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A comparison of forced oscillatory measurements to bronchoalveolar lavage

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2012

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Introduction

The respiratory system of horses is divided in two main parts: upper and lower respiratory system. The upper airway system of horses consists of nose, para-nasal sinuses and nasal cavity, larynx, trachea and guttural pouch. The uniqueness of the equine species is that they are exclusively nasal breathers as the result of a tight seal which is located between the soft palate and the laryngeal cartilages. Due to this anatomical structure upper airway obstruction is particularly troublesome in this species, because horses cannot bypass an obstruction by mouth breathing. (BEECH, 1991)

The lung belongs to the lower respiratory system and is a huge and sensitive organ. It has an enormous surface area of 2000m². This surface is mostly covered by only a single layer of epithelial cells and is challenged every day by dust particles, viruses, bacteria and allergens (BEECH, 1991). Due to domestication, stables and fodder, horses can suffer from several respiratory diseases. Some of these disorders are sub-clinical and only result in poor performance of the affected horses. This thesis will later deal in detail with the latter problems from a diagnostic point of view.

If we want to examine the respiratory tract of horses, we have a number of options to do so. Some of them are simple and easy to perform but others need special knowledge and equipment.

The simplest method is the clinical examination. We can use our senses, such as vision, smell and touch to check the respiratory organs. It is essential to follow the same procedure every time, starting with an observation from a distance, followed by close observation, and then hands-on physical examination (MUNROE and WEESE, 2011). We can percuss the thoracic wall and listen to changes in the percussion sounds and we can auscultate over the lungs and trachea and look for abnormal breathing sounds.

For further investigations we have different equipment what we could use.

We could perform thoracic ultrasonography, which is very useful to identify pleural fluid or peripheral pulmonary disease. We are also able to determine the character of the fluid, e.g. exudates or transudates and its location (MUNROE and WEESE, 2011). Ultrasound waves are

not transmitted through air-filled lung tissue, so the technique is not reliable for diseases located deep in the lung. (TAYLOR et al., 2010)

Another possibility would be thoracic radiography. To entirely visualize the lung in adult horses, we need to perform 4 x-rays, imaging 4 overlapping areas, the caudodorsal, caudoventral, craniodorsal and cranioventral areas respectively. Generally, the lighter the exposure, the denser the lung appears (BEECH, 1991). The Diernhofer-Triangle should be visible on the caudoventral images when the x-ray beam is optimally positioned. Nevertheless x-ray can give us a good idea how the lung looks like. A lot of diseases will have a negative or non-pathognomic result on the x-ray image, so in many cases thoracic radiography by itself is not sufficient for establishing the diagnosis.

In order to get a good idea how the airways look from the inside, we can perform an endoscopic examination. Endoscopy of the lower airways is a fundamental component of the diagnostic work (McGorum et al., 2007). We can visualize an increased amount of mucus easily and evaluate it on a scale. This scale has the section a,b, c and d. Section a) describes the accumulation and reaches from 0 – 5, whereas 0 means there is none and 5 means extreme; Section b) describes the localization and stickiness and has a range from 1 – 5, where each number describes a location, e.g. 1 ventral and 5 threading; Section c) is the apparent viscosity and also has a 1 – 5 range, where 1 means fluid and 5 viscous. The last Section is d) which describes the color, it also reaches from 1 – 5, whereas 1 is stated as yellow, 3 is white and 5 colorless.

Furthermore we can evaluate the quality structure of the carina, which should have a 'sharp' appearance. (BARAKZAI, 2007)

With the help of an endoscope we also can perform bronchoalveolar lavage (BAL) and tracheal lavage (TL). For this sample collection we need an endoscope which is between 1.8 meters and 2.4 meters long. (MUNROE and WEESE, 2011)

If we want to investigate the findings from ultrasound, x-ray or endoscopy more precisely, we can take biopsy samples from the lung in special cases. This would be only useful in chronic parenchymal disorders and interstitial problems. For a parenchymal lung biopsy we

can either use a percutaneous route using a biopsy needle, or by transbronchial route via an endoscope introducing a grasping biopsy wire. (TAYLOR et al., 2010)

Next to the useful ways of examining the lung in a 'traditional' way, newer methods are available to focus on the lung function of horses. These lung function tests were first used in human patients, but they are used more and more frequently in equine patients as well. So far they are mostly used for research, but their practical use is increasing. Compared to humans it is not possible to perform so called 'static tests' where it is necessary that the patient holds his/her breath. So we only can –in a not anesthetized horse – perform 'dynamic tests' where the breathing system is in motion. A good example for dynamic measurement is resistance, which requires flow. For receiving an expressive result it is best to describe the mechanical properties of the lung – pressure and flow – together. Lung mechanical tests focus on the relationship between these two variables. (HOFFMANN, 2002)

One method of pulmonary function test is the measurement of pleural pressure changes during respiration. We have two ways of performing this test: either by insertion of a blunt cannula through the intercostals space or by placement of a balloon catheter into the thoracic esophagus. The pressure changes during respiration are recorded and the maximal pressure change between in- and expiration is calculated. (RADOSTITS et al., 2007)

Another method of lung function testing in horses is the plethysmography, also called 'Flow-metrics'. It provides information by simultaneously measurement of the flow at the airway opening (nose) and the body surface (plethysmographic) without the use of intrathoracic pressures. Furthermore, it provides information about the breathing pattern, rib-abdomen synchrony, and changes in end-expiratory lung volume. The sensitivity of this method is similar to conventional testing and is used for improving sensitivity with regard to inflammatory airway diseases. (HOFFMANN, 2002)

Impulse oscillometry is a potentially clinically useful test of pulmonary function testing. It measures impedance of the respiratory system and provides information about the resistance and reactance. The impulse oscillometry has the great advantage of being more sensitive to changes in pulmonary function than measurements of pleural pressure changes and is relatively easy to perform.

The horse must wear a facemask containing a pneumotachograph for measurement of respiratory volumes and tubing to the horse. The tube is then attached to a loudspeaker, which generates square wave signals, producing harmonics between 0 – 10 Hz. A computer program then analyzes the information provided by the system and indices of pulmonary resistance and reactance are determined. (RADOSTITS et al., 2007)

The basis of the impulse oscillometry is the forced oscillation technique. We can measure the response of the respiratory system to external forces (pressure or flow). The pressure-flow arising from spontaneous breathing is largely ignored using this method. The basics of oscillometry arose from electrical models that describe impedance. The impedance to airflow is the sum of three effects: Resistance (pressure due to friction), elastance (pressure due to tissue recoil), and inertance (pressure related to acceleration of flow). These three forces will act in series. Oscillometry is completely non-invasive (HOFFMANN, 2002). In summary, an inordinately high pressure output relative to a given flow signifies increased impedance (HOFFMANN, 2002). Depending on the kind of obstruction the impedance is signified by exaggerated phase mismatching between pressure and flow signals. We also have the advantage of controlling the forcing frequency, the input of energy (e.g. the amplitude of the flow) and the exact waveform. When we can detect impedances in central (large or upper) and peripheral (small) airways, constriction is in series. Can we detect impedances in airways of similar generation the constriction is in 'parallel' and the frequency dependence of the respiratory system is increased. If we can find series and parallel impedances it is a form of 'heterogeneity', which would be characteristic for some diseases. (HOFFMANN, 2002)

Chapter 1 – The History of oscillatory measurements

The oscillatory measurements were first discovered and used in the human medicine. DuBois et al. are presumably the most quoted researchers in this field as they described the forced oscillation technique (FOT) first in 1956 as a possibility to perform lung function tests in humans. Instead of a loudspeaker they used a pump to produce frequencies. They described the frequencies of the pump as a sine wave and consequently the flow signal too, because their tests were based on the assumption that the respiratory system acts in a linear way. DuBois and coworkers were not the first who described the proportion of pressure to flow as impedance, but the first ones who used it in a lung function test (HOFFMAN, 2002).

DuBois et al. (1956) compared the respiratory system to an electric circuit and transferred the information from the electrical engineering on the respiratory system.

1968 Fisher et al. first applied the forced oscillation technique to examine pulmonary obstruction. They discovered that this method was highly sensitive to obstructions in the respiratory system and other influences. For example obesity or anesthesia had no influence on the result; the resistance increased if there was an obstruction.

Nowadays the forced oscillation technique is used routinely in human medicine.

With regard to horses it is still not used on a regular basis, but the FOT is on the rise. Young and co-workers were the first ones who used FOT in horses in the early nineties, later van Erck and co-workers and recently Hoffman and Mazan with co-workers. At first it was used in anesthetized horses but since then it has frequently been used in awake animals as well.

The forced oscillation technique is already a very helpful tool with regard to pulmonary obstructions in animals.

This thesis deals with the question if this procedure could be used to diagnose subclinical diseases as well.

Chapter 2 – Introduction to the forced oscillation technique (FOT), the bronchoalveolar lavage (BAL) and radiography in horses

1.1 The physical background of the respiratory mechanic

Respiration is a simple change of muscle contraction and relaxation. The lung always plays a passive role during respiration and reacts to the pressure changes in the thorax. During inspiration predominantly the diaphragm and partially the external intercostal muscles contract which causes a low pressure in the thorax. The lung automatically expands due to the low pressure, and the air flows into the airways. During expiration – in a healthy horse - the diaphragm and the external intercostal muscles relax, as a consequence the pressure in the thorax increases and the gas is pushed out of the lung. (REINHOLD, 1997a)

Put in other words breathing is a permanent change of pressure and volume within every breathing cycle.

These changes depend on three major factors (HOFFMAN, 2002):

- The elastic or static features of the lung and chest wall – referred to as Elastance (E) or Compliance (C) later on
- The limitation of flow by friction within the bronchi – later on it will be called Resistance (R)
- The Inertance (I) of the gas (air) and the lung tissue

The organism always tries to maintain the tidal volume even if the horse is severely ill. In case of respiratory illness the work load of the muscles – also for expiration where the internal intercostal muscles contract to support the breathing - increases and therefore the pressure and flow change as well. In simple words pressure and flow are the basic factors for determining the lung function in horses (McGorum, 2007).

Expressed in a simplified mathematic formula the total pressure would be the sum E and I (REINHOLD, 1997a):

$$\Delta P_{total} = \Delta P_{elastic} + \Delta P_{inertia}$$

1.2 Elastance and Compliance – the elastic properties of the respiratory System

Elastance (E) and Compliance (C) are parameters which provide information about the elasticity of the lung. (McGorum, 2007)

It can be thought of as the elastic resistance. This elasticity is dependent on two factors, the elastic fibers in the lung and the surface tension in the alveoli and terminal bronchi. The surface tension is reduced by surfactant. . (FROSOLONO et al., 1970; KAUP and DROMER, 1985, 1986 quoted after Reinhold, 1997a)

Both parameters can be calculated (McGorum, 2007). The reciprocal of the Elastance is the Compliance (Hoffman, 2002):

$$C = \frac{V}{P_{elastic}}$$

The Compliance describes the ductility of the lung tissue, which is given as the proportion of the volume of the respiratory gas in the thorax and the corresponding pressure (REINHOLD, 1997a).

Comroe (1968, quoted after Reinhold) described the relation between volume and pressure as non-linear, meaning that Compliance and Elastance depend on the current conditions in the lung and thus change during a breath.

1.3 Resistance – Resistive properties of the respiratory System

The parameter which is easiest to measure is the pleural pressure. If the pleural pressure is coupled with measurement of flow the Resistance (R) is assessable (McGORUM, 2007). With other words, R is entirely dependent on flow and provides information about the relationship between viscous pressure and flow (HOFFMAN, 2002). The resistive component includes central and peripheral airways, lung tissue and chest wall resistance

(SMITH et al., 2005). Lung tissue and chest wall resistance normally play a tangential role and are therefore negligible (RITZ et al., 2002). The airflow rate within the airway system is dependent on the cross section of the airways. The higher the branching of the airways, the smaller the cross section, but the number of airways increases with every branch and so the total cross section increases (sum of all single cross sections). Due to the larger total cross section, the airflow rate in the periphery is drastically decreased. (REINHOLD, 1997a)

A slight decrease of the diameter of the airways leads to a high increase of the flow resistance within the airway.

McGorum (2007) and Reinhold (1997a) use the formula of Poiseuille for the calculation of the Resistance, where the resistance is proportional to the length L in a cylindrical tube and inversely proportional to the fourth power of the radius r:

$$R = \frac{8 \pi \mu L}{r^4}$$

In this formula μ stands for the physical properties of a gas (or the viscosity of a liquid).

Resistance is a measureable parameter in the airway and will be very sensitive to any kind of obstruction or dilatation, because it is dependent among others on the radius.

1.4 Inertance – The inertia properties of the respiratory System

The Inertance (I) is a combination of the inertia of the air column and the inertia of the lung tissue. It is characterized by the proportion of inertive pressure change to the volumetric flow rate (V'') (REINHOLD, 1997a):

$$I = \frac{P_{Inertia}}{V''} \text{ in } \frac{[kPa]}{[\frac{1}{s^2}]}$$

V'' is the derivative of the flow with respect to time:

$$V'' = dV'/dt = d^2V/dt^2$$

1.5 Summary of the physical basics

The mechanical properties of the respiratory system are influenced by diverse factors, e.g. the elastic structures of the lung tissue, superficial active substances in the alveoli, the activity of the respiratory muscles, the secretion of mucus or the change of the diameter of the airways.

We have to characterize static and dynamic conditions. Static conditions are phases of relaxed muscles, dynamic phases are given if the muscles contract. The airflow has to overcome the formerly described resistor; consequently C, R and I are variable parameters during the dynamic phase. Compliance describes the elastic forces, whereas Resistance describes the frictional forces. All these factors act in series. (HOFFMAN, 2002)

2.1 The basics for the usage of the forced oscillation technique (FOT) as diagnostic tool

According to Oostveen et al. external signals are used to determine the mechanical response of the respiratory system. The examiner uses known, forcing waveforms to investigate the respiratory system on the basis of the linear system, which was described earlier.

Reinhold (1997b) suggests to look at the horses' respiratory system from an electrical engineering point of view. In that case we have to see the pneumatic resistors as follows:

- Resistance, R as the real resistor

- Inductivity, L as the inductive resistor
- Capacity, C as the capacitive resistor

2.2 Resistance R – The real resistor

Basically flow, resistance and pressure follow Ohm's law:

$$U=R*I$$

In case of respiration U is the pressure, R is the resistance and I is the flow rate. Focusing only on R it is possible to explain this flow resistance with the model of a fine-mesh. While breathing through a fine-mesh more power is needed than usual during breathing, but not all of the used power will be used as kinetic (mechanical) energy. Some of this energy will be lost, since due to the higher work load a higher temperature is produced which uses up energy. Pressure and flow are in the same phase, meaning they reach their maximum and their minimum at the same time. (REINHOLD, 1997b)

2.3 Inductivity L – The inductive resistance

The inductive resistance describes the inertia within the respiratory system. Pressure and flow are not in phase anymore, but pressure leads flow. In other words pressure is required to move flow. (HOFFMAN, 2002)

If we want to express this mathematically, we will have a sine oscillation, where the maximum of the pressure curve will be before the maximum of the flow curve. This phase difference can be expressed by the phase angle. (REINHOLD, 1997b)

The pressure signal leads flow by 90 degrees, meaning that the phase angle will be positive (+90°). (HOFMAN, 2002)

Here, we can imagine an air column within a tube, which would mimic the large airways, with negligible resistance. In this example the tube will act as a source of inertance. (HOFFMAN, 2002; REINHOLD, 1997b)

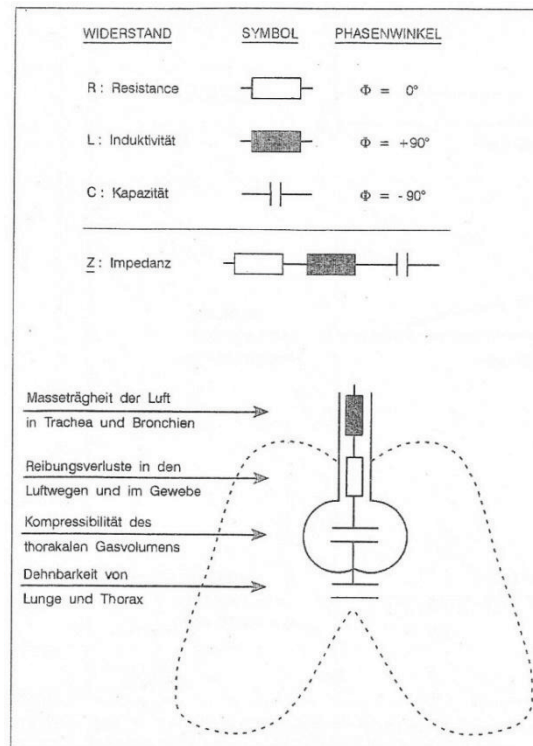
2.4 Capacity C – The capacitive Resistance

We have to imagine the capacitive resistance like a balloon. The balloon in the respiratory system would be the thorax wall and the elastic airways. If we filled the balloon with gas, we would have the inverse effect of the inductivity. First the airflow would arise and then followed by pressure when the balloon is full. If we describe this also in a sine oscillation, the maximum of the flow curve will be reached before the maximum of the pressure curve. This then will lead to a negative phase angle (-90°). (REINHOLD, 1997b)

2.5 Summary of the basics for usage

If we talk about resistance in the airways, we have to distinguish between energy storage units and energy consumption units. We can allocate the resistive resistor R to the latter, whereas the capacitive resistance and the inductive resistance belong to the energy storage units. The energy storage units are called Reactance (X). (SMITH et al., 2005)

As the whole airway system builds a very complex resistance, new metrics for the airway mechanics have been defined for the forced oscillation technique. These new metrics are independent, physiologic parameters within the lung function analysis. Although partly the same definitions are used, these parameters are not identical with conventional parameters of the airway mechanic. (REINHOLD, 1997b)



Picture 1: Electric Analogues to the resistors of the respiratory System (REINHOLD,1997b, schematized and modified by Reinhold after Flügel, 1979 and Petro, 1989)

3. Respiratory Impedance

The key concept of the forced oscillatory respiratory mechanics is termed as the 'impedance' (Z). (OOSTVEEN et al., 2003)

Impedance is a complex variable. This means that it is a combination of real, inductive and capacitive resistances. Put simply: Z can be conceived as a generalization of resistance, because it embodies both the in-phase and out-of-phase relationships. Every complex variable contains two components, the modulus and the related phase angle. With the spectral analysis or Fourier-Analysis these variables can be described. (REINHOLD, 1997b; OOSTVEEN et al., 2003)

The real part of Z is the in-phase component (Resistance R). On the other hand the out-of-phase relationship is expressed by the imaginary part (Reactance X). Both are functions of the oscillation frequency (f). R describes the dissipative mechanical properties of the respiratory system, whereas X is related to the energy capacity. (OOSTVEEN et al., 2003)

If R and X are known for a given frequency the impedance can be calculated as follows (REINHOLD, 1997b):

$$|Z| \sqrt{R^2 + X^2}$$

And we can calculate the phase angle in the following way:

$$\tan \Phi = \frac{X}{R} \text{ or } \Phi = \tan^{-1} \frac{X}{R}$$

If we only know $|Z|$ and Φ , we can calculate R and X:

$$R = |Z| \cos \Phi$$

$$X = |Z| \sin \Phi$$

4. Influences on the results

Van Erck et al. (2004) described in their study the influence of increased dead space and air leaks in the facemask, the influence of mask design and the influence of the head position on the results of oscillometric measurements. They found that the absence of an airtight seal did not alter the breathing pattern of the horses, but air leaks led to huge changes in R.

The reactance was significantly lower when air leaks were present, whereas X was significantly higher with air leaks. Also the dead space had a huge influence on the results. With maximal dead-space, R showed an extreme shift downwards compared to R measured with minimal dead-space.

The mask design had no influence on the measuring results, neither had the alteration of the head position.

5. Coherence

The “coherence” is a sign of quality during the measurements. Forced oscillation data must be filtered of noise to provide a reliable measurement. The perfect coherence would be 1. The more interference and irregularities we have, the lower is the coherence. For avoiding any interference forced oscillation data are collected at higher frequencies. The minimal acceptance for the coherence is 0.9. Possibilities to reduce the problem of interference are a careful sedation or repeated testing. The forced oscillation is therefore minimally repeated four times. (LANDSER et al., 1976; HOFFMANN, 2002)

The input of flow and the pressure are evaluated in a linear regression. The coherence can be described as the relation between input and output of the linear system. (HOFFMANN, 2002; MICHAELSON et al., 1975)

Reasons for a poor coherence could be leaks at the facemask (or the tube), swallowing, glottis closure irregular breathing or acute hyperventilation. (OOSTVEN et al., 2003)

6. Physiological and Pathological Results

In a healthy respiratory system Resistance (R) and Reactance (X) (together the Impedance (Z)) are not frequency dependent. (YOUNG et al., 1997). Information concerning the pattern of bronchoconstriction can be achieved at lower frequencies between one and two Hz. (HOFFMAN et al., 1998) In other words Resistance measured at one to two Hz provides information about the peripheral and central airways. R measured at higher frequencies represents the diameter of the airways. (HOFFMAN, 2002)

The conclusion is that frequency dependence is an abnormal finding and reflects tissue and airway pathology. Increased Resistance reflects upper airway pathology and a decreased Resistance peripheral airway pathology. (HOFFMAN, 2002) But it is not simple to evaluate the magnitude of frequency dependence, the ratio of R maximum and R

minimum is a useful index in horses. Obviously it is not enough to know the Impedance at only one frequency but investigate the Impedance over the whole length of the measurements. (HOFFMAN, 1999)

Also a decreased Reactance is a symbol of lower airway pathology.

7. Clinical Application of FOM

FOM in horses has basically the same applications as in the human medicine. In human medicine the most common pulmonary obstructive disease is asthma. FOT is the most used diagnostic tool for this disease. The equivalents to human asthma are non-infectious inflammatory airway diseases (IAD) in horses. (HOFFMAN, 2002; Deaton et al., 2007)

IAD has many different names, for example recurrent airway obstruction (RAO), chronic obstructive pulmonary disease (COPD) or heaves; but no matter how they are called, they all cause bronchoconstriction which is measurable with FOT. (DEATON et al., 2007; SMITH et al., 2005; Young et al., 1997)

Recurrent airway obstruction is a performance-limiting, allergic respiratory disease of horses characterized by chronic cough, nasal discharge, and respiratory difficulty. (RUSH and MAIR, 2004)

Not only is FOM a useful tool for the diagnosis of these diseases but also for the monitoring of the response to the treatment (SMITH et al., 2005), to screen the disorder (ERCK et al., 2004), to obtain information about the severity of the disease (HOFFMAN et al., 1998) and to get information about the quantitative respiratory function (COUETIL et al., 2001).

8. Reasons for usage of FOM in horses

FOM has the huge advantage that it does not need cooperation of the patient and is – as said before – completely non-invasive. Therefore it is a great tool to monitor a patient over a long time and repeat the examinations without a major effort. Small changes are

easy visible, and in increase as well as decrease in lung function can be achieved quick and simply. Drugs can be evaluated much better if they have a positive influence on the health or if there is a need to change them.

If a poor performance is related to IAD this is also easy diagnosed with the help of FOM.

9. Reasons for BAL in horses

Samples from the distal airways have not been contaminated with organisms from the upper respiratory tract or secretions before collection and are therefore more representative of the small airways, the parenchymal and alveolar secretions. (RADOSTITS et al., 2007) Bronchoalveolar lavage fluid composition correlates with the pulmonary function in horses. (COUETIL et al., 2001)

It is possible to detect chronic or acute lung diseases, regardless of there being an infectious or noninfectious cause. (RADOSTITS et al., 2007)

According to Radostits et al. (2007) there is no difference in the cytological composition of the first and the subsequent aspirates.

10. Reasons for thoracic radiographs of horses

A chest x ray is a procedure used to evaluate organs and structures within the chest for symptoms of disease. They enable the examiner to get a view on the heart, large vessels, lungs and bones of the thorax.

A non-pathognomic result should show clear structures of the lungs, the Diernhofer triangle should be visible, the heart should not be increased or decreased in size and in the physiological position; lungs should be in the range of the physiological borders. The lung structure should be even over the whole image and none of the structures should be more radio-dense or radio-lucent than normal.

Chapter 3 – Material and Method

1. The horses

For the experiments four Hungarian warm-blooded horses were used. Three of the horses were mares, one was a gelding. None of them showed any clinical signs of a respiratory disease and the horses tolerated the equipment for the experiment. The warm bloods had an age of 4-14 years \pm 1 year. The weight of the standard bred horses was 450-590kg \pm 40kg.

The horses were owned by the Large Animal Clinic in Üllö, Hungary or by their employees. All horses were housed and cared for in the clinic stable during the time of the experiment.

2. The pre-examination and the test procedure

The horses were examined daily during the experiment. The basic clinical values were all in a normal range, no nasal discharge was visible, cough was not inducible or in a normal pattern, no signs of dyspnoe were recognizable and auscultation over the lungs and trachea was without any pathologic findings, the lymph nodes had a physiological size, shape and structure and the capillary refill time was under two seconds.

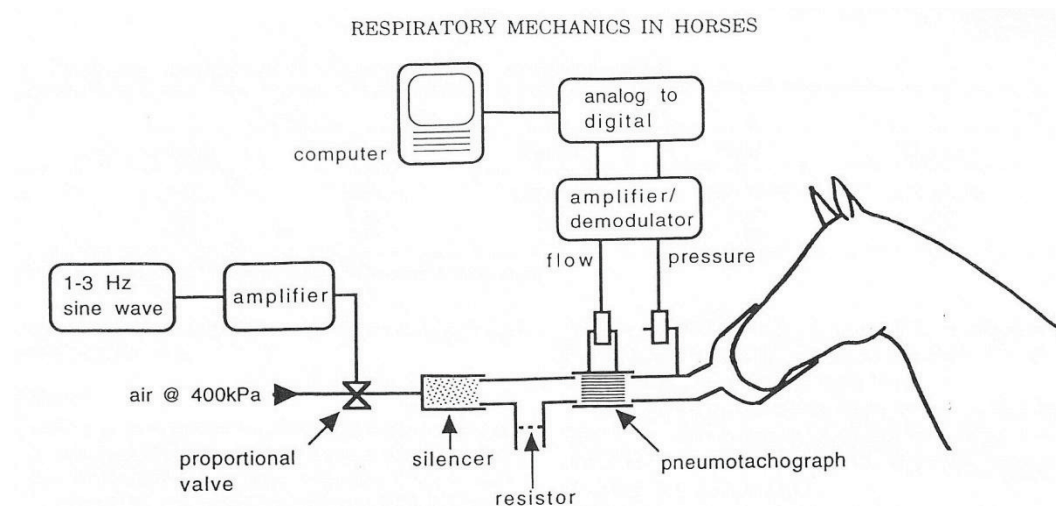
The forced oscillatory measurements were performed on day one of the experiment, after the clinical examination had not rendered any suspicious findings. The horses had to withstand the noise of the loudspeaker and accept the facemask. All horses tolerated the mask and the noise very well and the measuring results were without abnormal findings.

3. The experimental set up

For the experiment the horses were gently restrained in an approximately 1x2 meter stock with a halter and a lead rope held by an assistant.

The pulmonary mechanics were measured by forced oscillation technique (FOT). We used the 'Equine Oscillatory Mechanics V2.2' developed by Simon Young in 2003. We connected the machine to a Laptop and build it into a single box. The computer system we used for the data collection was based on 'labview'. The tube length from the speaker to the mask measured 6.09 meters and was 5.5 cm in diameter. The frequency range reached from 1 Hz to 7 Hz.

The whole apparatus had different parts: a function generator to produce sinusoidal voltage waveform, a T-piece for applying the oscillating air flow via the speaker to the face mask, face mask, a differential pressure transducer for measuring the mask pressure relative to the atmospheric pressure, heated pneumotachograph Fleisch no. 4 to measure the airflow to the mask and the software for the data collection (YOUNG and TESAROWSKI, 1994).



Picture 2: Experimental set-up (YOUNG and TESAROWSKI, 1994)

4. Additional examinations

Blood samples of all horses were taken at day two of the experiment and were sent to the laboratory for hematology- and biochemistry examination; Fibrinogen was controlled as well.

5. The endoscopic examination and the BAL

The endoscopic examination was performed on the sedated horses with the flexible endoscope model Olympus CF-VL, produced in Hamburg, Germany; it was 197 cm long and 1.3 cm in diameter. The endoscopic examination was tolerated well by the horses. The ventral meatus, Pharynx, Larynx, Trachea and Carina were observed. The structure, mucus content, the apparent viscosity, the localization (of mucus) and the color of the respiratory tract and if present mucus were evaluated.

After the careful examination of the respiratory tract, a BAL was performed to obtain information about the cytological character in the lower lungs. The fluid analysis included the measurement of cell number and type.

When performing BAL a plastic catheter, more precisely BIVONA catheter of company Kruuse was inserted. The cuff was inflated to make the tube stay in place. First we injected 60 ml of a 0.7% Lidocain solution to reduce the coughing effect. The Lidocain was administered as the tube entered the rostral trachea.

Then we administered sterile Saline; 300 ml in total. We injected five 60 ml syringes sterile Saline trough the plastic tube. Afterwards we pushed one syringe filled with air through the tube to ensure that all fluid was instilled.

After the horse had taken a few breaths we aspirated the fluid.

The samples were first stored in the fridge and after all examinations were done the samples were sent to the laboratory for the cytological examination altogether.

In the laboratory one slide of each sample was fixed and prepared to have a diagnostically conclusive result. For preparing the smear, 10 ml aliquots were centrifuged for 10 min with 2000 cycles per minute. The supernatant was then decanted. From the remaining fraction a drop was transferred to a clean slide, using a plastic transfer pipette. The slides were then air-dried, fixated by methanol for 6 min and stained with methylene blue solution also for 6 min. Afterwards the slide was carefully rinsed and observed under the microscope by 1000 magnification by an experienced clinical

pathologist who did not have information about the medical history, physical exam or endoscopic exam results. (TILLEY et al. 2012)

6. The radiography

At day four and five of the experiment radiographic images were taken from the lungs of the test horses. The horses were not sedated for this examination.

The x-rays were taken with an Optimus, tube SRO 2550 produced by Philips Medical Systems, Hamburg, Germany with a distance of 1.5 meters. The images were viewed and processed with Easy Vision software from Philips Medical Systems. Exposure factor were 120 mV and 16mAs.

Eight thoracic radiographic images were taken of every horse; caudodorsal, caudoventral, craniodorsal and cranioventral perspective from each side.

Chapter 4 – Results

1. Interpretation of the FOM data

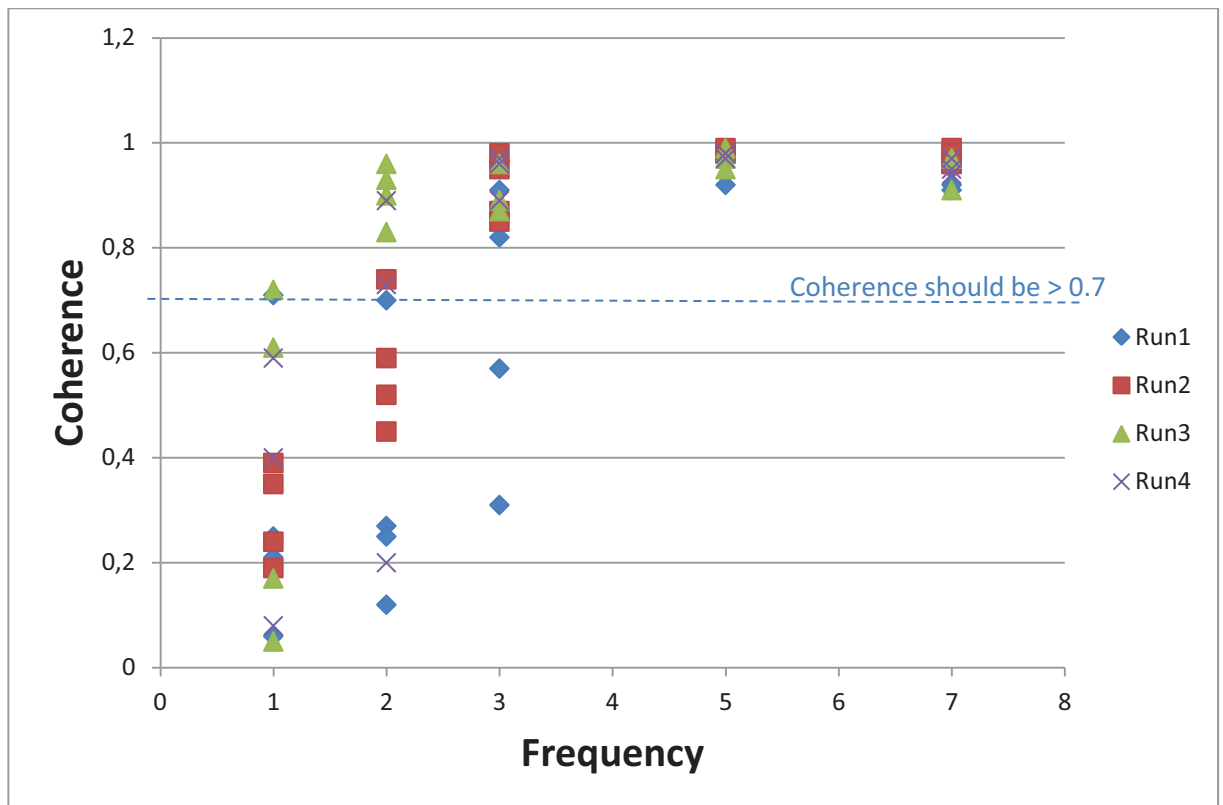
As explained earlier only those measuring results can be used which have a good coherence. So it has to be decided which of the minimum 4 runs would be most represent able, meaning having the highest coherence over all the measured frequencies.

The system used divides the measuring period in four different parts (30 seconds with lower frequencies; 10 seconds with higher frequencies) and compares the results. The results can differ strongly if coherence was bad during the measuring period. In the experiment values with a coherence of 0.7 or higher were used, if the horses tolerated the facemask without struggle, although authors like Hoffman (2002) suggested a higher coherence.

We can clearly see in picture 3 that the coherence is increasing with higher frequencies. In this table the data of all the horses in every run are visible.

The blue perforated line in picture 3 shows the minimal acceptance which was tolerated during the experiment. All results which are below this line will be discarded in the further evaluation of results.

As a reminder: the closer the coherence gets to 1 the better the quality of the measurement.



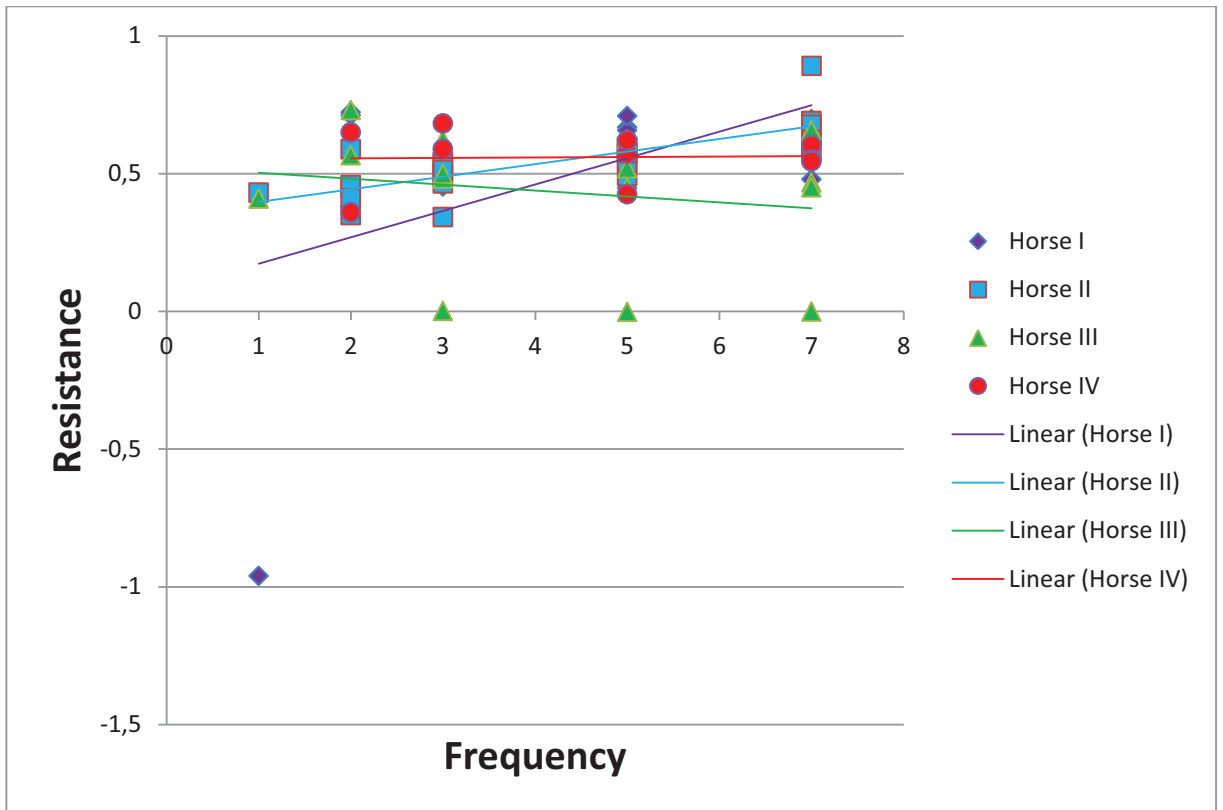
Picture 3: Frequency dependence of Coherence

If we put all measurements in graphics we can easily see that neither Reactance nor Resistance are frequency dependent in the test horses (Picture 4 and 5).

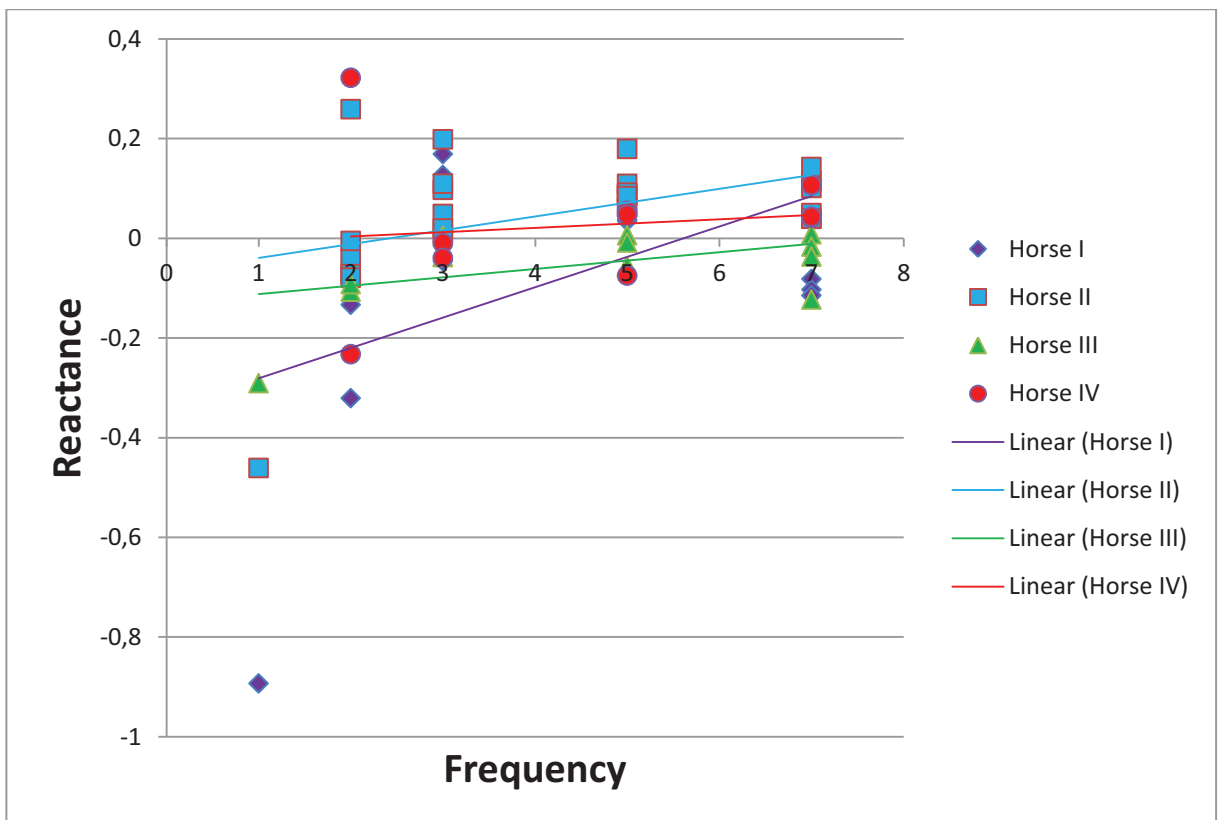
In the same graphs we can see the trendline for every horse during the used measurements. Especially in horse IV, but also in the other horses we have a straight or relatively straight Impedance course.

In case of an obstruction the trend line would obviously surge or decline with the frequency.

This leads to the conclusion that from the FOT point of view all horses are healthy or rather do not show any signs of disease.



Picture 4: Frequency dependence of Resistance



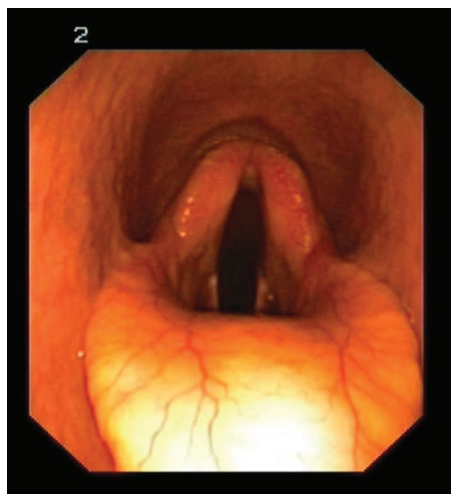
Picture 5: Frequency dependence of Reactance

2. Interpretation of the findings of the respiratory tract and the endoscopy

The external inspection was without any suspicious findings in all horses. One horse (IV) had a minimal serous discharge on the first day which was evaluated as physiological. Shape and form of noses were physiological according to the species and no nasal stridor was detected. The exhaled air was expired with a normal strength, the outcome was symmetric and temperature and odor were in a physiologic range.

Nasal plane, nasal openings and surfaces were intact and both sides were symmetrical with movable alae.

During endoscopy we saw different clinical pictures. The grading system for the the laryngeal function after: Proceedings of the Havermeier Foundation Monograph Series No. 11 (Equine Recurrent Laryngeal Neuropathy) 2003, p 96 (quoted after Barakzai, 2007) was used to evaluate the Larynx.



Picture 6: Cartilages of a healthy horse

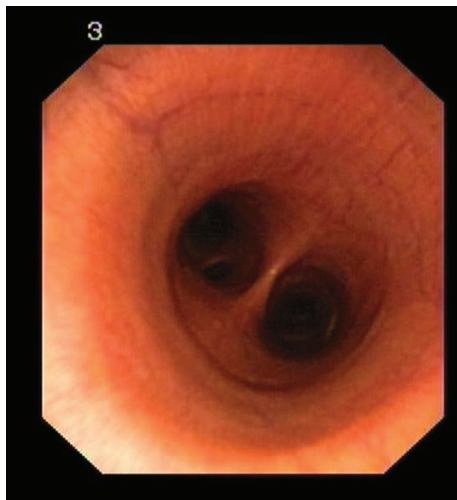
Test horse I showed a pathological finding on the Larynx, it was graded with 3 subgraded with 2 on the left arytenoids cartilage. The mucus membranes were physiological but there was a mucus accumulation in the trachea of score 2 (as explained in the introduction), meaning there were moderate larger blobs which were located ventrally, score 1, the apparent viscosity was graded with 2-3 and the color was evaluated as score 3.

The carina was nice and sharp without any abnormalities.

Test horse II showed a totally healthy respiratory tract. The Larynx was graded with 1, there was no mucus at all, with other words graded as 0 and the carina was in physiological shape and condition.

Test horses' III Larynx was graded with 1, the fluid accumulation also with 1, which means that small little blobs were present, the apparent viscosity was graded with 1 and the color was graded 4-5.

Also here the carina was in perfect condition with a sharp margin.



Picture 7: Normal carina

Test horse IV, the youngest of the test horses, had a Larynx graded with 1, but she had an enlargement of the lymphoid follicles on the walls and roof of the nasopharynx and particularly around the dorsal pharyngeal recess.



Picture 8: Larynx of horse IV with enlarged lymphoid follicles

Beside this finding horse IV was unsuspecting. There was no fluid in the respiratory tract and the carina looked just as fine as in the other horses.

From the endoscopic point of view no lower airway disorder was detectable.

3. Interpretation of the BAL

According to Hoffmann (2008) the reference values for BAL cytologic differential cell count of the horse is as follows:

Macrophages 50-70%

Neutrophils < 5%

Lymphocytes 30-50%

Eosinophils < 0,1

Others, e.g. the University of Leipzig tolerate Neutrophil counts up to 9% and Eosinophil counts up to 1%.

Deviating parameters in table 1 (next page) are marked red.

Table 1: BAL results

	Reference value	Horse I	Horse II	Horse III	Horse IV
Macrophages	50-70%	25%	40.5%	27%	51.5%
Neutrophils	< 5%	38%	26%	40.5%	5%
Lymphocytes	30-50%	37%	33.5%	32%	43%
Eosinophils	< 0.1	0%	0%	0.5%	0.5%
Comments			Sporadic phagocytized plant debris is found in the macrophages, seldom hemosiderin	Beside a mean neutrophilia eosinophil granulocytes are sporadically present. Some macrophages show reactivity.	Weak lymphocytosis, sporadically eosinophilic granulocytes can be detected.
Diagnosis		Mean neutrophilia	<ol style="list-style-type: none"> 1. Mild neutrophilia 2. Minimal old hemorrhage 	<ol style="list-style-type: none"> 1. Mean neutrophilia 2. Minimal eosinophilia 3. Slight phagocytic activity 	<ol style="list-style-type: none"> 1. Mild lymphocytosis 2. Minimal eosinophilia

With the help of the table one can easily see that in two cases the macrophages are drastically decreased and at the same time the neutrophils are increased. In horse II the macrophages are slightly decreased and a mild neutrophilia is present. It seems that there is a correlation between the decrease of macrophages and the increase of neutrophils. This is the typical cytological picture of an inflammation.

In two cases the eosinophils were slightly increased and in horse number IV a relative lymphocytosis is present.

4. Interpretation of the radiographic images

None of the horses showed any signs of disease on the x-ray image. All structures were in normal condition according to the age and the species. The Diernhofer triangle was clearly visible in all cases, the lungs were in normal position and so was the heart. The density of the lungs was averagely.

5. Interpretation of the Hematology and Biochemistry

The horses' blood was examined for white blood cells and biochemistry parameters. For a better overview the reference values are seen in the table. These values are the standards of the laboratory of the large animal clinic in Üllö, Hungary. Deviating parameters are marked red.

Table 2: Hematology and Biochemistry results

	Reference Value	Horse I	Horse II	Horse III	Horse IV
Hematology					
Neutrophil granulocytes (segmented)	45-55%	80	70	58	54
Neutrophil granulocytes	3-5%	6	4	6	4

(jugend)					
Lymphocytes	30-40%	14	20	34	34
Eosinophil granulocytes	2-4%	-	6	2	8
Fibrinogen	0.5-4 g/l	2.02	1.78	1.89	1.92
Biochemistry					
Albumin	27-40 g/l	33.79	32.39	33.81	34.14
Protein	60-85 g/l	82.6	81.1	85.2	80.6
Globulin	30-40 g/l	48.8	48.71	51.39	46.46
Alkaline-phosphatase (ALKP)	150-320 NE/l	226	331	378	325
Gamma glutamyl- transferase (GGT)	10-60 NE/l	12	15	17	10
Glutamate- dehydrogenase (GLDH)	<20 Ne/l	3.3	3.4	5.3	2.7
Amilase	15-50 NE/l	22	43	15	46
Lipase	40-80 NE/l	48	51	54	50
Glucose	3-4.5 mmol/l	4.77	3.76	5.02	4.19
Triglycerid	0.1-0.4 mmol/l	0.48	0.44	0.62	0.48
Lactic acid	1-1.8 mmol/l	2.26	2.30	2.81	2.56
Creatinkinase (CK)	70-160 NE/l	421	290	273	375
Lactatdehydrogenase (LDH)	150-400 NE/l	832	500	470	433
Carbamid	3.6-8.6 mmol/l	5.3	6.9	5.5	5.1
Creatinin	70-160 µmol/l	118	163	131	141

Horses I and II have a pathological hematology profile. A drastic increase of segmented neutrophils is visible and a clear decrease of lymphocytes. This phenomenon is called 'right shift'. A right shift is characteristic for a chronic inflammation.

Increased eosinophils can be a sign for allergy or parasites but not necessarily. So an increase of eosinophils alone is not enough for a diagnosis.

In the biochemistry profile all horses showed an increase of globulin. There are several reasons for a globulin increase and it is too unspecific to make a diagnosis just based on globulin, but globulin gives us information about the general health status. If globulin is high, the body is fighting against something.

Test horses II and III had a slightly increased ALKP level. Although ALKP is present in every cell, only hepatic and bone ALKP appears in the blood. But again it is not specific enough to give a diagnosis.

6. Summary of the results

Based on the results, it can be said that all horses had some type of lower airway disorder, basically RAO or IAD.

All the results which were taken into account from the FOM clearly showed that there were no obstructions in the respiratory system of the examined horses.

The radiography showed no signs of a respiratory disease either.

The BAL and the hematology on the other hand showed chronic inflammations in the bodies. The BAL is a specific tool for the diagnosis of the lower respiratory system and the hematology for a general health condition of the body. Three of the horses had a pathological result in the BAL, showing signs of a subclinical airway disease. Two of these horses were suspicious regarding allergies.

Two of the horses which had a pathological result in the BAL also had a pathological result in the hematology, also showing signs of a chronic inflammatory disease.

Chapter 5 – Discussion and Conclusion

Pulmonary function tests in comparison to BAL were performed already, but there were differences to our tests.

Fraipont et al. (2011) examined the pulmonary function with impulse oscillometry and BALF of well performing and poor performing endurance horses. They found out that the group of poor performing horses had a definite subclinical respiratory disease, but there was no difference for their mechanic breathing performance along the two groups.

Richard et al. (2009) also performed impulse oscillometric measurements, but their test group was free of any clinical signs. They found a correlation between the eosinophil and mast cell count of the BALF in the whole tested population.

Hoffman (2002), Couetil et al. (2007) and Bedenice et al. (2008) used FOM to verify presence or absence of mechanical disturbances in the respiratory system. They all found a correlation between the BALF and the FOM. In their test groups horses were already diagnosed with IAD or RAO. Couetil et al. (2007) defined IAD with a chronic, intermittent cough (although the absence of cough does not automatically mean that there is no disease), an increased mucus production and a lower performance or exercise intolerance. RAO was defined by Kutasi et al. (2011) as a chronic neutrophilic pulmonary inflammation accompanied by a mild cough up to strong dyspnoe and associated with the presence of dust or allergens.

The test group used for this thesis was clinically healthy and had not already been diagnosed with RAO or IAD. Only a small number of horses could be used, because horses should not have been stabled before, should be as young as possible but calm enough to withstand the FOM without sedation. This was important for the experiment as for example Gerber et al. (2003) proofed that all horses which were kept in a standard stable, meaning straw bedding and hay fodder, showed evidence of IAD.

As most of the horses could not tolerate the FOM calmly enough, the clinics' own horses were used, which were kept mainly outside before the experiment.

We have a different number of forced oscillation measurements for the horses, because only those data were used which had a coherence above 0.7. Other data were discarded.

The enlargement of the lymphoid follicles in test horse IV was according to Barakzai (2007) an extremely common finding in young horses, which is called Pharyngeal lymphoid hyperplasia (PLH). This condition is of no clinical importance in young horses as the follicle decrease in size with age.

Based on our findings all horses had some type of lower airway disorders (RAO or IAD). This shows that although there were negative results in all horse with FOM, BAL still showed different type of inflammations.

In conclusion, the FOT is a great method for evaluating the lung function of the airway system, but not for detecting subclinical inflammations without obstructions. The BAL cannot be replaced by the FOT and is still more accurate as the FOT in cases of RAO or IAD.

References

- Barakzai S.** (2007): Handbook of Equine Respiratory Endoscopy. UK: Elsevier Limited. p. 35-91
- Beech J.** (1991): Equine respiratory disorders. Pennsylvania, USA: Lea & Febiger. p. 10-51
- Couetil L.L.,** Rosenthal F.S., DeNicola D.B., Chilcoat C.D. (2001): Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am J Vet Res* 2001 62: 538–546
- Deaton C.M.,** Deaton L., Jose-Cunilleras E., Vincent T.L., Baird A.W., Dacre K., Marlin D.J. (2007): Early onset airway obstruction in response to organic dust in the horse. *J Appl Physiol* 102: 1071-1077s
- DuBois A.B.,** Brody A.W., Lewis D.H., Burgess B.F.Jr (1956): Oscillation mechanics of lungs and chest in man. *J Appl Physiol* 8: 578-594
- Fisher A.B.,** DuBois A.B., Hyde R.W. (1968): Evaluation of the forced oscillation technique for the determination of resistance to breathing. *J Clin Invest.* 1968 September; 47(9): 2045–2057.
- Flügel E.** (1978): Reelle, induktive und kapazitive Strömungswiderstände. Workshop Bad Reichenhall, 14. Januar 1978. In: Nolte, D., V. Korn (1979): Oszillatorische Messungen des Atemwiderstandes. München-Deisenhofen: Dustri, pp. 4-15
- Frosolono M.F.,** Charms B.L., Pawlowski R., Slivka S. (1970): Isolation, characterization, and surface chemistry of a surface-active fraction from dog lung. *J. Lipid Res.* 11, 439
- Hoffman A.M.** (1999): Programme of lung function testing horses with suspected small airway disease. *Equine Vet Educ* 11: 322-328
- Hoffman A.M.,** Mazan M.R., Ellenberg S. (1998): Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. *Am J Vet Res* 59: 176-181
- Hoffman A.M.** (2008): Bronchoalveolar Lavage: Sampling Technique and Guidelines for Cytologic Preparation and Interpretation. *Vet Clin Equine* 2008 24: 423-435
- Hoffmann A.M.** (2002): Clinical Application of Pulmonary Function Testing in Horses. Ithaca, New York, USA: International Veterinary Information Service. In: *Equine Respiratory Diseases*. P. Lekeux (Ed.). URL: http://www.ivis.org/special_books/lekeux/hoffman/ivis.pdf Downloaded: 28.02.2012
- Kaup F.J.,** Drommer W. (1985): Das Surfactantsystem der Lunge. Teil I: Morphologie, Zusammensetzung und Funktion des Surfactantsystems bei Mensch und Tier – eigene Befunde und Literaturübersicht. *Berl. Münch. Tierärztl. Wschr.* 98, 73-80
- Landser F.J.,** Nagels M., Demedts M., Billiet L., Van der Woestijne K.P. (1976): A new method to determine frequency characteristics of the respiratory system. *J. Appl. Physiol.*, 1976 41: 101-106
- McGorum B.C.,** Dixon P.M., Robinson N.E., Schumacher J. (2007): *Equine Respiratory Medicine and Surgery*. Philadelphia, USA: Elsevier Limited: 211-594
- Michaelson E.D.,** Grassman E.D., Peters W.R. (1975): Pulmonary mechanics by spectral analysis of forced random noise. *J. Clin. Invest.*, 1975 56: 1210-1230
- Munroe G.A.,** Weese J.S. (2011): *Equine Clinical Medicine, Surgery, and Reproduction*. London, UK: Manson Publishing Ltd. p. 381-389

- Ostveen E.**, MacLeod D., Lorino H., Farré R., Hantos Z., Desager K., Marchal F. (2003): The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 2003; 22: 1026-1041
- Radostits O.M.**, Gay C.C., Hinchcliff K.W., Constable P.D. (2007): *Veterinary Medicine; A textbook of the diseases of cattle, horses, sheep, pigs, and goats*. Great Britain: Elsevier Limited, 2007. pp. 485-490
- Reinhold P.** (1997,a): Atmungsmechanik beim Tier 1. Mitteilung: Physiologische Grundlagen und konventionelle Untersuchungsmethoden – Übersichtsarbeit. *Berl. Münch. Tierärztl. Wschr.* 110, 251-256
- Reinhold P.** (1997,b): Atmungsmechanik beim Tier 2. Mitteilung: Die forcierte Oszilloresistometrie – Übersichtsarbeit. *Berl. Münch. Tierärztl. Wschr.* 110, 257-266
- Ritz T.**, Dahme B., DuBois A.B, Folgering H., Fritz K.G., Harver A., Kotses H., Lehrer P.M., Ring C., Steptoe A., van de Woestijne K.P. (2002): Guidelines for mechanical lung function measurements in psychophysiology. *Psychophysiology* September 2002, Volume 39, Issue 5, p. 546–567
- Rush B.**, Mair T. (2004): *Equine Respiratory Diseases*. Iowa, USA: Blackwell Science Ltd. P. 189-193
- Smith H.J.**, Reinhold P., Goldman M.D. (2005): Forced oscillation technique and impulse oscillometry. *Eur Respir Mon* (2005) 31: 72-105
- Taylor F.G.R.**, Brazil T.J., Hillyer M.H. (2010): *Diagnostic Techniques in Equine Medicine*. UK: Elsevier Limited. p. 238-239
- Tilley P.**, Sales Luis J.P., Branco Ferreira M. (2012): Correlation and discriminant analysis between clinical, endoscopic, thoracic X-ray and bronchoalveolar lavage fluid cytology scores, for staging horses with recurrent airway obstruction (RAO). *Research in Veterinary Science* 93: 1006–1014
- Van Erck E.**, Votion D., Art T., Lekeux P. (2004): Measurement of respiratory function by impulse oscillometry in horses. *Equine vet. J.* 36 (1) 21-28
- Young S.S.**, Tesarowski D., Viel L. (1997): Frequency dependence of forced oscillatory respiratory mechanics in horses with heaves. *J Appl Physiol* 82: 983-987
- Young S. S.**, Tesarowski D. (1994): Respiratory mechanics of horses measured by conventional and forced oscillation techniques. *J Appl Physiol* June 1, 1994 76:(6) 2467-2472

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