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Early detection of pulmonary dysfunction in the horse - an introduction

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Summary

Equine pulmonary function testing has long focused on horses with clinical signs of recurrent airway obstruction (RAO). The most widely used method is based on esophageal balloon pressure and pneumotachograph measurements. The disadvantages of this traditional pulmonary function test are its invasiveness, considerable cost and lack of sensitivity: airway obstruction can only be detected and measured, once it has become relatively severe and is clinically apparent. Volumetric capnography, on the other hand, is an effort-independent method of pulmonary function testing, which promises superior characteristics and performance. The goal of the work was to validate this method by the use of ultrasonic spirometry and capnography in the awake, spontaneous breathing horse.

Keywords: pulmonary function, volumetric capnopgraphy, pulmonary function test, Pulmology

Zur Früherkennung von Lungenfunktionsstörungen beim Pferd - eine Einführung

Seit langem wurden Lungenfunktionsprüfungen bei Pferden mit der klinischen Symptomatik einer rezidivierenden Atemwegsobstruktion (RAO) in die Untersuchung mit einbezogen. Die am häufigsten angewandte Methode zur Lungenfunktionsprüfung beim Pferd basiert auf Interpleuraldruckmessungen mit der Ösopagusballonsonde und Flow-/Volumenmessungen mit dem integrierten Pneumotachographen. Der Nachteil dieser herkömmlichen Methode zur Lungenfunktionsprüfung beim Pferd liegt in seiner Invasivität, Aufwendigkeit und Mangel an Sensitivität. Diese Methode erfasst Lungenfunktionseinbußen nur, wenn sie sich im fortgeschrittenen Stadium befinden und sich durch klinische Symptomatik bemerkbar machen. Die volumetrische Kapnographie ist eine ohne die Co-Operation des Probanden auskommende, nicht invasive Methode der Lungenfunktionsprüfung beim Pferd. Das Ziel der Arbeit war, diese Methode mit Hilfe der klinisch einfach zu applizierenden Ultraschall-Spirometrie und Kapnographie zu validieren. Der Artikel gibt eine Übersicht über die verschiedenen Stufen in der Validierung der volumetrischen Kapnographie beim Pferd und verweist auf die korrespondierende Literatur

Schlüsselwörter: Lungenfunktion, volumetrische Kapnographie, Lungenfunktionstest, Pulmologie

Recurrent airway obstruction (RAO), also known as heaves, is a form of the syndrome known as equine chronic obstructive pulmonary disease (COPD) and is a hypersensitivity to the dust, molds, mites and spores in the environment of a susceptible horse (*Marti* et al. 1991; *Halliwell* et al. 1993). It is now clear that equine 'heaves' is characterized by reversible airway constriction that is due to bronchospasm apart from mucous swelling and secretion. In this regard, it more closely resembles human asthma than human COPD, which is a progressive disease with little reversibility that is usually related to smoking.

Recurrent airway obstruction plays a crucial role in equine medicine. In a study of Swiss and German horses, in which the diagnosis was based on abnormal lung sounds, and the presence of airway secretions containing neutrophils, the incidence of lower airway disease was 54 %, respectively 52 % (*Bracher* et al. 1991, *Herholz* 1993).

The main lesion of RAO in horses is bronchiolitis characterized by diffuse epithelial hyperplasia, mucus plugging of airways and neutrophilic, lymphocytic and plasmacytic infiltrates and hypertrophy of bronchiolar musculature (*Thurlbeck* and *Lowell* 1964; *Gerber* 1973, *Robinson* et al. 1996). In many instances the onset of disease is insidious and the owner is unaware of exactly when clinical signs started (*Herholz* 1993). Clinical signs range from intolerance to exercise in the performance horse to expiratory dyspnoea, chronic purulent nasal discharge, cough and weight loss in the chronic respiratory patient.

From a functional point of view, respiratory diseases exert their main deleterious effect through factors that alter ventilation to perfusion (VA/Q) ratios throughout the lung. The VA/Q matching of the lung is known to be vital to ensure efficient gas exchange and oxygenation of all body systems. A widening of the range of VA/Q ratios is associated with an increase in the number of alveoli which are either overventilated or underventilated with respect to their perfusion and diffusion capacity. The differences may be evenly distributed throughout the lung or confined to individual lobes and segments. VA/Q disturbances may occur long before clinical manifestations of respiratory disease appear.

Objective quantification of pulmonary function in humans with lower airway disease is currently clinical routine, especially in the early stages of the disease. The most important condition for spirometry in humans are voluntary, sometimes difficult breathing maneuvers, which require patient co-operation. Quantification of pulmonary functional impairment in horses by spirometry is difficult due to the lack of co-operation. In particular difficult to detect and important with regard to the prognosis are early and mild stages of pulmonary parenchyma and airway disease.

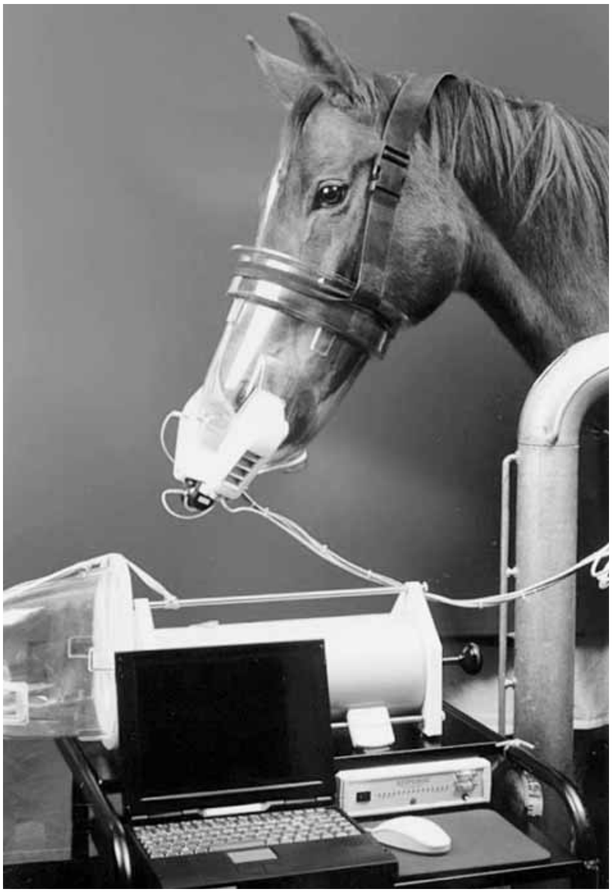

Fig. 1 Ultrasonic spirometry and capnography as a clinically easy applicable tool for generation of volumetric capnograms in the horse
Ultraschallspirometrie und -kapnographie als klinisch einfach anwendbare Methode zur Erstellung volumetrischer Kapnogramme beim Pferd

Pulmonary function tests in horses are dependent on involuntary breathing and meet almost the same requirements as in human neonates and infants. Volumetric capnography is an effort independent method of pulmonary function testing and has not been validated in the awake, spontaneous breathing horse.

A review of literature in equine pulmonary function testing

Equine pulmonary function testing has traditionally involved horses with clinical signs of lower airway obstruction (*Willoughby* and *McDonell* 1979; *Robinson* et al. 1996, *Herholz* et al. 2000) and has centered on the evaluation of breathing mechanics via measurements of maximal changes in transpulmonary or pleural pressure, dynamic lung compliance (Cdyn) and pulmonary resistance (RL) using the pneumotachograph-esophageal balloon method (*Derksen* et al. 1982; *Deegen* and *Stadler* 1986). Earlier investigators (*Spörri* and *Leemann* 1964; *Sasse* 1971; *Muylle* and *Oayert* 1973) performed transpulmonary pressure measurements directly by placing a catheter within the intrapleural space. Respiratory mechanics have also been measured by forced oscillation techniques (*Young* et al. 1997). The correlation between the results of forced oscillation technique and the pneumotachograph-esophageal balloon method was good (*Young* and *Tesarowski* 1994; *Mazan* et al. 1999). Tidal breathing flow-volume loops (TBFVL) have been measured by integrated pneumotachography (flowtransducer combined with a pneumotachograph for simultaneous measurements of air flow rate and inspiratory and expiratory volumes). In non-cooperative patients, including human infants (*Banovcin* et al. 1995; *Lodrup Carlsen* et al. 1997; *Paetow* et al. 1999), dogs (*Amis* and *Kurpershoek* 1986; *Amis* et al. 1986), cats (*McKierman* et al. 1993) and horses (*Schatzmann* et al. 1974; *Connally* and *Derksen* 1994; *Petsche* et al. 1994; *Guthrie* et al. 1995) TBFVL have been used to detect airway obstruction. It has been suggested that peak in- and expiratory flows rather represent the elastic than resistive properties of the lungs and are increased in horses with lower airway obstruction (*Gillespie* et al., 1966, *Petsche* et al., 1994).

Gas dilution methods (*Denc-Sikiric*,1976; *Willoughby* and *McDonell* 1979) as well as pletysmography (*Leith* and *Gillespie* 1971;*Reinhard* et al. 1972; *Attenburrow* 1990 provide measurements of end-expiratory lung volumes (functional residual capacity, FRC). In horses with obstructive pulmonary disease increased FRC have been reported compared to normal horses (*Leith* and *Gillespie* 1971; *Denac-Sikiric* 1976), most likely as a result of air trapping in the alveoli (*Robinson* et al. 1996).

Arterial oxygen tension (PaO_2) and alveolar to arterial oxygen tension gradient ($AaPO_2$) have also been widely used to assess pulmonary gas exchange, especially in clinical practice (*Meister* et al. 1976; *Willoughby* and *McDonell* 1979; *Klein* and *Deegen* 1986).

The short-comings of these established pulmonary function tests are their invasiveness and lack of sensitivity. These measures only detect airway obstruction once it has become relatively severe and is clinically apparent (*Willoughby* and *McDonell* 1979; *Bayly* 2000; *Couetil* et al. 2001).

Airway obstruction is clinically apparent at rest in horses with RAO in exacerbation or crisis, typically occurring within 24 hours of exposure to hay (*Fairbairn* et al. 1993), but not in animals with RAO in remission (*Robinson* 2001). This clinically asymptomatic form of equine lower airway disease is important, however.

The multiple-breath nitrogen washout test may provide a sensitive method to detect clinically inapparent RAO as it remains abnormal in mildly affected horses (*Gallivan* et al. 1990).
Forced expiration is one the most useful and commonly used pulmonary function tests for the early detection of lower airway disease in humans (*West* 1997). Recently, a test for airflow obstruction in horses using forced expiration maneuvers has been established which was able to detect mild peripheral airway obstruction (*Couetil* et al. 2000). However, these methods require expensive equipment and considerable technical assistance and are limited to the larger referral type veterinary clinics.

Flowmetric comparisons of respiratory inductance plethysmography and pneumotachography has been shown to provide a defensible method for quantifying airway obstruction in the horse without the need for invasive instrumentation (*Hoffmann* et al. 2001). However, this method has only been validated in a small population of horses with severe RAO. Further studies are required to determine the repeatability and sensitivity to identify early stages of lower airway disease, as well as the feasibility of this system for field measurements.

Ultrasonic spirometry in combination with capnography as a tool for measurements of volumetric capnograms opened up a method for simple, reliable, economical and rapid pulmonary function testing in equine clinical practice (*Buess* et al. 1993, *Herholz* et al. 1997, 2001a). Pulmonary function testing in horses with ultrasonic spirometry can be performed by the investigator and one person for technical assistance.
Ultrasonic spirometry is based on the measurement of 2 absolute transit times of sound waves across a tubular sensor from which the flow velocity and volume of breathing can be determined (Figure 2). The inherent advantages of this method compared to conventional spirometry (integrated pneumotachography) are its linearity and speed, its independence of gas composition, temperature and pressure (*Buess* et al. 1995).

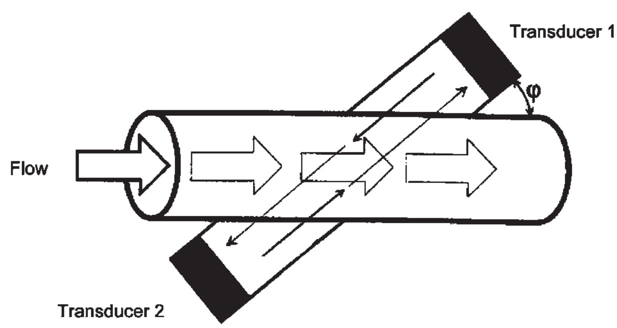

Fig. 2 Measurement of ultrasonic transit times
Messung der Ulztaschall-Transitzeiten

The effect of the face mask on respiration has been investigated by *Holcome* et al. (1996). A face mask was designed and fitted to the ultrasonic flow sensor (*Buess* et al. 1993, *Herholz* et al. 1997, 2001a), which does not affect respiration and meets the criteria of the American Thoracic Society (ATS; the dead space of the face mask was less than 10 % of the tidal volume, the flow resistance not measurable during tidal breathing at rest and 6 mbar at a flow rate of 100 L/s).

To provide an overview, different pulmonary function tests in the horse, the measurement techniques, advantages and disadvantages of the respective tests are listed in Table 1.

The plot of expired CO_2 concentration versus expired volume is called the volumetric capnogram or single breath diagram for CO_2 (SBD-CO_2). *Fowler* (1948) divided the concentration volume curve into three distinct phases, relating each phase to the anatomic compartments of the respiratory system in humans. Phase I is the relatively carbon dioxide free washout of the proximal conducting airways. Phase II is the transitional portion of the curve characterized by a rapid upswing as the alveolar gas surges mouthward and mixes within the conducting airways. Phase III represents the gases from the acini or peripheral alveolated airway and is known as the alveolar plateau.

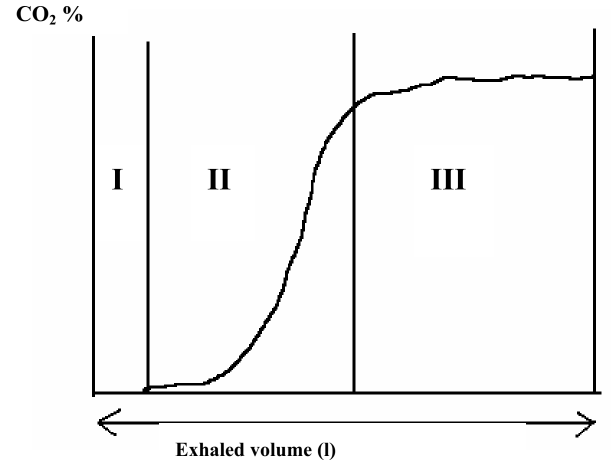

Fig. 3 The three phases of the volumetric capnogram
Die drei Phasen des volumetrischen Kapnogramms

There are two different approaches in evaluating volumetric capnograms. The first one is to analyze the shape of the CO_2-volume curve in healthy individuals and those with lower pulmonary disease. The second approach is to analyze pulmonary function indices derived from the volumetric capnogram. These are different dead space indices (e.g. dead space fraction according to Bohr's equation,. physiological and alveolar dead space fractions) and indices of efficient CO_2 elimination from the lungs.

Challenges in research

Starting on working with ultrasonic spirometry and capnography pulmonary function testing for the observer the most obvious occurring change was the shape deformity of volumetric capnograms with increasing degree of lower airway obstruction. The aim of the author and co-workers was to investigate, whether the shape analysis of volumetric capnograms is a useful tool to describe pulmonary functional disturbances and to discriminate horses with different degrees of lower airway obstruction. The authors were interested in the variability and reliability of volumetric capnography indices of horses breathing at rest and after application of a centrally acting respiratory stimulant (Lobelin®). The validity of these indices in diagnosing different degrees of lower airway obstruction were investigated and compared to the clinical examination. Also the precision was investigated with which clinical signs and the ^{99m}Tc-DTPA clearance from the lung as an index of alveolar integrity can predict pulmonary functional disturbances in horses with different degrees of lower airway obstruction. It was hypothized that age, sex and usage of the horses have significant effects on pulmonary function estimated by volumetric capnography and have to be considered

Table I.(pp 368-369) Equine pulmonary function tests; measuring technique, measured and derived pulmonary function indices, advantages and disadvantages of the different techniques.
Lungenfunktionstests beim Pferd, Messtechniken, Lungenfunktionsindizes, Vor- und Nachteile der unterschiedlichen Verfahren

Pulmonary function test	Measuring technique	Measured / derived pulmonary function indices	advantage	disadvantage
Pulmonary function indices reflecting mechanical properties of the lungs				
Integrated pneumotachography	Fast response measuring system that consists of a resistance and a differential pressure transducer to detect the pressure drop across the resistance occurring during gas flow	– flow – volume (used to generate tidal breathing flow volume loops – TBFVL)	– not invasive	– limited linear measurement range – dependent on gas composition – dependent on gas temperature – relative large measurement resistance
Ultrasonic spirometry	Measurement of 2 absolute transit times of sound waves across a tubular sensor from which the flow velocity and volume of breathing can be determined	– flow – volume, – molecular mass of the gas mixture (used to generate TBFVL)	– high linearity (up to a flow rate of 100L/s) and speed – independent on gas composition – independent on gas temperature and pressure – no frequent and easy calibration	– limited linear measurement range
Integrated pneumotachography combined with the esophageal balloon method	An esophageal balloon catheter is placed to the level of the midthorax of the horse and connected to a differential pressure transducer. The opposite pole of the pressure transducer is connected to a side port in the gas-collection mask of the pneumotachograph	– Transpulmonary pressure measurements (ΔPpl) – pulmonary resistance (RL), RL=ΔVol/ΔPpl; Vol=volume – dynamic lung compliance (Cdyn), Cdyn = ΔPpl/ΔV; V = flow – work of breathing	– does not require patient co-operation	– invasive – requires a referral type clinic – limited sensitivity
Body plethysmography	Horse is placed inside an airtight, inexpansible box having it breathe through a mouthpiece leading to the outside	– Functional residual capacity (FRC) – airway resistance, – tissue resistance, – pulmonary blood flow – compliance		– not easily adaptable to the equine temperament – requires a referral type clinic – expensive
Inductance plethysmography (combined with pneumotachography)	Two sensors are secured at the 11th intercostal space and 18th rib; relative changes in phase angle i.e. thoracoabdominal asynchrony are used as a measure of airway obstruction	– Flow estimated from the respiratory effort measured at the body surface and flow measured at the airway opening by pneumotachgraphy are subtracted. – The discrepancy between respiratory effort and resultant flow at the airway opening represents airway resistance. – FRC	– no need of invasive instrumentation	– prone to errors in the measurement of volume or flow, because of gas compression during obstructions – repeatability of measurements has not been investigated – sensitivity of the method to detect early stages of pulmonary dysfunction in RAO horses has not been investigated
Forced expiration (FE)	FE is induced after lungs are inflated to total lung capacity by exposing the horses airways to a vacuum reservoir via a nasotracheal tube. Flow is measured indirectly by computing the changes in pressure in the vacuum reservoir	– forced vital capacity (FVC) – forced expiratory volume in one second (FEV_1) – forced expiratory volume at x % of exhaled vital capacity ($FEF_{x\%}$) – peak expiratory flow (PEF)	– sensitive method to detect early stages of lower airway obstruction in the horse	– invasive (requires intubation) – patient co-operation essential – requires sedation of the horse – expensive, requires significant energy supply – requires a referral type clinic

Forced oscillation technique	A sinusoidal airflow of the desired frequency is generated by a pneumatic valve connected to a compressed airline. The oscillating airflow is applied to the horse's respiratory system by using a plastic T-piece. A resistor attached to the side arm of the T-piece directs most of the oscillating flow into the horse while the horse breathes relatively normal. Mask pressure relative to atmospheric pressure is measured with a differential pressure transducer.	– total respiratory impedance (Zrs) – respiratory resistance (R)	– noninvasive – patient cooperation not essential	– expensive, requires significant energy supply – method was not sensitive to detect early changes in respiratory mechanics of RAO horses – requires a referral type clinic
Pulmonary function indices reflecting mechanical properties as well as ventilation / perfusion matching of the lungs				
Gas dilution methods	A known quantity of an inert gas (helium,He; sulfur hexafloride, SF6) is introduced into an airtight spirometer and the resultant concentration is determined with a mass spectrometer. The horse rebreathes from the spirometer until the mixing of the inert, insoluble gas is complete. Knowing the initial spirometer volume, initial and final inert gas concentrations, functional residual capacity can be calculated.	– Functional residual capacity(FRC)		– expensive – method was not sensitive to detect early stages of pulmonary dysfunction in RAO horses – prone to measurement errors, because an absolutely airtight rebreathing system is not easily adaptable in the conscious horse – requires a referral type clinic
Multiple breath nitrogen washout	Inspiratory port of a nonrebreathing valve: O2 supply, expiratory port: 600 L spirometer. At end-inspiration the horse breathes 100% O2, the expired N2 concentration is measured using a fast response N2 analyzer. The washout is continued until end tidal N2 is less than 1 %. The N2 washout of poorly ventilated alveoli is more slowly and therefore an indicator of bronchial obstruction	– FRC – end-tidal N_2 concentration of the final breath – end-tidal N_2 concentration when the cumulative expired volume is equal to body weight – lung clearance index – N_2 clearance delay – ventilatory efficiency	– sensitive to detect early stages of pulmonary dysfunction in RAO horses	– expensive requires a referral type clinic
Arterial blood gas analysis	arterial blood samples are analyzed by a blood gas analyzer	– Arterial oxygen pressure (PaO^2) – Arterial carbon dioxide pressure ($paCO_2$) – Alveolar to arterial oxygen tension gradient ($A\text{-}aPO_2$)	– does not require a referral type clinic – expenses within an acceptable range	– invasive – hypoperfused alveoli are not detected – limited sensitivity – analysis must be performed within 2 hours and blood samples have to be stored on ice
Ultrasonic spirometry combined with capnography	Ultrasonic spirometry : see above Capnography: expired CO_2 is analyzed with a fast response CO_2 infrared transducer which is attached to the ultrasonic flow head	– expired volume – expired CO_2 volume – airway dead space – dead space according to Bohr – physiological dead space – alveolar dead space – indices of effective CO_2 elimination	– noninvasive – patient co-operation not essential – expenses within an acceptable range – sensitive to detect early stages of pulmonary dysfunction in RAO horses – does not necessarily require a	– requires the analysis of at least 60 breaths

For references see text

in interpreting pulmonary function testing results. Furthermore, one assumed that by considering the effects of age, sex and usage of the horses on pulmonary function the ability of tidal breathing flow volume loop (TBFVL) indices to detect even minor pulmonary functional changes is increased compared with studies which did not consider these effects.
Finally, the goal was to show that volumetric capnography indices identify pulmonary dysfunction in asymptomatic RAO horses by using the before mentioned interpretative strategies.

The shape analysis of volumetric capnograms

The shape of the volumetric capnogram is modified by airway obstruction and the evaluation of this deformation could allow an indirect measurement of bronchial patency in horses with RAO.

In healthy humans the volumetric capnogram has a nearly rectangular shape (*Ulmer* 1970, *Fletcher* 1980). In human patients with pulmonary disease changes occurring in the shape of the volumetric capnogram are a blurring of the transition between Phase II and III and increased Phase III slopes (*Ollson* et al. 1980; *Fletcher* 1986; *Schwardt* et al.1991, 1994).

Wolff et al. (1989) described the difficulty in interpreting and distinguishing different phases of the volumetric capnogram

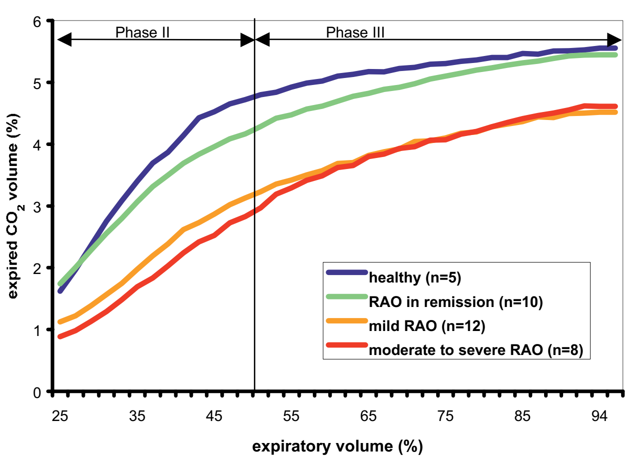

Fig. 4 The shape of volumetric capnograms in horses with different degrees of lower airway obstruction (Herholz et al. 2001b).
Form des volumetrischen Kapnogramms bei Pferden mit RAO unterschiedlichen Grades (Herholz et al. 2001 b).

of spontaneously breathing humans with most severe lower airway obstruction. The division of the volumetric capnogram into strict definitions of phases I, II, and III is arbitrary to some extent as the expirogram is a continuous curve influenced by both convection and diffusion (*Fletcher* et al. 1986).

Based on the proposition of the volumetric capnogram in horses with RAO, analysis of different phases was performed as suggested by *Worth* (1986) in humans. It was shown, that statistical analysis of the volumetric capnogram is a precise method for the description of shape deformities of the CO_2-volume curve in horses with different degrees of lower airway obstruction (*Herholz* et al. 2001b, Fig. 4).

Aspects of measurements reliability and validity

More than any other respiratory disease, RAO is characterized by functional and clinical variability: symptoms may be absent, mild or severe. Although pulmonary function tests have been used to evaluate horses with clinically normal lungs and those with chronic lung disease, few comprehensive studies of equine respiratory function are available and the range of reported normal values is large (*Spörri* and *Denac* 1970; *Muylle* and *Oyaert* 1973; *Orr* et al. 1975; *Willoughby* and *McDonell* 1979; *Guthrie* et al. 1995).

When you measure a variable in a number of individuals, the results will rarely be the same in all subjects and the scatter can be due to the biological variability and experimental errors. It is known that equipment, technique, time of the day, season, medications and the actual state of pulmonary health of the patient each influence the results of pulmonary function tests (*Snider* et al. 1967; *Thiruvengadam* et al. 1969; *Hruby and Butler 1975; Rozas and Goldman 1982). Therefore,* the investigator should attempt to control most of the factors known to affect the variability of pulmonary function tests for results that reflect the true lung function of an individual.

However, the precision of pulmonary function tests in spontaneous breathing horses is limited owing to high breath-to-breath variability in tidal volumes and flow rates (*Gallivan* et al. 1990; *Herholz* et al. 1997). Because the tidal breath volume and frequency are closely related to CO_2 elimination from the lung, multiple breaths should be considered for precise interpretation of pulmonary function tests.

The selection of five (*Ohnesorge* et al. 1998) or ten (*Couetil* et al. 2001) breath cycles can lead to imprecise results and might not reflect the actual state of pulmonary health in a given patient. The averaged measurements over at least 60 breath cycles, have been shown to assure good repeatability (*Herholz* et al. 2001a,c) of pulmonary function indices derived from volumetric capnography. Assuming a respiratory frequency of 16/min, the corresponding measurement times would be about four minutes; during that time conscious horses tolerate a face mask without problems. On the other hand, up to 974 breaths were required for precise calculation of the slope of phase II and III of the volumetric capnogram. Although the model of the statistical shape analysis is precise in the description of shape deformities, it is not necessarily a useful test to discriminate horses with different degrees of lower airway obstruction (*Herholz* et al. 2001a, c).

In order to determine the variability of pulmonary function at levels of ventilation above normal tidal breathing, pulmonary function tests have been performed during exercise (*Art* and *Lekeux* 1988; *Connally* and *Derksen* 1994). Exercise testing may be contraindicated due to age, lack of fitness, the presence of other pre-existing conditions (e.g. lameness) or temperament.

An alternative approach is the use of respiratory stimulants, e.g. lobeline hydrochloride. In the study of *Marlin* et al., (2000) the lobeline-induced hyperpnoea was highly reproducible, with no significant effect on breathing mechanics.
In the evaluation of volumetric capnography indices stimulating respiration with lobeline gave no advantage in the repe-

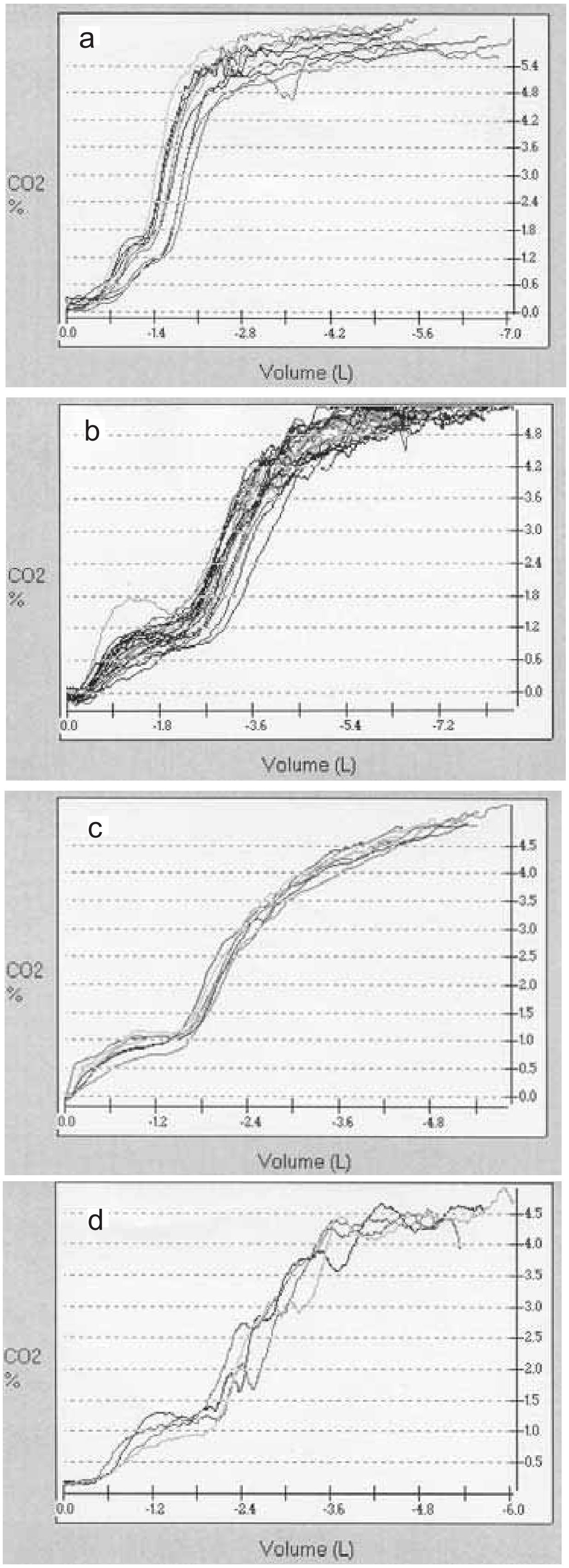

Fig. 5a-d Intra-individual breath-to-breath variability and inter-individual variability of volumetric capnograms in horses with different degrees of lower airway obstruction. a: healthy, b: RAO in remission, c: mild RAO, d: RAO in exacerbation.
Intraindivuelle Atemzugsvariabilität und interindividuelle Variabilität des volumetrischen Kapnogramms bei Pferden mit RAO unterschiedlichen Grades

atability of these indices or in differentiating between horses with different degrees of RAO (*Herholz* et al. 2001c). The quality of measuring devices is often described by the reliability or repeatability and the validity of a diagnostic test is described by the terms 'sensitivity' and 'specificity'. Diagnostic tests are not always precise, may give false positive or false negative results and lead to an incorrect classification of an animal as ill or healthy. The degree of misclassification can be quantified by comparing the diagnostic methods available with a 'Gold-standard' or to what is considered to be the best experienced method at hand. Strictly defined the Gold-standard is a diagnostic test with a 100% accuracy. The sensitivity of a test is the probability that a truly diseased animal will be classified as diseased using the test. The specificity of a test, is the probability that a truly non-diseased animal will be classified as non-diseased with the same test.

To the authors knowledge, there are no studies available describing the sensitivity and specificity of pulmonary function tests. Although far away from a Gold-standard, the clinical examination of the respiratory tract is the best experienced diagnostic method at hand. The validity of volumetric capnography pulmonary function indices has been established compared to the clinical examination as a reference method (*Herholz* et al. 2001d).

Further efforts should be done in the validation of diagnostic tests, since a Gold-standard might not be the best suited method in practice, but complex, expensive and time-consuming.

However, the validity of volumetric capnography indices estimated by *Herholz* et al. (2001d) can be regarded as a trend validation with reasonable results compared to the clinical examination.

Clinical, pathophysiological and functional relationships

To diagnose airway obstruction and evaluate the response to treatment, the clinician must judge the severity of respiratory distress based on clinical signs. These include contraction of the abdominal muscles at the end exhalation, flaring of the nares, respiratory frequency, the presence of cough and nasal discharge, excess mucoid exudates and neutrophilic inflammation within the airways. Clinical signs are regularly used to differentiate horses with airway obstruction from controls (*Bracher* et al. 1991, *Herholz* 1993, *Tesarowski* et al. 1994) and to determine the response to treatment (*Dieckmann*1987; *Traub-Dargatz* et al. 1992; *Herholz* 1993, *Rush* et al. 1998). It is currently impossible for the clinician to draw functional conclusions from the results of the clinical examination of the respiratory tract and it seems inappropriate to rely on the arterial blood gas analysis, only (*Spörri* and *Denac* 1970).
Excellent studies on the relationship between clinical, structural and functional changes in horses with RAO have been performed (*Viel* 1983; *Nymann* et al. 1991; *Rodrigues* et al. 2000).

The accuracy with which clinical signs predict the degree of airway obstruction has been a challenge for further research and was investigated by *Robinson* et al. (2000) and *Herholz* et al. (2002b). Although clinical symptoms (nasal flare, abdominal movement) identified the worsening of airway obstruction, changes in pulmonary function estimated by maximal change in pleural pressure during tidal breathing, pulmonary resistance and dynamic compliance were considerable before they were first recognized by the clinical evaluator (*Robinson* et al. 2000). *Robinson* et al. (2000) concluded, that there can be important pulmonary functional disturbances before the onset of clinical signs, airway disease is probably under-diagnosed and a convenient pulmonary function test would improve the diagnosis and evaluation of the response to therapy.

It was shown by the author, that the subjective, estimated viscosity of tracheobronchial mucus and the percentage of polynuclear neutrophils in the cytology of tracheobronchial secretions was significantly related to pulmonary function indices derived from the volumetric capnogram not only in horses with severe clinical signs of airway obstruction, but also in horses with RAO in remission and in horses with mild symptoms of lower airway obstruction (*Herholz* et al. 2002b). One approach in assessing the pathophysiology in pulmonary gas exchange is the indirect measurement of alveolar epithelial integrity by the clearance rate of ^{99m} technetium-diethylenetriaminepentaacetate (^{99m}Tc-DTPA) aerosol from the lung (*Votion* et al. 1999).
The normal alveolar-capillary membrane acts as a semi-permeable membrane and is therefore vital for efficient gas exchange (*Jones* et al. 1982). The rate of the ^{99m}Tc-DTPA clearance was negatively and significantly correlated with pulmonary function indices derived from the volumetric capnogram (*Herholz* et al. 2001d).

The correlations found by the authors were dependent on the clinical status of pulmonary health which demonstrates the association between the epithelial alveolar integrity estimated by ^{99m}Tc-DTPA clearance and dead space ventilation detected by volumetric capnography.

Aspects of population criteria in diagnosing different degrees of pulmonary disease

Studies of the influence of factors such as age, sex and usage on pulmonary function have not been performed in healthy horses and are required in order to interpret measurements made in those with respiratory disease.

In adults and older children, it is well established that ethnic group is an important determinant of lung function (*American Thoracic Society* 1991; *Stocks* et al. 1997) with both lung volumes and forced expiratory flows tending to be lower in black, than white subjects. Several studies have suggested that pulmonary function is diminished, and respiratory illness more common in boys than girls during both infancy and childhood (*Tepper* et al. 1986). In adults a significant sex times age interaction term showed that healthy elderly men do lose pulmonary function at a faster rate when compared with healthy elderly women (*Griffith* et al. 2001).

Few studies focussing on the age, sex and usage-specific effects on equine pulmonary function have been published over the last twenty years. One study reported on the effect of age on pulmonary function in a population of five ponies (*Derksen* et al. 1982). *Aguilera-Tejero* et al. (1998) investigated the effect of age on arterial blood gases in each sixteen healthy young and aged horses.
It was therefore hypothesized, that age, sex and usage of the horses is a considerable source of variation in the results of pulmonary function indices and that disease related differences in pulmonary functional indices may consequently be obscured by these effects.

The author has done research to investigate the before mentioned effects on volumetric capnography indices (*Herholz* et al. 2001e, 2002c,d). Furthermore, it has been shown, that the differentiating power of already established TBFVL pulmonary function indices increases by considering these effects (*Herholz* et al. 2002e).

Accounting for the significant effects of age, sex and use of the horses in inhomogeneous populations optimized the differentiating ability of pulmonary function indices (*Herholz* et al. 2002c,d,e). By using this interpretative strategy we have shown, that different lung function tests can detect airway obstruction in horses with mild, even clinically inapparent lower airway disease.

Conclusion

Statistical analysis of the volumetric capnogram is a precise method for the description of shape deformities of the CO_2-volume curve in horses with different degrees of lower airway obstruction. To average measurements over at least 60 breath cycles has been shown to assure good reliability of volumetric capnography indices. The sensitivity and specificity of volumetric capnography indices in diagnosing different degrees of lower airway obstruction compared to the clinical examination was reasonable and was highest for the physiological dead space / tidal volume ratio and the index A1/A2 as an index of effective CO_2 elimination.
Pulmonary functional indices derived from volumetric capnograms correlate with the severity of clinical signs and the alveolar integrity estimated by the ^{99m}Tc-DTPA clearance in horses with varying degrees of lower airway disease. In a multivariate statistical model the significant effects of age, sex and usage of the horses on pulmonary function were considered, which enabled us to identify pulmonary dysfunction estimated by volumetric capnography in horses with clinically asymptomatic and mild RAO.

Volumetric capnography by the use of ultrasonic spirometry and capnography offers a simple, non-invasive, effort independent, economical and sensitive method for pulmonary function testing in the awake, spontaneous breathing horse. Strategies presented by the author opened up new horizons in the interpretation of pulmonary function testing results in horses with lower airway obstruction.

Outlook

Future research should involve studies on the pathogenesis and pathophysiology of RAO with the use of volumetric capnography, which proved to detect even minor functional changes of the lungs: One of the major functional abnormalities in human asthma is the bronchial hyperreactivity and the capacity for acute airway narrowing. The potential severity of acute airway narrowing in the individual patient is likely to be reflected by the maximal response to inhaled bronchoconstrictor challenges. Indeed, it has been demonstrated that the maximal degree of airway narrowing (e.g. response to metacholine) is associated with the severity of asthma symptoms (*James* et al. 1992). A study in rats (*Pétak* et al. 1997) demonstrated, that an intravenous Metacholine infusion induced purely airway constriction, whereas aerosolized Metacholine administration gave rise to constriction both in the airways and in the parenchyma. It will be interesting to study respiratory functional responses to intravenous and aerosolized Metacholine using volumetric capnography in horses with RAO and to correlate the severity of the responses to clinical symptoms.

Volumetric capnography enables to interpret the significance of pathophysiological and functional changes caused by allergens, in particular recombinant mould allergens (*Eder* et al. 2000), in RAO horses. A previous study suggested that recombinant mould allergens may be involved in the pathogenesis of RAO, as affected horses had significantly higher serum IgE levels against some recombinant mould allergens than healthy animals. Bronchial provocation with these allergens and detection of functional changes with volumetric capnography will determine the role of these recombinant allergens in the pathogenesis of RAO. These investigations may finally lead to set up bronchoprovocation tests with recombinant allergens which allow to predict horses susceptible for RAO.

It is of pathophysiological interest to relate volumetric capnography pulmonary functional indices to objective measurements of the rheological properties of mucus (*Gerber* et al. 2000) in horses with RAO. Possibly, the observed relationship between volumetric capnography indices and subjective, endoscopically made estimation of mucus viscosity (*Herholz* et al. 2002b) can be confirmed by these objective rheological measurements.

Currently, the ^{99m}Tc-DTPA clearance is the most sensitive indicator of lung damage in the horse (*Votion* et al. 1999). It will be of major interest to look at the sensitivity and specificity of volumetric capnography indices compared to the ^{99m}Tc-DTPA clearance as a Gold-standard in larger populations of horses with different usage.

Abbreviations

$AaPO_2$: alveolar to arterial oxygen tension gradient
Cdyn: dynamic lung compliance
COPD: chronic obstructive pulmonary disease
FEV1: forced expiratory volume in one second
FRC: functional residual capacity
PaO_2: arterial oxygen tension
RAO: recurrent airway obstruction
RL: pulmonary resistance
SBD-CO_2: single breath diagram for carbon dioxide
TBFVL: tidal breathing flow volume loops
^{99m}Tc-DTPA: ^{99m} technetium-diethylenetriaminepentaacetate
VA/Q: ventilation to perfusion ratio

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