Università degli Studi di Milano

PhD Course in Veterinary and Animal Science

XXXII Cycle

Department of Veterinary Medicine

OSCILLOMETRY
IN EQUINE ASTHMA

PhD Candidate: Dr. Luca Stucchi

Supervisor: Prof. Francesco Ferrucci

Co-supervisor: Prof. Jean-Pierre Lavoie

Coordinator: Prof. Valeria Grieco

Academic Year 2018/2019

INDEX

PART A: LITERATURE REVIEW

PART B: RESEARCH PROJECT

ABSTRACT

The present project investigated the use of oscillometry as a mean for the diagnosis of equine asthma, evaluating two different techniques: the impulse oscillometry system (IOS) and the Forced Oscillation Technique (FOT).

The aim of the first study was to evaluate whether the IOS could be sensitive enough to discriminate amongst healthy and asthmatic horses in different clinical conditions.

Seven severely asthmatic (SEA) horses in disease exacerbation, 7 asthmatic horses in clinical remission and 7 control horses from a cohort of experimental age-matched animals underwent IOS testing. Only data at 3, 5 and 7Hz with coherence >0.85 at 3Hz and >0.9 at 5 and 7Hz were considered in the study. Mean, inspiratory and expiratory resistance (R) and reactance (X) and the difference between inspiratory and expiratory X (ΔX) were calculated at each frequency. The three groups were compared using one-way ANOVA and Dunnett's multiple comparison test or Kruskal-Wallis test and Dunn's multiple comparison test.

Significant differences were found between SEA horses in exacerbation and control horses for all R parameters at 3Hz. For X, significant differences were present between exacerbation horses and control horses at each frequency for mean, inspiratory and expiratory parameters. Between controls and remission horses differences were present for X7 and for inspiratory X3, X5 and X7. Regarding Delta X, values in exacerbation horses were significantly higher than remission or control horses.

Results indicate that, as reported in humans during tidal expiratory flow limitation (EFL), X during the expiratory phase is more negative than during inspiration in exacerbated horses. Difference in inspiratory X appears to be promising to discriminate between healthy horse and SEA horses in clinical remission.

The aim of the second study was to evaluate the application of a FOT device specially designed for horses and his ability in discriminate between healthy subjects, SEA horses in exacerbation and moderate asthmatic horses (MEA).

4 SEA horses, 4 MEA horses and 4 controls from a clinical population were selected, age-matched. Data of FOT measurement at 2, 3, 4, 5 and 6 Hz were considered in the study. Mean, inspiratory and expiratory resistance (R) and reactance (X) and the difference between inspiratory and expiratory X (ΔX) were calculated at each frequency. The three groups were compared using Kruskal-Wallis test and Dunn's multiple comparison test.

Statistical comparison showed significant differences between SEA and control group for R at 3Hz and for R at 2 Hz in expiration phase. Regarding X, several differences were found between controls and SEA horse at frequencies ranging from 2 to 6 Hz in all the phases of the breath. Finally, comparison for ΔX showed that values in SEA horses were significantly higher than MEA or control horses, indicating that in SEA horses in exacerbation the reactance during expiratory phase is worse than during inspiration. No differences were found between controls and MEA horses.

Results showed that the new FOT device was able to discriminate between healthy and SEA horses. EFL in SEA horses was identified also by this system. Further studies are required in order to increase the sensitivity in discriminate between healthy and MEA horses.

PART A:
LITERATURE
REVIEW

1. EQUINE ASTHMA

1.1 Definition

The definition of “Equine Asthma” has been recently introduced to describe the non-septic inflammatory disease of the airways of the horse (Lavoie, 2015). In fact, even if the presence of a chronic inflammation of the respiratory system in horses stabled has been recognized for centuries (Markam, 1656), the classification of the different clinical presentations of the disease has been reviewed several times.

The syndrome characterized by reversible bronchospasms in horses fed hay is historically known as “heaves” or “broken wind”. In the half of the 1900, the definition of ‘equine emphysema’, based on the macroscopic appearance of the lungs at necropsy, was introduced (Obel and Schmiterlow, 1948), but later abandoned, as hyperinflation is due to air trapping rather than disruption of the alveolar walls.

The introduction of bronchoalveolar lavage (BAL) cytology allowed clinicians to identify the presence of a large amount of neutrophils in the airways of affected horse, and so the term chronic obstructive pulmonary disease (COPD) was adopted, in the light of similarities with human COPD. However, COPD in human is characterized by pulmonary emphysema, that is not a feature of the horse lungs (Robinson, *et al.*, 1996). In the meantime, BAL allowed to identify the presence of neutrophils, mast-cells and eosinophils also in horses with milder inflammatory condition, not characterized by bronchospasm, and defined as “chronic bronchiolitis” (Nyman *et al.*, 1991)

Therefore, in the late ‘80s, a new different classification was introduced: the term “recurrent airway obstruction” (RAO) should be used to describe the horses affected by reversible bronchoconstriction due to the inhalation of environmental antigens, and “inflammatory airway disease (IAD), for these horses with an inflammation of the lower airways in absence of dyspnea (Derksen *et al.*, 1996, Couëtil *et al.*, 2007).

Nevertheless, more recent studies (Michela Bullone and Lavoie, 2015; Leclere, Lavoie-Lamoureux and Lavoie, 2011) showed several similarities between RAO, IAD and different phenotypes of human asthma. With these premises, it has been proposed to define IAD as a mild/moderate form of “equine asthma” (MEA), while "severe equine asthma” (SEA)" would be used for RAO (Couëtil *et al.*, 2016).

1.2 Epidemiology

Prevalence

In literature only few data are present concerning epidemiological studies on SEA. Hotchkiss *et al.* (2007) reported estimated disease prevalence in the UK of 14%, and it is generally considered representative of the northern hemisphere countries. Concerning MEA, instead, several studies investigated the prevalence of the disease, and it varies from 70-80% in racehorses (Christley *et al.*, 2010; Nolen-Walston *et al.*, 2013) and 20-30% in sporthorses (Robinson *et al.*, 2006).

Risk factor

Horses with SEA are usually 7 years of age or older (Couetil and Ward, 2003). Concerning gender and breed, conflicting results are present in literature, but all sex and breeds can be affected. Furthermore, residence in an urbanized environment, respiratory infections and exposure to hay/straw early in life seem to be significant risk factors for RAO (Hotchkiss *et al.*, 2007). For MEA, instead, the incidence reduces with the increase of age, and young racehorses (< 4 y.o.) result to be more affected (Chapman *et al.*, 2000).

A genetic predisposition, even if this is a common belief between horsemen, has been documented only in German and Lippizaner horses (Marti *et al.*, 1991), but the inheritance of SEA has not been fully clarified. The gene codifying the interleukin (IL)-4 receptor (IL4R) seems to play a role in the expression of SEA (Gerber, Tessier and Marti, 2015).

1.3 Aetiopathogenesis

It is clearly evident that the exposure to airborne organic dust, that is commonly present in stables and in the hay of feeding, has a key role in the induction of SEA. Numerous potentially proinflammatory agents are present in stable dust, including bacterial endotoxins, molds, peptidoglycan, proteases, microbial toxins, forage mites, plant debris and inorganic dusts. Furthermore, high levels of potentially toxic gases such as ammonia may also be present in poorly ventilated stables (Pirie, 2014). Nevertheless, it is universally accepted that the inhaled molds play the principal role in the pathogenesis of the disease. It has been demonstrated that the inhalation of an extract *of Aspergillus fumigatus* and *Faenia rectivirgula* induces an increase of neutrophils in the airways and an exacerbation of symptoms in SEA affected horses, but not in controls (McGorum *et al.*, 1993).

Furthermore, a different phenotype of SEA exists, historically known as summer-pasture associated obstructive pulmonary disease (SPAOPD). The horses affected by this condition have the same clinical and pathological features of RAO, but the exacerbation phase can be triggered by exposing the animals to open pasture, instead of dusty stables. In this cases the aetiological agents are unknown, even if a role of inhaled pollen has been suggested (Rodrigues Costa *et al.,* 2000)

Several studies showed that, in the development of airway inflammation and bronchoconstriction in SEA, the hypersensitivity reaction plays central role, even if the full immunological mechanisms is not fully clarified (Robinson, *et al.*, 1996).

Differently from human asthma, in which there is an early allergic response, SEA is predominantly characterized by a delayed response, by an increase in CD4+ T cells in BALF and the recruitment of neutrophils into the airways about 5-6 hours after allergen exposure (McGorum *et al.*, 1993), Nevertheless, the studies that attempted to identify the Th-type failed to reach an univocal response. Some paper demonstrated an increase in IL-4 and IL-5 level, and a decrease in IFN, suggesting the activation of Th-2 mechanism. Other studies, instead, identified an increase in IFN, supporting a Th-1 or a mixed Th1/Th2 answer. The disagreement among the results may reflect the complexity of the immune response in SEA, suggesting that different T-cell subpopulations could be involved in different phases (Pirie, 2014).

Inflammation, airway obstruction, mucus accumulation and tissue remodeling are the most important features that contribute to the pathophysiology of SEA. Airway inflammation is characterized by massive neutrophil chemotaxis as well as enhanced proteolytic activity and increased oxidative stress (Art *et al.*, 2006.). Bronchospasm in severe asthmatic horses is related to defections in the noradrenergic and nonadrenergic-noncholinergic (NANC) inhibitory systems, hypercontractility related to increased expression of neurokinin receptors and enhanced neurokinin-induced bronchoconstriction as well as increased release of endothelin-1 (Yu *et al.*, 1994). In SEA horses mucus hypersecretion is associated to altered viscoelasticity and reduced mucociliarity clearance (Lugo *et al.*, 2006). Finally, the small airway walls of SEA horses undergo to an increase in thickness of the bronchiolar epithelium, submucosa and smooth muscle, contributing to airway obstruction (Leclere *et al.*, 2011; Bullone *et al.*, 2017).

Also for MEA horses, environmental dust seems to have a central role in the development of the symptoms (Holcombe *et al.*, 2001; Millerick-May *et al.,* 2013). However, differently from SEA, the aetiology seems to be multifactorial. In fact it has been demonstrated that also the intense training, in young racehorses, can be a trigger for the development of MEA. The exercise-related stress could be a challenge for the immunity of the respiratory system, in addition to cold air and the dust of the track. Moreover, the possible presence of blood in the lumen of the airways, consequent to some episodes of exercise-induced pulmonary hemorrhage (EIPH), can induce an inflammatory reaction of the lung and can act as a pabulum for bacterial colonization (Mc Kane and Slocombe, 2010).

The role of some infectious agents in the aetiology of MEA has been also investigated. In fact, the degree of pulmonary inflammation is directly related to the number of colony forming unit (UFC) of *Streptococcus* spp (Chapman *et al*., 2000) isolated in tracheal wash. Nevertheless, the presence of bacteria could be the results of secondary colonization rather than a primary aetiological factor. Finally, some studies investigated the role of *Equine Herpesvirus* type 2 (EHV-2) in the development of MEA. Even if an association has been demonstrated, the pathogenic role of a ubiquitously virus such as EHV2 is still debatable (Fortier *et al.,* 2009).

1.4 **History**

Horses affected by SEA have commonly a history of cough (84%) and nasal discharge (54%). Nevertheless, these are nonspecific signs of the disease (Dixon *et al.,* 1995). Episodes of respiratory distress at rest are the required symptom of SEA but, depending on the severity of the inflammation, horses can exhibit only mild respiratory signs. The season is also important: typically, horse with RAO exhibit the symptoms in the autumn-winter, when they are kept more time in the stable, while horse with SPAOPD are more likely to show the respiratory distress during spring-summer, when they are kept in paddock (Seahorne *et al.*, 1996) Poor performance can be marked in exacerbation horses, or absent in asymptomatic patient. In chronic affected patient, generalized cachexia may occur, due to significant mismatch between peripheral tissue oxygenation and breathing work energy consumption, anorexia and systemic inflammatory response (Mazan *et al.*, 2004).

The history of horses affected by MEA is also characterized by cough, nasal discharge and poor performance, but in the absence of respiratory effort at rest (Couëtil *et al.,* 2016). As cough and nasal discharge are not specific, and the horses affected are mainly racehorses, often the only symptom is the impaired athletic capacity.

1.5 Clinical Presentation

At physical examination, in horses with SEA the most consistent finding is the abnormal breathing pattern, characterized by increased expiratory effort, also revealed by nasal flaring and recruitment of accessory abdominal muscle during the expiratory phase. Chronic affected animals can develop hypertrophy of the external abdominal oblique muscles resulting in the characteristic ‘heaves line’. Thorax auscultation is usually characterized by the presence of end-expiratory wheezes as well as early inspiratory crackles, and auscultation area may be enlarged because of lung hyperinflation (Pirie, 2014). However, horses in remission of the symptoms exhibit little to no clinical signs of respiratory disease except for poor performance and can be difficult to differentiate from horses with MEA.

At clinical examinations, horse with MEA, instead, are mainly asymptomatic. Signs of respiratory disease such as nasal discharge and fever do not appear to be associated with MEA. Thoracic auscultation is usually normal; for the most part, MEA is subclinical and can be undetected unless coughing is present or tracheal exudate is detected by endoscopy (Burrell *et al.*, 1996).

1.6 **Diagnostic test**

Respiratory endoscopy

The endoscopic examination represents an important diagnostic test in the diagnosis of SEA and MEA. At pharyngeal level, the presence of pharyngeal lymphoid hyperplasia (PLH) can be identified, and can be classified in grade from 0 to 4 depending on severity (Baker *et al.*, 1987). This finding is often associated with cough and tracheal mucus in young horses with MEA (Christley *et al.*, 2010). At tracheal level, mucus accumulation is a frequent finding both for SEA and MEA, and can be classified, based on quantity, from 0 to 5 (Gerber *et al.,* 2004). The amount of tracheal mucus has been associated with lower airway inflammation (Koblinger *et al.,* 2011). Another indication that can be obtained from endoscopy is the measure of interbronchial septum or *carena.* It can be classified between 0 and 4 and, even if SEA horses seem to have enlarged *carena*, a direct association with lung inflammation has not been reported (Koch *et al.*, 2007).

Finally, the endoscope is an important instrument for the execution of other diagnostic examination, such as broncho-alveolar lavage and tracheal wash, endobronchial ultrasonography and bronchial biopsies.

Bonchoalveolar lavage

During endoscopic examination, it can be possible to obtain samples of lavage fluids at different levels of the respiratory tree, such as tracheal wash (TW) and bronchoalveolar lavage (BAL). For MEA horses, most of the studies has been conducted on TW (Burrell *et al.*, 1996; Chapman *et al.*, 2000; Christley *et al.*, 2010); nevertheless, as the association between cytological examination of TW and lung inflammation is poor (Malikides *et al.*, 2003), the gold standard for diagnosis of SEA and MEA is considered the cytological examination of BAL (Couëtil *et al.*, 2016).

A BAL sample can be obtained by means of a flexible endoscope or a catheter with a blind technique. After sedation, endoscope or catheter are passed from nostril to the *carena;* here a 0.2-0.5% lidocaine solution is sprayed in order to avoid coughing reflex. Then, the endoscope or catheter are passed into the bronchial tree until they are wedged with the diameter of the bronchi. A 250-500 ml physiological solution is then instilled and immediately aspired (Hodgson and Hodgson, 2007). The sample obtained can subsequently undergo cytological and microbiological examination.

Laboratory Examination

Macrophages constitute the 60-70% of inflammatory cells in a normal BAL, followed by lymphocytes 30%, neutrophils 5%, mast-cell 2% and eosinophils 1% (Hare and Viel, 1998). In SEA horse, the characteristic finding is the elevated count of neutrophils (>25%). Nevertheless, BALF cytology and particularly neutrophil count do not correlate with the severity of clinical airway obstruction and lung dysfunction (Jean *et al.*, 2011).

For MEA horses, instead, the increase can be not only for neutrophils percentage, but also for eosinophils or mast-cells. A recent consensus statement established that, for diagnosis of MEA, a horse should have a BAL cytological profile consistent with (Couëtil *et al.*, 2016):

- Neutrophils > 10% and/or
- Eosinophils > 5% and/or
- Mast-cells > 5%

Sample collected from BAL and TW can also be submitted to microbiological examination, even though the best sample for microbiological isolation is TW. Bacterial isolation can be considered significant when UFC count is more the 10^3.

Hemogram and serum biochemistry are usually within the normal range. Recently, a systemic inflammatory reaction has been reported for MEA, highlighting increased serum levels of some acute phase proteins such as haptoglobin and serum amyloid A (M. Bullone *et al.*, 2015).

Blood gas analysis

In SEA horses, arterial blood gas analysis shows a marked hypoxaemia during exacerbation, depending on increased dead space ventilation and related pulmonary gas exchange compromising. Increased dead space results from alveolar hyperinflation and subsequent compression on pulmonary capillaries that prevents adequate perfusion. Hypercapnia, instead, is not a usual finding in SEA horses, because of the increase in respiratory rate and total ventilation (Nyman *et al.,* 1991).

In MEA horses the PaO_2 remain within normal limits, while the $PaCO_2$ can be higher the normal, suggesting an alteration in ventilation/perfusion ratio (Couëtil and De Nicola, 1999). Moreover, a study demonstrated that MEA horses presented a more pronounced hypoxaemia during exercise compared to controls (Sanchez *et al.,* 2005).

Diagnostic Imaging

Radiographic examination of the thorax may reveal an increased bronchointerstitial pattern. Flattening of the diaphragm is indicative of alveolar hyperinflation, and may be reversible when affected horses are in clinical remission (Seahorn and Beadle, 1993).

Thoracic ultrasonography of RAO-affected horses is usually unrewarding, although the surface of the visceral pleura may have irregular echogenicity (Couetil, 2014). Recently, transendoscopic endobronchial ultrasonography (EBUS) has been validated in horses. This technique allows non-invasive and reliable estimation of airway smooth muscle remodeling in SEA (Bullone *et al*., 2015). However, the equipment expensiveness and the technical difficulties limit its use only for research purpose.

Endobronchial biopsy

During endoscopy, an endobronchial biopsies can be easily collected by means of a transendoscopic forceps. A semiquantitative histological score has been recently developed in SEA horses, showing a good correlation between severity of central airway remodeling and airway obstruction (Bullone *et al.,* 2014; Bullone *et al*., 2016)

Pulmonary function tests

Pulmonary function tests can allow quantification of lung dysfunction, using various techniques. There are four methods that can be used in the horse to evaluate lung mechanics (Couetil, 2014):

1. measurement of pleural pressure changes in relation to airflow during tidal breathing or ‘conventional lung mechanics’,

2. measurement of airflow during forceful exhalation or ‘forced expiration’,

3. evaluation of the pressure–flow relationship while an oscillating source of flow is applied to the respiratory system during tidal breathing (‘forced or impulse oscillometry’),

4. measurement of thoracic and abdominal volume changes by plethysmography in relation to airflow during tidal breathing (‘flowmetrics’).

Conventional lung mechanics has been performed for decades on the horse and it is still commonly applied in research setting (Gillespie *et al.,* 1966). It can be performed with the use of a facemask, a flowmeter and an esophageal balloon connected to a differential pressure transducer, with horse breathing at rest. By the flowmeter, it is possible to measure tidal volume, respiratory frequency, peak inspired flow, peak expired flow and inspiratory and expiratory time. The esophageal pressure provides an accurate estimate of pleural pressure. The maximal change in pleural pressure during tidal breathing (ΔP_{plmax}) is associated with mechanical properties of the lungs and represents a good estimation of the degree of obstruction of the airways. Addition of a measure of tidal volume allows calculation of pulmonary resistance (R_L) and dynamic compliance (C_{dyn}). The latter reflects the elastic properties of the lungs and the magnitude of obstruction of the small peripheral airways. An increase in R_L is indicative of airway obstruction that can be the result of narrowing of the upper and/or lower airway. Dynamic compliance decreases if the lungs become stiffer, for example as a result of fibrosis or pulmonary edema, or when there is diffuse peripheral airway obstruction such as occurs in horses with SEA (Marlin and Deaton, 2007)

The conventional lung mechanic is considered as gold standard for the diagnosis of airway obstruction in SEA horse, but the sensitivity for milder obstruction is low (Couetil *et al.*, 2001). For MEA horses, the only significant increase in resistance has been seen in adding dead space for increasing CO_2 rebreathing (Pirrone *et al.,* 2007). The main disadvantage of this technique is the requirement of an esophageal balloon catheter that makes this method time consuming and unattractive for the owner.

Forced expiration (FE) is a sensitive test of airflow obstruction, where a reduction in forced expiratory flow during late FE is an early indicator of mild airway obstruction. Nevertheless, even if it can be performed in standing, sedated horses, it implies the application of a nasotracheal tube and mechanical ventilation, and it is not applicable in a clinical setting (Couetil *et al.*, 2000).

Flowmetrics or inductance plethysmography is non-invasive, portable in the field and shows satisfactory correlation with standard lung mechanics (Hoffman *et al.,* 2007).

Forced or impulse oscillometry techniques have the advantage of being non-invasive and more sensitive of conventional mechanics, and have been used both in research and clinical cases. This technique will be better described in the next chapter.

Another means of detecting airway obstruction is by testing airway reactivity, measured as an exaggerated narrowing of the airways in response to inhaled irritant, using one of the methods previously described. Horses with MEA, characterized by eosinophil and mast cell increase in BAL, present airways hyperresponsiveness (Hoffmann *et al.,* 1998). SEA horses, instead, have increased susceptibility to develop airway obstruction in response to a wide range of specific (i.e. mold or pollen) and non-specific (cold air, dusts, etc..) triggers. The reason is unknown but may include reduced airway caliber as a result of airway remodeling, impaired inhibitory mechanisms that normally limit smooth muscle contraction, and sensitization of cholinergic nerves and smooth muscle by inflammatory mediators (Robinson, 2001). Nevertheless, SEA horses that are kept in pasture have an airway responsiveness that is indistinguishable from controls (Fairbairn *et al.,* 1993, Votion *et al*., 1999).

1.7 Treatment

The therapeutic strategy in the management of equine asthma might be focused on the environmental reduction of the antigens and the pharmacological controls of the symptoms. Several studies are present in literature regarding SEA; the treatment approach of MEA should be borrowed from SEA.

Environmental control

Antigen avoidance is the most important feature in the successful management of horses with SEA. The best way to achieve a full remission of clinical signs is to maintain the affected horses at pasture for the all time. When it is not possible, the stable dust must be reduced. The hay and the straw should be changed with low-dust feed and bedding material, such as pellet or cubed hay, hay silage, wood shaving or shredded paper. Moreover, the ventilation of the stable must be ameliorated, with the aim to remove the airborne dust (Lavoie, 2007). The antigen avoidance strategy allows a reduction of clinical signs within 3 days, progressive improvement of lung function, regression of airway neutrophilic inflammation and reduction of IL-8 mRNA expression. Also the increase in the diameter of bronchial smooth muscle gradually reduced, but only of about 30% compared to healthy horses, indicating the incomplete reversibility of airway remodeling (Leclere *et al.,* 2012)

Medications

Similarly to human asthma, the elective drugs that are effective in the symptoms reduction in horses with equine asthma are corticosteroids and bronchodilators, often administered in association. Both of the drugs can be administered by two different route, systemic (intravenous, intramuscular or oral) and topical (inhalation).

Dexamethasone is the most commonly used corticosteroid for the treatment of equine asthma. Several papers reported its use in SEA horses both by parenteral (0.04-0.1 mg/Kg) and oral (0.04-0.066 mg/Kg) administration, and its efficacy in relief of symptoms, improvement of airway function and reduction of airway inflammation has been documented. (Rush *et al.,* 1998, Lavoie *et al.,* 2002, Cornelisse *et al.,* 2004). Other corticosteroids that have been reported to be effective in SEA treatment are oral prednisolone (1-2 mg/kg), triamcinolone acetonide (0.09 mg/kg) and isoflupredone acetate (0.03 mg/kg) (Lapointe *et al.*, 1993, Picandet *et al.,* 2003, Leclere *et al.,* 2010).

Many studies also reported the efficacy of corticosteroid inhalation in horses affected by SEA. This route of administration has the advantages of delivering high drug concentrations locally to the airways and of reducing systemic absorption, minimizing systemic side effects (Duvivier *et al.,* 1997). There are several devices that allow the administration of drugs by means of nebulization, with different method of delivery and different application on the muzzle of the horse.

Beclomethasone dipropionate (1000-3750 μg q12h), administered by means of different devices, showed variable efficacy compared to parenteral dexamethasone according to different studies (Rush *et al.,* 2000; Couetil *et al.,* 2006). Fluticasone propionate (2-6 mg q 12h) administration, although resulting in a slightly reduced efficacy in improving airway obstruction, is associated with significant lower side effects on adrenal function compared to parenteral dexamethasone and represent the most used inhaled corticosteroid (Robinson *et al.*, 2009). The administration of fluticasone induces a reduction in airway smooth muscle remodeling, even if this reduction is not complete, and pulmonary neutrophilia remain elevated also after long-term administration, such as other corticosteroids (Leclere *et al.,* 2012).

Concerning bronchodilators, several drugs have been used in relieving symptoms in SEA horse, with different route of administration and mechanism of action.

Clenbuterol (0.8-3.2 μg/kg per os q12h) is the most ancient of β2-adrenergic agonists. The problems connected with its systemic administration are related to the risk of adverse effects at higher dose, such as tachycardia and sweating (Thomson and McPherson, 1984). The use of new molecules for inhalation therapy, such as fenoterol, albuterol, pirbuterol and salmeterol, can obviate this problem, even if the rapid desensitization and down-regulation of β2-adrenoreceptors is a concern of all these drugs. This effect can be reversed by corticosteroid administration (Abraham *et al.,* 2002). A recent study (Bullone *et al.*, 2017) showed that long term treatment with combinations of inhaled salmeterol and fluticasone is more effective than fluticasone alone in controlling clinical signs and airway neutrophilia, while no differences were detected regarding the effect on airway remodeling. Other classes of bronchodilators that showed an efficacy on SEA horses are anticholinergics and antimuscarinics. The administration of parenteral N-butylscopolammonium bromide (0.3 mg/kg) demonstrated an effective and rapid bronchodilatory effects (Couetil *et al.*, 2012), even if the time of bronchodilation is very short. Ipratropium bromide is an antimuscarinic drug that can be administered by inhalation, and can be used as an additive of β2-adrenergic agonists therapy (Duvivier *et al.*, 1999).

A recent metanalysis reviewed the efficacy of corticosteroids and bronchodilators in the treatment of SEA horses. Results showed that long-term treatments with inhaled corticosteroids (fluticasone) and long-acting β2-adrenergic agonists (salmeterol) may represent the first choice for treating equine asthma (Calzetta *et al.,* 2017).

Concerning MEA, as the presence of bacteria is considered a risk-factor for the development of the symptoms, the administration of antibiotics was largely diffuse, even if only 50% of racehorses diagnosed with MEA showed a resolution after a single course of antibiotic therapy (Fogarty and Buckley, 1991), and so this is not currently recommended. The inhalation of amikacin (3.3 mg/Kg) by means of nebulization showed efficacy in the reduction of clinical score, of neutrophils percentage and of UFC/ml in the tracheal wash of racehorses affected by poor performance syndrome related with MEA (Ferrucci *et al.,* 2013).

1.8 **Prognosis**

Horses with MEA have a good prognosis for return to previous level of performance. The pharmacological treatment and the environmental changes permit to obtain a complete resolution of the syndrome (Couetil, 2014).

SEA horses, instead, show a median survival time after first diagnosis of 8 years, with a survivor percentage of 87% after three years. After treatment, 21% of owner believed that horse is healed, but 79% of patients have recurrent episodes of airways obstruction. Concerning athletic prognosis, 74% of SEA horses continue with their previous performance, instead of 21% that have to be retired (Aviza *et al.,* 2011).

2. FORCED OSCILLATION MEASUREMENT

2.1 Introduction

As described for the first time by Dubois *et al.,* 1956 oscillometry is the study of lung mechanical function by the application of external forces to the respiratory system. Forced oscillatory mechanics is a technique that allows to measure the dynamic lung function evaluating the answer to external forces superimposed to the respiratory system, that can be pressure or flow, filtered from the pressure-flow signals obtained from spontaneous breathing (Hoffman, 2002). As the measure of oscillometry is independent from the respiratory flow originating from normal breathing, it does not need the cooperation of the patient, and in men it requires only the maintenance of an airtight seal of the lips around a mouthpiece and breathing normally through the measuring system with a nose-clip occluding the nares. For this reason oscillometry can be easily performed in pediatric, adult and geriatric populations, independent of severity of lung disease, and it has also a promising application in veterinary medicine (Smith, Reinhold, and Goldman 2005).

By means of a facemask, air currents are imposed to the respiratory system using an extern source of energy, that can be a loudspeaker or air pressured, with the aim to produce a significant oscillation of pressure. At the level of the opening of the airways, such as the mouth for human and the nostrils for animals, it is possible to measure the answers of flow pressure, by means of a pneumotachograph, of the respiratory system, excluding the data obtained from tidal breathing (Fig. 1) (Hoffman, 2002).

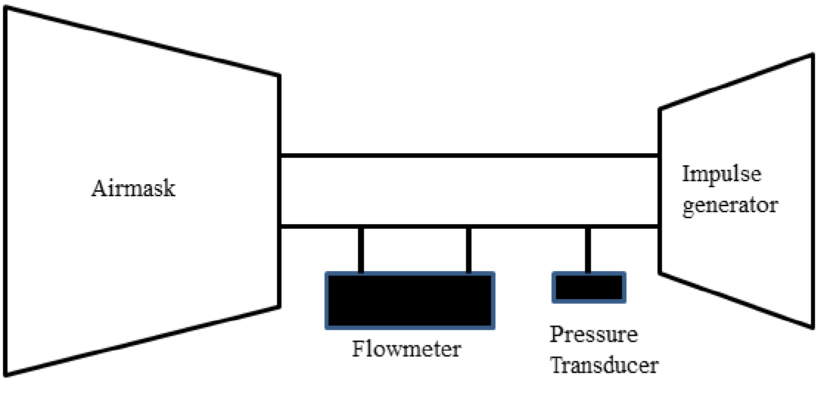


Figure 1. Schematic representation of an oscillometry system.

These data are used to calculate impedance (Z_{rs}) of the respiratory system, which is a measure derived from electrical modeling of the airways. Respiratory impedance is the sum of all the forces (resistance, R_{rs} and reactance, X_{rs}) opposing the oscillations and is calculated from the ratio of pressure and flow at each frequency (Otis *et al.*, 1956).

Respiratory R_{rs} includes all the components of the respiratory tract that contribute to friction, essentially the resistance to flow in the airways, which depends on airway caliber and the architecture of the airways. X_{rs}, instead is the out-of-phase component of lung impedance and represents the capacitive and inertive properties of the airways. Capacitance is a measure of the elasticity of the airway, whereas inertance represents the forces of the moving air column. X_{rs} reflects the stiffness of the lung and their distensibility (Oostveen *et al.*, 2003). In healthy subjects, R_{rs} is almost independent of oscillation frequency, but may increase slightly at higher frequencies due to the upper airways shunt effect. Proximal airways obstruction elevates R_{rs} evenly independent of oscillation frequency. In distal airways obstruction, R_{rs} is highest at low oscillation frequencies and falls with increasing frequency (Clement *et al.,* 1983)

At low frequencies, reactance is primarily composed by capacitance properties, as at higher frequencies it reflects more the inertance. Therefore, as the ability of the lungs to store capacitive energy is due to the properties of the small airways, reactance at low frequencies can provide important information about the lower part of the respiratory system (Bickel *et al.*, 2014). The capacitance has a negative value and inertance a positive values, so at lower frequencies the X_{rs} would be negative and at higher frequencies positive. When the ability of the distal airways to store energy in reduced, for example in pulmonary fibrosis in men, because of the stiffness of the lung, or in emphysema, because of hyperinflation that reduces lung elastic recoil, reactance at low frequencies becomes more negative, even if is nonspecific for the type of limitation (Smith, Reinhold, and Goldman 2005).

Where the capacitance and the inertance have the same value, the X_{rs} is 0, and this value is defined as frequency of resonance (f_{res}). This value increases in patient with lower airways obstructive disease (Smith, Reinhold, and Goldman 2005).

Coherence (Co) is another parameter that is useful to evaluate the accuracy of oscillometry parameters. Co can be a value comprised between 0 and 1 and is based on a relationship between the airflow entering the lungs and the pressure wave reflected back from the respiratory system. High values of coherence are requested to obtain a valid measurement of forced oscillation (Bickel *et al.,* 2014).

More recently, the advance of technology allowed differentiating between the inspiratory and expiratory phase of the breath, obtaining data of within-breath FOT. The comparison between the two phase brought to the identification of some characteristic of resistance and reactance, that can be worse or better during inspiration than during expiration (Dellacà *et al.,* 2004).

Currently, in literature two methods are described to generate oscillation input: the forced oscillation technique (FOT), that can be based on single frequency, multiple frequencies or multiple random frequencies, and the impulse oscillation system (IOS), that is based on a single impulse generated from a loudspeaker in response to an electrical squarewave introduced into the respiratory system (Hoffman, 2002). Then, after the application of the Fast Fourier Transform (FFT), the results are measured across multiple frequencies (Smith, Reinhold, and Goldman 2005). The advantage of FOT is the ability to focus energy at the only frequencies reflecting the condition of lung periphery, even if it requires more energy to reach this zone. IOS has the advantage of that multiple frequencies are conducted simultaneously, and it is faster than FOT, even if lot frequencies are of little interest for diagnostic aim (Hoffman, 2002).

In human medicine, FOT and IOS have been applied in a large number of healthy and pathological subjects, and several papers are present in literature. Both the techniques have been studied in asthma, COPD, cystic fibrosis, broncho-pulmonary dysplasia (BPD), central airway obstruction, adult interstitial lung disease and occupational and environmental irritant exposure. It has also been applied as a mean to evaluate the response to bronchodilators in patients with asthma and COPD (Bickel *et al.,* 2014). This allowed identifying some peculiarity of the different diseases that can be identified by means of forced oscillation. Briefly, the R_{rs} is an indicator of the airway caliber; the ratio between R at 5Hz and R at 20 Hz reflects the frequency dependence of R_{rs}, that during the disease becomes higher; a low values of X_{rs} represents some abnormality in lung parenchyma or airways; the difference between inspiratory and expiratory X indicates the expiratory flow limitation, a major determinant of dynamic hyperinflation and exercise limitation (Mikamo *et al.,* 2016).

Application of the FOT and IOS has been described also in different animal species. The standard IOS device developed for humans has been validated in calves and in pigs (Reinhold *et al.*, 1998; Klein and Reinhold, 2001). Data from FOT and IOS are also available for dogs (Clercx *et al.*, 1993), animals models as mice, rabbits or lambs (Zannin *et al.*, 2014; Sudy *et al.*, 2019), and horses (see chap. 2.2).

The most useful frequency range for clinical evaluation of respiratory impedance is different on the basis of the species, dependent on the animal size. The principle is that the lower airways can be investigated by means of the lower frequencies. In calves the resonant frequency is between 5–12 Hz, depending on body weight, while is lower than 3 Hz in horses. Similarly with what happen in humans, in veterinary medicine the lower airways obstruction is characterized by a low reactance at lower frequencies an increase in R_{rs} (Smith, Reinhold, and Goldman 2005).

2.2 Oscillometry in horses

In horse respiratory medicine, both FOT and IOS have been applied in the last 30 years, but only few works have been published, and their use still remain confined to research unit, due to the expensiveness of the devices and the complexity of the data processing.

The first report of the application of a FOT device on horses is from Young and Hall, 1989. They measured for the first time the impedance using forced random noise method of healthy ponies from 3 to 40 Hz using a FOT apparatus from human medicine adapted to the horse respiratory system. Moreover, they compared the results with some measurement performed on healthy men. They so could first describe the differences between men and horse; in fact, from 3 to 40 Hz the inertial forces are greater than elastic forces in horses compared to men, suggesting that the resonant frequency must be lower than 3 Hz (differently from men in whom it is 7 Hz).

In the same year Art *et al.*, 1989, always applying pseudorandom noise method, measured inertance from 2 to 26 Hz of healthy ponies. They obtained for the first time the f_{res} of the respiratory system of equine patient, calculated as 1.51±0.11 Hz.

Young and Tesarowski (1994) firstly described the application of a FOT device with oscillation generated from an air compressor. They measured respiratory mechanics at 1, 2 and 3 Hz in adult Standardbred horses, considering values of Co $\geq$ 0.8 at 1 Hz and $\geq$ 0.9 at 2 and 3 Hz. They also compared the results with conventional mechanic measurement. Differently from Art *et al.*, 1989, in this work the f_{res} resulted 2.40±0.25 Hz. They also observed a difficult in obtain good coherence values at 1Hz, because of the nearness with breathing frequency.

In 1997 the same group published another paper, reporting for the first time the values of FOT measurement at 1.5, 2, 3 and 5 of SEA horses before and after bronchodilation, together with the data concerning resistance and reactance (Young, Tesarowski, and Viel 1997). They showed a negative frequency dependence of resistance in asthmatic horse in exacerbation. Moreover, as the limit of frequencies relating to the lower airways is considered the double of resonant frequency (Michaelson *et al.*, 1975), they suggested that the most specific frequencies for evaluating the lungs of horses were between 1 and 5 Hz.

Hoffman and Mazan, 1999 tried to resume the clinical significance of the measurement of FOT in asthmatic horses. They reported clinical data obtained by means of FOT at frequencies from 1 to 7 HZ in SEA, MEA and control horse, They showed significant difference in X and R at lower frequencies in SEA horses compared to controls or MEA horses. They also report significant higher values of R in MEA horses compared to control at 1, 2 and 3 Hz. Moreover, they described the application of FOT with bronchoprovocation test, and they demonstrated the ability of this technique to identify bronchial hyper-reactivity. Even if they did not report data concerning values of R and X, from their graphs it can be argued that R ranged from about 0.4 to 0.6 cm H_20*l/s for control horses, from about 0.6 to 0.8 cmH_20*l/s for MEA horses and from 0.8 to 1.0 cmH_20*l/s for SEA horses, and X ranged from about -0.4 to 1.0 cm H_20*l/s for controls and MEA horses, and from -0.6 to 0.8 cm H_20*l/s.

In 2007, FOT has been used to measure the pulmonary function of SEA horses after inhalation of dust. In this study, SEA horses were in remission of the symptoms and data of R and X were not significantly different to control horses (Deaton *et al.,* 2007).

Finally, data from FOT has been published in 2013 (Onmaz, Stoklas-Schmidt and Van Den Hoven, 2013) for the evaluation of daily variability of respiratory mechanics in SEA horses, demonstrating the influence of ambient and management factor on FOT parameters.

Concerning IOS, the first reports about its application are from Lekeux and her group (Van Erck *et al.*, 2003, 2004a, 2004b, 2006). In these papers they validated an IOS device developed for human medicine and adapted to the horse. In the first work the frequencies evaluated ranged from 5 to 20 Hz. They compared the results with conventional mechanic measurements and they found a good correlation between R_{rs} and R, and between X_{rs} and C_{dyn}. Moreover, IOS showed to be more sensitive of conventional mechanic during methacholine bronchoprovocation test. In the second and third study they standardized and validated the IOS device with an *in-vitro* and *in vivo* study, working on frequencies between 5 and 35 Hz. They analyzed the influence of head position, mask design, biometrical parameters and they evaluated repeatability. Finally, they assessed IOS values from normal horses and they obtained results from SEA horses, describing lower airway obstruction as characterized by negative frequency dependence of resistance, frequency dependence of reactance and negative X_{rs} values throughout the frequency range. The last paper evaluated also horse with recurrent laryngeal neuropathy, demonstrating the influence of upper airways obstruction on R_{rs} parameters.

In 2006 also Klein and colleagues evaluated the same IOS device on sedated horses (Klein, Smith, and Reinhold, 2006). Differently from previous papers, they concentrated their attention on frequencies at 1, 5 and 10 Hz, considering only these frequencies as representative of lower airways of the horses. They also considered coherence values that were not reported in previous works. For the first time, the within-breath analysis were performed, and data concerning inspiratory and expiratory R and X were reported. This kind of analysis allowed identifying the influence of sedation on inspiratory resistance.

Finally, in 2009 IOS has been applied also on MEA horses. The frequencies evaluated were from 1 to 20 Hz, and both inspiratory and expiratory parameters were evaluated. Results showed that MEA affected horses exhibited significantly higher values of R and lower values of X. No coherence values were reported.

Recently, other authors reported data obtained from IOS in some research protocols evaluating SEA horses (Bullone *et al.*, 2014), evaluating only frequencies ≤10 Hz. The values of X3 and R3 have been associated with the remodeling of the airways.

PART B:
RESEARCH
PROJECT

1. AIM OF THE PROJECT

As explained in the literature review section, the oscillometry technique seems to be a very attractive tool in the diagnostic protocol of Equine Asthma.

Currently, the gold standard for the diagnosis of SEA and MEA are considered the cytological examination of BAL or the measurement of conventional lung function (Couëtil *et al.*, 2016). Nevertheless, these procedures are relatively invasive and, even if they are sensitives, they have poor specificity. Moreover, in SEA horses in remission of the symptoms, BAL does not allow to discriminate between healthy and affected horses; in fact airway neutrophilia reduces after environmental changes, even if the remodeling of airways still remain (Leclere *et al.*, 2012). The conventional method of lung function testing is considered another good mean for the diagnosis of airway obstruction, even though the sensitivity for mild obstruction is low (Pirrone *et al.*, 2007). The main disadvantage of this technique is the requirement of an esophageal balloon catheter, which makes this method time consuming and unattractive for the owner. The FOM techniques have the advantages to be totally non-invasive and they do not require the collaboration of the patient.

The IOS device described in literature showed a good sensitivity in discriminate between SEA horses in exacerbation and control, and also between MEA horses and controls (Van Erck, 2004b; Richard *et al.*, 2009). Nevertheless, no data are available concerning SEA horses in remission of the symptoms. Moreover, in human medicine, besides evaluating X_{rs} and R_{rs}, also the parameter of Delta X (ΔX), that represent the difference between the inspiratory and expiratory reactance, is considered an important value to take into account in the diagnosis process (Dellacà *et al.*, 2004), but it has never been applied in equine respiratory medicine.

Aim of the first study of this project was then to evaluate whether the IOS could be sensitive enough to discriminate amongst healthy, SEA horse in exacerbation and SEA horse in clinical remission of the disease, calculating also the parameter of ΔX. An abstract of this study has been presented at the International Conference on Equine Exercise Physiology (ICEEP) in November, 2018 at Lorne, Victoria (AUS), and has been published as Conference Paper on Comparative Exercise Physiology (Stucchi *et al.*, 2018).

The disadvantages of the oscillometry, instead, are related mainly to the high-cost equipment (currently only one IOS system is available, and a previous device for FOT is no more commercialized), and at the moment only few reference center can have this availability, and to the difficulty in data interpretation, due to the complexity of the results obtained. In human medicine, instead, the oscillometry technique has growth in popularity and currently there are different devices on the market, with low-cost technology and relying on largely automatic data processing algorithms, suitable for the application on a large number of patients (Dellacà *et al.*, 2010).

So the aim of the second study was to evaluate a new FOT device, developed by the Department of Electronic, Information and Bioengineering of Politecnico di Milano, based on these technological advances, specially assembled for horses, which could overcome the limit of the currently available respiratory mechanical measurement. The device has been essayed on SEA, MEA and control horses in order to evaluate its reliability in measurement and its sensitivity to discriminate between the different clinical conditions.

2. MATERIALS AND METHODS

2.1 Study 1

Sample selection

To perform study 1, 7 SEA horses in exacerbation of the symptoms, 7 SEA horses in clinical remission and 7 healthy controls were selected, age-matched. All cases were selected from a well-characterized population of asthmatic horses of the research herd of the Equine Asthma Laboratory, Faculty of Veterinary Medicine, University of Montréal. Horses in exacerbation were kept in stable and feed hay; horses in remission of the symptoms were kept at pasture 24h/day for at least 6 month and feed pelleted hay. Control horses were selected from the teaching herd of the Faculty of Veterinary Medicine of the University of Montréal and for history and clinical examination were considered free from any respiratory disease. The study was conducted in compliance with guidelines of the Canadian Council on Animal Care.

IOS Measurement

Every horse were restrained in stock and underwent to IOS measurement by Equine IOS MasterScreen (Jaeger, Würzburg, Germany), as described by Van Erck *et al.*, (2004a). Briefly, the latter consisted in a plastic mask adapted to fit on the muzzle of the horse that was sealed by a rubber tape. The mask was attached by a tube to a loudspeaker that was used to produce the impulses, and to a pneumotachograph placed directly in front of the face mask (Fig. 2)

Figure 2. A horse wearing the mask attached to the IOS device during measurement.

The pressure and flow answers of the respiratory system superimposed to the animal tidal breathing were measured. Prior to each experiment, the system was calibrated by means of a 2 l calibration pump forcing known volumes of air through the pneumotachograph. At least 3 measurements for each horse were performed, during 30 seconds for each measurement, and the mean value of the three measurements was considered. All horses were already trained to this kind of procedure.

The data were then processed by two software (LabManager version 4.53; Jaeger, and FAMOS imc; Meβsysteme, Berlin, Germany) using Fast-Fourier transformation. With these software it was possible to obtain the mean resistance (R), reactance (X) and coherence (Co) of the respiratory system at all frequencies of impulse (from 0.1 to 20 Hz). It was also possible to split the parameters in the inspiratory and expiratory phase. For this study, only value at 3, 5 and 7 Hz have been evaluated, with values of Co >0.85 at 3Hz and 0.9 at 5 and 7 Hz. Moreover, the difference between inspiratory and expiratory X (ΔX) at each frequency has been calculated.

Statistical Analysis

Data concerning the mean, inspiratory and expiratory R and X and the difference between inspiratory and expiratory X (ΔX) calculated at each frequency for the three group were collected on an electronic sheet (Microsoft Excel, Redmont, USA). Data distribution was evaluated by means of Shapiro-Wilk normality test. If data were distributed normally, the comparison between the three groups was performed by means of one-way ANOVA and Dunnett's multiple comparison test. If data were not-normally distributed, the comparisons were performed by Kruskal-Wallis test and Dunn's multiple comparison test. Statistical analysis was performed by statistical software (Prism Graphpad, San Diego, USA). Statistical significance was set at $p<0.05$

2.2 Study 2

Sample selection

To perform study 2, 4 SEA horses in exacerbation of the symptoms, 4 MEA horses and 4 healthy controls were selected, age-matched. All cases were selected from clinical patients admitted at the Equine Unit of the Veterinary Teaching Hospital of the University of Milan. Horses were identified as SEA, MEA or controls by means of history and clinical examination; other concomitant respiratory diseases were excluded. The study was approved by the University of Milan Ethic Committee (OPBA 48/19) and all horse owners signed informed consent.

FOT Measurement

FOT measurement was performed on all horses by means of a device specially assembled for horses. The device was provided with a brushless direct current motor (BLDC), coupled with a propeller and encapsulated into a custom 3D printed housing that helps channeling the airflow from the external environment into a adapted horse mask (Equine AeroMask, Trudell, Canada), while simultaneously reducing turbulences and cooling down the motor. A sensor unit, made of a pressure and a flow sensor, was placed at the entrance of the horse mask to guarantee the correct generation of the sinusoidal pressure waveforms. Due to the great values of flow expected, the pneumotachograph used to acquire the flow signal was specifically built, using a 3D printed casing and a ceramic mesh inert to moisture. The device has been designed to be powered either with a proper AC/DC power supply or with a portable battery, allowing the acquisition of data even in a non-controlled environment (Fig. 3).

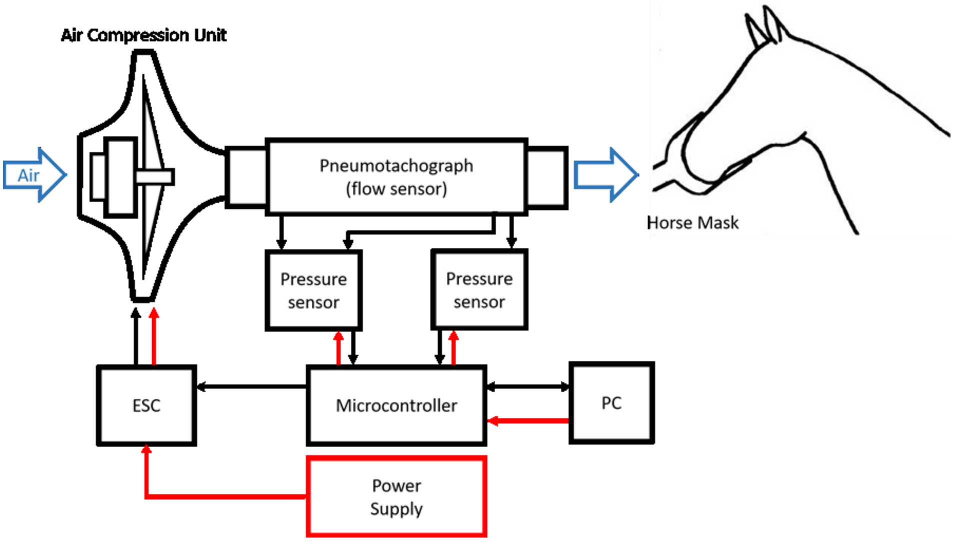
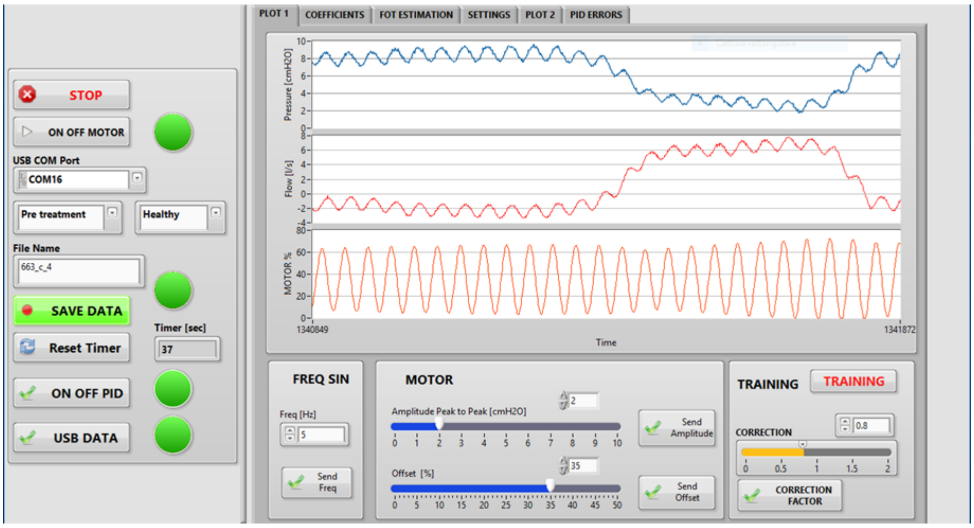


Figure 3. Schematic view of the FOT system.

The motor was driven by an Electronic Speed Controller (ESC), a compact electronic circuit specifically designed for BLDC motor controlling that is in turn controlled by a low-level control unit based on a programmable microcontroller of the PSoC family (Cypress Semiconductors, USA), that also handles the acquisition of the data and the communication with a high-level user interface. The system was able to generate an oscillatory pressure waveform at different frequencies by controlling the motor speed. The union between the two controllers has been required to provide the proper waveform at different frequencies and to be insensitive to the breathing pattern of the subject.

Furthermore, a user-friendly graphic interface (GUI) (Labview, National Instruments, USA) in continuous communication with the low-level control unit, allowed the clinician to handle the device, to real-time visualize data acquired, to set the necessary parameters and to save data during the acquisition (Fig. 4).



USB COM Port: COM16

Pre treatment

Healthy

File Name: 663_c_4

Timer [sec]: 37

Freq [Hz]: 5

Amplitude Peak to Peak [cmH2O]: 2

Offset [%]: 35

CORRECTION: 0.8

Figure 4. Graphic interface of the FOT device

The measurement was performed at frequencies from 1 to 6 Hz, during 30 s for each frequency. For all the horses, this was the first time they performed FOT. (Fig. 5)

Figure 5. A horse undergoing FOT measurement. Notice the FOT device attached to the airmask.

Data of flux and pressure obtained were collected on an electronic sheet (Matlab, MathWorks, USA) and the whole breath, inspiratory and expiratory R and X of the respiratory system were calculated. The data were filtered following visual inspection and the flow-shape index (Marchal *et al.,* 2004), using only breath without artifacts. Moreover, the difference between inspiratory and expiratory X (ΔX) was calculated.

Statistical Analysis

Data concerning the mean, inspiratory and expiratory R and X and the ΔX calculated at each frequency for the three group were collected on an electronic sheet (Microsoft Excel, Redmont, USA). As the number of the groups was too small, was not possible to evaluated data distribution. The comparison between the three groups was performed by means of Kruskal-Wallis test and Dunn’s multiple comparison test. Statistical analysis was performed by statistical software (Prism Graphpad, San Diego, USA). Statistical significance was set at $p<0.05$

3. RESULTS

3.1 Study 1

Horses in the SEA group in exacerbation were composed by 4 gelding and 3 mares, average age 11.9±3.4. SEA horses in remission were 1 gelding and 6 mares, aged 16.4±5.0. Control group was composed by 7 mares, with an average age of 13.1±3.5.

IOS Measurement

The results of IOS measurement are reported in Tab. 1 and Fig. 6, 7, 8.

	CONTROLS		REMISSION		EXACERBATION	
	AVERAGE [Kpa/l/s]	S.D.	AVERAGE [Kpa/l/s]	S.D.	AVERAGE [Kpa/l/s]	S.D.
R3	0,064	0,021	0,085	0,034	0,139	0,024
R5	0,074	0,022	0,084	0,031	0,091	0,009
R7	0,092	0,024	0,090	0,033	0,080	0,012
X3	0,012	0,006	-0,003	0,014	-0,095	0,055
X5	0,020	0,006	-0,001	0,020	-0,066	0,034
X7	0,023	0,011	-0,003	0,020	-0,048	0,024
CO3	0,930	0,020	0,942	0,025	0,904	0,034
CO5	0,960	0,008	0,966	0,021	0,954	0,015
CO7	0,980	0,008	0,976	0,017	0,969	0,015
R3i	0,059	0,019	0,090	0,030	0,131	0,037
R5i	0,065	0,020	0,086	0,031	0,090	0,017
R7i	0,080	0,021	0,091	0,030	0,078	0,019
X3i	0,015	0,009	-0,006	0,015	-0,038	0,014
X5i	0,022	0,005	-0,005	0,017	-0,030	0,014
X7i	0,027	0,010	-0,005	0,018	-0,018	0,027
CO3i	0,930	0,021	0,937	0,028	0,933	0,041
CO5i	0,960	0,008	0,956	0,022	0,968	0,032
CO7i	0,970	0,010	0,980	0,015	0,983	0,014
R3e	0,068	0,024	0,083	0,034	0,137	0,026
R5e	0,081	0,024	0,083	0,030	0,088	0,011
R7e	0,095	0,026	0,090	0,034	0,078	0,014
X3e	0,008	0,005	-0,002	0,015	-0,113	0,067
X5e	0,018	0,008	0,001	0,021	-0,075	0,039
X7e	0,022	0,013	-0,002	0,022	-0,053	0,026
CO3e	0,950	0,017	0,954	0,019	0,920	0,029
CO5e	0,970	0,010	0,972	0,019	0,963	0,015
CO7e	0,990	0,009	0,980	0,014	0,970	0,018
Delta X3	0,008	0,007	-0,005	0,010	0,075	0,057
Delta X5	0,003	0,004	-0,006	0,010	0,046	0,031
Delta X7	0,008	0,009	-0,003	0,014	0,035	0,033

Table 1. Average values of R, X, Co and ΔX obtained from the three groups.

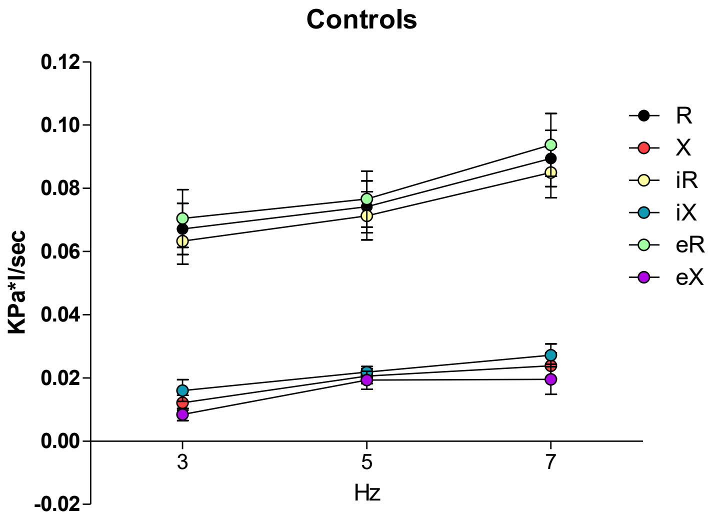


Figure 6. Mean values of R and X of control group

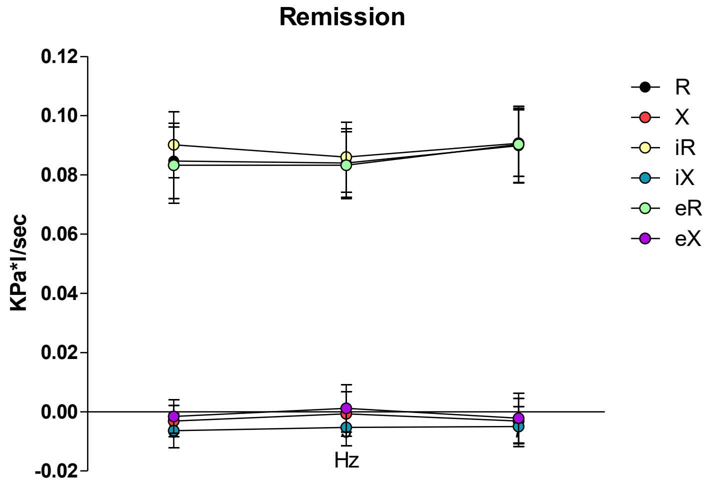


Figure 7. Mean values of R and X of remission group

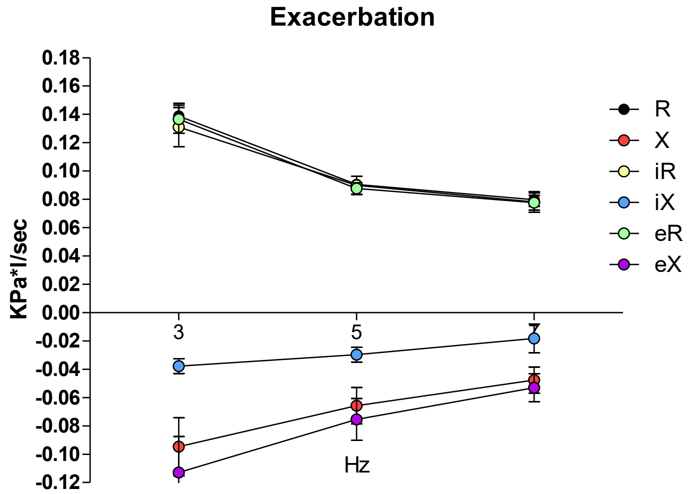


Figure 8. Mean values of R and X of exacerbation group

Statisical Analysis

Shapiro-Wilk normality test showed a normal distribution for all the parameters except for R3i and ΔX7. Statistical comparison between groups showed significant differences between SEA horses in exacerbation and control horses for R at 3Hz, for mean, inspiratory and expiratory parameters (Fig 9)

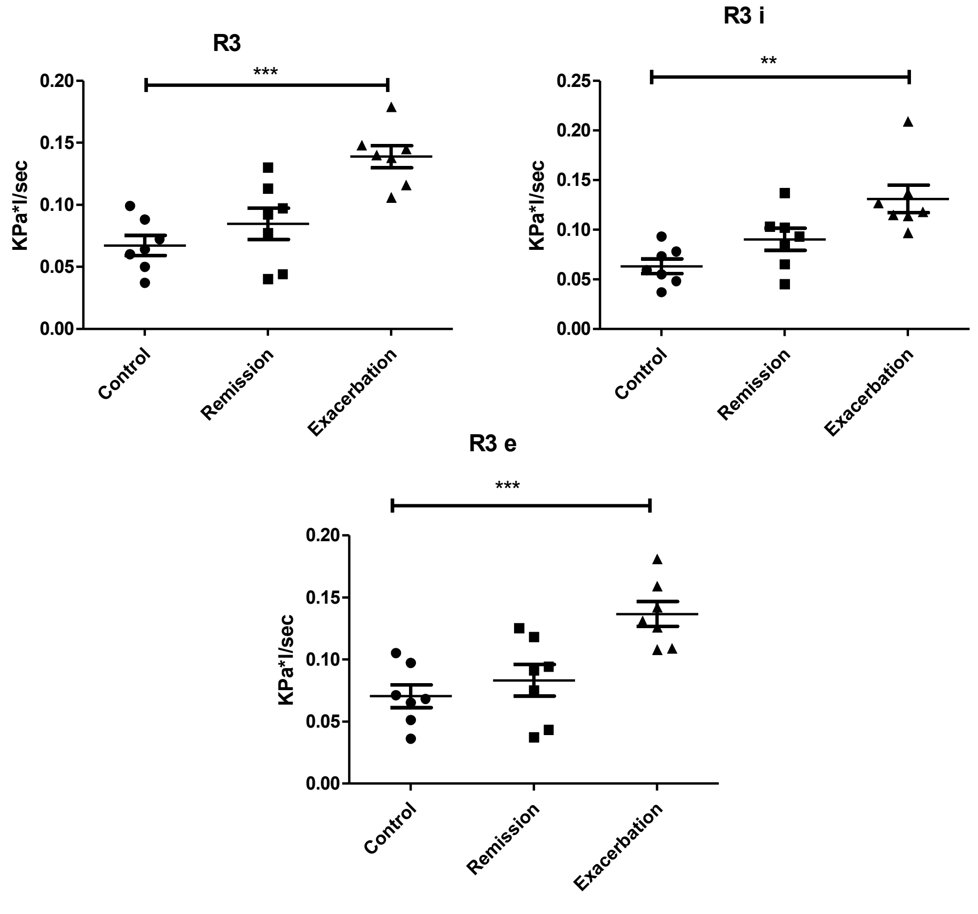


Figure 9. Resistance at 3Hz (**= p<0,01, ***=p<0,001)

For X, significant differences were present between exacerbation horses and control horses at each frequency for mean, inspiratory and expiratory parameters. Between controls and remission horses, instead, differences were present for X7 and for inspiratory X3, X5 and X7. (Fig. 10, 11, 12)

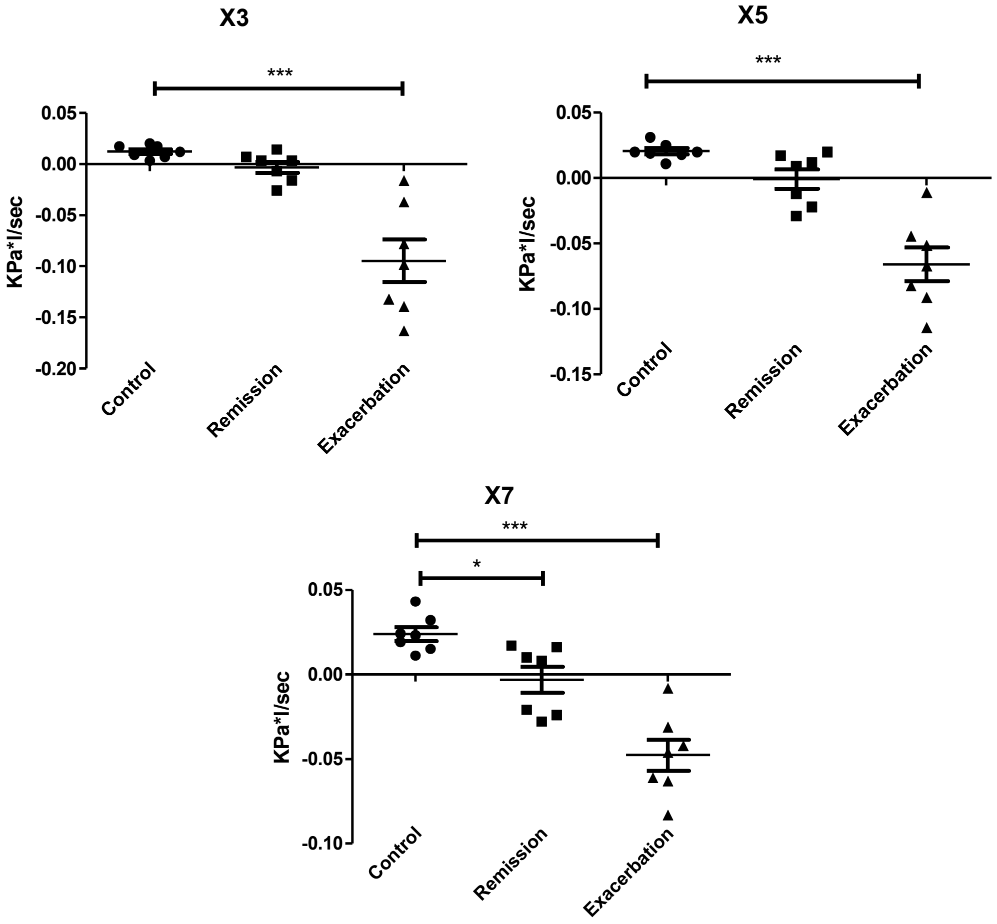


Figure 10. Whole breath reactance at 3, 5 and 7 Hz (*= p<0,05, ***=p<0,001)

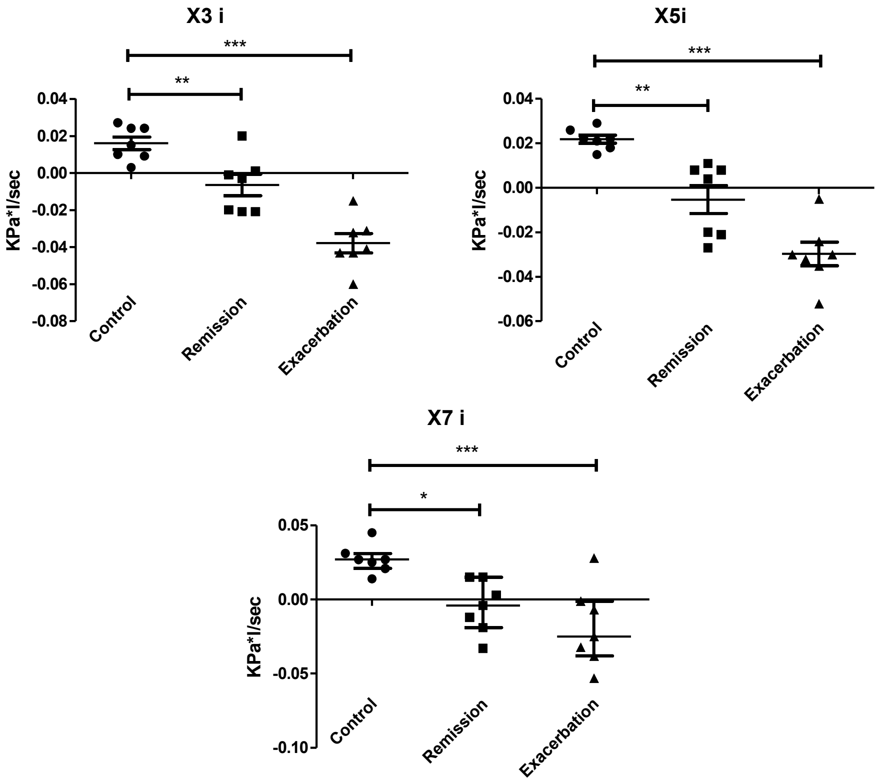


Figure 11. Inspiratory reactance at 3, 5 and 7 Hz (*= p<0,05, **=<0,01, p***=p<0,001)

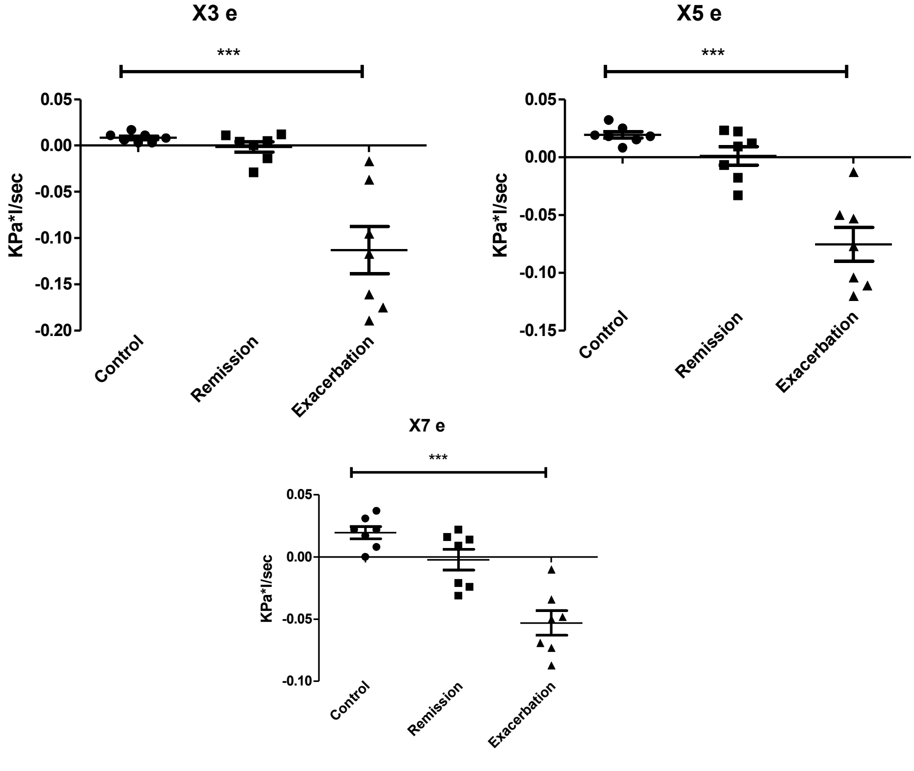


Figure 12. Expiratory reactance at 3, 5 and 7 Hz (p***=p<0,001)

Regarding Delta X, values in exacerbation horses are significantly higher than remission or control horses, indicating that in SEA horses in exacerbation the reactance during expiratory phase is worse than during inspiration (Fig. 13)

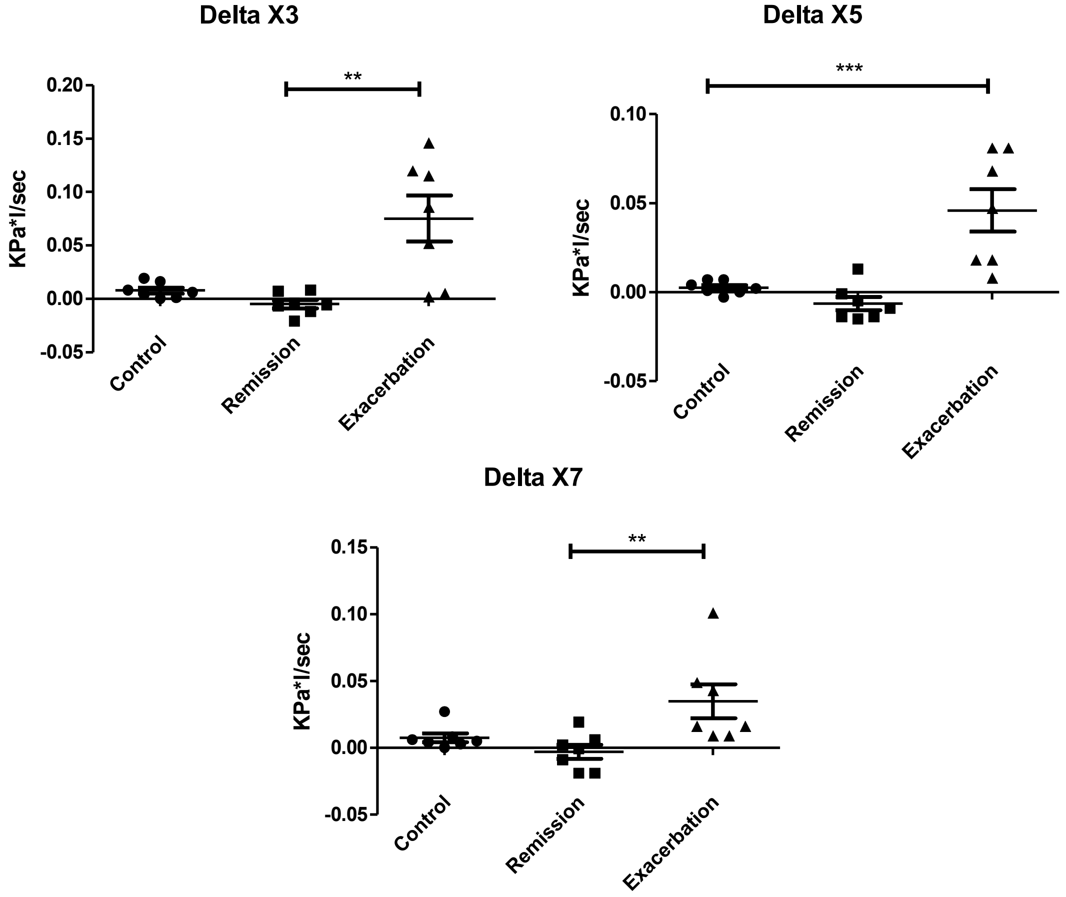


Figure 13. ΔX for all frequencies (**=p<0,01, ***=p<0,001).

3.2 Study 2

Horses in the SEA group was composed by 4 geldings, meadian age 17.5 y.o., interquartile (I.Q.) range 12.5-22.5. MEA horses were 2 males gelding and 2 mares, meadian aged 5.0 y.o., I.Q. range 3-11.5. Control group was composed by 1 males and 3 mares, with a median age of 11.5 y.o., I.Q. range 6.25-13.0.

FOT Measurement

All the horses well accepted the procedure and no horses showed any sign of discomfort or stress during the measurement. As the results obtained from measurement at 1 Hz has very low quality signal, the data were discharged and they were not used for statistical analysis. The results of FOT measurement are reported in Tab.2 and Fig. 14, 15, 16.

	CONTROL				MODERATE				SEVERE			
	MEDIAN (cmH2O*l/s)	I.Q. RANGE			MEDIAN (cmH2O*l/s)	I.Q. RANGE			MEDIAN (cmH2O*l/s)	I.Q. RANGE		
R2	0,455	0,439	-	0,468	0,534	0,421	-	0,637	1,091	0,845	-	1,238
R3	0,553	0,531	-	0,579	0,566	0,507	-	0,626	1,109	0,949	-	1,202
R4	0,624	0,622	-	0,632	0,674	0,537	-	0,808	1,031	0,920	-	1,063
R5	0,689	0,675	-	0,704	0,592	0,563	-	0,651	0,920	0,779	-	1,099
R6	0,736	0,694	-	0,803	0,635	0,577	-	0,712	0,770	0,706	-	0,807
X2	0,031	0,018	-	0,041	-0,021	-0,039	-	-0,004	-0,291	-0,357	-	-0,271
X3	0,157	0,145	-	0,170	0,105	0,088	-	0,123	-0,373	-0,507	-	-0,283
X4	0,180	0,171	-	0,182	0,087	0,066	-	0,111	-0,534	-0,617	-	-0,374
X5	0,232	0,220	-	0,243	0,133	0,123	-	0,160	-0,433	-0,476	-	-0,401
X6	0,208	0,184	-	0,228	0,139	0,119	-	0,184	-0,408	-0,445	-	-0,296
R2i	0,386	0,337	-	0,435	0,441	0,315	-	0,527	0,555	0,302	-	0,834
R3i	0,490	0,449	-	0,524	0,517	0,467	-	0,559	0,817	0,576	-	1,017
R4i	0,563	0,547	-	0,587	0,483	0,444	-	0,527	0,899	0,756	-	0,971
R5i	0,613	0,605	-	0,620	0,511	0,472	-	0,577	0,786	0,618	-	1,007
R6i	0,635	0,576	-	0,719	0,597	0,522	-	0,646	0,644	0,566	-	0,705
X2i	0,024	-0,001	-	0,033	-0,050	-0,075	-	-0,017	0,051	-0,130	-	0,258
X3i	0,127	0,116	-	0,146	0,068	0,037	-	0,112	-0,173	-0,317	-	-0,111
X4i	0,154	0,137	-	0,161	0,072	0,039	-	0,110	-0,327	-0,390	-	-0,241
X5i	0,204	0,192	-	0,217	0,119	0,098	-	0,161	-0,313	-0,341	-	-0,304
X6i	0,191	0,167	-	0,199	0,119	0,109	-	0,161	-0,265	-0,320	-	-0,174
R2e	0,504	0,485	-	0,525	0,606	0,524	-	0,703	1,426	1,280	-	1,540
R3e	0,616	0,602	-	0,626	0,618	0,540	-	0,726	1,252	1,130	-	1,345
R4e	0,682	0,676	-	0,686	0,672	0,598	-	0,783	1,138	1,025	-	1,149
R5e	0,730	0,710	-	0,745	0,645	0,629	-	0,710	1,016	0,879	-	1,182
R6e	0,790	0,766	-	0,836	0,678	0,624	-	0,792	0,846	0,760	-	0,888
X2e	0,025	-0,006	-	0,049	-0,005	-0,005	-	-0,001	-0,552	-0,594	-	-0,461
X3e	0,173	0,165	-	0,181	0,118	0,095	-	0,131	-0,495	-0,610	-	-0,443
X4e	0,168	0,154	-	0,183	0,087	0,039	-	0,143	-0,652	-0,756	-	-0,456
X5e	0,247	0,244	-	0,251	0,121	0,113	-	0,146	-0,566	-0,575	-	-0,536
X6e	0,204	0,185	-	0,227	0,149	0,097	-	0,208	-0,481	-0,536	-	-0,336
Delta X2	-0,016	-0,018	-	-0,008	-0,052	-0,081	-	-0,011	0,627	0,395	-	0,811
Delta X3	-0,032	-0,050	-	-0,021	-0,031	-0,106	-	0,048	0,294	0,288	-	0,310
Delta X4	-0,032	-0,044	-	-0,013	-0,031	-0,051	-	0,003	0,277	0,215	-	0,318
Delta X5	-0,038	-0,055	-	-0,027	0,007	-0,020	-	0,030	0,219	0,181	-	0,252
Delta X6	-0,041	-0,062	-	-0,012	0,020	-0,012	-	0,026	0,191	0,121	-	0,233

Table 2. Median values of R, X and Co obtained from the three groups

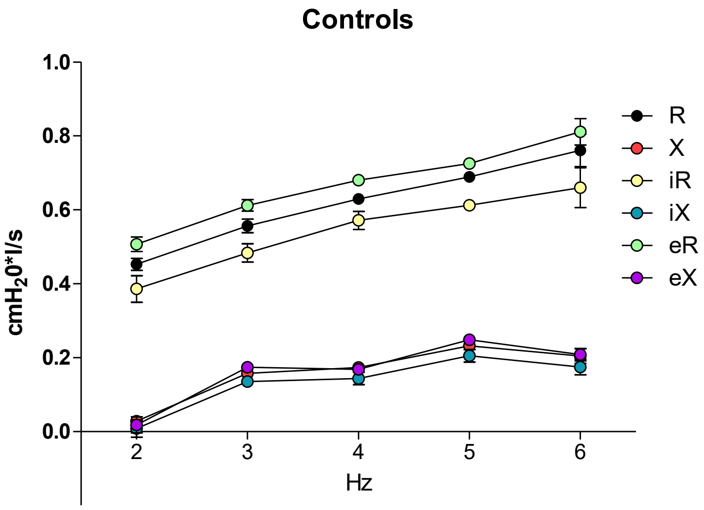


Figure 14. Mean value of R and X of control group

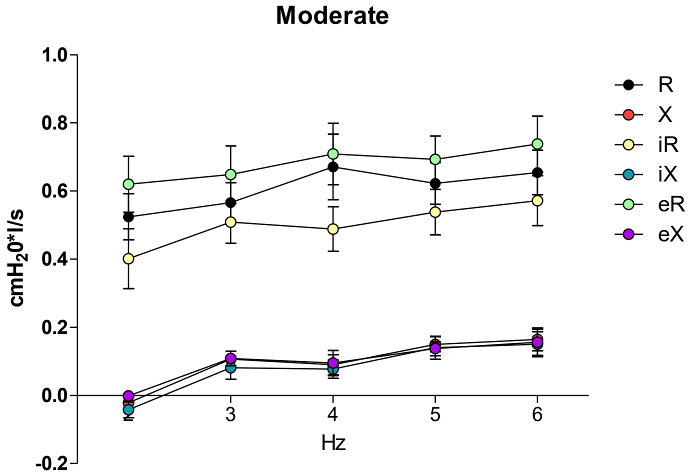


Figure 15. Mean value of R and X of MEA group

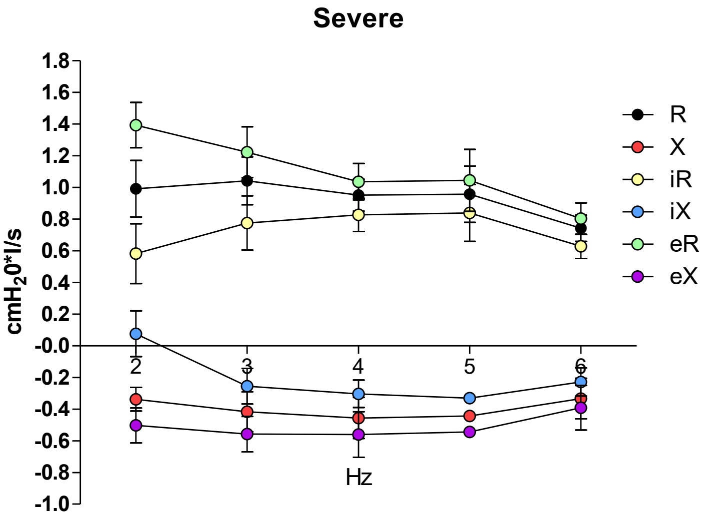


Figure 16. Mean value of R and X of SEA group

Statisical Analysis

Statistical analysis between groups showed significant differences between SEA and control group for R at 3Hz, for mean and expiratory parameters, and for R at 2 Hz in expiration phase (Fig. 17)

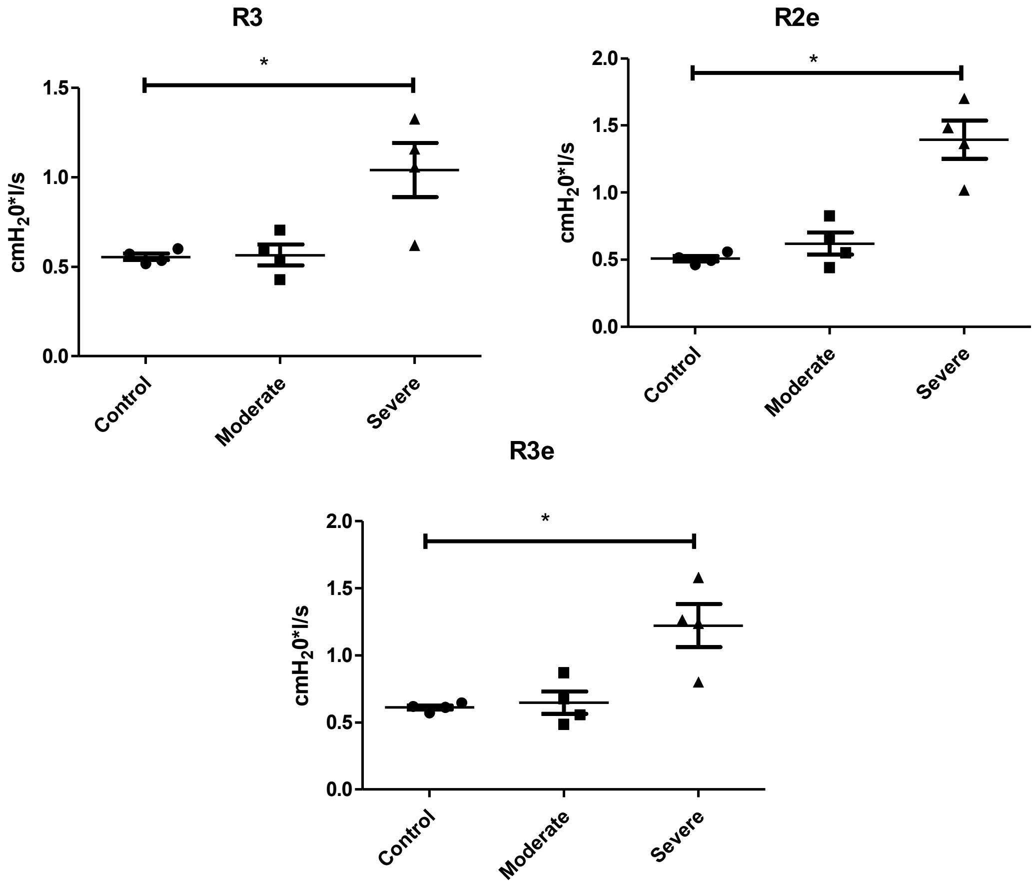


Figure 17. Whole breath resistance at 3Hz and expiratory resistance at 2 and 3 Hz (*=p<0,05)

Regarding X for the whole breath, difference were found between controls and SEA horse at frequencies ranging from 2 to 5 Hz. (Fig. 18)

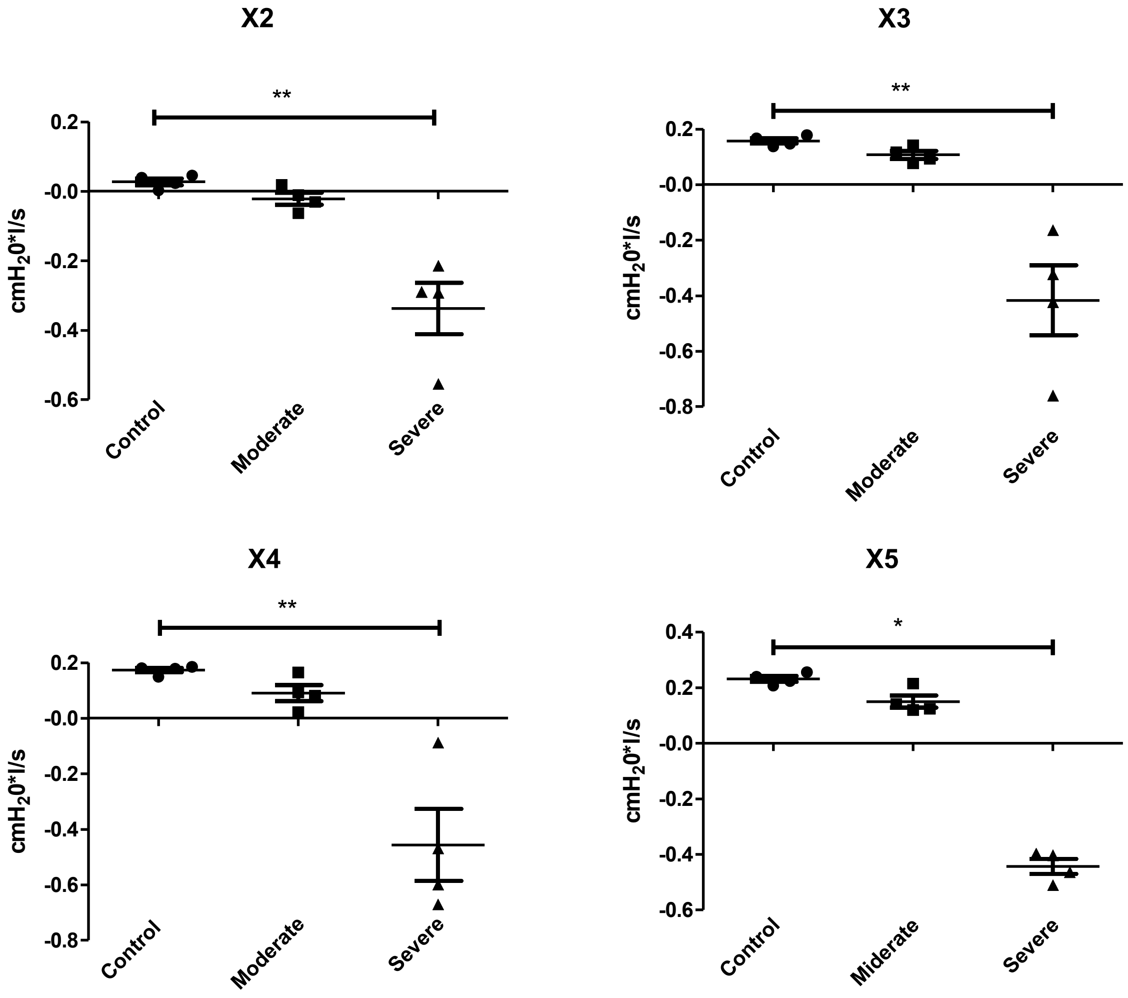


Figure 18. Whole breath reactance (*=p<0,05, **=p<0,01)

Concerning inspiratory X, SEA group and control group differed significantly from 3 to 5 Hz. (Fig. 19).

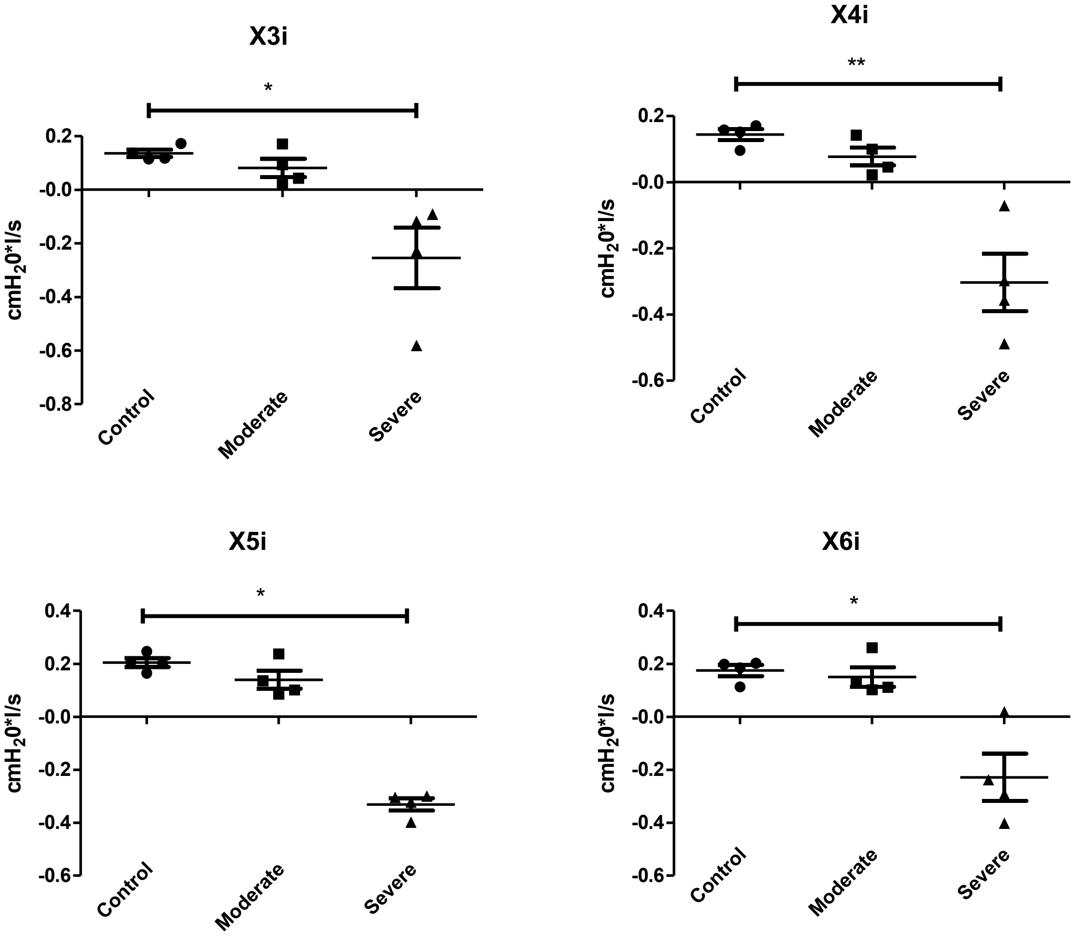


Figure 19. Inspiratory reactance from 2 to 6 Hz (*=p<0,05, **=p<0,01)

Evaluating expiratory X, instead, differences were found between SEA group and control group for all the frequencies (2 to 5 Hz). (Fig. 20, 21).

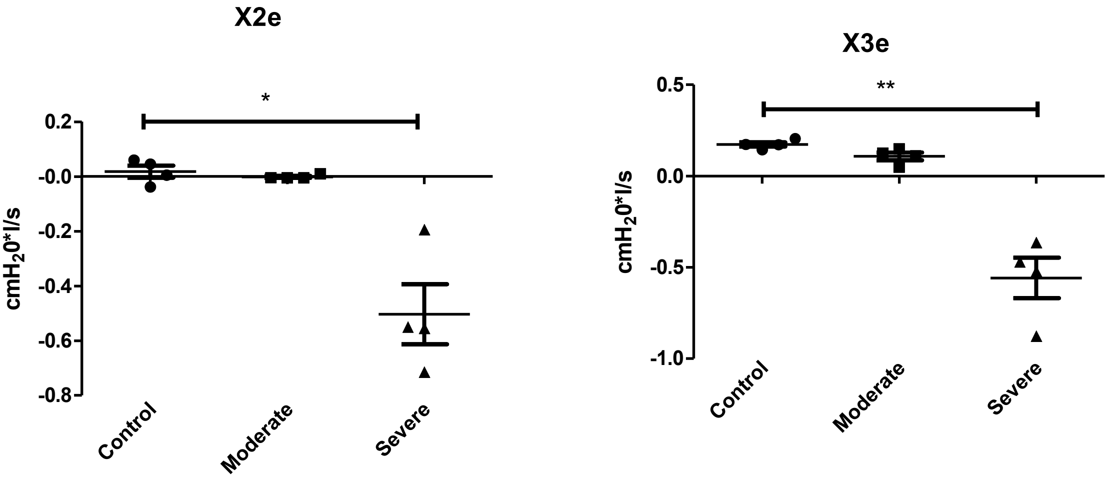


Figure 20. Expiratory reactance for 2 and 3 Hz (*=p<0,05, **=p<0,01)

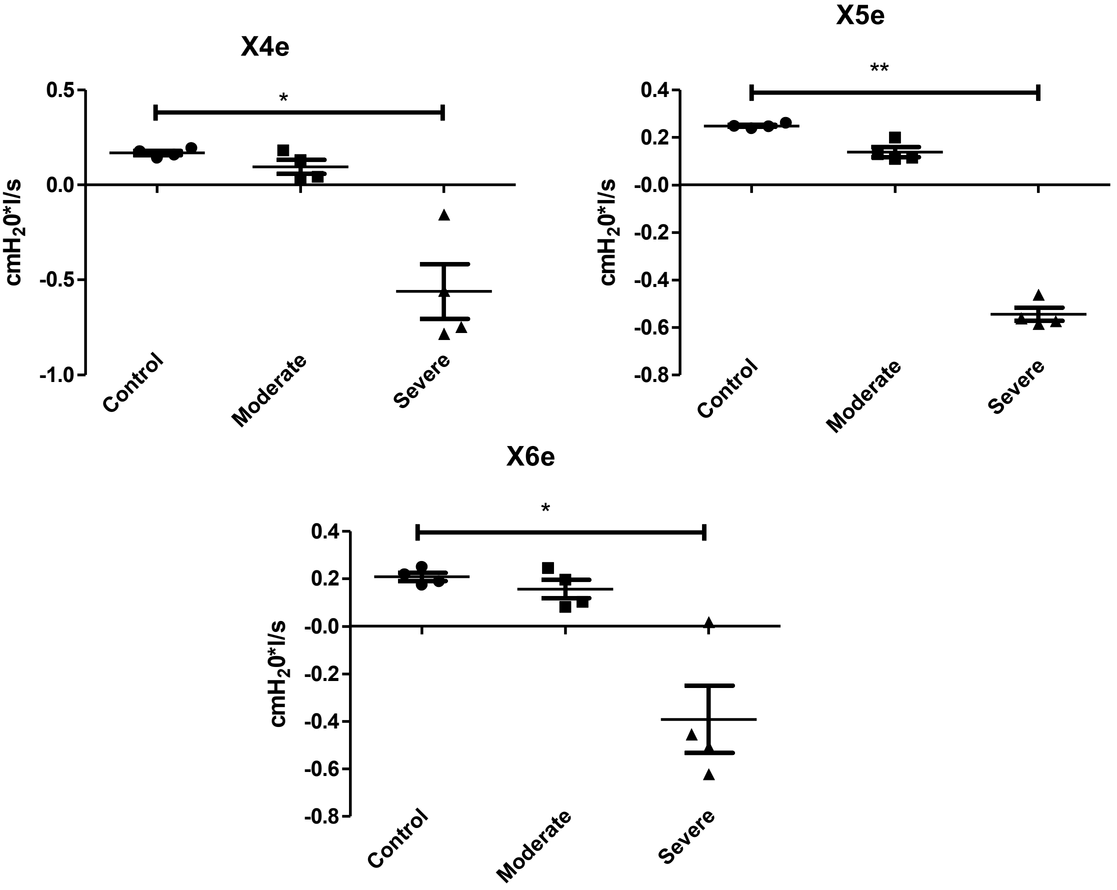


Figure 21. Expiratory reactance from 4 to 6 Hz (*=p<0,05, **=p<0,01)

Finally, comparison for ΔX showed that values in SEA horses were significantly higher than MEA or control horses, indicating that in SEA horses in exacerbation the reactance during expiratory phase is worse than during inspiration (Fig. 22)

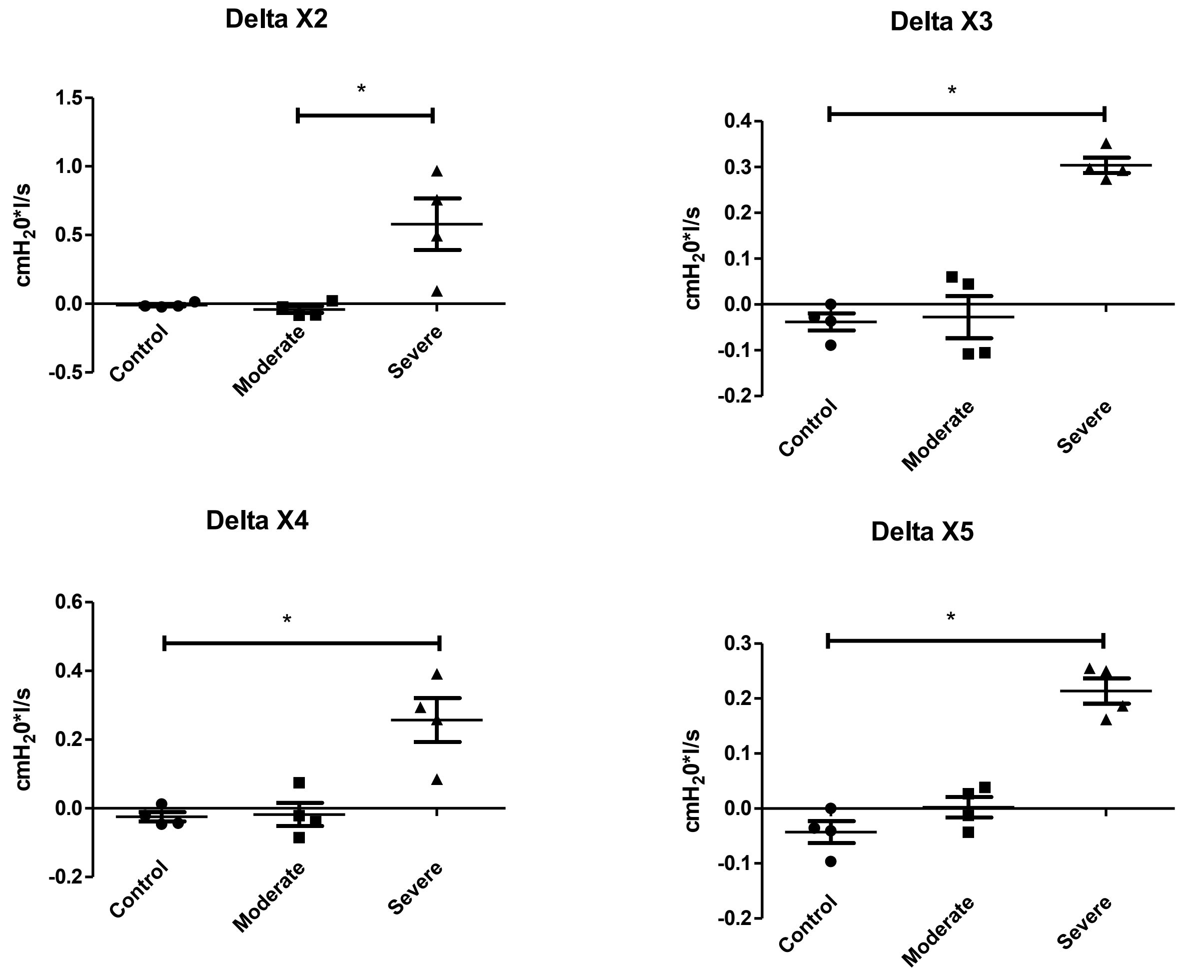


Figure 22. ΔX from 2 to 5 Hz (*=p<0,05)

4. DISCUSSION

The aim of the present project was to in-depth investigate the diagnostic mean of forced oscillation in presence of equine asthma. In the first study the sensitivity of IOS in identifying SEA horses in remission of the symptoms was evaluated, also with the help the new parameter ΔX. In the second study we evaluate a new FOT device and his ability to discriminate horses affected by MEA. The results obtained from both the study highlighte several features that are newsworthy to discuss.

Concerning study 1, the study sample was selected from a population of experimental horses affected by SEA. In this way, all horses were in a well-carachterized stage of the disease and under a strict control of the environment, in order to avoid any kind of interference. Moreover, all horses were already trained for the IOS measurement, and no behavioral changes have been registered during the procedure. A relaxed condition during IOS measurement is foundamental, in order to avoid any changes in respiratory breathing pattern (Van Erck *et al.*, 2004b). It is important to notice that human patients who undego IOS measurement describe an unpleasant sensation during the procedure, and could be presumed that this sensation can be felt also by the horses (Smith, Reinhold, and Goldman 2005). The average age of the SEA groups was 11.9±3.4 and 16.4±5.0. for remission and exacerbation respectively, that are considered a normal age for SEA manifestation (Couetil and Ward, 2003).

The frequencies evaluated for this study were only 3, 5 and 7 Hz, even if IOS device can produce spectra of frequencies from 0.1 to 20 Hz. The aim was to focus the attention only on the frequencies lower than 10 Hz that are considered the most representative of the lower airways (Klein, Smith and Reinhold, 2006). We did not consider the frequencies lower than 3 Hz because they could be influenced by higher harmonics of spontaneous respiratory frequency, and the quality of data could be low (Peslin and Fredberg, 1986). For the same reason, we decided to use high values of coherence (>0.85 at 3Hz and 0.9 at 5 and 7 Hz), that are similar to the values used in a previous study of IOS on normal horses (Klein, Smith and Reinhold, 2006). In the other studies on the application of IOS on asthmatic horse, no values of coherence are reported (Van Erck et al., 2003, 2004a, 2004b, 2006).

Looking at the results of IOS measurement, it is possible to observe that the average value of R at 3 Hz of the control horses was lower compared to 7 Hz, and the average values of reactance are all positive at all frequencies. This is similar to what reported in literature, considering that the frequency of resonance (the point when the reactance cross the 0 line) has been calculated at 2.4 Hz in normal horses (Young and Tesarowski 1994).

In the SEA group in exacerbation, instead, the value of R at 3 Hz was higher than at 7 Hz, showing a negative frequency dependence of the resistance, that is typical of SEA horses (Young, Tesarowski and Viel 1997). Moreover, the value of reactance are negative at all frequencies, showing a frequency of resonance that is higher than 7 Hz, that is comparable to what reported for severly asthmatic horse (Van Erck *et al.*, 2004b). The SEA group in remission showed a mixed behavior, because the values of R have a trend similar to controls, but the values of X have all a negative sign.

Statistical results showed that, as expected, between controls and SEA group in exacerbation, IOS identified several significant differences. Concerning R, the differences were present at 3 Hz, for whole breath, inspiratory and expiratory phase. This is similar to what reported in a recent work (Bullone *et al.,* 2014). In the other previous work on SEA horses, difference were found at 5 Hz (Van Erck *et al.*, 2004b); nevertheless, in that paper the frequency of 3 Hz was not investigated. The increase in R at low frequencies in SEA horses in exacerbation is indicative of the presence of the obstruction of the lower airways (Clement *et al.*, 1983), which is typical of SEA horses respiratory crisis. The difference for inspiratory and expiratory R, instead, has never been reported in previous studies.

SEA group in exacerbation showed also significant lower values of all the X parameters, at all frequencies and for all the phase of the breathing. This is in according to the previous reported data (Van Erck *et al.*, 2004b; Bullone *et al.*, 2014). However, this is the first work describing differences also for the within-breath analysis. The reactance at low frequencies reflects the peripheral airway obstruction, even if is nonspecific for the type of limitation (Smith, Reinhold and Goldman, 2005). In men reactance is reduced in asthma exacerbation, in COPD or in emphysema (Bickel *et al.*, 2014). During an exacerbation attack in SEA horses, the obstruction is primarily due to the bronchospasm, and it could be hypothesized that it represents the cause of reactance drop.

Moreover, it is possible to notice that ΔX in SEA group in exacerbation was significantly higher than in controls or in SEA horses in remission. It means that the expiratory X was significantly lower then inspiratory X in exacerbated asthmatic horses. This is the first time of this report in horses, and a comparison with what happens in man is needed. In fact in man the increase in ΔX has been validated as a parameter of the presence of Expiratory Flow Limitation (EFL) in COPD patients (Dellacà *et al*., 2004). The EFL occurs when the airflow stops to increase despite the increase in expiratory effort and it is a condition of human patient affected by pulmonary obstruction. It represents the narrowing of some of the airways (chock point) after the peak of expiration (Pedersen and Butler, 2011). During a measurement with FOT, the forced oscillations would penetrate to those alveoli where flow was not yet limited, but would not pass through the choke points that were established in parallel. This would cause a fall in X (Dellacà *et al.*, 2004). In a similar way, exacerbation of SEA horse is characterized by an early peak of expiration, and a consequent decrease in the expiratory flow (Petsche *et al.,* 1994). It could be argued that this is due to the presence of some choke points that cause a drop in expiratory X and a consequent increase in ΔX. The presence of EFL is considered an important cause of pulmonary hyperinflation and exercise intolerance in man, which are features also for SEA horses (Nyman *et al.*, 1999).

Finally, the SEA horses in remission differed from controls for X7 and for inspiratory X at all frequencies. This is the first report of the sensitivity of IOS in discriminate between healthy and SEA horses remission. It must be noticed that remission in the horses of the study was induced by a long-term maintenance at pasture and a well-controlled change in environmental management, and this strategy should bring SEA horses in a clinical condition that is similar to healthy subjects. Neverthless, in a previous work, SEA horses kept in pasture with antigen avoidance maintained a residual increase in bronchial smooth muscle mass compared to controls (Leclere *et al.*, 2012). It can be hypothesized that the remission condition do not influence the resistance parameters, in absence of increased respiratory effort, but that the presence of a residual remodeling of the airways affects the elastic properties of the lung, and consequently the reactance values.

The difference found only in inspiratory values could be explained beacuse SEA remission horses do not complain bronchospasm, and the absence of “choke point” during expiration, which affected exacerbated group, do not influence expiratory X. A similar pattern was reported in human patient with interstitial pneumonia, and the lower values of inspiratory reactance has been justified by the presence of pulmonary fibrosis (Mori *et al.*, 2013). As pulmonary fibrosis is present also in SEA horses (Bullone *et al.*, 2018), it could be speculated that.the presence of a residual remodeling could explain the differences in inspiratory X.

Concerning study 2, the sample has been selected from a population of hospitalized patients, on the basis of history and clinical sign. Although SEA horses in exacerbation were well characterized, as well as horses from control group, the selection of MEA group might be influenced by some bias. In fact, as MEA is often a subclinical disease, the selection based only on clinical sign could be inaccurate. Moreover, differently from SEA, that is characterized by accumulation of neutrophils in the airways, in MEA affected horses the inflammation can be mediated by also other cell opulation, such as mast-cells or eosinophils (Couëtil *et al.*, 2016). The influence of the different cell type on the FOT results is currently unknown.

The new FOT device for horses showed several advantages. First of all, it was very light, it had a good portability and the positioning of the motor producing airflow directly connected on the airmask allowed a very easy of use. Moreover, the custom-friendly software was very easy to use, allowing a direct control of the measurement and the possibility of intervention of the operator on the flux, the amplitude of signal and the frequencis, and also on the data storage.

All horses tried the FOT measurement for the first time; by the way, no horse showed any sign of discomfort, and the measurement could be performed in a relaxed way. The device produced just a little of noise but was very well accepted from all the horses. Comparing to IOS, it took more time for a registration (3 minute vs 30 second), but it did not need any repetition (3 times for IOS).

The frequencies considered for the measurement (2-6 Hz) were selected on the basis of the previous literature on FOT (Hoffman and Mazan, 1999). The values of coherence were not calculated because the system used a different method to evaluate the quality of signal (Marchal *et al.,* 2004). However, the data with poor quality have been discharged. As the measurement at 1 Hz showed several artifacts, probably due to the resonance with the breathing frequency of the horses, the data wer not used for the analysis (Smith, Reinhold and Goldman, 2005).

Looking at the values of FOT measurement, values of R ranged from 0.4 to 0.7 cm H_20*l/s for control horses, from 0.5 to 0.6 cmH20*l/s for MEA horses and from 0.7 to 1.0 cmH20*l/s for SEA horses, and X ranged from about 0.02 to 0.2 cm H20*l/s for control horse, from -0.02 to 0.1 cm H20*l/s for MEA horses, from -0.3 to -0.4 about cm H20*l/s. These values are comparable to what reported from Hoffman and Mazan, 1999. This result means that the FOT device produced reliable measurement.

Also the behaviour of the result of R, with a negative frequency dependence in SEA group, similar to what happened in study 1, is consistent with the previous study on FOT (Young, Tesarowski and Viel, 1997), and represent the increase of R at lower frequencies due to peripheral obstruction.

Concerning the values of X, instead, in the control group all the values of X at 2 Hz were positive, implying a frequency of resonance at a lower frequency. This is quite different from previously reported from Young and Tesarowski, 1994 (2.4 Hz). Nevertheless, in one of the first studies on FOT (Art *et al.*, 1989), authors suggested a value 1.51±0.11 Hz for frequency of resonance. As the frequency of resonance could be influenced by the body size, biometrical parameter of the horses sample would be an useful report (Smith, Reinhold and Goldman, 2005). As for study 1, for SEA horses, instead, the X values were all negative, showing a frequency of resonance > 6 Hz.

As observed in study 1, statistical analysis showed several differences between control group and SEA horses. Concerning resistance, SEA horses had significant higher values at lower frequencies (R2e, R3, R3e), and significant lower values of X at almost all frequencies and all the phase of breathing. These results are comparable with previously reported by Hoffman and Mazan, 1999, and with the data obtained in study 1. Then, it is possible to affirm that the FOT device was able to detect the respiratory obstruction in SEA horses.

The analysis on ΔX showed higher values in SEA horses compared to controls or to MEA group, in the same manner of Study 1, so the presence of EFL during exacerbation could be highlighted also by the FOT device.

Concerning the MEA group, instead, in the present study no differences were found compared to controls. This is in contrast to what described by Hoffman and Mazan, 1999, that reported a difference for R at 1, 2 and 3Hz. This would be an expected result, because MEA is characterized by inflammation of the lower airways, and R should be influenced by the airways caliber (Smith, Reinhold and Goldman, 2005). Also Richard *et al.*, 2009, measuring respiratory mechanic with IOS, reported a difference between control and MEA horses for R from 1 to 7 Hz, and also for X at frequencies >5 Hz. Neverthless, considering that X represent the elastic properties of the lung and that in MEA horses the bronchospasm and the remodeling are not so severe such as in SEA, differences for this parameter were not expected. For this reason, the reported data from Richard *et al.*, 2009 at high frequencies might not reflect the condition of the lower airways. It could be supposed that in the present study it was not possible to highlight the differences between MEA and controls because of the small number of the sample, which could have affected the statistical results.

5. CONCLUSION

The present study brought several novelties in the study of the application of forced oscillation in the diagnosis of asthma in equine patients.

First of all, this is the first time the within-breath analysis is described in the SEA patients, both in remission and in exacerbation of the symptoms. This kind of analysis allowed to identify the greater alteration of the expiratory phase in the horses affected by acute brosbchosapsm compared to inspiratory phase, and the identification of the possible presence of EFL similar to what happen in human COPD patients. Moreover, for the first time IOS allowed to discriminate between healthy subjects and SEA horses in a stable remission of the symptoms, a situation that can be hard to detect with other diagnostic techniques, such as BAL or convemtional mechanic. For this reason, oscillometery could be a useful tool in the clinical practice.

From results of study 2, it can be stated that the new FOT device was easy to use, well-accepted by the horses and the measurement obtained were reliable. Moreover, it was able to identify the differences between SEA horses and healthy subjects in a correct way. The application of the most recent expertises and technologies already adopted in human medicine, such as the 3D print, the custom-friendly interface and the possibility of portability of the device, together with the results of the present project, could bring in the near future to the application of this kind of device on a larger scale of clinical patients.

Neverthless, the present project presents some limitation: first of all, in both the study some useful data of the samples, such as biometrical parameters, the results of conventional mechanics or of BAL cytology, are missing. The comparison of the results of oscillometry measurement also with these data would increase the meaningful significance of this technique. Further studies are needed in order to evaluate these data. Moreover, the sample number in study 2 is small, and this could have influenced the statistical results. The evaluation of the new FOT device on a larger number of asthmatic patients would increase the significance of the first results obtained.

For the future, the attention would be focused on the analysis of MEA horses. In fact, in this preliminary work, FOT lacked to identify any alteration in these patients, even if previous studies showed some sensitivity of FOT in evaluating low-grade lung inflammation. Nevertheless, it would be very useful to work increasing the sensitivity of the device, in order to obtain a non-invasive system that could be able to diagnose a subclinical disease such as MEA.

It could be hypotizised that the little differences between healthy subject and MEA horses is due to the absence of bronchospasm in MEA horses, that instead is a feature of SEA and that causes the large alterations in FOT measurement. Neverthless, some authors suggested the presence of exercise-induce bronchospasm in horses affected by MEA (Couetil *et al.*, 2016). For this reason in the future the FOT measurement before and after exercise in MEA horses could be an interesting field to investigate.

REFERENCES

Abraham, G., Brodde, O.E. and Ungemach, F.R. 2002 “Regulation of equine lymphocyte beta-adrenoceptors under the influence of clenbuterol and dexamethasone.” Equine Veterinary Journal 34, 587-593

Art, T., Franck, T., Lekeux, P., de Moffarts, B., Couetil, L., Becker, M., Kohnen, S., Deby-Dupont, G. and Serteyn, D. 2006. “Myeloperoxidase concentration in bronchoalveolar lavage fluid from healthy horses and those with recurrent airway obstruction” Canadian Journal of Veterinary Research 70, 291-296

Art, T., Lekeux, P., Gustin, P., Desmecht, D., Amory, H. and Paiva, M. 1989. “Inertance of the Respiratory System in Ponies Inertance of the Respiratory System in Ponies,” 534–40.

Aviza, A., Ainsworth, D.M., Eicker, S.W., Santiago, M.A., Diversandg, T.J., and Perkins, A. 2001 “Outcome of horses diagnosed with and treated for heaves (recurrent airway obstruction” Equine veterinary Education 13 (5) 243-246

Baker, G.J. 1987 “Diseases of the Pharynx and the Larynx” In: Robinson NE “Current Therapy in Equine Medicine” 2nd Edition, Saunders, 607-608

Bickel, S., Popler, J., Lesnick, B., and Eid, N. 2014. “Impulse Oscillometry: Interpretation and Practical Applications.” Chest 146 (3): 841–47. https://doi.org/10.1378/chest.13-1875.

Bullone, M., and Lavoie, J.P. 2015. “Asthma ‘of Horses and Men’-How Can Equine Heaves Help Us Better Understand Human Asthma Immunopathology and Its Functional Consequences?” Molecular Immunology 66 (1): 97–105. https://doi.org/10.1016/j.molimm.2014.12.005.

Bullone, M., Beauchamp, G., Godbout, M., Martin, J.G., and Lavoie, J.P. 2015. “Endobronchial Ultrasound Reliably Quantifies Airway Smooth Muscle Remodeling in an Equine Asthma Model.” PLoS ONE 10 (9): 1–20. https://doi.org/10.1371/journal.pone.0136284.

Bullone, M., Chevigny, M., Allano, M., Martin, J.M., and Lavoie, J.P.. 2014. “Technical and Physiological Determinants of Airway Smooth Muscle Mass in Endobronchial Biopsy Samples of Asthmatic Horses.” Journal of Applied Physiology 117 (7): 806–15. https://doi.org/10.1152/japplphysiol.00468.2014.

Bullone, M., De Lagarde, M., Vargas, A., and Lavoie, J.P. 2015. “Serum Surfactant Protein D and Haptoglobin as Potential Biomarkers for Inflammatory Airway Disease in Horses.” Journal of Veterinary Internal Medicine 29 (6): 1707–11. https://doi.org/10.1111/jvim.13602.

Bullone, M., Hélie, P., Joubert, P., and Lavoie, J.P. 2016. “Development of a Semiquantitative Histological Score for the Diagnosis of Heaves Using Endobronchial Biopsy Specimens in Horses.” Journal of Veterinary Internal Medicine 30 (5): 1739–46. https://doi.org/10.1111/jvim.14556.

Bullone, M., Joubert, P., Gagné, A., Lavoie, J.P., and Hélie, P. 2018. “Bronchoalveolar Lavage Fluid Neutrophilia Is Associated with the Severity of Pulmonary Lesions during Equine Asthma Exacerbations.” Equine Veterinary Journal 50 (5): 609–15. https://doi.org/10.1111/evj.12806.

Bullone, M., Vargas, A., Elce, Y., Martin, J.G. and Lavoie, J.P. 2017. “Fluticasone/Salmeterol Reduces Remodelling and Neutrophilic Inflammation in Severe Equine Asthma.” Scientific Reports 7 (1): 1–13. https://doi.org/10.1038/s41598-017-09414-8.

Burrell, M.H., Wood, J.L.N., Whitwell, K.E., Chanter, N., Mackintosh, M.E. and Mumford, J.A. 1996. “Respiratory Disease in Thoroughbred Horses in Training: The Relationships between Disease and Viruses, Bacteria and Environment.” Veterinary Record 139 (13): 308–13. https://doi.org/10.1136/vr.139.13.308.

Calzetta, L., Roncada, P., di Cave, D., Bonizzi, L., Urbani, A., Pistocchini, E., Rogliani, P., and Matera, M.G. 2017. “Pharmacological Treatments in Asthma-Affected Horses: A Pair-Wise and Network Meta-Analysis.” Equine Veterinary Journal 49 (6): 710–17. https://doi.org/10.1111/evj.12680.

Chapman, P. S., Green, G., Main, J.P., Taylor, P.M., Cunningham, F.M., Cook, A.J., and Marr, C.M. 2000. “Retrospective Study of the Relationships between Age, Inflammation and the Isolation of Bacteria from the Lower Respiratory Tract of Thoroughbred Horses.” The Veterinary Record 146 (4): 91–95. https://doi.org/10.1136/vr.146.4.91.

Chapman, P., Green, C., Main, J. 2000. “Retrospective study of the relationship between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses” Veterinary Record 146, 91-95

Christley, R.M., Hodgson, D.R., Rose, R.J., Wood, J.L.N., Reid, S.W.J., Whitear, K.G., and Hodgson, J.L. 2010. “A Case-Control Study of Respiratory Disease in Thoroughbred Racehorses in Sydney, Australia.” Equine Veterinary Journal 33 (3): 256–64. https://doi.org/10.2746/042516401776249796.

Clement, J., Landser, F.J., Van de Woestijne, K.P. 1983 “Total resistance and reactance in patients with respiratory complaints with and without airways obstruction.” Chest 83:215-220

Clercx, C., Gustin, P., Landser, F.J., and Van de Woestijne, K.P. 1993 “Measurement of total respiratory impedance in dogs by the forced oscillation technique” Veterinary Research Communications, 17: 227-239

Cornelisse, C.J., Robinson, N.E., Berney, C.E., Kobe, C.A., Boruta, D.T., and Derksen, F.J. 2004 “Efficacy of oral and intravenous dexamethasone in horses with recurrent airway obstruction.” Equine Veterinary Journal 36(5):426-30

Couëtil, L.L. 2014. “Inflammatory diseases of the lower airway of athletic horses” In: Hinchcliff KW, Kaneps AJ, Geor RJ “Equine sport medicine and surgery”, 2nd Edition, Saunders, 605-618

Couetil, L.L., and Ward, M.P., 2003. “Analysis of risk factors for recurrent airway obstruction in North American horses: 1,444 cases (1990-1999)” Journal of American Veterinary Medicine Association 223, 1645-1650.

Couetil, L.L., Art, T., de Moffarts, B., Becker, M., Melotte, D., and Jaspar, F. 2006 “Effect of beclomethasone dipropionate and dexamethasone isonicotinate on lung function, bronchoalveolar lavage fluid cytology, and transcription factor expression in airways of horses with recurrent airway obstruction.” Journal of Veterinary Internal Medicine 20(2):399-406.

Couëtil, L.L., Cardwell, J.M., Gerber, V., Lavoie, J.P., Léguillette, R., and Richard, E.A. 2016. “Inflammatory Airway Disease of Horses-Revised Consensus Statement.” Journal of Veterinary Internal Medicine 30 (2): 503–15. https://doi.org/10.1111/jvim.13824.

Couëtil, L.L., Denicola, D.B. 1999 “Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease” Equine Veterinary Journal 30, 77-82

Couëtil, L.L., Hoffman, A.M., Hodgson, J., Buechner-Maxwell, V., Viel, L., Wood, J.L.N. and Lavoie, J.P. 2007. “Inflammatory Airway Disease of Horses. ACVIM Consensus Statement.” Journal of Veterinary Internal Medicine 21 (2): 356–61. https://doi.org/10.1892/0891-6640

Couetil, L.L., Rosenthal, F.S. and Simpson, C.M. 2000 “Forced expiration: a test for airflow obstruction in horses.” Journal of Applied Physiology 88, 1870-1879.

Couëtil, L.L., Rosenthal, F.S., and DeNicola, D.B. 2001. “Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease.” American Journal of Veterinary Research 62:538–46.

Deaton, C.M., Deaton M.L., Jose-Cunilleras, E., Vincent, T.L., Baird, A.W., Dacre, K., and Marlin, D.J. 2007. “Early Onset Airway Obstruction in Response to Organic Dust in the Horse.” Journal of Applied Physiology 102 (3): 1071–77. https://doi.org/10.1152/japplphysiol.00264.2006.

Dellacà, R.L., Gobbi, A., Pastena, M., Pedotti, A., and Celli, B. 2010. “Home Monitoring of Within-Breath Respiratory Mechanics by a Simple and Automatic Forced Oscillation Technique Device.” Physiological Measurement 31 (4): 10–24. https://doi.org/10.1088/0967-3334/31/4/N01.

Dellacà, R.L., Santus, P., Aliverti, A., Stevenson, N., Centanni, S., Macklem, P.T., Pedotti, A., and Calverley, P.M.A. 2004. “Detection of Expiratory Flow Limitation in COPD Using the Forced Oscillation Technique.” European Respiratory Journal 23 (2): 232–40. https://doi.org/10.1183/09031936.04.00046804.

Derksen, F.J., Scott. J.S., Miller. D.C., Slocombe, R.F., Robinson, N.E. 1985 “Bronchoalveolar lavage in ponies with recurrent airway obstruction (heaves)” American Rev Respiratory Disease 132(5):1066-70.

Dixon, P., Railton, D., McGorum, B. 1995 “Equine pulmonary disease: a case control study of 300 referred cases. Part 2: Details of animals and of historical and clinical findings” Equine Veterinary Journal 27:422–7.

Dubois, A., Brody, A., Lewis, D., et al. 1956 “Oscillation mechanics of lungs and chest in man.” Journal of Applied Physiology 8:587-594.

Duvivier, D.H., Bayly, W.M., Votion, D., Vandenput, S., Art, T., Farnir, F. and Lekeux, P. 1999 “Effects of inhaled dry powder ipratropium bromide on recovery from exercise of horses with COPD.” Equine Veterinary Journal 31, 20-24.

Duvivier, D.H., Votion, D., Vandenput, S., and Lekeux, P. 1997 “Aerosol therapy in the equine species.” Veterinary Journal 154(3):189-202.

Fairbairn, S.M., Page, C.P., Lees, P. and Cunningham, F.M. 1993 “Early neutrophil but not eosinophil or platelet recruitment to the lungs of allergic horses following antigen exposure.” Clinical and Experimental Allergy 23, 821-828

Ferrucci, F., Stucchi, L., Salvadori, M., Stancari, G., Conturba, B., Bronzo, V., Ferro, E., and Zucca, E. 2013. “Effects of Inhaled Amikacin on Racehorses with History of Poor Performance and Comparison with Intravenous Administration.” Ippologia 24 (3–4).

Fogarty, U., Buckley, T. 1991 “Bronchoalveolar lavage findings in horses with exercise intolerance” Equine Veterinary Journal 23, 434-437

Fortier, G., Van Erck, E., Fortier, C., Richard, E., Pottier, D., Pronost, S., Miszczak, F., Thiry, E., Lekeux, P. 2009 “Herpesviruses in respiratory liquids of horses: Putative implication in airway inflammation and association with cytological features” Veterinary Microbiology 139, 34-41

Gerber, V., Straub, R., Marti, E., Hauptman, J., Herholz, C., King, M., Imhof, A., Tahon, L., Robinson, N.E. 2004 “Endoscopic scoring of mucus quantity and quality: observer and hose variance and relationship to inflammation, mucus viscoelasticity and volume”, Equine Veterinary Journal 36(7), 576-582

Gerber, V., Tessier, C., and Marti, E. 2015. “Genetics of Upper and Lower Airway Diseases in the Horse.” Equine Veterinary Journal 47 (4): 390–97. https://doi.org/10.1111/evj.12289.

Gillespie, J., Tyler, W, and Eberly, V. “Pulmonary ventilation and resistance in emphysematous and control horses.” J Appl Physiol 1966;21:416–22.

Hare, J.E., and Viel, L. 1998 “Pulmonary Eosinophilia Associated With Increased Airway Responsivness in Young Racing Horses” Journal of Veterinary Internal Medicine 12, 163-170

Hoffman, A.M. 2002. “Clinical Application of Pulmonary Function Testing in Horses.” In: Equine Respiratory Diseases, P. Lekeux (Ed.) Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA

Hoffman, A.M., and Mazan, M.R. 1999. "Programme of lung function testing horses suspected with small airway disease" Equine veterinary Education 11 (6) 322-328

Hoffman, A.M., Oura, T.J., Riedelberger, K.J. and Mazan, M.R. 2007 “Plethysmographic comparison of breathing pattern in heaves (recurrent airway obstruction) versus experimental bronchoconstriction or hyperpnea in horses.” Journal of Veterinary Internal Medicine 21, 184-192

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

Jean, D., Vrins, A., Beauchamp, G. and Lavoie, J.P. 2011. “Evaluation of variations in bronchoalveolar lavage fluid in horses with recurrent airway obstruction.” American Journal of Veterinary Research 72, 838-842.

Klein, C., Reinhold, P. “Analysis of respiratory mechanics by impulse oscillometry in non-sedated and diazepam-sedated swine. Res Vet Sci 2001; 70: 181–189.

Klein, C., Smith, H.J. and Reinhold, R. 2006. “The Use of Impulse Oscillometry for Separate Analysis of Inspiratory and Expiratory Impedance Parameters in Horses: Effects of Sedation with Xylazine.” Research in Veterinary Science 80 (2): 201–8. https://doi.org/10.1016/j.rvsc.2005.06.005.

Koblinger, K., Nicol, J., McDonald, K., Wasko, A., Logie, N., Weiss, M., Léguillette, R. 2011 “Endoscopic Assessment of Airway Inflammation in Horses” Journal of Veterinary Internal Medicine 25, 1118-1126

Koch, C., Straub, R., Ramseyer, A., Widmer, A., Robinson, N.E., Gerber, V. 2007 “Endoscopic scoring of the tracheal septum in horses and its clinical relevance for the evaluation of lower airway health in horses” Equine Veterinary Journal 39(2), 107-112

Lapointe, J.M., Lavoie, J.P., and Vrins, A.A. 1993 “Effects of triamcinolone acetonide on pulmonary function and bronchoalveolar lavage cytologic features in horses with chronic obstructive pulmonary disease.” American Journal of Veterinary Research 54: 1310–1316

Lavoie, J.P. 2007 “Recurrent Airway Obstruction (RAO) and Summer Pasture Associated Pulmonary Disease (SPAOPD)” In: McGorum, B.C., Dixon, P.M., Robinson, N.E., Shumacher, J. “Equine Respiratory Medicine and Surgery” Saunders, 565-589

Lavoie, J.P. 2015. “Is the Time Primed for Equine Asthma?” Equine Veterinary Education 27 (5): 225–26. https://doi.org/10.1111/eve.12332.

Lavoie, J.P., Leguillette, R., Pasloske, K., Charette, L., Sawyer, N., Guay, D., Murphy, T. and Hickey, G.J. 2002 “Comparison of effects of dexamethasone and the leukotriene D4 receptor antagonist L-708,738 on lung function and airway cytologic findings in horses with recurrent airway obstruction.” American Journal of Veterinary Research 63, 579-585

Leclere, M., Lavoie-Lamoureux, A., and Lavoie, J.P. 2011. “Heaves, an Asthma-like Disease of Horses.” Respirology 16 (7): 1027–46. https://doi.org/10.1111/j.1440-1843.2011.02033.x.

Leclere, M., Lavoie-Lamoureux, A., Gelinas-Lymburner, E., David, F., Martin, J.G. and Lavoie, J.P. 2011. “Effect of Antigenic Exposure on Airway Smooth Muscle Remodeling in an Equine Model of Chronic Asthma.” American Journal of Respiratory Cell and Molecular Biology 45 (1): 181–87. https://doi.org/10.1165/rcmb.2010-0300OC.l

Leclere, M., Lavoie-Lamoureux, A., Joubert, P., Relave, F., Lanctot Setlakwe, E., Beauchamp, G., Couture, C., Martin, J.G., and Lavoie, J.P. 2012. “Corticosteroids and Antigen Avoidance Decrease Airway Smooth Muscle Mass in an Equine Asthma Model.” American Journal of Respiratory Cell and Molecular Biology 47 (5): 589–96. https://doi.org/10.1165/rcmb.2011-0363OC.

Leclere, M., Lefebvre-Lavoie, J., Beauchamp, G. and Lavoie, J.P. 2010 “Efficacy of oral prednisolone and dexamethasone in horses with recurrent airway obstruction in the presence of continuous antigen exposure.” Equine Veterinary Journal 42, 316-321.

Lugo, J., Harkema, J.R., DeFeijter-Rupp, H., Bartner, L., Boruta, D. and Robinson, N.E. 2006 “Airway inflammation is associated with mucous cell metaplasia and increased intraepithelial stored mucosubstances in horses” Veterinary Journal 172, 293-301

Malikides, N., Hughes, K.J., Hodgson, D.R., and Hodgson J.L., 2003 “Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses. 2. Evaluation of the diagnostic significance of neutrophil percentage.” Australian Veterinary Journal 2003;81:685–7.

Marchal, F., Schweitzer, C., Demoulin, B., Choné, C., and Peslin, R. 2004. "Filtering Artefacts in Measurements of Forced Oscillation Respiratory Impedance in Young Children." Physiological Measurement 25 (5): 1153–66. https://doi.org/10.1088/0967-3334/25/5/006.

Markham G., 1656. "Markhams Maister-peece: Containing All Knowledge Belonging to the Smith, Farrier, or Horse-leech" W. Wilson, London

Marlin, D.J., and Deaton, C.M. 2007 "Pulmonary Function Testing" In: McGorum, B.C., Dixon, P.M., Robinson, N.E., Shumacher, J. "Equine Respiratory Medicine and Surgery" Saunders, 211-233

Marti, E., Gerber, H., Essich, G., Oulehla, J. and Lazary, S. 1991. "The genetic-basis of equine allergic diseases. 1. Chronic hypersensitivity bronchitis" Equine Veterinary Journal 23, 457-460

Mazan, M.R., Deveney, E.F., DeWitt, S., Bedenice, D. and Hoffman, A. 2004 "Energetic cost of breathing, body composition, and pulmonary function in horses with recurrent airway obstruction" Journal of Appied Physiology 97, 91-97.

McGorum, B.C., Dixon, P.M., and Halliwell, R.E.W., 1993 "Phenotypic analysis of peripheral-blood and bronchoalveolar lavage fluid lymphocytes in control and chronic obstructive pulmonary-disease affected horses, before and after natural (hay and straw) challenges" Veterinary Immunology and Immunopathology 36, 207- 222

McKane, S.A., Slocombe, R.F. 2010 "Experimental mild pulmonary inflammation promotes the development of exercise-induced pulmonary haemorrhage" Equine Veterinary Journal 42(38), 235-239

Michaelson, E.D., Grassman, E.D., and Peters, W.R. 1975 "Pulmonary mechanics by spectral analysis of forced random noise." Journal of Clinical Investigation 56:1210-1230

Mikamo, M., Fujisawa, T., Oyama, Y., Kono, M., Enomoto, N., Nakamura, Y., Inui, N., Sumikawa, H., Johkoh, T., and Suda, T. 2016. "Clinical Significance of Forced Oscillation Technique for Evaluation of Small Airway Disease in Interstitial Lung Diseases." Lung 194 (6): 975–83. https://doi.org/10.1007/s00408-016-9949-1.

Millerick-May, M.L., Karmaus W., Derksen, F.J. 2011 "Particle mapping in stables at an American Thoroughbred racetrack" Equine Veterinary Journal 43, 599–607

Mori, K., Shirai, T., Mikamo, M., Shishido, Y., Akita, T., Morita, S., Asada, K. et al. 2013. “Respiratory Mechanics Measured by Forced Oscillation Technique in Combined Pulmonary Fibrosis and Emphysema.” Respiratory Physiology and Neurobiology 185 (2): 235–40. https://doi.org/10.1016/j.resp.2012.10.009.

Nolen-Walston, R.D., Harris, M., Agnew, M.E., Martin, B.B. Reef, V.B., Boston, R.C., and Davidson, E.J. 2013. “Clinical and Diagnostic Features of Inflammatory Airway Disease Subtypes in Horses Examined Because of Poor Performance: 98 Cases (2004-2010).” Journal of the American Veterinary Medical Association 242 (8): 1138–45. https://doi.org/10.2460/javma.242.8.1138.

Nyman, G., Björk, M., and Funkquist, P. 1999 “Gas exchange during exercise in standardbred trotters with mild bronchiolitis.” Equine Veterinary Journal Supplement (30):96-101.

Nyman, G., Lindberg, R., Weckner, D., Bjork, M., Kvart, C., Persson, S.G.B., Gustafsson, H. and Hedenstierna, G. 1991 “Pulmonary gas-exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis.” Equine Veterinary Journal 23, 253-260.

Obel, N.J., and Schmiterlow, C.G., 1948. “The action of histamine and other drugs on the bronchial tone in horses suffering from alveolar emphysema (heaves)”. Acta Pharmacologica Toxicologica 4, 71-80

Onmaz, A.C., Stoklas-Schmidt, C., and Van Den Hoven, R. 2013. “Daily Variability of Forced Oscillometry Parameters in Horses Suffering Recurrent Airway Obstruction, a Pilot Study.” Veterinary Research Communications 37 (1): 11–17. https://doi.org/10.1007/s11259-012-9541-y.

Oostveen, E., MacLeod, D., Lorino, H., Farré, R., Hantos, Z., Desager, K., and F. Marchal. 2003. “The Forced Oscillation Technique in Clinical Practice: Methodology, Recommendations and Future Developments.” European Respiratory Journal 22 (6): 1026–41. https://doi.org/10.1183/09031936.03.00089403.

Otis, A., McKerrow, C., Bartlett, R., et al. 1956 “Mechanical factors in distribution of pulmonary ventilation.” Journal of Applied Physiology 8:427-443.

Pedersen, O.F., and Butler, J.P. 2011 “Expiratory flow limitation.” Comprehensive Physiology 1(4):1861-82. doi: 10.1002/cphy.c100025.

Peslin, R., and Fredberg, J. 1986 “Oscillation mechanics of the respiratory system.” In: Macklem P, Mead J, eds. Handbook of Physiology, Section 3: The respiratory system. Vol III, Mechanics of breathing. Bethesda, American Physiological Society, pp. 145–166

Petsche, V.M. , Derksen, F.J. , and Robinson, N.E. 1994 “Tidal breathing flow-volume loops in horses with recurrent airway obstruction (heaves).” American Journal of Veterinary Research 55(7):885-891

Picandet, V., Leguillette, R., and Lavoie, J.P. 2003 “Comparison of efficacy and tolerability of isoflupredone and dexamethasone in the treatment of horses affected with recurrent airway obstruction (“heaves”). Equine Veterinary Journal 35: 419–424

Pirie, R. S. 2014. “Recurrent Airway Obstruction: A Review.” Equine Veterinary Journal 46 (3): 276–88. https://doi.org/10.1111/evj.12204.

Pirrone, F., Albertini, M., Clement, M.G., and Lafortuna, C.L. 2007. “Respiratory Mechanics in Standardbred Horses with Sub-Clinical Inflammatory Airway Disease and Poor Athletic Performance.” Veterinary Journal 173 (1): 144–50. https://doi.org/10.1016/j.tvjl.2005.07.020.

Reinhold, P., Smith, H.J., Langenberg, A., Lekeux, P. 1998 “Measurement of respiratory impedance in healthy calves using the impulse oscillation technique - physiological and methodological aspects.” Veterinary Journal 155: 27–38.

Richard, E.A., Fortier, G.D., Denoix, J.M., Art, T., Lekeux, P.M., and Van Erck, E. 2009. “Influence of Subclinical Inflammatory Airway Disease on Equine Respiratory Function Evaluated by Impulse Oscillometry.” Equine Veterinary Journal 41 (4): 384–89. https://doi.org/10.2746/042516409X366121.

Robinson, N.E. 2001 “International Workshop on Equine Chronic Airway Disease.” Michigan State University 16–18 June 2000. Equine Veterinary Journal 33: 5–19

Robinson, N.E., Derksen, F.J., and Olszewski, MA. 1996. “Review the Pathogenesis of Chronic Obstructive Pulmonary Disease of horses” British Veterinary Journal 152, 28303: 283–306.

Robinson, N.E., Karmaus, W., Holcombe, S.J., Carr, E.A., and Derksen, F.J. 2006. “Airway Inflammation in Michigan Pleasure Horses: Prevalence and Risk Factors.” Equine Veterinary Journal 38(4):293-9..

Rodrigues Costa, L.R., Seahorn, T.L., Moore, R.M., Taylor, H.W., Gaunt, S.D., and Beadle, R.E. 2000. "Correlation of Clinical Score, Intrapleural Pressure, Cytologic Findings of Bronchoalveolar Fluid, and Histopathologic Lesions of Pulmonary Tissue in Horses with Summer Pasture-Associated Obstructive Pulmonary Disease." American Journal of Veterinary Research 61 (2): 167–73.

Rush, B.R., Raub, E.S., Rhoads, W.S., Flaminio, M.J., Matson, C.J., and Hakala, J.E 1998 "Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively." American Journal of Veterinary Research 59(8):1039-43

Rush, B.R., Raub, E.S., Thomsen, M.M., Davis, E.G., Matson, C.J., and Hakala JE. 2000 "Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction." Journal of American Veterinary Medicine Association 217(3):359-64.

Sánchez, A., Couëtil, L.L., Ward, M.P., and Clark, S.P. 2005 "Effect of Airway Disease on Blood Gas Exchange in Racehorses" Journal of Veterinary Internal Medicine 19, 87-92

Seahorn, T., Groves, M., and Harrington, K. 1996 "Chronic obstructive pulmonary disease in horses in Louisiana." Journal of American Veterinary Medicine Association 208:248–51.

Smith, H.J., Reinhold, P., and Goldman, M.D.. 2005. "Forced Oscillation Technique and Impulse Oscillometry." Lung Function Testing, 72–105. https://doi.org/10.1183/1025448x.00031005.

Stucchi, L., Ferrucci, F., Dellacà, R.L., Lafortuna, C.L., Bullone, M., Pompilio, P.P., Tabozzi, S., and Lavoie, J.P., 2018 "Within-breath input impedance by impulse oscillometry in severely asthmatic horses" Comparative Exercise Physiology 14 (Supplement 1):S1-S114 DOI:10.3920/cep2018.s1.

Südy, R., Fodor, G.H., Dos Santos Rocha, A., Schranc, Á., Tolnai, J., Habre, W.,and Peták, F. 2019. "Different contributions from lungs and chest wall to respiratory mechanics in mice, rats, and rabbits." Journal of Applied Physiology (1985) 1;127(1):198-204. doi: 10.1152/japplphysiol.00048.2019

Thomson, J.R. and McPherson, E.A. 1984 “Effects of environmental-control on pulmonary-function of horses affected with chronic obstructive pulmonary-disease.” Equine Veterinary Journal 16, 35-38

Van Erck, E., Votion, D., Art, T., and Lekeux, P. 2006 "Qualitative and quantitative evaluation of equine respiratory mechanics by impulse oscillometry.", Equine veterinary Journal 38 (1) 52-58

Van Erck, E., Votion, D., Art, T., and Lekeux, P. 2004a. “Measurement of Respiratory Function by Impulse Oscillometry in Horses.” Equine Veterinary Journal 36 (1): 21–28. https://doi.org/10.2746/0425164044864714.

Van Erck, E., Votion, D., Kirschvink, N., Genicot, B., Lindsey, J., Art, T., and Lekeux, P. 2004b. “Influence of Breathing Pattern and Lung Inflation on Impulse Oscillometry Measurements in Horses.” Veterinary Journal 168 (3): 259–69. https://doi.org/10.1016/j.tvjl.2003.09.010.

Van Erck, E., Votion, D.M., Kirschvink, N., Art, T., and Lekeux, P. 2003. “Use of the Impulse Oscillometry System for Testing Pulmonary Function during Methacholine Bronchoprovocation in Horses.” American Journal of Veterinary Research 64 (11): 1414–20. https://doi.org/10.2460/ajvr.2003.64.1414.

Votion, D.M., Vandenput, S.N., Duvivier, D.H., Lambert, P., van Erck, E., Art, T. and Lekeux, P.M. 1999 “Alveolar clearance in horses with chronic obstructive pulmonary disease.” American Journal of Veterinary Research 60, 495-500

Young, S.S., and Hall, L.W. 1989. “A Rapid, Non-invasive Method for Measuring Total Respiratory Impedance in the Horse.” Equine Veterinary Journal 21 (2): 99–105. https://doi.org/10.1111/j.2042-3306.1989.tb02106.x.

Young, S.S., and Tesarowski, D. 1994. “Respiratory Mechanics of Horses Measured by Conventional and Forced Oscillation Techniques.” Journal of Applied Physiology 76 (6): 2467–72. https://doi.org/10.1152/jappl.1994.76.6.2467.

Young, S.S., Tesarowski, D., and L. Viel. 1997. “Frequency Dependence of Forced Oscillatory Respiratory Mechanics in Horses with Heaves.” Journal of Applied Physiology 82 (3): 983–87. https://doi.org/10.1152/jappl.1997.82.3.983.

Yu M., Wang Z., Robinson N.E., and Leblanc P.H. 1994 “Inhibitory nerve distribution and mediation of NANC relaxation by nitricoxide in horse airways” Journal of Applied Physiology76: 339–344

Zannin, E., Ventura, M.L., Dellacà, R.L., Natile, M., Tagliabue, P., Perkins, E.J., Sourial, M., Bhatia, R., Dargaville, P.A., Tingay, D.G. 2014 “Optimal mean airway pressure during high-frequency oscillatory ventilation determined by measurement of respiratory system reactance.” Pediatric Research 75(4):493-9. doi: 10.1038/pr.2013.251