biopsy have been reported historically in association with the procedure (Savage et al., 1998). Therefore, lung biopsy should not be considered an innocuous procedure and warrants post-biopsy monitoring for at least 24 hours (Hewson and Viel, 2002). In a previous survey among internal medicine diplomates in the United States a variable percentage of respondents felt there were contraindications to perform of this technique, which included neonatal septicemia (68%), pulmonary abscessation (65%), pleuropneumonia (55%) and pneumonia (42%), EIPH (41%), and RAO (26%) (Savage et al., 1998). Lung biopsy is contraindicated in animals experiencing dyspnea, a marked elevation in respiratory rate, or paroxysmal coughing, since these patients are at greater risk for complications due to the potential for pulmonary tissue lacerations by the biopsy needle in the presence of increased chest excursions.

1.1.1.4 **Serology and Molecular diagnostic tests**

Serological tests are suitable for revealing the cause of an active progressive infectious disease or a delayed recovery, for demonstrating carrier status and, in certain cases, for distinguishing between antibody titers resulting from vaccination, acute infection or clinical disease. In certain cases, chronic respiratory infections may be demonstrated already by the presence of the antigens, while in other cases the determination of antibody titers or the testing of paired sera is necessary. In general, a fourfold increase of titers between the level measured in the acute phase and 14–21 days thereafter is considered indicative of an acute infection (Pusterla et al., 2006). Molecular diagnostic methods are used primarily for the diagnosis of diseases caused by bacteria, fungi or viruses that are difficult to culture, or whenever they are necessary for establishing a rapid diagnosis or possibly for the prevention of disease outbreaks. Depending on the type of pathological processes induced by the suspected infectious agent, these tests may be performed with whole blood or with different airway samples (Pusterla et al., 2006).

1.1.2. **Diagnostic Imaging**

1.1.2.1 **Respiratory endoscopy**

Endoscopy of the upper airway is the primary diagnostic tool for the evaluation of the upper respiratory tract and can be beneficial in making some assessment of lower airway disease as well (Parente, 2002, Davidson and Martin, 2003; Franklin et al., 2006). The naso-pharynx can be carefully assessed for any evidence of anatomic abnormalities or discharges. If upper airway noise is suspected, it is important to visualize the function of the pharyngo-laryngeal structures prior to sedation and without the use of a nose twitch in order to better assess any irregularities (**Figure 4**).

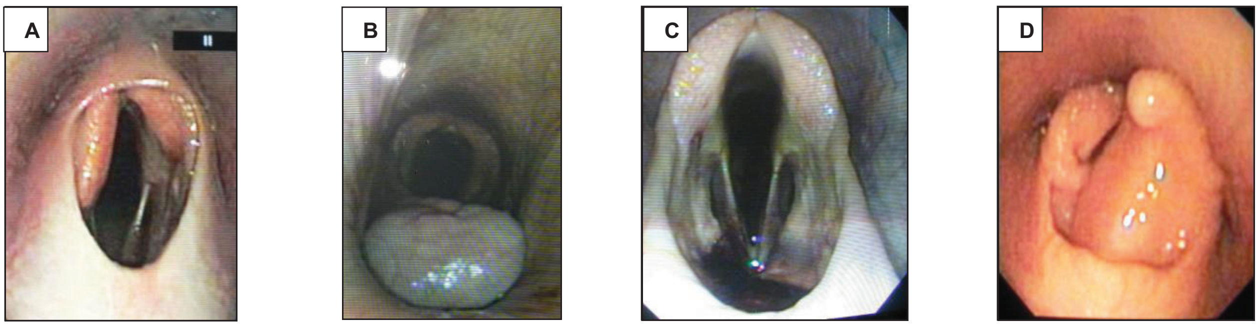

Figure 4. Different types of anatomic and functional abnormalities of the upper airway. A. Fourth branchial arch developmental abnormality, B. Subepiglottal cyst with epiglottal entrapment, C. Dorsal displacement of the soft palate. Note the blood in the laryngeal opening, upper airway obstruction causing lower airway bleeding in this case, D. Epiglottis retroversion.

The trachea should be inspected for any hyperemia, as well as the quality and amount of any secretions. The normal horse will usually cough 2-3 times during passage of the bronchoscope, in contrast to a dramatic repetitive cough response by horses with airway hypersensitivity. The presence of edema should be determined by assessing the sharpness of the tracheal bifurcation at the carina as well as the large bronchial divisions, which will appear thickened and blunted when edema is present. Along with the appearance of mucosal edema, bronchospasm becomes evident during endoscopy, characterized by protrusion of the cartilaginous rings into the lumen of the airways and a significant reduction of the airway lumen diameter (**Figure 5**). The latter is readily observed in horses with a high degree of airway hypersensitivity such as in heaves for example. The evaluation of the airways for any of the above abnormalities is very subjective and dependent on the experience of the observer.

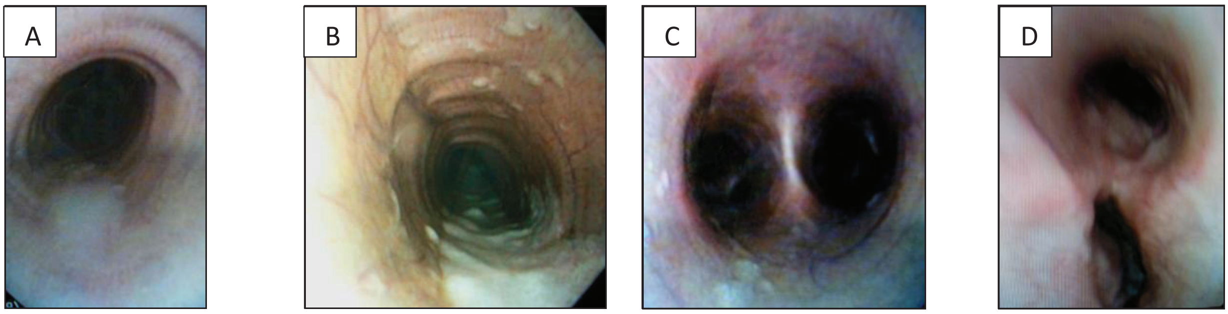

Figure 5. Lower airway endoscopy. A: Small amount of tracheal secretion (IAD). B. Large amount of airway secretion. Hyperaemic mucus membrane (RAO with secunder Streptococcal infection). C. Edema of the tracheal bifurcation (RAO). D. Bronchoconstriction of bronchi (RAO).

Therefore, semi-quantitative scoring systems have been developed in order to standardize the reports of clinical examination findings and endoscopically-determined airway abnormalities (Hare and Viel, 1998, Hewson and Viel, 2002). In foals with compromised respiratory function, endoscopic examination should preferably be performed unsedated, as alpha-2 agonist drugs increase upper airway resistance and cause hypoxemia in horses (Lavoie et al., 1992). Small diameter endoscopes (11 mm or less) are less traumatic and easier to pass through the nasal cavities of foals (Léguillette et al., 2002). The endoscope can also be used at the site of infection to take samples for cytologic examination or bacterial culture.

1.1.2.2 **Thoracic ultrasonography**

Thoracic ultrasonography is one of the most readily available and widely used diagnostic techniques for the evaluation of the thoracic cavity in horses (Reef, 2002). The portability and versatility of the diagnostic ultrasound equipment, the lack of exposure to ionizing radiation and the breadth of diagnostic information obtained in horses with thoracic disease, have made diagnostic ultrasound a routine part of the examination in horses with thoracic diseases (Reef et al., 2004). There are characteristic abnormalities detected upon ultrasonographic examination of the thorax that help the ultrasonologist differentiate a variety of pulmonary pathologies; in particular pulmonary atelectasis, consolidation, necrosis, and pulmonary abscess (Reimer, 1990; Reef, 1991). The sonographic findings in horses with pulmonary edema, exercise induced pulmonary hemorrhage, chronic obstructive pulmonary disease, and horses with scarring from previous pleuropneumonia are usually very similar and other diagnostics, like radiography are needed to differentiate between the different conditions (Reimer, 1990; Reef, 1991). Similarly, the sonographic findings in horses with pulmonary fibrosis, granulomatous pneumonia, fungal pneumonia and metastatic neoplasia are not diagnostic for the disease process (Reimer, 1990; Reef, 1991) (**Figure 6.**).

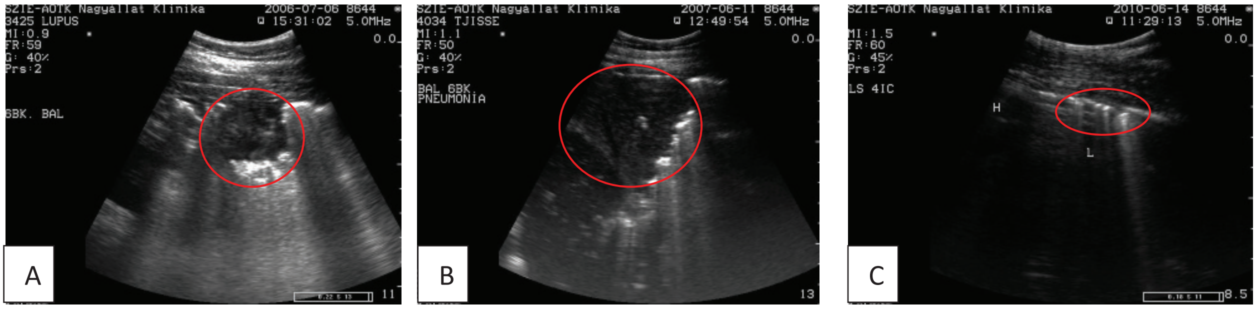

Figure 6. Different ultrasonographic abnormalities of the lungs. A: Pulmonary abscess (Streptococcus zooepidemicus), B: Lung consolidation (Streptococcus zooepidemicus), C: Nodular fibrosis (Equine Multinodular Pulmonary Fibrosis-EHV-5, L:lung, H: abdominal cavity)

A lung biopsy and/or culture of the affected lung parenchyma are needed to definitively differentiate between these conditions. The site for thoracocentesis can be determined and the response to treatment can be monitored by ultrasonography. Masses can be identified in the lung or mediastinum and an ultrasound guided biopsy obtained (Reef, 2002). Thoracic ultrasonography is probably less informative than radiography for pneumonia in foals (Ramirez et al., 2004). Various probes can be used for this purpose, the major restriction in neonates being the width of the transducer, which should be small enough to fit between the ribs (Léguillette et al., 2002).

1.1.2.3 **Thoracic radiography**

Thoracic radiography is often used in veterinary medicine as a complementary method for the diagnosis of respiratory diseases and other thoracic disorders (Farrow, 1981; Butler et al., 1993; Lester and Lester, 2001; Bedenice et al., 2003). Although taking a thoracic radiograph is expensive, it can be accomplished fast and relatively easily. Radiography of the equine thorax may require as many as eight projections however; the cranial ventral thorax is superimposed by the shoulders and front limbs. Radiographs of the cranial thorax are not often diagnostic even when using the most sophisticated radiographic equipment and techniques.
The interpretability of good-quality radiographs may be influenced by the animal's position, the phase of respiration, the projection, the selected values and the applied films and grids as well (Toal and Cudd, 1986; Lamb and O'Callaghan, 1989).
In the horse, the morphology typical of the species may result in a unique appearance of the pathological changes. Therefore, thorough knowledge of the anatomic characteristics is especially important when establishing a radiological diagnosis (Lamb et al., 1990).
A factor hampering the evaluation of radiographs is that the radiographic signs of pulmonary diseases are nonspecific (**Figure 7**). Different thoracic disorders may produce similar radiological signs, and a given pathological condition may have multiple different radiographic manifestations (Toal and Cudd, 1986; Lamb et al., 1990).

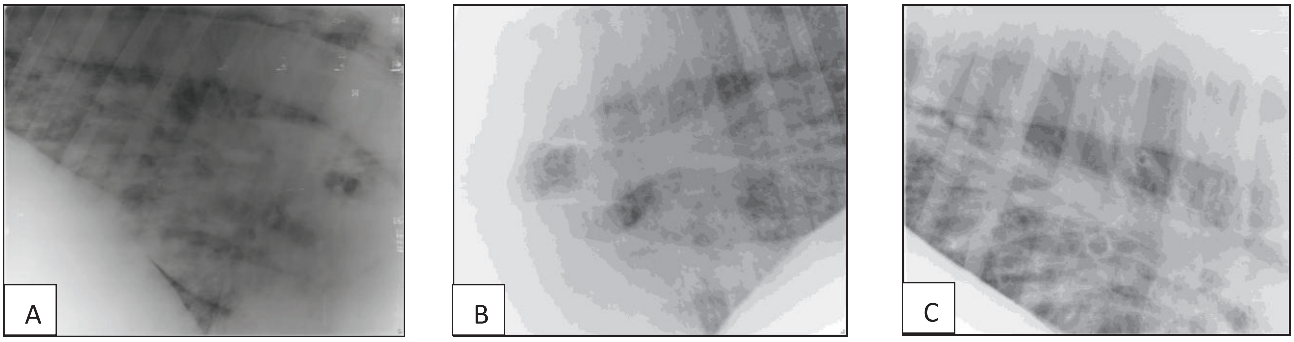

Figure 7. Different types of non-specific but suggestive radiologic patterns. A: nodular interstitial pattern (Equine multinodular pulmonary fibrosis), B: miliary interstitial-alveolar pattern (hematogen Staphylococcus pneumonia), C: bronchial pattern with bronchiectasis (Recurrent airway obstruction)

It is fundamentally important that the specialist evaluating radiographs should have abundant radiological experience, as the recognition of pathological changes and the determination of physiological status are uncertain (Toal and Cudd, 1986).

Thoracic radiography in foals

(Kutasi, O., Reményi, B., Horváth, A., Szabó, F., Machay, K., Szmodits, Zs., Paár, L., Szenci, O.: Csikók mellkasi röntgenvizsgálata : Irodalmi áttekintés, Magyar Állatorvosok Lapja, 129. 579-589, 2007.)

As the second experiment of my thesis focuses on postnatal thoracic radiography of foals, in this section I would like to introduce this diagnostic modality more in detail.

For the radiographic imaging of the thorax of a newborn or small-sized foal, a 35×43 cm cassette and a right and a left laterolateral projection are usually sufficient. In horses with diffuse pulmonary diseases a one-sided laterolateral imaging may provide adequate data, but in other cases two-sided laterolateral projections may be necessary for determining the sagittal location of the lesion. When evaluating radiographs, it must be taken into account that, because of the divergent X-rays, structures located at a greater distance from the film appear to be larger, are poorer in detail and their borders are less distinct (Lester and Lester, 2001). In addition to the laterolateral view, a ventrodorsal (or a so-called orthogonal) projection may also be used when the horse is examined in dorsal recumbency. However, performing of ventrodorsal radiographs is often more stressful for the foals, and the additional information possibly provided by ventrodorsal radiographs is redundant or may even render the establishment of a diagnosis more difficult because of its poor comparability with the laterolateral radiographs. The use of ventrodorsal projections may be justified to enable the accurate diagnosis of certain heart diseases as well as pleural and mediastinal changes (Lester and Lester, 2001).

In newborn foals positioned in lateral recumbency both a right and a left laterolateral radiograph should be taken. When the foal is in lateral recumbency, its forelimbs should be brought in a slightly anteriorly extended position, which makes it possible to image also the cranial areas of the thorax and lungs **(Figure 8.)** (Lamb et al., 1990; Lester and Lester, 2001). A possible overextension of the limbs may result in torsion of the thorax, which may affect the radiological appearance of the organs and render the interpretation of radiographs much more difficult (Toal and Cudd, 1986). Laterolateral radiographs do not pose any risk to healthy newborn foals, but in foals with respiratory diseases it should be taken into account that lateral recumbency may impair proper ventilation of the ventral lung half (Butler et al., 1993; Lester and Lester, 2001).

In older foals, it is much easier to take a radiograph in standing position **(Figure 9.)**; however, in this case it is much more difficult to pull the forelimbs in cranial direction; depending on the size of the animal, the number of radiographs to be taken may have to be increased in order to obtain information about the entire area of the lung. Although the standing posture makes it easier for the horse to breath, because of the large mass of the triceps brachii muscle it is often difficult to obtain a well-interpretable radiograph of the cranial lung lobe.

Positioning (standing or recumbent) must always be selected on the basis of the foal's age and the severity of its disease, and it must be taken into consideration that foals of poor general condition often tend to become recumbent.

Figure 8. Thoracic radiography performed with the foal in lateral recumbency.

Figure 9. Thoracic radiography with the foal in standing position.

Radiographs must be taken at the instant of maximum inspiration. Namely, the air content of the lung can be judged the most reliably if the radiograph is taken at the peak of inspiration (Butler et al., 1993). Because of the reduced air content of the lung, radiographs taken in the expiration phase may result in the appearance of a misleading pulmonary pattern and, thus, a false diagnosis (Lamb et al., 1990). The phase of respiration can be determined on the basis of the size and radiodensity of the triangular area bordered by the caudal margin of the heart, the caudal vena cava and the diaphragmatic line, as well as of the arch of the diaphragmatic line. On radiographs taken during expiration this area is smaller and more radiodense, while in the phase of inspiration the diaphragmatic line is less arched (Toal and Cudd, 1986).

In diseased foals, the elevated respiratory rate may result in movement artifacts that may be mistaken for pathological changes (Butler et al., 1993). 'Rare earth' type radiographic intensifying screens are used with the appropriate film to facilitate the elimination of such artifacts. An X-ray apparatus suitable for thoracic radiography in foals of small body size should have the following performance parameters: 80–100 kVp and 5–20 mA. The parameters most commonly used for the imaging of thoracic organs are 80 kVp, 5 mA, 100 cm focus-film distance and 8:1 or 10:1 grid ratio (Farrow, 1981, Lester and Lester, 2001). These data may be changed in a proportional manner in foals of larger body size. If a longer exposure time has to be selected, the use of a larger (100–150 cm) focus-film distance and 12–15 cm chest-cassette distance is recommended. When using a grid, consideration should be given to increase the exposure time (Lamb and OCallaghan, 1989).

Quality of the radiograph

On properly positioned radiographs, the soft-tissue organs (e.g. the heart), the organs filled with air (e.g. the lung) and the third rib (which is projected to the third thoracic vertebra) must be well visible.

On a radiograph taken after proper positioning, cranially the cranial lung lobe, caudally the diaphragmatic line and the entire caudal lung lobe, dorsally the spinous processes of the thoracic vertebrae, and ventrally the sternum are imaged. The position of the humerus and the possible torsion of the thorax because of pulling forward the forelimbs must be checked. The latter may be indicated by the arrangement of the costal arches on different levels, as this may result in a misleading increase of soft-tissue shadow mainly in the hilar region.

The *phase of respiration*, that is the most important factor influencing the interpretability of the soft-tissue shadow, may be determined on the basis of the triangle behind the heart.

The presence of *movement artifacts* impairs the interpretability of radiographs; therefore, radiographs exhibiting movement artifacts, indicated primarily by the indistinct rib margins and the blurred trabecular structure of the tubular bones, must be repeated (Toal and Cudd, 1986; Bedenice et al., 2003).

Evaluation of radiographs

We should be familiar with the physiological thoracic radiography findings in foals of different age as well as with the characteristics of pathological lung patterns produced by different diseases. The terminology of radiography is highly varied. The grading and the description of different lung patterns may also vary by author. Patterns corresponding to different disease entities can not always be accurately distinguished from each other, and sometimes different patterns may even occur together. Evaluation of a change is often based on the examiner’s subjective judgment and experience. Of the many different lung patterns, only the most important ones are overviewed below.

Physiological lung pattern. In healthy foals, completely clear and thoroughly ventilated lung fields can be seen already 12 hours after birth. By that time, the fluid filling the lungs during the fetal life has almost completely disappeared and resorbed. On thoracic radiographs taken earlier than that, it cannot be decided whether the alveolar, interstitial or alveolar-interstitial pattern is caused by a pathological process or the still incomplete fluid resorption (**Figure 10.)** (Lamb et al., 1990). Therefore, after 12 hours of age those lungs can be considered physiological in which the branching of the pulmonary vascular pattern, most closely resembling the venation of a leaf, is well visible. The bronchovascular anatomic elements can be recognized down to the third-order branches. The caudal margin of the heart is not sharply delineated but the caudal vena cava and the diaphragm have sharp margins (Lester and Lester, 2001).

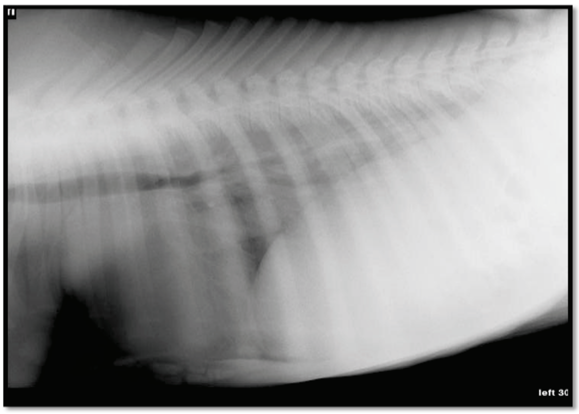

Figure 10. Alveolar-interstitial pattern on a thoracic image of a 30 mins old foal caused by the residual fetal lung fluid.

Interstitial pattern. The interstitial pattern is typical of the early stage of all diseases characterized by increased radiodensity. As a result of the pathological process, the pattern typical of the physiological lung disappears, and the fluid or cellular infiltrate accumulating in the interstitium masks the fine vascular pattern (Bedenice et al., 2003).

Depending on the course of disease, the interstitial pattern may vary from an irregular hatched to a jagged appearance, which is sometimes interwoven with a *nodular* pattern. These areas can often be interspersed with physiological, radiolucent lung areas (Lamb and O'Callaghan, 1989).
Despite the fact that many pulmonary diseases start to develop in the interstitium, it may easily occur that an interstitial lung pattern is physiological and does not have pathognomonic value (Butler et al., 1993).
This is why the interstitial lung pattern is the commonest cause that leads to a false diagnosis. This happens when the radiograph is taken at the end of expiration or when an increase in abdominal volume occurs, or if the lung of a foal lying on one side become relatively atelectatic (Lester and Lester, 2001).

Alveolar pattern. The development of this pattern may be caused by cellular infiltration, infiltration of the lung by blood or water, the absence of air or by the combination of all these three factors. The result is a homogeneous increase in the radiodensity of the lung tissue. Initially the borders of the lung lobes become opacified (a cloud-like shadow develops). The background is much more radiodense (whitish or of increased opacity), and the details of the vascular pattern disappear. In some cases, radiolucent bands appear against a surrounding radiodense grayish-white alveolar area; these bands represent smaller or larger bronchi still filled with air. These radiological signs are designated as air bronchograms (Lamb et al., 1990, Lester and Lester, 2001).

Bronchial pattern. The bronchial pattern is not typical of neonates but may appear in young foals and, therefore, its knowledge is important for the correct evaluation of thoracic radiographs. The diseases of bronchi may be acute or chronic, and of allergic or inflammatory origin. The bronchial pattern may result from fluid accumulation or cellular infiltration in the wall of the bronchus or in the peribronchial space. Because of the thickening of the bronchial walls and the constriction of airways, this pattern resembles a doughnut in cross section and a tube with thickened walls in longitudinal section.
The linear pattern of the bronchi is the most easily distinguishable at the base of the heart. Chronic diseases are characterized by the development of mineralization or bronchiectasis. The peribronchial pattern is usually less pronounced, and results from infiltrative processes taking

place around the bronchi (Toal and Cudd, 1986; Lamb and O'Callaghan, 1989; Bedenice et al., 2003).

If these changes are accompanied by pathological processes involving the interstitium, this is termed as bronchointerstitial pulmonary disease. Some authors discuss this pattern as a type of the interstitial pattern (Bedenice et al., 2003).

1.1.2.4 **Other diagnostic imaging modalities**

1.1.2.4.1 **Scintigraphy**

Since the introduction of scintigraphy as a diagnostic imaging tool in the 1960's (Croll, 1994), nuclear medicine has provided a wide range of sophisticated methodologies to study the physiology and disorders of the human lung. Air distribution from conducting airways to parenchyma called lung ventilation and blood distribution through the pulmonary artery ramifications called lung perfusion may be studied with scintigraphy. In contrast to other pulmonary function tests that measure the function globally, lung scintigraphy has the unique capability to provide topographical analysis of the lung function. Furthermore, the ventilation-perfusion relationship may be determined. Scintigraphy is also used for alveolar clearance measurements, where the integrity of the alveolar epithelium membrane can be assessed by measuring its permeability to 99mTc-DTPA. When determining of the involvement of inflammatory cells in lung diseases neutrophils, eosinophils and platelets are labelled with 111-In or 99mTc (Coyne et al, 1987; Daniel et al, 1992). The most common scintigraphic methods to detect pulmonary infection and/or inflammation are Gallium-67 (67-Ga) citrate and labelled leukocytes scanning. Mucociliary clearance is a non-specific mechanism that aims at keeping the lung free from environmental contaminants (e.g., bacteria, aeroallergen, dust). This defence mechanism may also be studied by scintigraphy. The inhaled route for respiratory drugs has gained a major interest for treating equine respiratory disorders (Duvivier et al., 1997). Scintigraphy allows to visualise and quantify the distribution of radioactive aerosol within the different parts of the respiratory tract. Therefore, it might significantly advance the technique of aerosolised drugs (Votion, 2002).

1.1.2.4.2 **Computer tomography and magnetic resonance imaging**

The size of the animal over 150 kg makes these modern diagnostic imaging techniques impracticable for examination of the thorax. General anaesthesia necessary to perform these examinations further complicates the methodology and poses an increased risk for complications in respiratory distress.

1.1.3 **Pulmonary function tests**

With the recent view that lung disease is widespread and impacting significantly on performance in horses (Viel, 1997), there is a strong need for screening tests that address these problems. Typical respiratory signs that prompt these investigations include chronic or intermittent cough, excess secretions, or exaggerated respiratory rate or effort during exercise or recovery. Lung function tests are used to determine the functional significance of these specific respiratory signs in particular to differentiate obstructive from non-obstructive conditions (Hoffmann, 1997). In horses with more overt signs of lower airway obstruction (i.e., heaves), lung function testing has a different role. They are used to assess disease severity, bronchodilator effects, breathing strategy, and the basis for refractory cases. More recently there is growing interest for the early detection of lung disease in horses with poor performance, which do not exhibit respiratory signs. Highly sensitive lung function tests can help detect lung disease in these competitive horses and aid in developing a unique strategy for treatment where there are drug restrictions.
There are many different types of tests that have been applied to the horse for research or clinical applications, however most of these tests require specialized equipment and personnel (Derksen and Paradis, 1999). These include tests of diffusion capacity (Aguilera-Tejero et al., 1994), lung volumes - functional residual capacity (FRC) by nitrogen washout (Gallivan et al., 1990) or helium dilution (O'Callaghan, 1991), ventilation-perfusion inequality (O'Callaghan, 1991), alveolar clearance (Votion et al., 1999), muco-ciliary clearance (Willoughby et al., 1991), collateral ventilation (Robinson and Sorenson, 1978), indirect calorimetry (Morris, 1991), and mechanics of breathing and ventilation during exercise (Morris, 1991), for example. These studies have revealed important species-specific information concerning gas exchange and mechanical function in equids, with and without severe obstructive lung disease (heaves or

RAO). Unfortunately, there are few studies concerning pulmonary function in low-grade sub-clinical respiratory disease, the kind of dysfunction observed in competitive horses that we are often asked to evaluate.

Lung mechanical tests are the most direct approach to the diagnosis of lower airway dysfunction as a cause of poor performance. Many performance horses will not be able or willing to perform high intensity exercise on the treadmill, so tests of lung mechanics at rest, as in humans, has become an important addition to the performance evaluation (Hoffmann, 2002).

1.1.4 **Atropine test**

Reduction of respiratory distress after administration of a bronchodilator confirms the presence of bronchospasm, the major cause of airway obstruction in heaves. Intravenous atropine (0.02 mg/kg) should relieve respiratory distress within 15 minutes in a horse with recurrent airway obstruction or summer-pasture associated recurrent airway obstruction. A single atropine dose is safe but, the dose should not be repeated or there is a risk of intestinal stasis. In rare cases, horses with chronic interstitial pulmonary disease will present with classical signs of heaves but these animals will not respond to a bronchodilator (Robinson, 2002).

1.2 **Objectives**

Our objectives were:

1. to assess the types and frequency of ancillary diagnostic techniques used routinely by first opinion veterinarians when evaluating chronic respiratory cases
2. to evaluate the diagnostic value of different techniques and examination types routinely used in the diagnostic workup of chronic equine lower airway cases in both stable and clinical circumstances.
3. to estimate the prevalence of different chronic equine lower airway diseases among horses admitted to a Hungarian referral clinic.
4. to test the usefulness of thoracic radiography during the postpartum adaptation period to visualize the clearance of fetal lung fluid.
5. to establish the earliest time when normal foals have clear, radiolucent lung fields on thoracic radiographic images.
6. to characterize the radiographic pattern of this clearance.

Chapter 2. Diagnostic approaches for the assessment of equine chronic pulmonary disorders

(Kutasi, O., Balogh, N., Lajos, Z., Nagy, K., Szenci O.: Diagnostic approaches for the assessment of equine chronic pulmonary disorders. Journal of Equine Veterinary Science, accepted with revision for publication)

2.1 **Introduction**

After establishing a definite diagnosis in as many pulmonary cases as possible, a significant number of horses are always left with no definitive diagnosis even when using current understanding and available ancillary diagnostic techniques (Dixon et al., 2003). Although an accurate history and especially bronchoscopy can confirm the presence of pulmonary disease, pulmonary cytology forms a mainstay for diagnosing the specific chronic pulmonary disease using the criteria previously described in the literature (Dixon et al., 1995).

Chronic lower airway disorders can be of several origins such as allergy, hypersensitivity, infections, toxicity, loss of pulmonary vascular integrity, or neoplasia. One of the most commonly diagnosed chronic lower airway diseases is recurrent airway obstruction (RAO) (Dixon et al., 1995), which is believed to be caused by an allergic reaction to inhaled molds and shares similarities with the non-eosinophilic forms of asthma in humans (Ward and Couëtil, 2005). Airway obstruction, inflammation, mucus accumulation, and tissue remodeling have been shown to contribute to the pathophysiology of RAO (Lavoie, 2007). Airway obstruction causing typical labored breathing is reversible by controlling dust in the environment or using bronchodilators (Lavoie, 2007). A mild form of lower airway inflammatory disease commonly encountered in young athletic horses has been recognized as a separate entity from RAO and is termed inflammatory airway disease (IAD) (Moore et al., 1995; Robinson, 2001; Couëtil et al., 2007). In the majority of cases, RAO and IAD may be differentiated on the basis of clinical grounds; however, some have argued that, over time, horses with IAD may progress into RAO (Viel, 1997; Couëtil, 2002). In the pathogenesis of IAD, a variety of etiological agents might be involved, such as respirable organic and inorganic particles in stable dust (Holcombe et al., 2001), immunological factors, and infectious agents (Couëtil et al., 2007; Wood et al., 2005). Although IAD is a non-septic inflammation of the lower airways without any evidence of systemic signs of infection, in a previous study, a clear association was demonstrated between some infectious agents and the prevalence of IAD (Wood et al., 2005). Infections causing lower airway disease in adult horses include viral, bacterial, fungal, and parasitic agents, and they more typically occur after a predisposing effect that suppresses pulmonary immunity like long-distance transport or strenuous exercise, resulting in systemic signs (Rush and Mair, 2004). Exercise-induced pulmonary hemorrhage (EIPH) occurs in the majority of racehorses and is observed sporadically in many other sport horses that require strenuous exercise for short periods of time (Rush and Mair, 2004; Couëtil and Hinchcliff, 2004). Proposed pathophysiological mechanisms include high pulmonary vascular pressures during maximal exercise as well as pulmonary inflammation or obstruction of the upper or lower airways (Cook et al., 1988; Langstemo et al., 2000; Rush and Mair, 2004). Other lower airway disorders like granulomatous, neoplastic diseases, or interstitial pneumonias are relatively rarely diagnosed in horses (Rush and Mair, 2004). Differentiation between the above listed lower airway respiratory disorders on the basis of their flexible and ambiguous definitions can sometimes be difficult or even impossible. Clinical signs and etiology may overlap, or one of these disorders may induce the other. Since treatment and prognosis can significantly differ, an appropriate diagnosis is always necessary.

Our objectives were to evaluate the diagnostic value of different techniques and examination types used routinely in the diagnostic workup of chronic equine lower airway cases by field veterinarians and in clinical circumstances. Another aim of this study was to estimate the prevalence of different equine lower airway diseases among horses admitted to a Hungarian referral clinic.

2.2 **Materials and methods**

The retrospective study was performed at the Clinic for Large Animals, Faculty of Veterinary Science, Szent Istvan University between July 2005 and August 2008. In total, 100 horses (25 stallions, 39 geldings, and 36 mares) of different breeds—61 Hungarian Half-breeds, 10 other European Half-breeds, 9 Lipizzaner, 5 Friesians, 4 Thoroughbreds, 4 ponies, 4 Arabians, and 3 American Breeds— and age 1-17 years (mean 9,1 ± 2,8 years) with chronic respiratory symptoms like cough, nasal discharge, dyspnea, or poor performance were involved in this study. Chronicity of at least 4 weeks was the minimum requirement for inclusion in the study.

Most of the patients (76%) were referred for a second opinion. The same standardized examination protocol was followed in all cases.

Examination protocol

History

A special questionnaire was developed for taking the history. Breed, age, gender, usage of the horse and a complete history with presenting signs, disease process, duration and type of previous treatments and stabling conditions were recorded. Then based on these data a simple scoring system was established to evaluate the stabling technology and disease process for statistical analysis (**Table 1**). The month of clinical admission for examination and the month/s of disease establishment or exacerbations were noted. Referring surgeons were also questioned about the diagnostic techniques that they used in each particular respiratory case and also about their suspected diagnosis.

Table 1. Simplified history questionnaire focusing on differentiation between environmental induced and infectious disorders

Score	Duration of disease	Course of disease	Stabling	Infection	Treatment with steroid anti-inflammatory drug
0	>4 weeks	continuous signs	pasture	fever, companion animals were affected	no or negative reaction
1	>6 weeks		hypoallergenic bedding and soaked hay		no treatment
2	>8 weeks	remission-exacerbation	simple stabling	no fever, no other horse affected	positive reaction

Clinical examination

A general physical examination was carried out about 60 minutes after the horse arrived at the clinic. The main findings with regard to the respiratory tract were evaluated with clinical scores on the basis of the methods developed by Naylor et al. (1992) and Traub-Dargatz et al. (1992) with slight modifications (**Table 2**). The sum of the numbers assigned to the different symptoms was used to generate the general clinical severity score.

Table 2: Clinical severity scoring system (according to Naylor et al. 1992, and Traub-Dargatz et al.1992 with modifications)

Score	Respiratory rate	Respiratory effort	Lung auscultation	Cough	Nasal discharge
0	<20	no	normal	no	no or serous
1	20-30	increased	increased bronchial sounds	induced, strong	mucinous
2	30<	expressed intercostals muscle contraction and abdominal lift	local wheezes and crackles	spontaneous, frequent or bouts	mucopurulent
3		flared nostrils and anal movement	generalized wheezes and crackles or reduced lung sounds despite deep breath		

Respiratory tract (RT) endoscopy

In the majority of the cases, RT endoscopy (CF-VL, Olympus GmbH, Hamburg, Germany) was performed without sedation to obtain the most information about the function of both the lower and upper airways. In noncooperative animals, sedation with detomidin (10 µg/bwt; Domosedan inj.; Orion Pharma, Espoo, Finland) in combination with butorphanol (10 µg/bwt; Alvegesic inj.; Alvetra u. Werfft GmbH, Wien, Austria) was used. The nasal passages, pharynx, larynx, and guttural pouches were inspected and the upper respiratory tract (URT) was evaluated with score 0 if negative and with score 1 if any functional disorder was suspected. The volume of the respiratory secretion (RS) present in the cranial thoracic trachea was semi-quantitatively described according to the grading system by Gerber et al. (2004). The nature of the RS was also recorded as mucoid, mucopurulent, purulent, or hemorrhagic. Tracheal and bronchial respiratory mucosa was also examined for evidence of inflammation, i.e., for bluntness of the normally sharp carina and for the presence of hyperemia. End expiratory bronchoconstriction or bronchial collapse was also noted.

Respiratory (tracheal) secretion cytology and culture

RS was collected transendoscopically through the work channel using a sterile 2 m long plastic catheter (PW1V, Olympus GmbH, Hamburg, Germany). Within 1 hour of collection, an air dried smear of RS was prepared and fixed with a fixative, and a differential cell count of 100 cells was performed on a Diff-Quick (Reagens Kft., Budapest, Hungary) stain preparation. We sent sample for bacteriology when secretion was macroscopically considered purulent or the history had described a previous suspected respiratory tract infection or results of clinical examinations were suspicious of infectious origin. Samples were injected to a transport media and sent for culturing to a specialized veterinary microbiology laboratory.

Bronchoalveolar lavage fluid (BALF) cytology and culture

In each case, BALF was obtained via a BIVONA catheter (Bivona Medical Technologies Inc., Gary, USA) under sedation as previously described. To reduce the physical irritation of the mucous membrane, 0.5% lidocaine solution (Lidokain inj.; Richter Gedeon Nyrt., Budapest, Hungary) was sprayed on the carina, and then 350 ml of lukewarm saline was instilled and aspirated. The amount of fluid gained back, its transparency, color, and the presence of a foamy layer were recorded. Within 30 min of collection, BALF cytospin cell preparations were made. Romanowsky stain (Diff-Quik; Reagens Kft., Budapest, Hungary) was used, while keeping in mind that this stain has been found to be inadequate for detecting pulmonary mast cells (Hughes et al., 2003, Leclere et al., 2006). Differential cell counts were performed on 300 cells by a board certified clinical pathologist blinded to the clinical and endoscopic findings. Values given by Derksen et al. (1989) were used as references.

In 67 cases supplementary laboratory examinations (48/67), further diagnostic imaging procedures (67/67) or bronchodilator test (10/67) with 0,02 mg/kg intravenous atropine (Atropinum sulfuricum inj., Egis, Budapest, Hungary) were performed (**Table 3**).

Table 3. Supplementary diagnostic procedures in selected cases. (performed as described by Lekeux P. et al. (2001))

Type of examination	**Number of tested animals**	**Indication**
Thoracic radiography	51	moderate or severe clinical signs
Arterial blood gas analysis	43	moderate or severe resting dyspnoea or tachypnoe
Thoracic ultrasonography	35	distorted lungborders on percussion or positive thoracic radiography
Hematology	20	history of fever, depression or weight loss
Serology	12	history of fever or more horses affected simultaneously nearby
Culture on BALF	12	history of fever or suspected respiratory infections or diffuse abnormal lung patterns on thoracic radiographies
Bronchodilator (atropine) administration	10	severe dyspnoea
Treadmill endoscopy	6	supposed dynamic URT disorders based on history or resting endoscopy findings
Molecular diagnostic tests	3	fever, non responsive to antibiotic treatments and interstitial radiographic pattern
Lung biopsy	1	non responsive to any treatment, nodular interstitial radiographic pattern

Diagnostic criteria used to classify cases

Recurrent airway obstruction (RAO/Heaves)

RAO was defined as chronic neutrophilic pulmonary inflammation associated with the presence of hay and/or straw in the affected horses' environment and with clinical manifestations varying from mild cough to severe dyspnea at rest. The BAL fluid of horses with RAO showed moderate to severe neutrophilia (>20% cells) and decreased lymphocyte and alveolar macrophage counts (Derksen et al., 1985, Couëtil et al., 2001). Summer pasture associated RAO (SPA-RAO) is clinically indistinguishable from RAO except that affected horses develop signs while maintained on pasture (Couëtil et al., 2007).

Inflammatory airway disease (IAD)

By definition, horses with IAD might show poor performance, exercise intolerance, or coughing, with or without excess tracheal mucus, but without showing depression, fever, or increased respiratory efforts at rest (Couëtil et al., 2007). It is common in young racehorses and decreasing in frequency with increasing age (Wood et al., 2005) but non-racehorses of all ages can have IAD (Couëtil et al., 2001; Couëtil et al., 2007). The most commonly encountered BAL fluid cytologic profiles are characterized by increased total nucleated cell count with mild neutrophilia, lymphocytosis, monocytosis Moore et al, 1995; Couëtil et al., 2001; Couëtil et al., 2007), or eosinophilia (Hare and Viel, 1998; Hoffman, 1999). Although neutrophilic inflammation is commonly observed in BAL fluid from horses both with RAO and IAD, the neutrophilia is usually less pronounced in cases of IAD (i.e., <20%).

Infectious disorders (ID)

Manifestations of infection such as lymphadenitis, fever, depression, decreased appetite, and weight loss are usually present in lower airway diseases of bacterial, viral, fungal, or parasitic origin (Couëtil et al., 2007). Diagnosis is based on a positive culture with concurrent suggestive cytological findings (intracellular bacteria or fungal spores and signs of neutrophilic degeneration, like swollen nuclei or karyolisis) of tracheal wash fluid or a rise in antibody titer over the course of the disease within 14-21 days in suspected viral infection or a positive result of other molecular diagnostic tests.

Upper respiratory tract functional disorders (URTFD) with small airway inflammation (SAI)

Upper airway endoscopy at rest or during exercise allows for the identification of significant upper airway diseases. Concurrent abnormal BAL cytology reflects lower airway inflammation.

Horses with mild URTFD, expiratory dyspnea at rest, and BAL cytology of neutrophils of more than 20% were classified as RAO cases and URTFD was evaluated as coincidence findings.

Exercise Induced Pulmonary Hemorrhage (EIPH)

Exercise-induced pulmonary hemorrhage occurs primarily in horses performing short periods of high intensity work. The diagnosis is based on finding blood upon bronchoscopy (Raphel et al., 1982) or by detecting increased hemosiderin content within alveolar macrophages (Fogarty, 1990; Richard et al, 2010).

Chronic interstitial lung diseases of non-infectious origin and neoplasia

The interstitial lung disease is generally unresponsive to antimicrobial and anti-inflammatory therapy. Thoracic radiographs commonly show severe, diffuse or nodular interstitial pattern. A trans-thoracic lung biopsy is the definitive test for diagnosing chronic interstitial lung disease or neoplasia (Nolen-Walston, 2002).

Undifferentiated pulmonary disorders

This group was composed of animals with detectable pulmonary disease where the diagnosis did not fall clearly into any of the above categories.

Statistical Analysis

To compare the history (Table 1) of horses with or without RAO, the Fisher test was used. To evaluate the usefulness or necessity of the different examination types used in the diagnostic workup of chronic equine lower airway and pulmonary cases, data were analyzed by using conditional inference tree methods (Nagy et al., 2010). First, we summed the historical and the clinical scores separately (scores are presented in Tables 1 and 2) and used these two new variables along with age, gender, and breed in a conditional inference tree model, which basically represents the decision making paradigm frequently used in field veterinarian practice. Then, we added all the measured variables (age, gender, breed of the horse, historical data listed in Table 1, month of admission, clinical parameters listed in Table 2, respiratory tract endoscopy, respiratory secretion cytology and bacteriology, BALF cytology, arterial blood gas and pH measurements, and x-ray and ultrasound findings) into another conditional inference tree model to see how much the decision making rule might improve by using these ancillary tests.

Conditional inference trees were constructed with c_{quad}-type test statistics and α = 0.10 with simple Bonferroni correction. Each split needed to send at least 3% of the observations into each of the two child nodes. All analyses were carried out using the R 2.7.2. Statistical Software (R Development Core team, 2007).

2.3 **Results**

Overall, out of the 100 horses used in this study, 76 cases were referred by 45 veterinarians for a second opinion. They performed physical examination in all cases. Respiratory tract endoscopy was carried out only in 22 cases with taking tracheal sample for culture and for cytology in 20 and 8 cases, respectively. Blood was taken for hematology on 20 occasions, and BALF sent for cytology on 6 occasions. Suspected diagnoses by field veterinarians were heaves (49/76) or respiratory infection (12/76) while the rest of patients were referred without any previous diagnosis.

Based on the BAL cytology, all of the examined 100 horses had some type and degree of lower airway disorder.

The case selection comprised horses with RAO (n = 54), IAD (n = 20), infectious pulmonary disease (n = 9), URTFD with SAI (n = 13, which consisted of idiopathic left laryngeal hemiplegia (n = 4), dorsal displacement of the soft palate (n = 4), pharyngeal collapse (n = 1), tracheal collapse (n = 1), subepiglottic cyst (n = 1), fourth branchial arch defect (n = 1), and arytenoid chondritis (n = 1)), and undifferentiated cases (n = 4). We did not group any animal as primary EIPH case, but we had horses with erythrophages in their BAL in all other groups except the undifferentiated one. During the examined period we did not diagnose any neoplasia or interstitial lung disease of non-infectious origin.

Chronic pulmonary disorders were more likely to be diagnosed during the warm months (87% of the cases were diagnosed between March and November), and most horses started to show symptoms or had exacerbated clinical signs also during this period. The distribution of the onset dates shows a trend for three main peaks during the year for both RAO and IAD patients: one peak in the beginning of spring, one second smaller peak in the middle of summer, and another peak at the end of the summer **(Figure 11)**. Clinical admission dates clearly follow the peaks of onset.

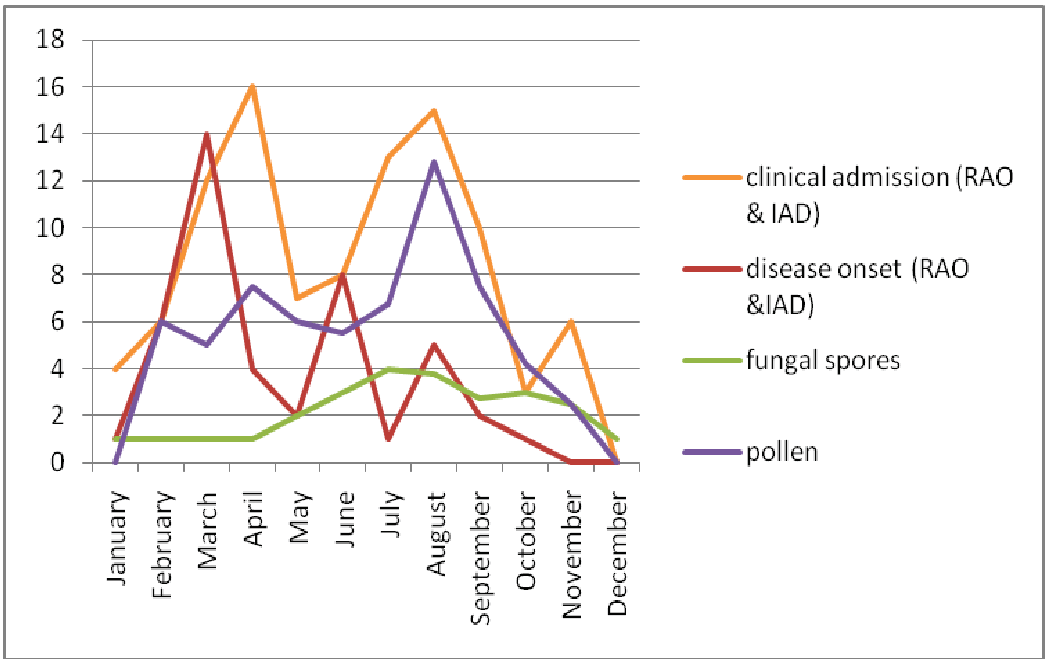

Figure 11. The time- distribution of disease onset, clinical admission and airborne pollutants (fungal spores, pollen).

The mean (SD) time span between the onset of the disease and the clinical admission was 4.4 (3.7) months, during which 66% of the animals were not treated at all or treated with no success (19%)

Horses were used for pleasure riding (48%) or sports (52%), and the majority of them (71%) were kept in stables with the traditional Hungarian stabling technology of feeding dry hay and bedding with straw. Some owners (24%) had already implemented changes in this technology and introduced new hypoallergenic materials for bedding and used soaked hay. Only five horses, all of them diagnosed with RAO, were kept on pastures, but these animals also had supplementary dry hay almost year round.

Horses with RAO were significantly older compared to horses with IAD ($p<0.001$), URTFD ($p=0.022$) or ID ($p<0.001$). The average (SD) ages were 10.8 (2.7), 6.3 (1.4), 8.3 (3.8) and 6.0

(3.3) years, respectively. Horses with IAD, URTFD or ID did not differ significantly from each other regarding age. Horses with RAO were 3.4 times more likely to have a duration of respiratory symptoms for more than 8 weeks (Fisher test, p=0.022), were 5.0 times more likely to show remission-exacerbation (Fisher test, p=0.002) and were 3.4 times more likely to show no fever (Fisher test, p=0.023) compared to horses diagnosed with other chronic pulmonary disorders.

Regardless of the final diagnosis, the most common presenting clinical sign was cough (63%), and the least common was poor performance (10%). Nasal discharge and dyspnea were recorded in 41% and 40% of the cases, respectively.

The result of the first tree model (**Figure 12**), where we used data usually available through history questionnaire and physical examination carried out by field practitioners in the classification of horses suffering from pulmonary disorders, suggests that horses with RAO will most likely to be found among horses with summed clinical scores greater than 4 and summed historical scores greater than 4. According to this tree model, 38 of the 54 RAO horses and 5 out of 9 ID horses could possibly be classified correctly as RAO or ID patient. However, 13 of the 46 non RAO patients were also classified with this tree model as horses suffering from RAO. None of the 20 IAD and 13 URTFD cases was classified correctly by this model.

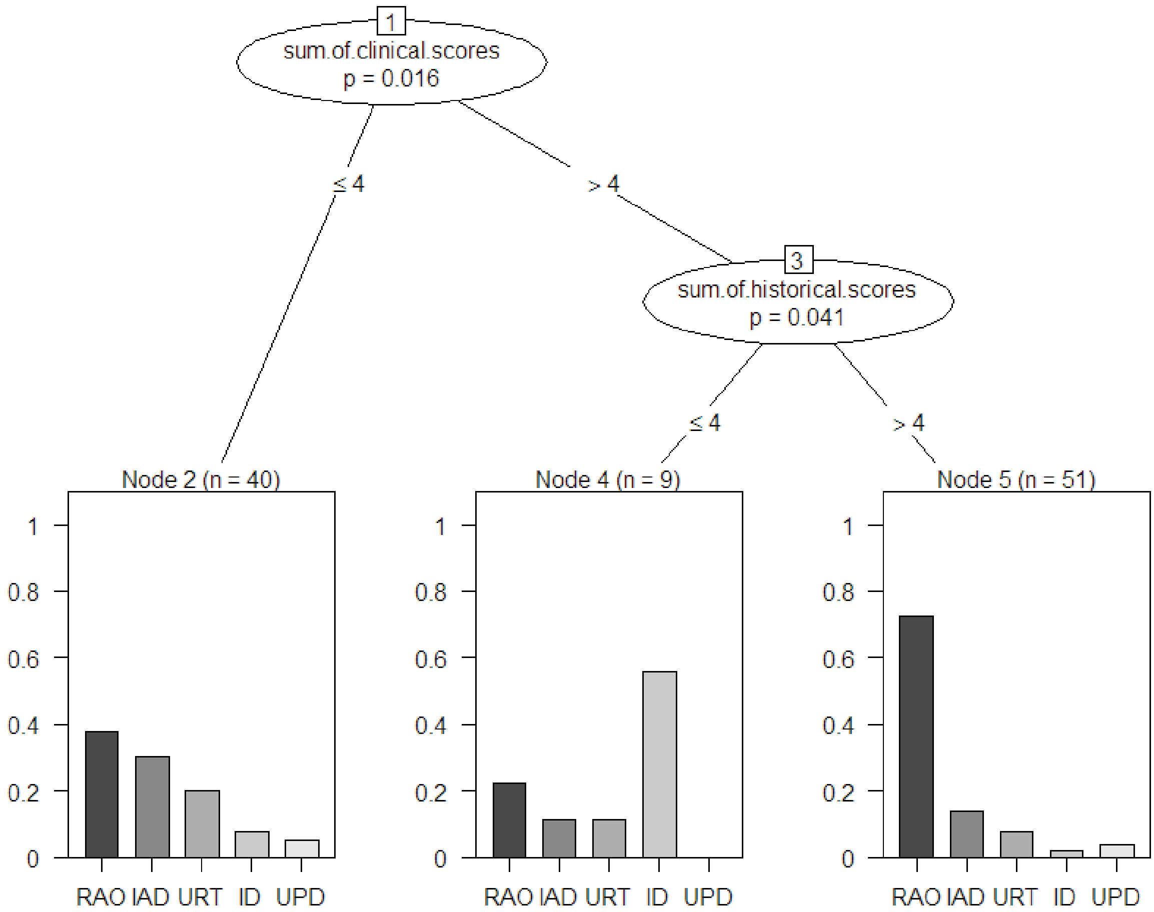

Figure 12. Conditional inference tree built by using data usually available through physical examination and history. RAO: Recurrent Airway Obstruction, IAD: Inflammatory Airway Disease, URT: Upper respiratory tract functional disorders with small airway inflammation, ID: Infectious disorders, UPD: Undifferentiated pulmonary disorders.

For the second tree model we added the data of ancillary diagnostic procedures (respiratory tract endoscopy: URT scoring and tracheal mucus grading, tracheal secretion cytology and bacteriology, BALF cytology, arterial blood gas measurements, as well as thoracic x-ray and ultrasound). Results of URT endoscopy, neutrophil percentage in the bronchoalveolar lavage fluid, history of previous infection and age variables were selected as the main diagnostic criteria by the model (**Figure 13**).

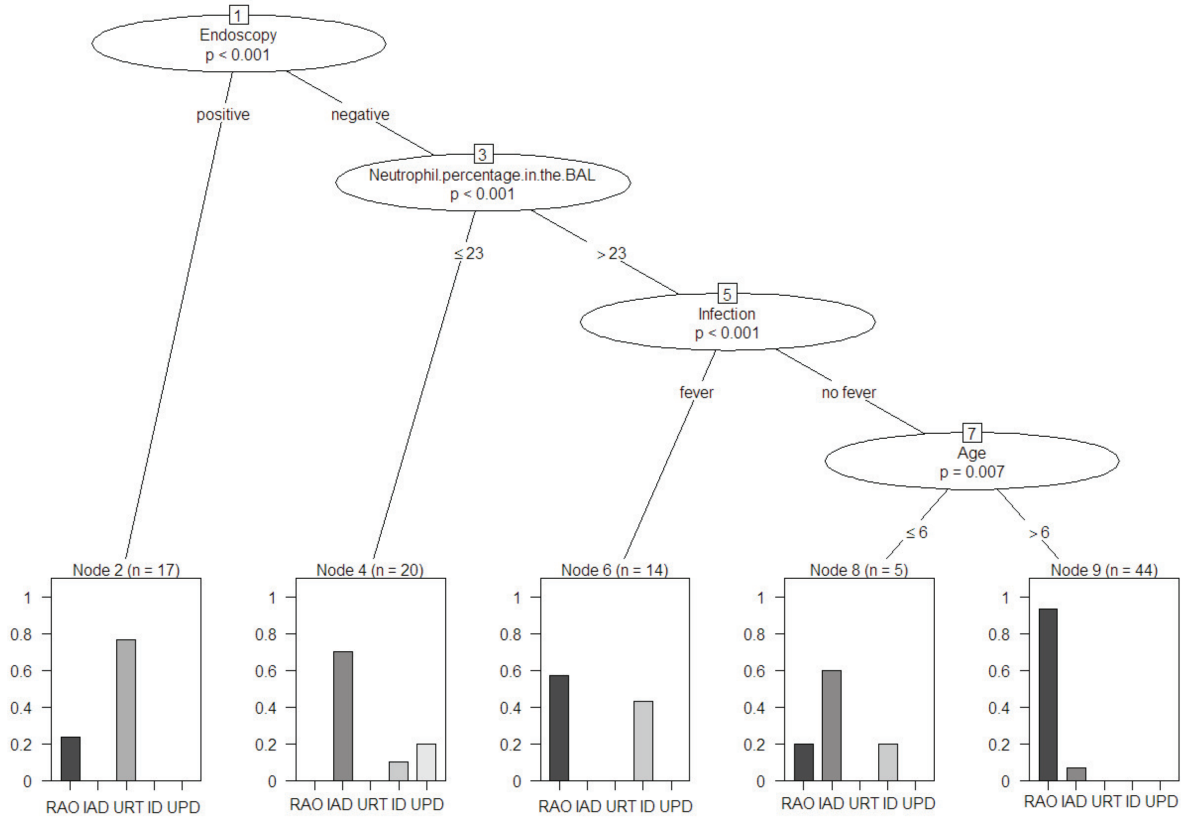

Figure 13. Conditional inference tree built by using data when added the results of ancillary diagnostic procedures. RAO: Recurrent Airway Obstruction, IAD: Inflammatory Airway Disease, URT: Upper respiratory tract functional disorders with small airway inflammation, ID: Infectious disorders, UPD: Undifferentiated pulmonary disorders

The first splitting criteria of the tree model resulted in a group of horses, where the endoscopy was positive for URTFD. 76.5% of these horses had URTFD and 23,5% was diagnosed with RAO. All of the URTFD horses, but only 7% of the RAO horses belonged to this group.

The second most important grouping variable was the neutrophil percentage in the bronchoalveolar lavage fluid. Where the endoscopy was negative and neutrophil granulocyte percentage was below 23%, none of the horses were diagnosed with RAO.

Among horses that had negative URT endoscopy and a neutrophil granulocyte percentage greater than 23%, RAO was the most prevalent if horses had a history without fever and were older than 6 years (93% of the horses suffering from RAO were found in this group).

Within the group of horses with negative URT endoscopy, a neutrophil granulocyte percentage greater than 23% and a history of fever, 57% of horses were diagnosed with RAO and 43% of them with infectious disorders (in total, 15% of horses diagnosed with RAO and 67% with ID were found among this group of horses).

With the help of this tree model, 41 of the 54 RAO horses, 14 of 20 IAD horses and all 13 URTFD horses were classified correctly as RAO, IAD or URT patient. Only 3 of the 46 non RAO patients were classified as horses suffering from RAO. None of the 9 ID cases was classified correctly by this model.

Due to the small sample size, we were not able to present statistically significant correlations, but the results of further laboratory, diagnostic imaging, and other supplementary findings giving relevant information are presented in **Table 4.** Finally 4 cases remained undifferentiated.

In contrast to this, 40% of RAO and 75% of ID patients were eventually misclassified by the field practitioners previously.

Table 4. Additional findings of laboratory, diagnostic imaging and other supplementary procedures

Type of Examination	**Findings**	**Number of positive cases / final diagnosis**				
		RAO	**IAD**	**ID**	**URTFD with SAID**	**undifferentiated**
Bronchoscopy	bronchoconstriction	9		1		1
	food particles in trachea				4	
	hemorrhagic mucus in trachea			2		
Tracheal cytology	septic inflammation	2		10	2	
Tracheal culture	Str. zooepid.(n=4), Str. equi(n=2), Klebs. pneum.(n=1), Actin. equuli(n=1), Staph. aureus(n=1)	2		6	1	
BALF cytology additional	hemosiderophages	1	2	2	1	
	intracellular plant or	4		2	5	

findings	pollen particles					
BALF culture	Str. zooepidemicus (1)			1		
Thoracic radiology	severe bronchial pattern	14				1
	increased radiolucency with concave diaphragm	3				
	bronchiectasis	2				
	increased interstitial-bronchial pattern	6		8	2	3
	increased interstitial pattern		2	1		
Thoracic ultrasonography	caudally displaced lungborders	5				
	cranially displaced lungborders			1		1
	comet tail echoes	5		8	3	4
	subpleural hypoechogenic areas			4		
Bronchodilator administration	positive	7				
	negative	1				2
Serology	EHV4 (n=2), equine virusarteritis (n=1)			2	1	
Hematology	anemia			1		
	lymphocytosis			1		
	eosinophilia					1
PCR	EHV5			1		
Lung biopsy	nodular fibrosis			1		

2.4 **Discussion**

According to our study, the most frequently occuring chronic pulmonary disorders in Hungary are of non-infectious origin, principally RAO, which is similar to previous data about horses presented for evaluation to North American (Rush and Mair, 2004) and British (Dixon et al., 1995) referral clinics. RAO and IAD have been reported worldwide, but their incidence is highly variable and may depend on regional climatic factors, in particular temperature and precipitation (Ward and Couëtil, 2005). The incidence of RAO is reported to be high in countries with a cool and wet climate and low in regions with a warm and dry climate (Bracher et al., 1991; Robinson et al., 1996; Ward and Couëtil, 2005). Although Hungary has a relative dry and warm continental climate compared to other western and middle European countries, RAO seems to be a very common respiratory disorder.

Regardless of the initiating cause, the most clinical admissions with respiratory disorders took place during the warm months, between March and November, when stables were open and most horses had some limited access to paddocks and pastures.

Seasonal occurrence can also be a typical feature of some diseases. As RAO is associated with exposure to hay and straw, as was previously described, it should be more common when horses are stabled during the winter (Bracher et al., 1991, Davis and Rush, 2002; Rush and Mair, 2004). Interestingly, in our study, RAO had a higher prevalence during the spring and summertime as compared with the winter season. Conversely, Couetil et al. (2003) described that horses were 1.6 and 1.5 times as likely to be examined for RAO during the winter and spring, respectively, than they were during summer. In our study, the rapidly increasing number of cases in early spring coincides with the rise in pollen levels, the peak in June follows the rise in the outdoor measured air-borne mold content, and the third peak overlaps the highest level of pollen particles (nepegeszseg.net, 2007). Although most of these horses were maintained in stable environment, we could not exclude that SPA-RAO induced by pollen allergy and other outdoor aeroallergens might coexist in such cases. Ward and Couetil (2005) have also described that the prevalence of RAO correlated with outdoor aeroallergen levels. According to the literature, however, RAO occurs during the winter or early springtime when horses are kept in closed stables and fed with dry hay (Robinson et al., 1996; Robinson, 2001; Rush and Mair, 2004, Ward and Couëtil, 2005). As evidenced by our results, strictly following the literature data with respect to the seasonal prevalence of RAO may result in a false diagnosis. Concurrent SPA-RAO complicates both evaluation and management of these horses and is a significant problem in Hungarian climatic and geographic conditions. Five horses in the RAO group were kept on pasture during the whole year and experienced exacerbation during the warm months, but the original onset of the disease occurred when these horses had previously been maintained in a stable environment. Although the field veterinarian directed these patients to pastures, unfortunately, environmental control was not successful in these cases because the climate of Hungary makes hay supplementation necessary even during the summer months. Both outdoor aeroallergens and dry hay supplementation might be responsible for these exacerbations. An unsuccessful management change in the history might complicate the establishment of a diagnosis.

As described in other studies, weeks to months may pass between the onset of clinical signs in the field and the time of clinical admission (Dixon et al., 1995; Couëtil and Ward, 2003). This might also influence our results regarding seasonal prevalence. Generally, more than 4 months passed during which the horses started representing clinical signs but were not examined, diagnosed, or treated at all. Subtle clinical signs usually do not alert owners and delay veterinary examination. This time delay might complicate diagnostic workup in several cases but makes RAO differentiation easier with recorded exacerbation-remission periods and relatively longer disease duration.

Details in the history about antecedent respiratory infection were not an exclusive feature of the cases in the ID group. We had horses in the IAD group that were referred as cases with suspected complications after some infection. Dixon et al. (1995) described that 19.2 % of COPD affected horses had an infection immediately preceding the current respiratory disorder; in addition, according to Couetil (2002), owners often report a history of infectious respiratory disease in the months preceding the diagnosis of IAD with several horses in the stable being affected. The role of infectious agents in the development of RAO and IAD is still not clear (Lavoie, 2007; Couëtil et al., 2007).

Although age is a helpful parameter when identifying cases of heaves, we also had horses in this group as young as 6 years of age. On the other hand our IAD group consisted of older horses than generally described in the literature (Couëtil et al., 2007; Chapman et al., 2000).

First opinion veterinarians usually have limited possibilities to perform special laboratory or any diagnostic imaging techniques. Based on our study, a specific questionnaire regarding history and a thorough clinical examination can be reliable in diagnosing horses with RAO. Hotchkiss et al. (2006) has previously demonstrated the usefulness of a well-constructed questionnaire in discriminating between horses with and without RAO. Another study also emphasized that the majority of cases with heaves can be correctly diagnosed on the basis of physical examination (Naylor et al., 1992)]. According to our study, these cases with typical history and unequivocal clinical signs of heaves accounted for 70% of the RAO patients, which accounted for only 38% of all cases. This reflects other previous data indicating that history taking and the results of physical examinations were not sufficient to establish a respiratory diagnosis (Couëtil et al., 2001; Hoffman and Mazan, 2003). None of the clinical signs were typical for any disorder. More severe respiratory symptoms were suggestive for RAO or ID, but history could help to differentiate between them.

Performing BAL and evaluating the cytology sample had a great impact on carrying out a successful diagnostic workup. Increased neutrophil counts are the main diagnostic criteria for RAO, but cut off values vary greatly among publications depending on the BAL technique used or the population studied (Richard et al., 2010). Since we had no previous data concerning our technique and horse population we decided to possibly include patients in the RAO group with neutrophils of greater than 20%. Finally we realized that our cases with heaves had more than 23% of neutrophils in their BALF. This finding is in agreement with the proposal that more than 25% neutrophils in BAL are necessary for a horse to qualify as being affected with RAO (Robinson, 2001). Neutrophilia in the BAL sample was prominent in the RAO horses as well as most of the ID affected horses, which complicated differentiation on the basis of cytology. BAL cytology results combined with no previous febrile period history and the age of the horse resulted in the identification of most of the RAO patients.

Although lower airway disorders cannot be differentiated on the basis of respiratory tract endoscopy, endoscopy is the unique method to define URTFD (Davidson and Martin, 2003; Franklin et al., 2006). Simple respiratory endoscopy at rest selected all URFTD cases. In our caseload, none of the animals presented with suspicion of upper airway dysfunction. Cough, nasal discharge, and dyspnea can be caused by upper airway inflammation and obstruction as well (Couëtil et al., 2007; Holcombe and Ducharme, 2004). Interestingly all of the horses with URFTD also had abnormal BAL cytology results indicating small airway involvement, which might also be responsible for the clinical signs. Small airway inflammation in these cases could have possibly been caused by the altered airflow dynamics causing decreased mucociliary clearance and more negative pressure in the lower airway segments resulting in mechanical irritation or hemorrhage. Mild chronic aspiration and secondary infections could contribute to the disease. In a previous study about URT functional problems, the authors also suggested their predisposing role in lower airway inflammation (Brakenhoff et al., 2006). Depending on each individual case, we have found various cytological findings with different increased cellular ratios. Increased neutrophil and exfoliated epithelial cell ratios can be explained by mechanical or septic inflammation. Neutrophilia could be a sign of concurrent RAO as well. Aspiration of foreign material and bleeding can cause an increased number of macrophages (Hewson and Viel, 2002). Horses with DDSP had either high lymphocyte or high neutrophil numbers in BAL fluid, both of which might be a sign of chronic viral or bacterial infection. Chronic bacterial or viral infections can cause upper airway inflammation simultaneously and may result in impaired function of the soft palate (Holcombe et al., 1999; Rush and Mair, 2004). In these cases, small airway inflammation might result from URT dysfunction or simply be a concurrent finding. Holcombe et al. (2000) demonstrated that upper and lower airway inflammations were both associated with stabling, but there was no direct correlation between them.

We performed further ancillary diagnostic tests in 67 animals but according to the inference tree method they were not necessary for grouping them reliably in 67% of cases. These methods were useful only to refine the final diagnosis. In all other cases (22/100), a very thorough and complex diagnostic workup using special laboratory tests and diagnostic imaging techniques was necessary to reach the final diagnosis, thereby making it unachievable for the field veterinarian.

Radiographic and ultrasonographic (US) evaluation of the chest facilitated differentiating mainly infectious conditions; all of them in this group had abnormal lung pattern and US findings. As was described previously, these imaging techniques are helpful in differentiating between horses in group IAD and ID (Couëtil et al., 2007); however, in the absence of clinical evidence of more extensive, infectious disease, thoracic radiographs neither refine nor improve the diagnosis of IAD, but only increase diagnostic costs (Mazan and Hoffman, 2005). None of the radiologic findings were pathognomonic, not even in the ID group; thus, in each case, further diagnostic procedures were necessary. Caudally displaced lung borders, increased radiolucency with concave diaphragm, and bronchiectasis were sequelae of severe RAO as demonstrated earlier (Lavoie et al., 2003, Lavoie, 2007).

Septic tracheal cytology or positive culture did not mean that animals could simply be grouped in the infectious group. Two horses with positive culture were placed in the URTFD group based on

endoscopic results, and two horses with a secondary infection were placed in the RAO group based on history, clinical signs, and positive bronchodilator test. When infections complicated suspected RAO cases, bronchoconstriction, as one of the causes of the respiratory signs and its severity, was evaluated with the atropin test (Robinson, 2002). Bacteria are commonly detected in airways of horses affected with heaves and in many cases these findings are caused by impaired clearance due to RAO (McPherson and Thomson, 1983, Dixon et al., 1995; Couëtil et al., 2001). Further, infectious cases could be isolated based upon serology and PCR, but hematology did not prove to be reliable. Blood gas parameters did not differ significantly between groups, being quite useful for evaluating the evolution stage of the inflammatory process rather than in the diagnostic workup (Ferro et al., 2002).
Finally, 4% of the patients were left without a specific diagnosis. In such cases, conclusions were drawn from the response to different treatment protocols. In two cases, we had contradictive results with history and clinical signs being typical for RAO but BAL cytology showing a low number of neutrophils. These horses improved with steroid treatment. These cases could be horses suffering from RAO in remission but since they did not fulfill the criteria of RAO definition we had to handle them separately. This also points out the fact that horses with RAO in remission are difficult to evaluate and final diagnosis can only be based on the characteristic history and clinical signs. There were cases resembling infectious disorders, but all cytology, cultures, serology, and PCR examinations were negative. BALF cytology showed moderately increased neutrophil number and bronchoconstriction tests were negative. They responded well for rest, anti-inflammatory and long term antimicrobial treatment. In the above listed undifferentiated cases results of further ancillary diagnostic tests were not specific for any lower respiratory disorder. Inappropriate staining technique might also be responsible for some of the unidentified cases.
We did not have any primary EIPH case, probably because we had not got any racing thoroughbreds for examination and also other sports where EIPH is rather common such as cutting, reining, polo or cross-country event are not widespread in Hungary. Hemosiderophages were found in some horses with all other types of disorders secondary to inflammation or obstruction of the airways. Frequent concurrent finding of hemosiderophages in BALF and tracheal muco-purulent secretion had earlier been demonstrated and supports the hypothesis of

correlation between EIPH and lower airway inflammation (Derksen et al., 2007; Ferruci et al., 2009).

The single chronic interstitial lung disease diagnosed was equine multinodular pulmonary fibrosis, but since equine herpes virus 5 was detected with PCR in this case we classified it as infectious disorder. Other chronic interstitial lung diseases were not identified, so they seem to account for minimal percentage of respiratory cases.

Capter 3. **Radiographic assessment of pulmonary fluid clearance in healthy neonatal foals**

(Kutasi, O., Horvath, A., Harnos, A., Szenci, O.: Radiographic assessment of pulmonary fluid clearance in healthy neonatal foals. Journal of Veterinary Radiology & Ultrasound, 50:6, 584-588. 2009.)

3.1 **Introduction**

Immediately after birth, the airways of foals are rapidly cleared of liquid to allow the onset of air breathing. This initiates a cascade of physiologic changes that enable the lung to adapt to gas exchange. Most studies about the adaptation period focus on liquid clearance because liquid retention in airways is a major cause of respiratory morbidity in newborn infants (Hooper et al.,2007).

The prevalence of pulmonary disease in hospitalized neonatal foals has been reported to be approximately 50%, with a 39% survival to discharge (Freeman and Paradis, 1992). Early recognition of respiratory abnormalities during the postnatal period is of special importance for successful management of critically ill foals (Wilkins, 2003). In most foals, physical examination is not adequate for precise identification of the cause or severity of respiratory dysfunction, even when clinical signs are present (Koterba 1990; Lester and Lester, 2001; Bedenice et al., 2003/1; Bedenice et al., 2003/2). Clinical signs have to be interpreted in conjunction with blood gas- and acid-base analysis, radiographic findings, and other laboratory values.

Thoracic radiographs of foals made immediately after birth are characterized by a pronounced interstitial-alveolar opacity with blurring of small vessels. This opacity is the result of incomplete lung inflation, the presence of residual fluid in the small airways, and uptake of fetal alveolar fluid into the lung interstitium (Lester and Lester, 2001). Foals with respiratory disease may have a similar radiographic pattern, but it typically persists beyond the normal absorption time.

Pulmonary fluid production decreases over the last few days before natural delivery (Dickson et al, 1986). Only a small fraction of pulmonary fluid is cleared from the airways and alveoli as the foal traverses the birth canal (Jain et al, 2006). After birth, pulmonary fluid moves rapidly through amiloride sensitive Na^+ channels in the epithelium to the pulmonary interstitium, where it is cleared by pulmonary lymphatics and vessels (Wilkins, 2003; Jain et al., 2006; Chua and Perks, 1999; Olver et al., 2004). Although aquaporin channels provide the main route for osmotically-driven water transport, alveolar fluid clearance in the neonate is not affected by aquaporin deletion (Verkmann, 2007). Colloid oncotic effects and Starling forces also do not play a key role in pulmonary fluid absorption in neonatal foals (Jain et al., 2006; Machay, 2004). In healthy mature lambs, the transepithelial component of lung liquid clearance requires 2-3 hours and drainage of fluid from the interstitium is complete by the 6th hour after birth (Bland et al., 1982).

As normal pulmonary fluid can complicate interpretation of thoracic radiographs of neonates with pulmonary disease, it is important to know the kinetics of fluid removal, and also the earliest time at which normal-term foals have normal lung pattern on thoracic radiographs. The purpose of this report was to characterize those criteria.

3.2 **Materials and Methods**

One hundred seventy six radiographs of eight newborn foals of different breeds (Hungarian halfbreds and Hungarian draughthorses) born in the years 2003 and 2004 were assessed by three independent reviewers who were unaware of the age and status of the foals. So that foals could be radiographed shortly after birth, mares about to give birth were evaluated every 2 hours using a foaling monitoring system (Flett Street Corporation, Gaithersburg, USA). After birth the heart rate, respiratory rate, and body temperature were measured, and auscultation over the heart and lung was performed. Arterial blood gas analysis was also performed within 15 minutes after birth and before each radiographic examination. Blood was collected anaerobically from the great metatarsal artery into heparinized (1000 IU/ml) plastic syringes. Blood samples were analyzed within 10 minutes after collection. The time after birth at which foals stood up for the first time, whether the foal was recumbent longer than 15 minutes on one side before radiography, and the time of first suckling were recorded. Venous blood was withdrawn from each foal within 24 hours for quantification of complete blood cell count, and various serum electrolytes, glucose, protein and various enzymes.

Right-to-left and left-to-right lateral thoracic radiographs were acquired within the first 30 minutes after birth and repeated thereafter at 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h. A ceiling-mounted X-ray machine (Optimus, tube SRO 2550, Philips Medical Systems, Hamburg, Germany) and a computed radiography system (Easy Vision, Philips Medical Systems, Hamburg, Germany) were used to obtain the thoracic radiographs.

The body weight of the foals ranged from 45 to 65 kg, but all radiographs were made at 80 kVp and 5mA at a 100 cm focus-film distance. Radiographs were made at peak inspiration with the foal in lateral recumbency with the forelimbs pulled cranially from over the lung field. After each exposure the radiograph was evaluated for adequacy of positioning, phase of respiration and exposure so that poor quality studies could be eliminated. Postprocessing of the images was based on a thorax algorithm. Deficient radiographs were discarded and corresponding images repeated.

To achieve an objective and comparable assessment, a scoring system was developed for radiographic evaluation. Three independent observers evaluated each radiograph and scored them individually. The general assessment of duration of clearance was followed by the evaluation of all four (craniodorsal-, cranioventral-, caudodorsal-, caudoventral-) lung quadrants individually to characterize the pattern of clearance.

In the general assessment, the lung patterns were graded from 1 to 3. Radiographs with marked pulmonary opacity were scored as 3, radiographs with less opacity but blurring of small vessels were scored as 2, and normal appearing thoracic radiographs were scored as 1. The average score for the 3 observers was computed at each time point; complete fluid clearance was defined as the time as which the average reached score equaled 1.

We compared left-to-right and right-to-left radiographs at each given time-point. If the score for each view was the same, the paired images were given a mark of 0 and if the score for each view was different, the paired images were given a mark of 1. To compare left versus right laterals a logistic mixed model using Penalized Quasi-Likelihood was built (R Development Core Team, 2007). The explanatory variables were the interpreter and time and the foal identifier was a random factor to take into account the correlated measurements.

Finally, a slightly different, more sensitive, scoring system was used to grade the four lung quadrants individually. The interstitial-alveolar opacity in each quadrant was graded as normal, mild, moderate or severe and assigned grades from 0 to 3. The individual grading of the quadrants was performed up to 6 hours and only on the left-to-right images.

To verify of the interpretations Kendall's coefficient of concordance (Wt) was calculated (Kendall, 1948). The coefficient was determined only for the evaluations in the first 4 hours because thereafter all images from all foals received a score 1 from all observers. Statistical analyses were performed using R 2.8.0 (Venables and Ripley, 2002).

3.3 **Results**

Mares had a gestation length between 329-340 days and uncomplicated parturition.

All foals were physically normal. Some moist crackles were heard in the first 30 minutes in all foals, but after this time only normal respiratory sounds were heard. A grade III-, systolic-, left-sided heart murmur could be heard during the first 24-48 hours in two foals. One foal stood within 30 minutes after birth, two within the first hour, and five between 60 and 120 minutes. Until being able to stand, foals were usually in sternal recumbency and periods of prolonged lateral recumbency were not observed.

Arterial pH, pCO_2 and pO_2 values improved in the first 3 hours with mean pH increasing from 7,295 (SD=0,030) to 7,359 (SD=0,062), with mean pCO_2 decreasing from 49,6 Hgmm (SD=7,55) to 44 Hgmm (SD=3,03) and with mean pO_2 increasing from 77 Hgmm (SD=7,55) to 95 Hgmm (SD=12,98). After this initial period pH decreased slightly, pCO_2 increased slightly, and pO_2 fluctuated.

No foal was eliminated because of any laboratory abnormality.

There was agreement among the interpreters both in the general evaluation (Wt = 0.898, $P < 0.001$) and in the individual quadrant evaluation (Wt=0.93-0.97, $P < 0.001$). According to each interpreter, all foals had a clear lung field by the 6th hour after birth.

Although there was agreement between observers, the subjectivity of the radiographic evaluation process still has an influence on the results (**Figure 14**). One observer evaluated one foal as still having vascular blurring at 4 hours while the two other radiologists thought lung clearing occurred by 3-4 hours.

When comparing left-to-right versus right-to-left radiographs, scores were interpreter-dependent (p=0.002). Clearing of the cranioventral and caudoventral lung field preceded that of the dorsal quadrants (**Figure 15**).

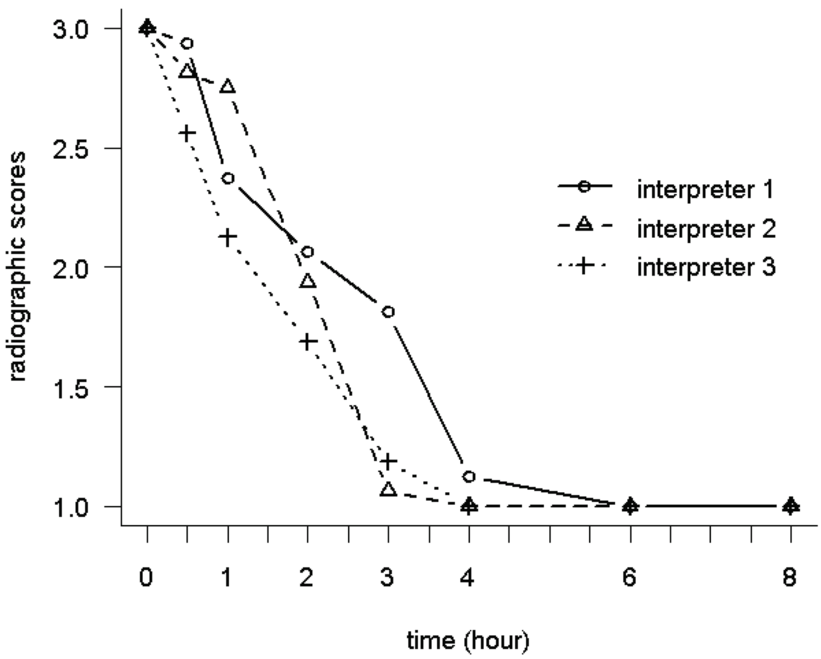

Figure 14. Clearance of thoracic radiographic opacity. Average scores of the three independent interpreters as a function of time after birth. Note differences between interpreters at some evaluation times.

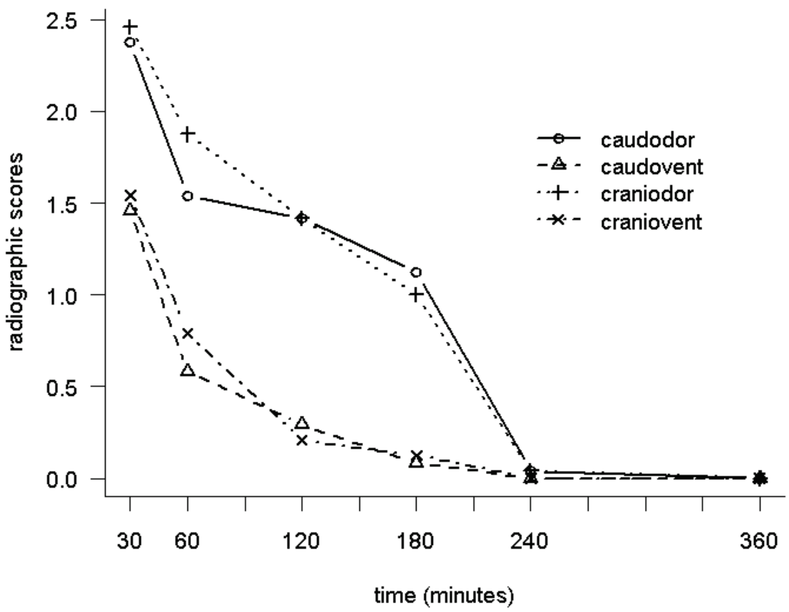

Figure 15. Patterns of radiographic clearance in different lung quadrants: ×: cranioventral, +: craniodorsal, ○: caudodorsal, Δ: caudoventral. Fluid cleared most rapidly from ventral lung fields.

3.4 **Discussion**

In human neonates there is rapid complete lung aeration (Yao and Lind 1976; Fawcitt et al., 1960). Previously, it was determined that normal foals had clear lungs within 12 hours of birth but the kinetics of clearance were not assessed (Lamb et al 1990). In newborn lambs delivered at term by Caesarean section, lung clearing occurred between one to four hours, with the cranial aspect of the lung clearing first (Fletcher et al., 1970).

In rabbits, the critical role of the dynamics of lung aeration with regard to physiologic changes was clarified (Hooper et al. 2007). Transpulmonary hydrostatic pressure generated by inspiration provided the predominant driving force for residual airway fluid clearance. Also, the degree of lung aeration at each time-point was variable with some lungs being well-aerated at 30 seconds, while others were only partially aerated at 2 hours (Hooper et al. 2007).

Whether the pulmonary opacity seen in these neonatal foals represented fluid, atelectasis, aspirated cells or an exudate was not determined. However, based on the normal adaptation period, we presumed the lungs were normal and that the opacity was due mainly to fetal lung fluid.

We followed the dynamics of postpartum clearance of equine pulmonary fluid from 30th minutes after birth until 72 hours and concluded that clearing of the equine lung occurs within 3 to 6 hours of birth. Foals with respiratory distress syndrome may have abnormal pulmonary radiographic opacity beyond the normal fluid clearance time (Wilkins 2003, Lester and Lester 2001). Delayed resorption of pulmonary fluid leading to increased pulmonary radiographic opacity was observed on thoracic images of infants (Kuhn et al., 1969). In equine neonates, physiologic postpartum fluid absorption seems to take longer period than in humans. The primary mechanism of lung liquid reabsorption is the change in ion transport induced by catecholamines during labor. In humans the vast bulk of this fluid leaves the lung before normal term birth (Alvaro and Rigatto, 2005). Since humans have relatively prolonged labor compared to horses enabling more time for absorption, this might be the cause of a much shorter radiographic lung clearance in infants. Fluid absorption time of this study was more similar to data from lambs. We did not acquire thoracic radiographs immediately after initiation of respiration, which would be of value to describe the kinetics of fluid resorption in more detail.

During the first hours after delivery, foals were in sternal recumbency. Considering the effect of gravity on fluid distribution, we suspected that fluid resorption would begin in the dorsocaudal region. To the contrary, fluid resprotion from the ventral quadrants preceded that of the dorsal quadrants (**Figure 16**).

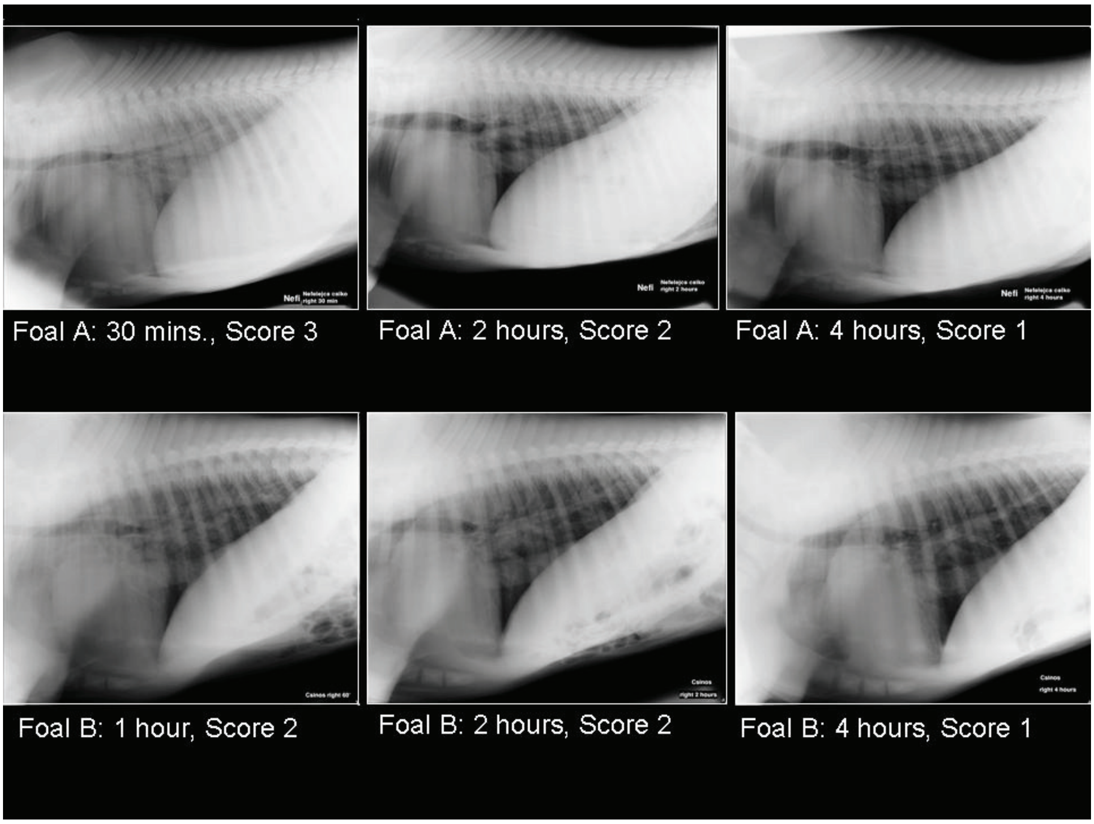

Figure 16. Series of left-to-right lateral thoracic raiographs of two foals (A and B). Note the fluid absorption pattern and change in radiopacity. The clearing of the ventral quadrants preceded that of the dorsal quadrants.

This pattern can be the result of the distribution of amiloride sensitive Na^+ channels in the lung epithelium of the newborn. It is also possible that the thoracic wall is more flexible ventrally, where it expands relatively faster. Similarly in human infants, the air first fills the inferior lung fields, surrounded by the only part of the ribcage that expands (Kuhn et al., 1969). In rabbits the spatial pattern of lung aeration is influenced by the motion of the diaphragm and the non-uniform way the chest wall deforms during inspiration (Hooper et al., 2007). There is no evidence for gravity-related movement of fluid, indicating that surface tension prevents significant movement of fluid between regions of the aerating lung (Hooper et al., 2007). The air/liquid interface moves toward the distal airways during inspiration because of transpulmonary pressure. Inspiratory activity and body position influences the process (Hooper et al., 2007).

In prolonged lateral recumbency, the dependent lung undergoes atelectasis. The nondependent lung is better aerated and characterized by better radiographic contrast, which is more conducive to detection of lung disease (Letser and Lester, 2001). For this reason, both left-to-right and right-to-left lateral radiographs were evaluated with respect to each other at each time point. Ventrodorsal projections would provide additional information on fluid lateralization, but these projections were not obtained because of the associated stress. We found significant differences between left-to-right and right-to-left lateral radiographs but lateral recumbency of the foal prior to radiography did not result in more pulmonary opacity on the dependent side, likely due to the short time foals spent in continuous lateral recumbency. In recumbent anesthetised horses, the maximum radiographic opacity of the dependent lung developed within 20 minutes of recumbency (McDonell and Hall, 1979). The increased opacity often improved within minutes of standing (Letser and Lester, 2001). Regardless of the effect of recumbency, there were still different scores for left-to-right versus right-to-left radiographic images in 5.7% of all evaluations. Apart from dependent atelectasis, other reasons for this difference could be distortion resulting from small changes in position of central beam or because radiographs were obtained at slightly different phases of respiration. The effect of body position was assessed in rabbits, where the dependent region of the lung aerated more slowly than non-dependent regions, leading to a non-uniform pattern of lung aeration (Hooper et al., 2007). This body position dependence is also more the result of restricted movements of the thoracic wall than of compression by mediastinal structures or to displacement of the diaphragm by abdominal contents.

Chapter 4. Final conclusions

We recorded that first opinion veterinary surgeons infrequently use ancillary diagnostic techniques when investigating chronic lower airway disorders. We conclude that taking the history and performing a clinical examination is not sufficient to establish a final diagnosis in these cases. Number of successful diagnostic workup in the field would be higher if first opinion veterinarians used respiratory tract endoscopy and broncholaveolar lavage as basic diagnostic tools in all chronic respiratory cases. These ancillary diagnostic procedures are easily performed in stable circumstances as well. Although tracheal secretions and blood samples more easily collected, results of TS cytology and hematology are less informative. Culturing tracheal lavage samples might add to the final diagnosis but results cannot be evaluated easily as secondary infections can complicate primary non-infectious disorders and false negative cultures may also occur. We also concluded that establishing a diagnosis in stable circumstances is impossible in approximately one quarter of cases, and it is still challenging in clinical settings.

It is also important to note that RAO appears to be widespread in Hungary, accounting for more than half of chronic pulmonary disorders. Finally, contrary to the current literature, it interestingly occurs mainly during the warm season. The high outdoor dust, air-borne mold and pollen levels and the necessary hay supplementation on pastures during the warm months complicates the optimal management of horses with RAO and commonly induce exacerbations in this period of the year.

Six hours after birth is the earliest time when normal foals reliably have clear lung fields on radiographs. Images at this time, and later, can be evaluated successfully and conclusions between physiologic and pathologic conditions can be drawn. Ventral lung cleared first, presumably because this region is bounded by the most flexible region of the thoracic wall. It is most likely that the free expansion of the thorax is important in lung aeration, which is more easily maintained in sternal recumbency or in a standing position.

Chapter 5. New scientific results

1. We estimated that patients with RAO had the highest prevalence among horses admitted to a Hungarian referral clinic with different chronic equine lower airway diseases.

2. Opposite to previously described scientific results we have found that RAO rather occurs during the warm months in Hungary and possible co-existence of SPA-RAO might complicate both diagnosis establishment and treatment.

3. According to the conditional inference tree method, age of the horse, history, clinical examination, respiratory tract endoscopy and bronchoalveolar lavage cytology proved to be the most valuable tools to define chronic lower airway pathology.

4. It was also concluded that in 22% of cases specific ancillary diagnostic modalities, unavailable for the field veterinarian, were needed to establish the final diagnosis.

5. We found that 4 % of chronic pulmonary cases are left with no definitive diagnosis even in clinical settings when using current understanding and all available ancillary diagnostic techniques.

6. We concluded that serial images taken with a computed radiographic system is useful to follow the postpartum clearance of fetal lung fluid.

7. We radiographicly followed the dynamics of postpartum clearance of equine pulmonary fluid from 30th minutes after birth until 72 hours and concluded that clearing of the equine lung occurs within 3 to 6 hours after birth.

8. We found that ventral lungfields cleared first, presumably because this region is bounded by the most flexible region of the thoracic wall. This stresses the importance of body-position in the postnatal period.

Chapter 6. References

6.1 **References to Chapter 1**

Aguilera-Tejero E., Pascoe J.R., Smith B.L., Tyler W.S., Woliner M. J.: Evaluation of a technique for detection of pulmonary hemorrhage in horses, using carbon monoxide uptake. Am. J. Vet. Res., 55. 1032-1036, 1994.

Ainsworth D.M., Eicker S.W., Yeagar A.E. Sweeney C.R., Viel L., Tesarowski D., Lavoie J.P., Hoffman A., Paradis M.R., Reed S.M., Erb H.N., Davidow E., Nalevanko M.: Associations between physical examination, laboratory, and radiographic findings and outcome and subsequent racing performance of foals with *Rhodococcus equi* infection: 115 cases (1984-1992). J. Am. Vet. Med. Assoc., 213. 510-515, 1998.

Bedenice D., Heuwieser W., Brawer R., Solano M., Rand W., Paradis M. R.: Clinical and prognostic significance of radiographic pattern, distribution, and severity of thoracic radiographic changes in neonatal foals. J. Vet. Intern. Med., 17. 876-886, 2003.

Butler J.A., Colles Ch. M., Dyson S. J.: Clinical radiology of the horse. The thorax. Blackwell Science Ltd., 1993. p. 483-528.

Couëtil L.L., Hoffman A.M., Hodgson J., Buechner-Maxwell V., Viel L., Wood J.L.N., Lavoie J.P. ACVIM consensus statement: Inflammatory airway disease of horses. J. Vet. Intern. Med., 21. 356-361, 2007.

Couetil, LL, Rosenthal FS, de Nicola DB, Chilcoat CD. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. Am. J. Vet. Res., 62. 538-46, 2001.

Coyne C.P., Kelly A.B., Hornof W.J., O'Brien T.R., Philp M.S., Lamb J.F.: Radiolabeling of equine platelets in plasma with 111In-(2-mercaptopyridine-N-oxide) and their in vivo survival. Am. J. Vet. Res., 48. 385-391, 1987.

Croll M.N.: Nuclear medicine instrumentation. Historic perspective. Semin. Nucl. Med., 24. 3-10, 1994.

Daniel G.B., Tucker R.L., Buckman T., Daniel S.L.: In vitro comparison of equine granulocytes labeled with 99mTc-hexamethylpropyleneamine oxime or 111In-oxine. Am. J. Vet. Res., 53. 871-876, 1992.

Davidson E.J., Martin B.B. Diagnosis of upper respiratory tract diseases in the performance horse. J. Vet. Clin. North Am. Equine Pract., 19. 51-62, 2003.

Derksen F.J., Brown C.M., Sonea I., Darien B.J., Robinson N.E.: Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. Equine Vet. J., 21. 23-26, 1989.

Derksen F.J., Paradis M.R.: Examination of the respiratory system. In: Colahan T.C., Mayhew I.G., Meritt M.A., Moore J.N. (editors) Equine medicine and surgery, Mosby, 1999, p. 440-447.

Duvivier D.H., Votion D., Vandenput S., Lekeux P.: Aerosol therapy in the equine species. Vet. J., 154. 189-202, 1997.

Farrow C. S.: Radiography of the equine thorax. Anatomy and technique. Vet. Radiol., 22. 62-68, 1981.

Franklin S.H., Naylor J.R., Lane J.G. Videoendoscopic evaluation of the upper respiratory tract in 93 sport horses during exercise testing on a high-speed treadmill. Equine Vet. J. Suppl., 36. 540-545, 2006.

Ferrucci F., Stancari G., Zucca E., Ayalon S., Falcone C., Ferro E. Specificity and sensitivity of ultrasonography and endoscopy for the diagnosis of exercise-induced pulmonary haemorrhage (EIPH) in 157 race horses. Vet Res Com (suppl). 33.185-188, 2009.

Fogarty U.: Evaluation of a bronchoalveolar lavage technique. Equine Vet. J., 22. 174-176, 1990.

Gallivan G.J., Viel L., McDonell W.N.: An evaluation of the multiple-breath nitrogen washout as a pulmonary function test in horses. Can. J. Vet. Res., 54. 99-105, 1990.

Grünig G., Hermann M., Howald B., Winder C., von Fellenberg R.: Partial divergence between airway inflammation and clinical signs in equine chronic pulmonary disease. Equine Vet. J., 21. 145-148, 1989.

Hare J.E., Viel L.: Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. J. Vet. Intern. Med., 12. 163-70, 1998.

Hart K.A., Barton M.H., Williams K.J., Flaminio M.J.B.F., Howerth E.W.: Multinodular pulmonary fibrosis, pancytopenia and equine herpesvirus-5 infection in a Thoroughbred gelding. Equine Vet. Edu., 20. 470-476, 2008.

Hewson J. and Viel L.: Sampling, microbiology and cytology of the respiratory tract. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Hoffman A. Outpatient lung function testing and the use of inhaled medications. In: Proceedings of the Second Dubai International Equine Symposium - Diagnosis and Treatment of Respiratory Diseases 1997; p. 299-313.

Hoffman A.M., Viel L., Juniper E., Prescott J.F.: Clinical and endoscopic study to estimate the incidence of distal respiratory tract infection in Thoroughbred foals on Ontario breeding farms. Am. J. Vet. Res., 54. 1602-1607, 1993.

Hoffman A.M., Viel L., Prescott J.F.: Microbiologic changes during antimicrobial treatment and rate of relapse of distal respiratory tract infections in foals. Am. J. Vet. Res., 54. 1608-1614, 1993.

Hoffman A.M.: Bronchoalveolar lavage technique and cytological diagnosis of small airway inflammatory disease. Equine Vet. Edu., 11. 208-214, 1999.

Hoffmann A.M.: Clinical application of pulmonary function testing in horses. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Kosch P.C., Koterba A.M., Webb A.I.: Developments in management of the newborn foal in respiratory distress 1: Evaluation. Equine Vet. J., 16. 312-318, 1984.

Koterba A. M.: Respiratory disease: Approach to diagnosis. In: Kosch P.C. (editor) Equine clinical neonatology. Lea & Febiger, 1990, p.153-172.

Lamb Ch. R., O'Callaghan W. O., Paradis M. R.: Thoracic radiography in the neonatale foal: A preliminary report. Vet. Radiol. and Ultrasound, 31. 11-16, 1990.

Lamb Ch. R., O'Callaghan W. O.: Diagnostic imaging of equine pulmonary disease. Comp. Cont. Edu. Pract. Vet., 11. 1110-1119, 1989.

Laumen E., Doherr M.G., Gerber V.: Relationship of horse owner assessed respiratory signs index to characteristics of recurrent airway obstruction in two Warmblood families. Equine Vet. J., 42. 142-8, 2010.

Lavoie J.P., Fiset L., Laverty S.: Review of 40 cases of lung abscesses in foals and adult horses. Equine Vet. J., 26. 348-352, 1994.

Lavoie J.P., Pascoe J.R., Kurpershoek C.J.: Effects of xylazine on ventilation in horses. Am. J. Vet. Res., 53. 916-20, 1992.

Lavoie J.P.: The respiratory system. In: Robinson N. E. (editor) Current therapy in equine medicine. Saunders, 1997. p. 401-467.

Leadon D., Farrelly B., Fogarty U., Buckley T.: Platelet counting in diagnosis of Rhodococcus equi. Vet. Rec., 123. 279-a, 1988.

Léguillette R., Roy M. F., Lavoie J.P.: Foal pneumonia. In: Lekeux P. (editor): Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Lester G. D., Lester N. V.: Abdominal and thoracic radiography in the neonate. Vet. Clin. Equine Pract., 17. 35-45, 2001.

Mair T. S., Gibs C.: Thoracic radiography in the foal. Equine Vet. Edu., 1. 56-61, 1989.

Mair T.S., Stokes C.R., Bourne F.J.. Cellular content of secretions obtained by lavage from different levels of the equine respiratory tract. Equine Vet. J., 19. 458-62, 1987.

Mansmann R.A. and Knight H.D.: Transtracheal aspiration in the horse. J. Am. Vet. Med. Assoc., 160. 1527-9, 1972.

Morris E.: Application of clinical exercise testing for identification of respiratory fitness and disease in the equine athlete. Vet. Clin. North Am. Equine Pract., 7. 383-401, 1991.

Mumford E.L., Traub-Dargatz J.L., Salman M.D., Collins J.K., Getzy D.M., Carman J.: Monitoring and detection of acute viral respiratory tract disease in horses. J. Am. Vet. Med. Assoc., 213. 385-90, 1998.

Norman W., Rantanen L., Gage M., Paradis M.S.: Ultrasonography as a diagnostic aid in pleural effusion of horses. Vet Radiol & Ultrasound, 22. 211-216, 1981.

O'Callaghan M.W.: Nuclear imaging techniques for equine respiratory disease. Vet. Clin. North Am. Equine Pract., 7. 417-33, 1991.

Parente E.J.: Video-endoscopy. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Pustrela N., Watson J.L., Wilson W. D.: Diagnostic approach to infectious respiratory disorders. Clin Tech. Equine Pract., 5. 174-186, 2006.

Ramirez S, Lester GD, Roberts GR. Diagnostic contribution of thoracic ultrasonography in 17 foals with Rhodococcus equi pneumonia. Vet. Radiol Ultrasound.; 45. 172-176, 2004.

Raphel C.F., Gunson D.E.: Percutaneous lung biopsy in the horse. Cornell Vet., 71. 439-448, 1981.

Reef V.B.: Ultrasonographic evaluation. In: Beech J. (editor) Equine respiratory disorders. Lea and Febiger, 1991. p. 69-88.

Reef V.B.: Ultrasonography. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Reef V.B., Whittier M., Griswold Allam L.: Thoracic ultrasonography. Clin Tech. Eqine Pract., 3. 284-293., 2004.

Reimer J.M. Diagnostic ultrasonography of the equine thorax. Compend. Contin. Edu. Pract. Vet., 12. 1321-1327, 1990.

Richard E.A., Fortier G.D., Lekeux P.M., Van Erck E. Laboratory findings in respiratory fluids of the poorly-performing horse. Vet J., 185.115-22, 2010.

Robinson N.E., Sorenson P.R.: Collateral flow resistance and time constants in dog and horse lungs. J. Appl. Physiol., 44. 63-68, 1978.

Robinson N.E. Recurent Airway Obstruction (heaves). In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, NY (www.ivis.org), 2002.

Roy M.F., Lavoie J.P.: Tools for the diagnosis of equine respiratory disorders. Vet. Clin. North Am. Equine Pract., 19. 1-17, 2003.

Savage C.J.: Evaluation of the equine respiratory system using physical examination and endoscopy. Vet. Clin. North Am. Equine Pract., 13. 443-62, 1997.

Savage C.J., Traub-Dargatz J.L., Mumford E.L.: Survey of the Large Animal Diplomates of the American College of Veterinary Internal Medicine regarding percutaneous lung biopsy in the horse. J. Vet. Intern. Med., 12. 456-464, 1998.

Sellon D.C., Levine J.F., Palmer K., Millikin E., Grindem C., Covington P.: Thrombocytosis in 24 horses (1989-1994). J. Vet. Intern. Med., 11. 24-9, 1997.

Toal R. L., Cudd T.: Equine neonatal thoracic radiography: A radiographic-pathologic correlation. Proc. Am. Assoc. Equine Pract., 3. 117-128, 1986.

Traub-Dargatz J.L., McKinnon A.O., Thrall M.A., Jones R.L., Bruyninckx W., Blancquaert A.M., Dargatz D.A.: Evaluation of clinical signs of disease, bronchoalveolar and tracheal wash analysis, and arterial blood gas tensions in 13 horses with chronic obstructive pulmonary disease treated with prednisone, methyl sulfonmethane, and clenbuterol hydrochloride. Am. J. Vet. Res., 53. 1908-16, 1992.

Viel L. Small airway disease as a vanguard for chronic obstructive pulmonary disease. Vet. Clin. North Am. Equine Pract., 13. 549-560, 1997.

Vörös, K. (szerk.): Belgyógyászati terápiás és diagnosztikai gyakorlatok. Állatorvostudományi Egyetem jegyzete, Budapest, 1998.

Votion D.M., Vandenput S.N., Duvivier D.H., Lambert P., van Erck E., Art T., Lekeaux P.: Alveolar clearance in horses with chronic obstructive pulmonary disease. Am. J. Vet. Res., 60. 495-500, 1999.

Votion D.M.: Scintigraphy. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Willoughby R.A., Ecker G.L., McKee S.L., Ridolls J.L.: Use of scintigraphy for the determination of mucociliary clearance rates in normal, sedated, diseased and exercised horses. Can. J. Vet. Res., 55. 315-320, 1991.

6.2 **References for Chapter 2**

Bracher V., von Fellenberg R., Winder C.N., Gruenig G., Hermann M., Kraehenmann A. An investigation of the incidence of chronic obstructive pulmonary disease (COPD) in random populations of Swiss horses. Equine Vet. J., 23.136-41, 1991.

Brakenhoff J.E., Holcombe S.J., Hauptman J.G., Smith H.K., Nickels F.A., Caron J.P. The prevalence of laryngeal disease in a large population of competition draft horses. Vet. Surg., 35. 579-83, 2006.

Chapman P.S., Green C., Main J.P.M., Taylor P.M., Cunningham F.M., Cook A.J. Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. Vet Rec., 146.91-95, 2000.

Cook W.R., Williams R.M., Kirker-Head C.A. Upper airway obstruction (partial asphyxia) as the possible cause of exercise-induced pulmonary hemorrhage in the horse: An hypothesis. J. Equine Vet. Sci., 8. 11-26, 1988.

Couëtil L.L. IAD: Cough, poor performance, mucus in the airways—What is so important about that? AAEP Proceedings, 48. 200-207, 2002.

Couëtil L.L., Hinchcliff K.W. Non-infectious diseases of the lower respiratory tract. In: Hinchcliff K.W., Kaneps A.J., Geor R.J. (editors) Equine sports medicine and surgery London: Saunders; 2004. p. 613-57.

Couëtil L.L., Hoffman A.M., Hodgson J., Buechner-Maxwell V., Viel L, Wood JLN, Lavoie JP. ACVIM Consensus statement: Inflammatory airway disease of horses. J. Vet. Intern. Med., 21. 356-361, 2007.

Couëtil L.L., Rosenthal F.S., DeNicola D.B., Chilcoat C.D. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. Am. J. Vet. Res., 62. 538-546, 2001.

Couëtil L.L., Ward M.P. Analysis of risk factors for recurrent airway obstruction in North American horses: 1,444 cases (1990-1999). J. Am. Vet. Med. Assoc., 223. 1645-1650, 2003.

Davidson E.J., Martin B.B. Diagnosis of upper respiratory tract diseases in the performance horse. J. Vet. Clin. North Am. Equine Pract., 19. 51-62, 2003.

Davis E., Rush B.R. Equine recurrent airway obstruction: pathogenesis, diagnosis, and patient management. Vet. Clin. North Am. Equine Pract., 18. 453-467, 2002.

Derksen F.J., Brown C.M., Sonea I., Darien B.J., Robinson N.E. Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. Equine Vet. J., 21. 23-26, 1989.

Derksen F.J., Scott J.S., Miller D.C., Slocombe R.F., Robinson N.E. Bronchoalveolar lavage in ponies with recurrent airway obstruction (heaves). Am. Rev. Respir. Dis., 132. 1066-1070, 1985.

Derksen F.J., Williams K.J., Uhal B.D., Slocombe R.F., De Feijter-Rupp H., Eberhart S. Pulmonary response to airway instillation of autologous blood in horses. Equine Vet J., 39.334-339, 2007.

Dixon P.M., McGorum B.C., Pirie R.S. Inflammatory airway disease: European clinicians' perspective. In: Hoffman A., Robinson N.E., Wade J.F. (editors) Havemeyer Foundation Monograph Series No. 9., Proceedings of a workshop on inflammatory airway disease: defining the syndrome. Newmarket: R & W Publications; 2003, p. 7-8.

Dixon P.M., Railton D.I., McGorum B.C. Equine pulmonary disease: a case controlled study of 300 referred cases. Part 1: Examination techniques, diagnostic criteria and diagnoses. Equine Vet. J., 27. 416-421, 1995.

Ferro E., Ferrucci F., Zucca E., DiFabio V., Castoldi S. Arterial blood gas analysis in 53 racehorses with a diagnosis of small airway inflammatory disease (SAID). J Equine Vet Sci., 22.165-168, 2002.

Ferrucci F., Stancari G., Zucca E., Ayalon S., Falcone C., Ferro E. Specificity and sensitivity of ultrasonography and endoscopy for the diagnosis of exercise-induced pulmonary haemorrhage (EIPH) in 157 race horses. Vet Res Com (suppl). 33.185-188, 2009.

Fogarty U. A bronchoalveolar lavage technique for routine diagnostic purposes. Equine Vet Edu., 2.102-104, 1990.

Franklin S.H., Naylor J.R., Lane J.G. Videoendoscopic evaluation of the upper respiratory tract in 93 sport horses during exercise testing on a high-speed treadmill. Equine Vet. J. Suppl., 36. 540-545, 2006.

Gerber V., Straub R., Marti E., Hauptman J., Herholz C., King M., Imhof A., Tahon L., Robison N. E.: Endoscopic scoring of mucus quantity and quality: observer and horse variance and relationship to inflammation, mucus viscoelasticity and volume. Equine Vet. J., 36. 576-582, 2004.

Gerber V., Swinburne J.E., Blott S.C., Nussbaumer P., Ramseyer A., Klukowska-Rotzler J. Genetics of recurrent airway obstruction (RAO). Deutsche Tieraerztliche Wochenschrift, 115.271-275, 2008.

Hare J.E., Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. J. Vet. Intern. Med., 12. 163-170, 1998.

Hewson J., Viel L. Sampling, microbiology and cytology of the respiratory tract. In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, NY (www.ivis.org), 2002.

Hoffman A.M. Bronchoalveolar lavage technique and cytological diagnosis of small airway inflammatory disease. Equine Vet. Edu., 11. 208-214, 1999.

Hoffman A.M., Mazan M.R. Airway obstruction and hyper-reactivity in horses with signs of inflammatory airway disease. In: Hoffman A.M., Robinson N.E., Wade J.F. (editors) Inflammatory airway disease: defining the syndrome. Newmarket R&W Publications 2003. p. 71-74.

Holcombe S.J., Derksen F.J., Stick J.A., Robinson N.E. Pathophysiology of dorsal displacement of the soft palate in horses. Equine Vet. J. Suppl., 30. 45-48, 1999.

Holcombe S.J., Ducharme G.D. Abnormalities of the upper airway. In: Hinchcliff K.W., Kaneps A.J., Geor R.J. (editors) Equine sports medicine and surgery London: Saunders; 2004. p. 613-657.

Holcombe S.J., Jackson C., Gerber V., Jefcoat A., Berney C., Eberhardt S., Robinson N. E.: Stabling is associated with airway inflammation in young Arabian horses. Equine Vet. J., 33. 244-249, 2001.

Holcombe S.J., Robinson N.E., Jackson C., Berney C., Gerber V., Jefcoat A. Stabling, airway inflammation, and dorsal displacement of the soft palate in young horses. AAEP Proceedings, 46. 254-5, 2000.

Horohov D.W., Mills W.R., Gluck M. Specific and innate immunity in the lung as it relates to equine RAO. In: Tessier C, Gerber V (editor) 4th world equine airways symposium (WEAS). Berne: Pabst Science Publishers, 2009, p. 106-108.

Hotchkiss J.W., Reid S.W., Christley R. Construction and validation of a risk-screening questionnaire for the investigation of recurrent airway obstruction in epidemiological studies of horse populations in Great Britain. Prev. Vet. Med., 75. 8-21, 2006.

http://www.nepegeszseg.net/pollenjelentes.php-2007.

Hughes K.J., Malikides N., Hodgson D.R., Hodgson J.L. Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses 1. Evaluation of cytological stains and the percentage of mast cells and eosinophils. Aust. Vet. J., 81. 681-684, 2003.

Langsetmo I., Fedde M.R., Meyer T.S., Erickson H.H. Relationship of pulmonary arterial pressure to pulmonary haemorrhage in exercising horses. Equine Vet. J. 32. 379-384, 2000.

Lavoie J.P. Recurrent airway obstruction (Heaves) and summer-pasture-associated obstructive pulmonary disease. In: Dixon P.M., McGorum B.C., Schumacher J., Robinson N.E. (editors) Equine Respiratory Medicine and Surgery. London: Saunders Co Ltd.; 2007, p. 565-591.

Lavoie J.P., Dalle S., Breton L., Pierre H. Bronchiectasis in three adult horses with heaves. J. Vet. Int. Med., 18. 757-60, 2004.

Leclere M., Desnoyers M., Beauchamp G., Lavoie J.P. Comparison of four staining methods for detection of mast cells in equine bronchoalveolar lavage fluid. J. Vet. Intern. Med., 20. 377-381, 2006

Lekeux P. Equine respiratory diseases. International Veterinary Information Service, Ithaca, (www.ivis.org), 2002.

Mazan M.R., Vin R., Hoffman A.M. Radiographic scoring lacks predictive value in inflammatory airway disease. Equine Vet. J., 37. 541-545, 2005.

McPherson E.A., Thomson J.R. Chronic obstructive pulmonary disease in the horse. 1: Nature of the disease. Equine Vet. J., 15. 203-206, 1983.

Moore R.B., Krakowka S., Robertson J.T., Cummins J.M. Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. Am. J. Vet. Res., 56. 562-567, 1995.

Nagy K., Reiczigel J., Harnos A., Schrott A., Kabai P. Tree-based methods as an alternative to logistic regression in revealing risk factors of crib-biting in horses. J. Equine Vet. Sci., 31. 21-26, 2010.

Naylor J.M., Clark E.G., Clayton H.M. Chronic obstructive pulmonary disease: Usefulness of clinical signs, bronchoalveolar lavage, and lung biopsy as diagnostic and prognostic aids. Can. Vet. J., 33. 591-598, 1992.

Nolen-Walston R.D., Sweeney C.R. Equine restrictive lung disease, Part 3: interstitial diseases. In: Lekeux P. (editor). Equine respiratory diseases international veterinary information service, Ithaca, NY (www.ivis.org), 2002.

R Development Core Team. R, A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.r-project.org; 2007.

Raphel C.F., Soma L.R. Exercise-induced pulmonary hemorrhage in Thoroughbreds after racing and breezing. Am J Vet Res., 43.1123-1127, 1982.

Richard E.A., Fortier G.D., Lekeux P.M., Van Erck E. Laboratory findings in respiratory fluids of the poorly-performing horse. Vet J., 185.115-22, 2010.

Robinson N. International workshop on equine chronic airway disease. Equine Vet. J., 33. 5-19, 2001.

Robinson N.E. Recurent Airway Obstruction (heaves). In: Lekeux P. (editor) Equine respiratory diseases. International Veterinary Information Service, Ithaca, NY (www.ivis.org), 2002.

Robinson N.E., Derksen F.J., Olszewski M.A., Buechner-Maxwell V.A. The pathogenesis of chronic obstructive pulmonary disease of horses. Br. Vet. J., 152. 283-306, 1996.

Rush B., Mair T. Equine respiratory diseases. 1st ed. Oxford: Blackwell Science Ltd; 2004.

Traub-Dargatz J.L., McKinnon A.O., Thrall M.A., Jones R.L., Bruyninckx W., Blancquaert A.M., Dargatz D.A.: Evaluation of clinical signs of disease, bronchoalveolar and tracheal wash analysis, and arterial blood gas tensions in 13 horses with chronic obstructive pulmonary disease treated with prednisone, methyl sulfonmethane, and clenbuterol hydrochloride. Am. J. Vet. Res., 53. 1908-1916, 1992.

Viel L. Small airway disease as a vanguard for chronic obstructive pulmonary disease. Vet. Clin. North Am. Equine Pract., 13. 549-560, 1997.

Ward M.P., Couëtil L.L. Climatic and aeroallergen risk factors for chronic obstructive pulmonary disease in horses. Am. J. Vet. Res., 66. 818-824, 2005.

Wood J.L.N., Newton J.R., Chanter N., Mumford J. The association between respiratory disease and bacterial and viral infections in British racehorses. J. Clin. Microbiol., 43. 120-126, 2005.

6.3 **References for Chapter 3**

Alvaro R.E., Rigatto H.: Cardiorespiratory adjustments at birth. In: Avery, G.B., MacDonald, M.G., Seshia, M.M., Mullett, M.D. (editors) Avery's neonatology: pathophysiology and management of the newborn, Lippincott Williams & Wilkins, Portland, 2005. p. 284–304.

Bedenice D., Heuwieser W., Solano M., Rand W., Paradis M.R.: Risk factors and prognostic variables for survival of foals with radiographic evidence of pulmonary disease. J. Vet. Intern. Med., 17. 868–875, 2003.

Bedenice D., Heuwieser W., Brawer R., Solano M., Rand W., Paradis M.R.: Clinical and prognostic significance of radiographic pattern, distribution, and severity of thoracic radiographic changes in neonatal foals. J. Vet. Intern. Med., 17. 876–886, 2003.

Bland R.D., Hansen T.N., Haberkern C.M., Haberkern C M., Bressack M.A.: Lung fluid balance in lambs before and after birth. J. Appl. Physiol., 53, 992–1004, 1982.

Chua B.A., Perks A.M.. The pulmonary neuroendocrine system and drainage of the fetal lung: effects of serotonin. Gen. Comp. Endocrinol., 113. 374–387, 1999.

Dickson K.A., Maloney J.E., Berger P.J.: Decline in lung liquid volume before labor in fetal lambs. J. Appl. Physiol., 61, 2266–2272, 1986.

Fawcitt I., Lind J., Wegelius, C.. The first breath: a preliminary communication describing some methods of investigation of the first breath of a baby and the results obtained from them. Acta Pediatr., 49. 5–17, 1960.

Fletcher B.D., Sachs B.F., Kotas R.V.: Radiologic demonstration of postnatal liquid in the lung of newborn lambs. Pediatrics, 46. 252–258, 1970.

Freeman L., Paradis M.R.: Evaluation of the effectiveness of equine neonatal care. Vet. Med., 87. 921–926, 1992.

Hooper S.B., Kitchen M.J., Wallace M.J., Yagi·N., Uesugi K., Morgan M.J., Hall C., Siu K.K.W., Williams I.M., Siew M., Irvine S.C., Pavlov K., Lewis R.A.: Imaging lung aeration and lung liquid clearance at birth. FASEB J., 21. 3329–3337, 2007.

Jain L., Douglas M.D., Eaton C.: Physiology of fetal lung fluid clearance and the effect of labour. Semin. Perinatol., 30. 34–43, 2006.

Kendall M.G.: Rank correlation method. Griffin and Company Ltd., London. 1948.

Koterba A.M.: Respiratory disease: approach to diagnosis. In: Koterba, A.M., Drummond, W.H., Kosch, P.C. (editors) Equine clinical neonatology, Lea & Febiger, Philadelphia, 1990. p. 153–177.

Kuhn J.P., Fletcher B.D., DeLemos R.A.: Roentgen findings in transient tachypnea of the newborn. Radiology, 92. 751–757, 1969.

Lamb Ch.R., O'Callaghan W.O., Paradis M.R.: Thoracic radiography in the neonatal foal: a preliminary report. Vet. Radiol. and Ultrasound, 31. 11–16, 1990.

Lester G.D., Lester N.V.: Abdominal and thoracic radiography in the neonate. Vet. Clin. North Am. Equine Pract., 17. 35–45, 2001.

Machay T.: Az újszülöttek kardioreszpiratoórikus adaptációja; adaptációs zavarok. Gyermekgyógyászati Továbbképző Szemle, 9. 211–216, 2004.

McDonell W.N., Hall L.W.. Radiographic evidence of impared pulmonary function in laterally recumbent anaesthetised horses. Equine Vet. J., 11. 24–32, 1979.

Olver R.E., Walters D.V., Wilson S.M.: Developmental regulation of lung liquid transport. Annu. Rev. Physiol., 66. 77–101, 2004.

R Development Core Team: R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, ISBN 3-900051-07-0. Available at http://www.R-project.org (accessed October 28, 2008). 2007.

Toal R.L., Cudd T.: Equine neonatal thoracic radiography: a radiographic-pathologic correlation. Proc. Am. Assoc. Equine Pract., 32. 117–128, 1986.

Venables W.N., Ripley B.D.: Modern applied statistics with S, Springer. New York. 2002.

Verkmann A.S.: Role of aquaporins in lung liquid physiology. Respir. Physiol. Neurobiol., 159. 324–330, 2007.

Wilkins P.A.: Lower respiratory problem of the neonate. Vet. Clin. North. Am. Equine Pract.19. 19–33, 2003.

Yao A.C., Lind J.: Current concepts on respiratory and circulatory adaptation of the fetus and newborn. Eur. J. Obstet. Gynecol. Reprod. Biol., 6. 347–358, 1976.

Chapter 7. Publications

IF: 6,636

7.1 **Publications related to the dissertation**

7.1.1 **Full-text papers published in peer-reviewed journals in English**

Kutasi, **O.,** Horvath, A., Harnos, A., Szenci, O.: Radiographic assessment of pulmonary fluid clearance in healthy neonatal foals. Journal of Veterinary Radiology & Ultrasound, 50. 584-588, 2009. **IF: 0,985**

Kutasi, **O.**, Balogh, N., Lajos, Z., Nagy, K., Szenci O.: Diagnostic approaches for the assessment of equine chronic pulmonary disorders. Journal of Equine Veterinary Science, accepted with revisions for publication **IF:0,515**

7.1.2 **Full-text papers published in peer-reviewed journals in Hungarian**

Kutasi, O., Reményi, B., Horváth, A., Szabó, F., Machay, K., Szmodits, Zs., Paár, L., Szenci, O.: Csikók mellkasi röntgenvizsgálata : Irodalmi áttekintés, Magyar Állatorvosok Lapja, 129. 579-589, 2007. **IF: 0.104**

Rózsa, S., Bakos, Z., Molnár, B., Biksi, I., Jármy, M., **Kutasi, O.,** Szenci, O.: Tüdõfibrosis megállapítása lóban. Esetismertetés. Magyar Állatorvosok Lapja, 124. 323-330, 2003. **IF:0.089**

7.1.3. **Papers in conference proceedings**

7.1.3.1 **Conference proceedings in English**

Kutasi, O., Remenyi, B., Horvath, A., Harnos, A., Szenci, O.: The postnatal clearance of the fetal lung fluid in newborn foals. 25th Symposium of the Veterinary Comparative Respiratory Society, Lafayette, USA, 2007.

Kutasi, O., Pataki, Zs., Balogh, N., Nagy, K., Szenci, O.: The role of the owner in the diagnosis and prognosis of RAO. SIVE, Bologna, Italy, 2007. www.ivis.org/proceedings/sive/2007/pdf/46.pdf

Kutasi, O., Pataki, Zs., Balogh, N., Lajos, Z., Harnos, A., Szenci, O.: Heaves from the clinician's point of view. 2nd ECEIM Congress, Naas, Ireland, J. Vet. Int. Med. 21. 4. 885. 2007.

Kutasi, O., Remenyi, B., Horvath, A., Harnos, A., Szenci, O.: The follow-up of the postnatal clearance of the fetal lung fluid in newborn foals. AVEF, Versailles, France, 2006. www.ivis.org/proceedings/avef/2006

Bakos, Z., Rózsa, S., **Kutasi, O**.: Digital luminescence radiography of the thorax in horses affected with recurrent airway obstruction. 1st ECEIM Congress, Leipzig, Germany, 2005.

Bakos, Z., Vörös, K., **Kutasi, O**.: Comparison of the caudal lung borders determined by percussion and ultrasound in healthy horses. 8th Congress of the World Equine Veterinary Association. Buenos Aires, Argentina. 2003.

7.1.3.2 **Conference proceedings in Hungarian**

Kutasi, O., Langer, D., Szenci, O., Balogh, N.: Egy új típusú inhalációs szteroid, a ciclesonid kipróbálása lovak kehességének gyógykezelésében: részleteredmény közlése Akadémiai beszámoló, 2008.

Kutasi, O., Pataki, Zs., Tóth, A., Langer, D., Paár, L., Balogh, N., Szenci, O.: A kehesség napjainkban Magyarországon. XV. Magyar Lógyógyászati Kongresszus, Sümeg, 2007.

Kutasi, O., Pataki, Zs.: A ló-asthma (RAO=recurrent airway obstruction) jelentősége hazánkban. Civilizációs betegség? Lótenyésztési Tudományos Napok, Debrecen, 2006.

Kutasi O., Reményi B.: Csikók mellkasi radiográfiája. XII. Magyar Lógyógyászati Kongresszus, Budapest, 2004.

7.2 **Publications non-related to the dissertation**

7.2.1 **Full-text papers published in peer-reviewed journals in English**

Kutasi, O., Vörös, K., Paár, L., Szenci, O., Sótonyi, P.: Common atrioventricular canal in a foal. Case report and literature review. Acta Veterinaria Hungarica, 55. 51-65, 2007. **IF: 0.474**

Porter, R.S., Leblond, Á., Lecollinet, S., Tritz, P., Cantile, C., **Kutasi, O.**, Zientara, S., Pradier, S., Galen, G., Speybroek, N., Saegerman, C.: Clinical diagnosis of West Nile Fever in equids by classification and regression tree (CART) analysis and comparative study of clinical appearance in three European countries, accepted for publication, Transboundary and Emerging Diseases **IF: 1.854**

Kutasi; O., Lecollinet; S., Bakonyi; T., Biksi; I., Ferenczi; E., Bahuon; C., Sardi; S., Zientara; S., Szenci, O.: Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile Virus in Hungary. Journal of Equine Veterinary Medicine, accepted for publication, **IF: 2,168**

Bakonyi T., Ferenczi E., Erdélyi K., **Kutasi O.**, Csörgő T., Seidel B., Weissenböck H., Brugger K., Bán E., Nowotny N.: Explosive spread and persistence of a neuroinvasive lineage 2 West Nile virus in 2008/2009, central Europe submitted for publication, Journal of General Virology, **IF: 3,26**

7.2.2 **Full-text papers published in peer-reviewed journals in Hungarian**

Bohák, Zs., Langer, D., **Kutasi, O.:** Lovak teljesítmény-élettana: irodalmi áttekintés Magyar Állatorvosok Lapja, 131. 579-585, 2009. **IF: 0.114**

Jakab, Cs., Izing, S., Veres, S., Talpag, B., Molnár, J., **Kutasi, O.:** A lovak daganatos és nem daganatos bőrelváltozásainak előfordulása és hisztopatológiája Magyar Állatorvosok Lapja, 129. 515-524, 2007. **IF: 0.104**

Filipsz, I. **Kutasi, O.,** Veres, K., Bodó, G.: Újszülött méncsikó köldök környéki, irreponabilis álsérvvel szövődött húgyhólyagrepedése: Esetismertetés. Magyar Állatorvosok Lapja, 128. 259-264, 2006. **IF:0.155**

Horváth, D., Horváth, A., **Kutasi, O.,** Bakos, Z., Szenci, O.: Az újszülött csikók immunglobulin-ellátottságának vizsgálata Magyar Állatorvosok Lapja, 127. 3-11, 2005. **IF:0.114**

7.2.3. **Papers in conference proceedings**

7.2.3.2 **Conference proceedings in English**

Kutasi, O., Bakonyi, T., Ferenczi, E., Erdélyi, K., Norbert, N.: Nyugat-Nílusi vírusfertőzések Magyarországon, Intermediate Congress of the World Equine Veterinary Association, Debrecen, Hungary, 2010.

Kutasi, O., Biksi, I., Bakonyi, T., Ferenczi, E., Lecollinet, S., Bahuon; C., Zientara, S., Szenci, O.: Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile Virus in Hungary. AAEP Annual Convention, Las Vegas, USA, 2009.

Kutasi, O., Sardi, S., Biksi, I., Bakonyi, T., Ferenczi, E., Lecollinet, S., Bahuon, C. Clinical and virological characterisation of the equine West Nile Virus outbreak in Hungary in 2008, BEVA Birmingham, Great Britain, 2009.

Kutasi, O., Langer, D., Molnar, B., Lorincz, M., Lajos, Z., Bodó, G., Szenci, O.: Streptococcus equi subspecies equi infections, complications and their treatments: experiences of a university clinic. 4. Leipziger Tierärztekongress, Blaue Hefte. Leipzig, Germany, 2008.

Posters

Kutasi, O., Langer, D., Molnar, B., Bakonyi, T., Szenci, O.: West Nile Virus in Hungary: the first detected equine case caused by A genetic lineage 2 virus. 3rd ECEIM Congress, Barcelona, Spain, J. Vet. Int. Med. 23. 885, 2009.

Kutasi, **O**., Bacsi, V., Langer, D., Harnos, A., Szenci, O.: Clinical efficacy of miracle drug called dimethyl sulphoxide: retrospective study. 10th Congress of the World Equine Veterinary Association. Moscow, Russia. 2008.

Langer, D**., Kutasi, O.,** Bodó, G., Szenci, O.: Exercise testing in the Lippizan driving horse.The Preperformance horse. Liege, 2008.

Kutasi, O., Ari, K.: Infarctive purpura haemorrhagica with severe anaemia. Voorjarsdagen Amsterdam, 2007.

7.2.3.2 **Conference proceedings in Hungarian**

Kutasi, O., Moravszki, L., Sárdi, S., Bohák, Zs.: Csikók mellkasi és hasűri röntgen és ultrahangdiagnosztikája, XVIII Magyar Lógyógyászati Kongresszus, Cegléd, 2010.

Búza, L.,, Denkinger, G., Domahidy, G., Holló-Szabó, P., Izing, S., Jozwiak, Á., Kolozsvári, T., Kunsági, Z., **Kutasi, O.**, Marton, Zs., Bükki, E.: A lovak takarmányozási eredetű idegrendszeri megbetegedései. Magyar Állatorvosok Világszervezete Lógyógyászati Konferencia, Zsibó 2008.

Bakonyi, T., Molnár, B., **Kutasi, O.**, Ferenczi, E., Dobos-Kovács, M., Erdélyi, K.: Nyugat-Nílusi vírusfertőzések 2007-ben Magyarországon, Akadémiai beszámoló 2007.

7.3 **Publications in non-scientific journals**

Kutasi, O.: Új ellenségünk: a Nyugat-Nílusi Vírus. Lovas Nemzet 14. 20-23, 2008.

Kutasi, O., Langer, D.: Gyomorfekély - a civilizált lótartás egy újabb átka. Lovas Nemzet 14. 24-28, 2008.

Kutasi, O.: Fejrázás: rossz szokás vagy betegség? Lovas Nemzet 13. 28-30, 2007.

Kutasi, **O**., Pataki, Zs.: Civlizációs betegség: A lovak kehessége 1. rész, Lovas Nemzet 13. 38-40, 2007.

Kutasi, **O**., Pataki, Zs.: Civlizációs betegség: A lovak kehessége 2. rész, Lovas Nemzet 13. 38-40, 2007.

Langer, D., **Kutasi, O.**, Bodó, G., Szenci, Ottó.: Sportlovak teljesítményvizsgálata. Lovas Nemzet 13. 22-25, 2007.

Chapter 8. Acknowledgements

First of all, I am deeply grateful to my supervisor, Prof. Szenci Otto, for his continuous help and encouragement throughout the years. I will always admire his experience in the field of scientific research.

I also warmly thank my colleagues at the Clinic for Large Animals for their support. Without their altruistic help during many days and nights of these years, I could surely not manage to perform the research protocol. I am grateful for the friendly atmosphere we always have at the clinic even during difficult and hard times.

I am forever indebted to the directory of the Dóra major Teaching Farm, Némedi István and Sári Viktor who contributed with their mares and newborn foals to this study. Their support goes back high in the past when I decided to choose this profession and was with me all the time.

I wish to thank to the field veterinarians and horse owners who trusted me and brought their horses to our clinic for respiratory evaluation.

I would like to acknowledge to Rényi Blanka, Lombay Béla, Rostás Tamás and Kiss Zsuzsanna for their assistance in evaluating and interpreting neonatal radiographic images, to Balogh Nándor to evaluate clinicopatholigic result and to Lajos Zoltán for the valuable help in microbiology questions.

Last but not least, I owe to my warmest gratitude to my sympathetic husband and my lovely daughter, my parents, grandma and to my whole family and also to my friends who stood beside me during the important moments of my life and helped me whenever it was needed. Their existence makes everything, even this PhD work, worthwhile.