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Which is the most appropriate in 2017: "Mild to Severe Equine Asthma" or heaves, RAO, equine COPD, IAD, tracheal IAD, bronchial IAD, small airway disease, chronic bronchitis, SPACOPD, SPOPD, Summer Heaves or Summer RAO?

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Keywords: Equine asthma, heaves, RAO, IAD, SPOPD

The numerous terminologies employed to describe inflammatory conditions of equine lower airways has led to great confusion in both the equine veterinary scientific and lay community. The terms were modified over time to encompass newly discovered features of equine lower airway diseases. In the age of "Omics", both the volume and rapidity of emerging discoveries now make this approach unsustainable.

Until recently, Heaves or RAO (recurrent airway obstruction) and IAD (inflammatory airway diseases) have been most frequently used to describe these conditions. While they are suitable in research settings as they are differentiated based on lung function measurements, there is considerable overlap between these syndromes, and discriminating clinical signs are not always easily identified. For instance, "respiratory distress" at rest, a distinctive feature of heaves which is absent in IAD, although obvious in appearance, when assessed, is subjective and variable between clinicians (i.e. tachypnea vs distress) and over time.

Non-infectious inflammatory lower airway diseases of horses (IAD, and heaves) share striking similarities with human asthma in terms of etiology, clinical presentation, tissue remodeling alterations and response to therapy. Asthma is a term easily understood by most horse owners, unlike current terminologies, and its easy translation into many different languages facilitates international dialogue on the disease. For these reasons, the medical term "Equine Asthma" has recently been proposed to facilitate communication between all stakeholders. As its clinical features (phenotypes), underlying causes and pathophysiological processes are likely to be multiple, we suggest that Equine Asthma be further defined to encompass currently known phenotypes (mild, moderate, severe, summer pasture, neutrophilic ...) and newly emerging endotypes (allergic, Th2, Th17 / mediated...). Based on severity, "Mild or Moderate Equine Asthma" would be used instead of Inflammatory Airway Disease (small airway disease, chronic bronchitis, and the mild forms of SPOPD) and "Severe Equine Asthma" would replace heaves, RAO, equine COPD, SPACOPD, SPOPD, Summer Heaves, Summer RAO (...).
Human asthma and COPD; really different diseases?

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keywords: airway hyperresponsiveness, eosinophilia, Th2 cytokines.

There has been controversy surrounding the issue of whether COPD and asthma have common roots or whether they are distinct disease processes since the 1960s. In the UK the concept of separate diseases was retained whereas in Holland the notion that the diseases were linked was argued, leading to the British and Dutch hypotheses, respectively. The truth lies somewhere in between. Asthma and COPD may both share airway hyperresponsiveness (AHR) to inhaled methacholine but AHR in the latter is more tightly correlated with loss of lung function. Loss of lung recoil in COPD may contribute to the increase in airway responsiveness since the tethering of the airways by the parenchyma is an important determinant of airway narrowing. Asthma and COPD may both develop fixed airway obstruction but the impairment in COPD is associated with a disappearance of airways from the lung whereas asthma has not been demonstrated to show this abnormality. Both diseases show airway remodeling with an increase in airway smooth muscle but COPD shows lesser changes in muscle than asthma. In both diseases the likelihood of experiencing an exacerbation is increased when previous exacerbations have been experienced. The exacerbations of asthma are predominantly virally triggered and show a seasonal variation which is different from COPD. Both diseases tend to be associated with eosinophilic airway inflammation when of the exacerbating phenotype. Adaptive immune mechanisms are generally CD4 T cell dependent whereas CD8 T cells are more prominent in COPD. Eosinophilia in asthma is interleukin-5 dependent and its neutralization by biologics reduces exacerbation rates. In contrast treatment of COPD patients with blood eosinophilia with a biologic (benrazilumab; an anti-IL-5 receptor antibody) did not reduce exacerbations in COPD so that the pathobiologic role of the eosinophil in COPD may be different from asthma. Innate lymphoid cells are an alternative, if unproven, source of IL-5 in COPD whereas CD4 Th2 cells are the major source in asthma.

In conclusion, although asthma and COPD share common clinical features there are fundamental differences in the structural changes that underlie the airway obstruction and the associated inflammation.
RAO/SPAOAD – severe equine asthma?

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Keywords: heaves, endotype, allergic

Justification for including Recurrent Airway Obstruction (RAO) and Summer Pasture Associated Obstructive Airway Disease (SPAOAD) in the overall term “equine asthma” (EA) is largely, but not exclusively, based on the biological appropriateness of the use of the term, considering its application in human medicine. In light of the increased recognition of various disease subtypes (endotypes) in human asthma, the appropriateness of the use of this broad term in horses is largely determined by a minimum set of criteria which are (a) shared by all human asthma endotypes and (b) recognised in all equine respiratory diseases for which the term “asthma” is proposed. Such criteria are limited to the presence of chronic, non-septic lower airway inflammation and reversible respiratory signs/functional changes consistent with variable airflow limitations in a defined (“at risk”) subset of the population. RAO/SPAOAD is largely differentiated from Inflammatory Airway Disease based on the presence of increased respiratory effort at rest, with “severe” EA proposed to define this subset [1]. In light of the heterogeneous nature of human asthma, any further efforts to identify areas of common ground between human asthma and severe EA necessitate comparisons with more defined human asthma endotypes, based on a more extensive array of criteria. This exercise is somewhat hampered by the recognition that disease heterogeneity also exists in EA, largely evidenced by varying immunological signatures [2]. However, rather than refuting the appropriateness of the use of the term asthma (and assuming the minimum set of inclusion criteria are met), such heterogeneity, being consistent with that seen in human asthma, supports its use. It does however also reveal the need to further characterise equine EA, based on criteria other than clinical severity. As well as facilitating comparisons with specific human asthma endotypes, such an exercise has the potential to inform the development of more targeted therapeutic approaches. Based on current knowledge, severe EA has distinct similarities with non-allergic asthma, late-onset asthma and severe asthma in humans [2].

References
Sport horse IAD - moderate equine asthma?

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Keywords: equine asthma, IAD, RAO

Many sports horses show occasional coughing and/or poor performance, but no increased respiratory effort at rest; markedly, but not severely increased BALF neutrophils, an increased tracheal mucus score and/or airway hyperresponsiveness. This presentation, which could be classified as « moderate equine asthma », was found in approximately 10-20% of sport horses in several studies investigating show jumpers or dressage horses in Switzerland and Germany. However, examination protocols were not consistent between studies, which were based on convenience samples, so the results allow only a rough estimate and may not be representative of different sport horse populations.

“Moderate” obviously implies a distinction from “mild” and “severe”. Findings as described above could potentially reflect incomplete remission from underlying severe equine asthma, i.e. recurrent airway obstruction (RAO). To further investigate this, a challenge with (poor quality) hay may be necessary, which is relatively straightforward at least in theory. In contrast, making the distinction between milder forms is more difficult, since inflammatory airway disease (IAD) spans mild and moderate equine asthma according to the recently revised consensus statement.

The studies in sports horses referred to above indicate that at least another 10-20 % of sports horses show « mild equine asthma ». This could be characterised by the same clinical signs as moderate equine asthma, but with only mild neutrophilic, eosinophilic, and/or metachromatic airway inflammation in the range defined as « equivocal » in the revised consensus.

Comparing sport horses vs. racehorses, we can expect that milder forms of lower airway disease are more relevant and therefore more often investigated and identified in the latter (see “racehorse IAD – mild equine asthma?”), resulting in reporting bias towards mild IAD in racehorses. The younger mean age could further lead to a larger proportion of milder forms of “racehorse IAD”, since there is evidence that severity of equine asthma is often progressive. Direct comparison of representative populations of racehorses vs. sport horses of different disciplines would be required to substantiate these speculations.

References available upon request
Racehorse IAD – mild equine asthma?
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Keywords: airway, inflammation, performance, cough, mucus

A clinical phenotype called inflammatory airway disease (IAD) is recognized as the second most common cause of poor performance in racehorses. The syndrome is characterized by chronic airway inflammation and is associated with mild clinical signs such as cough and excess tracheal mucus. Because IAD presents similarities with some subtypes of human asthma, the term "mild equine asthma" has been recently proposed.

Horses with mild asthma often present for decreased performance however, since many conditions can be implicated, non-respiratory causes must be ruled out. A diagnosis of mild asthma may be confirmed by endoscopy of the trachea post-racing to demonstrate excess mucus. A mucus accumulation score of 2 or higher (range 0-5) visualized approximately 1 hour post-racing has been associated with poor performance in both Standardbred and thoroughbred racehorses. Evidence of increased neutrophils, eosinophils or mast cells in bronchoalveolar lavage fluid (BALF) is also diagnostic of mild equine asthma. Eosinophils or mast cells are more commonly detected in young asthmatic racehorses whereas older racehorses are more often affected by neutrophilic asthma. Equine asthma in racehorses is also characterized by mild pulmonary dysfunction as evidenced by variable degree of airflow obstruction, airway hyperresponsiveness and impaired blood gas exchange during exercise.

There is mounting evidence that airway inflammation in racehorses is caused by exposure to dust although the role of infectious agents has not been ruled out definitively. In recent studies, we found associations between respirable dust exposure and airway eosinophilia in young thoroughbreds in training and airway neutrophilia in older racing Standardbreds (unpublished data). Taken together, clinical data associated with the IAD phenotype in racehorses support its inclusion in the equine asthma syndrome.

References:
Axonopathies
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Keywords: axon pathology; axonal transport; neurodegenerative diseases

Axon pathology is a common feature of many neurodegenerative diseases in humans and animals. It renders neurons functionally inactive, or less active if axon branches are lost, in a manner that is often irreversible. As neurons are polarized cells with axons and dendrites, and because most neuronal proteins are synthetized in cell bodies, mechanisms are required to direct axonal and dendritic transport. Many of the challenges axons face relate to their extreme length. Two obvious stresses that result are the need to transport material long distances from, and back to, the cell body, and the metabolic demand placed both on the cell body for macromolecular synthesis and on the axon for powering this transport process. Anterograde axonal transport has a role in supplying proteins and lipids to the distal synapse and mitochondria for local energy requirements, whereas retrograde transport is involved in the clearance of misfolded and aggregated proteins from the axon and the intracellular transport of distal trophic signals to the soma.

Axonal transport of proteins, organelles, vesicles or other cargoes is impaired in many of the neurodegenerative conditions and declines substantially during normal ageing. The consequences of reduced axonal transport are likely to include axon loss and reduced synaptic vesicle number in distal axons. A variety of methods used to study axonal transport impairment and axon degeneration in many different contexts show a diversity of morphology and topology that masks underlying similarities between some of the mechanisms. New genetic, pharmacological and imaging tools are now revealing these similarities.
The vagal bundle provides autonomic innervation to the airways and is composed of the parasympathetic (i.e. vagus nerve) and the sympathetic systems. The vagus nerve provides cholinergic innervation and the sympathetic system provides adrenergic innervation.

In the horse, the autonomic innervation of the airways is ipsilateral and more developed in the right side. The parasympathetic nervous system is anatomically larger and provides direct autonomic innervation to the upper and lower airways. The sympathetic nervous system provides direct innervation to the cardiovascular system primarily.

In the horse, the vagal bundle exhibits large connections between the sympathetic and parasympathetic trunks in the cranial thorax. These connections allow intermingling of fibers and the presence of cholinergic and adrenergic fibers in either trunk. By this means, the adrenergic fibers can reach the lungs via the vagus nerve and facilitate bronchial relaxation. The presence of adrenergic fibers in the vagal branches to the lower airways was demonstrated with the use of special stains on selected sections of the vagal bundle. Immunohistochemical staining techniques assisted in identifying the presence of neurotransmitters of the postganglionic sympathetic neurons by identifying enzymes intervening in the metabolism of the neurotransmitters of interest (i.e. tyrosine hydroxylase). In horses, other mechanisms for bronchial relaxation have been suggested (i.e. non-adrenergic non-cholinergic inhibitory system, systemically released catecholamines).

The equine larynx is likely innervated by cholinergic and adrenergic fibers intermingled in the cranial laryngeal nerve and the recurrent laryngeal nerve. The role of a newly described pararecurrent laryngeal nerve is yet to be determined.
Correlation of histopathology and clinical RLN

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Keywords: recurrent laryngeal neuropathy; axonopathy; ultrasound; endoscopy

Histopathology may be regarded as a gold standard in assessing muscle morphology (Dubowitz and Sewry, 2007). However, the small size and location of the CAD muscle in the horse renders serial muscle biopsy of this muscle technically challenging, and furthermore, may induce injury. Hence, objective, non-invasive methods of evaluating structure and function of the left CAD muscle using advanced imaging techniques are required that will enable monitoring of response to novel muscle-directed treatments, including functional electrical stimulation.

A study by Tulloch (2014) has demonstrated the relationship between intrinsic laryngeal muscle morphology, recurrent laryngeal nerve fibre density and laryngeal function at rest and exercise in horses with naturally occurring RLN and thus endorses the use of standing CT, TEU and laryngeal endoscopy for the evaluation of RLN severity.

The histopathology of RLN is very complex due to the concurrent denervation and reinnervation of the muscle and thus will vary between individuals and disease severity. By summarising the histopathology variables from the left and right CAD muscles a significant difference was identified between horses with resting grades 2 and 4, and 3 and 4. This is evidence that laryngeal muscle morphology at a microscopic level affects.

This study demonstrated the association between left recurrent laryngeal nerve fibre density, histopathology of the intrinsic laryngeal muscles, CT and ultrasonographic assessment of the intrinsic laryngeal muscles and laryngeal function at rest and exercise in horses with naturally occurring RLN. Standing CT and TEU of the larynx provide useful information on laryngeal muscle morphology and function and are likely to be beneficial in monitoring the effects of muscle/nerve directed treatments.
Clues to the pathophysiology of recurrent laryngeal neuropathy

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Keywords: recurrent laryngeal neuropathy; axonopathy; axonal transport; nucleus ambiguous

Descriptions of equine recurrent laryngeal neuropathy (RLN) were first made by Bouley, and Dupuy in the 1820s. Atrophy of equine laryngeal intrinsic muscles was commonly identified at necropsy in horses that ‘roared’. The pathophysiology remains enigmatic, as does the aetiology: genetic, acquired and environmental factors have each been proposed. In summary, RLN can be described as a distal axonopathy of (predominantly) the left recurrent laryngeal nerve with secondary neurogenic muscle atrophy of intrinsic laryngeal muscles. The consequence is a reduced size of the rima glottis, which becomes relevant at exercise and manifests as poor performance due to reduced PaO2. Certain issues currently remain unresolved – (1) actual disease prevalence; (2) polyneuropathy vs mononeuropathy; (3) selective involvement of specific motor units. These elements will be discussed.

Estimates of RLN prevalence in horses vary widely. Historically, horses were diagnosed on the basis of the sound of their inspiratory stridor made at exercise. More advanced techniques, in particular exercising laryngoscopy, laryngeal computed tomography and ultrasound reveal that these earlier methods underestimated disease prevalence. Indeed, when examined histologically, it is hard to find a normal horse. This has profound implications for the search for the genetic cause and for genetic modifiers.

Histopathological changes within the distal nerves of horses with RLN reveal collapsed myelin sheaths, increased relative myelin sheath thickness, regenerating Schwann cell membrane clusters and ‘onion-bulb’ formations indicative of demyelination and remyelination. More specific indicators of a primary axonopathy are the central axon fragments commonly seen within myelin digesting chambers, accumulation of axoplasmic organelles and margination of microtubules. Such defects are seen in human neuropathies with suspected axonal transport defects.

It is conceivable that RLN might have both an acquired and genetic basis: indeed, RLN has long been proposed to be related to stretch-induced trauma of the nerve as it passes around the aorta in the thorax perhaps similar to the induced neuropathy that occurs in humans with hereditary neuropathy with liability to pressure palsies associated with a mutation in peripheral myelin protein (PMP)-22. A combination of both a genetic and acquired component would seem to be necessary, since similar axonopathies are not known to be present in other athletic quadrupeds.
Peripheral nerve repair: Immune modulation

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Recurrent laryngeal neuropathy (RLN) or “Roaring” is a major cause of poor athletic performance affecting 8% of racehorses and a higher percentage of sport horses. The disease affects the ability of the nerve to conduct a signal from the brain to the muscle that opens the larynx or voice box at exercise - the CAD muscle. This CAD muscle is the only muscle that opens the larynx during exercise. In affected horses the impulses carrying this signal down the nerve travel more slowly and do not reach the muscles as effectively as in normal horses. This leads to a reduction in the size and strength of the CAD muscle causing collapse of the larynx with reduced airflow and abnormal noise production.

Currently current standard of care for RLN is the placement of a fixed and permanent laryngoplasty sutures – a “tie-back”. While this method is relatively successful in the treatment of airway obstruction in RLN affected horses, it does not restore function to the airway and can be associated with some risks such as coughing and failure of the suture to hold the airway open. Previous research has attempted to restore muscle function through nerve-muscle pedicle grafting to bring a new nerve supply to the affected CAD muscle. Although this technique showed good results it took up to 12 months to bring function back to the CAD muscle – too slow for career equine athletes. We propose a regenerative approach to restore normal laryngeal function in horses affected by RLN using an enhanced nerve graft. The approach would avoid interfering with the normal protective mechanisms of the airway and so also avoid the complications associated with the current treatment. Reinnervation techniques have been attempted in horses in the past but have not gained widespread popularity as the time to reinnervation has been too long to be relevant in clinical practice. Slow reinnervation also remains a problem in human patients treated by nerve graft.

We have begun to understand the basic mechanisms behind the role of a particular type of immune cell – the macrophage - in peripheral nerve repair. These cells are the major cell type migrating to the repair site and are the 'conductors of the orchestra', laying down tiny capillary networks along which other cell types can migrate. We have developed a sophisticated technique to isolate macrophages from the site of peripheral nerve injury, evaluated how changes in genes expressed by these macrophages change over time after injury and how gene that control the types of macrophages at the injury site affect repair after nerve graft.

The overall goal of the experiments is to change the type of macrophages at the site of nerve injury using a stable nerve specific hydrogel that supports nerve growth. This system is safe and biocompatible. We anticipate that will prevent the nerve degeneration and muscle atrophy, commonly observed in RLN, and restore full function. We hypothesize that by manipulating the microenvironment at the site of nerve graft, by changing the function of macrophages will allow regrowing nerves to cross the repair site more rapidly and functional recovery will be faster and better.
Genetics of recurrent laryngeal neuropathy

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Keywords: LCORL, NACP, ECA3, axonopathy

Equine recurrent laryngeal neuropathy (RLN) is a bilateral mononeuropathy. Although RLN etiology is unknown, a genetic component is suggested by the observation that RLN-affected sires produce more RLN-affected foals than do unaffected sires.¹ In Warmbloods, two protective loci have been identified on equine chromosome 21 (ECA21) by haplotype analysis.² In a GWAS of over 500 Thoroughbreds (TBs), we observed that on ECA3, haplotype associations for height and RLN were coincident (genetic correlation between traits was >90%). This haplotype included LCORL and NCPG, genes strongly associated with body height in horses and fetal growth rate in cattle. However, LCORL/NCPG accounted for only 6% of the variation in RLN.

Secondary genetic associations for RLN on ECA18 and X did not correlate with height. In TBs, there is extensive linkage disequilibrium across the ECA3 locus so that RLN and height may be influenced by two independent loci associated by linkage. To address this, we did two new investigations. In the TB cohort, we re-interrogated ECA3, ECA18, and X chromosomes by use of a custom-designed SNP panel. This ruled out an effect of these secondary loci on RLN. The second approach, an across-breed analysis, used a large cohort of Belgian draft horses in a new GWAS. Belgians were chosen because a) they are under strong selection for height and therefore fixed for the "tall" height allele at LCORL and NACP on ECA3³ and b) there is a higher frequency of RLN in draft horses⁴ than TBs⁵. Furthermore, if, in Belgian horses there are other size-associated genes, we should have been able to determine if an RLN locus also is associated with these novel height loci. This investigation confirmed that Belgians are fixed for height on ECA3 and revealed no additional loci contributing to RLN. Other novel height-associated loci are being further investigated but none of these appear to be associated with RLN. In summary, RLN is a complex genetic disease. The genetic contribution to RLN may be a unique effect of the LCORL/NCPG locus. By determining growth rate and body size these genes may also determine recurrent laryngeal nerve length, putting larger horses at greater risk for nerve injury or axonal transport defects. Alternatively, both LCORL and NCPG are genes encoding transcription factors so that a single variation in these genes could affect diverse functions, e.g. growth and axonal degeneration.

References
Room B

July 13th - AM
Function of the Upper Airway

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Horses are magnificent athletes that run in excess of 30 miles per hour. The adaptation to high speed was likely driven by the horses’ need to escape predators but now provides an advantage in performance. Such speed is achieved because horses have tremendous capacity to utilize oxygen. For example, while running at speeds that produce maximum heart rate, Thoroughbred and Standardbred racehorses have a maximal oxygen consumption (VO2max) of approximately 160ml/kg/min which is 40 times the value at rest. Such an expansion of oxygen consumption is far superior to the 6 to 8 fold increase found in endurance trained human athletes.

Oxygen delivery for this uniquely high rate of consumption requires a number of specific cardiovascular and respiratory responses. In order to accommodate the tremendous oxygen demand of skeletal muscles during intense exercise, the horse increases its minute ventilation by up to 40 fold. Tidal volume and respiratory frequency both increase to provide this increase in minute ventilation. In human athletes the proportionate increase in tidal volume is greater than the increase in respiratory frequency. Horses increase respiratory frequency initially. At maximum speed, increases in minute ventilation are achieved by expanding tidal volume. High airflow rates required to meet this ventilatory demand are created principally by diaphragmatic contraction, which produces very negative driving pressures within the upper airway. The horse’s upper airway must quickly prepare for these large changes in airflow and pressures by dilating and becoming more rigid or less compliant. Such accommodation is achieved by the basic structure of the upper airway and synchronous and coordinated contraction of upper airway muscles and constriction of capacitance vessels within the mucosa of the upper airway.

Obligate nasal breathing Obligate nasal breathing is a term used to describe a physiological obligation or predisposition to breathe through the nose as opposed to the mouth. Obligate nasal breathing is likely an adaptation especially useful in prey species, as it permits animals to feed while preserving the ability to detect predators by scent. The soft palate is tightly opposed to the base of the larynx, such that there is no communication between the oropharynx and the nasopharynx. The conformation of the soft palate and larynx protects the airway during mastication of feed. Horses lack the hypopharynx, the gap between the edge of the soft palate or uvula and epiglottis, as exists in people (Figure 2a and b). Oral breathing does occur in horses but is generally a sign of anatomic pathology, disease conditions such as dorsal displacement of the soft palate.

Basic Upper Airway Mechanics The airway consists of the nostrils, nasal cavity, sinuses, guttural pouches nasopharynx, soft palate, larynx, trachea, bronchi, bronchioles and alveoli. The equine respiratory system is uniquely adapted to produce a high rate of oxygen delivery to the alveolar surface during sustained high speed exercise. The extra thoracic (upper) airway is a conduit through which oxygen and carbon dioxide move between the environment and the alveoli. The flow of oxygen through this respiratory system is determined by the oxygen required for adenosine triphosphate conversion in the respiratory chains of the mitochondria. Narrowing of any of the anatomic regions between the nostril and the alveolar surface limits the flow of oxygen available for aerobic metabolism. Indeed, the structural design of the airway is optimized so there is just enough structure at each level to support the maximal oxygen flow rate with a small amount of redundancy at each level.

Tidal Volume The resting horse is unusual as it has biphasic exhalation and occasionally a biphasic inspiration. This biphasic flow pattern is lost at exercise or with excitement when both inspiration and exhalation are active. At rest, the horse breathes around and in excess of its functional residual capacity (FRC) which is approximately 25 liters in the 450 kg horse. Inhalation results in a lung volume higher than FRC and exhalation a lung volume lower than FRC. This differs from humans where FRC is the beginning of the respiratory cycle (18). Inhalation begins with passive recoil of the respiratory system followed by active contraction of the
diaphragm and external intercostal muscles. The first phase of exhalation is passive relaxation of the inspiratory muscles. Contraction of the abdominal and internal intercostal muscles supports the active phase of exhalation. Active exhalation immediately precedes passive inhalation. When the abdominal muscles relax passive inhalation must occur. Breathing around the mechanical equilibrium of the FRC rather than from it is likely mechanically advantageous in an animal with a stiff thoracic wall such as the horse.

Respiratory Frequency At walk and trot, respiratory frequency is not related to stride frequency. At the canter and gallop, stride frequency and respiratory frequency are synchronized in a 1:1 ratio in fit horses. The synchronization of gait and respiration, with inhalation when the forelimbs are nonweight bearing and exhalation during the support phase, may offer some mechanical advantage. In addition, although thoracic expansion occurs during inhalation at rest, walk, trot and slow canter, at faster speeds there are minimal changes of thoracic circumference with ventilation. During swallowing or sighing, normal horses may alter the 1:1 respiratory to stride synchronization. Horses may occasionally take a ‘big respiratory cycle’ (BRC) or a long, deep breath, with a duration of approximately two strides. The role of these BRC’s is unclear although they may be used to produce an improvement in pulmonary gas exchange at lower levels of fitness as the incidence of BRCs decreases with training.
Endoscopic Diagnosis in the Upper Airways

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Keywords: Endoscopy, resting endoscopy, over-ground endoscopy.

Resting endoscopic examination has for decades been used to identify abnormalities in the equine upper respiratory tract (URT). However, most abnormalities in the URT are only visibly during exercise. Over ground endoscopy (OGE) is today the golden standard when evaluating the upper airway and URT abnormalities in ridden horses, and in addition, OGE also implements the effect of the rider and environment. Abnormalities best diagnosed during exercise include displacement of the soft palate and pharyngeal collapse. During OGE the severity of laryngeal neuropathy can better be gauged.

In healthy ridden horses in different head-neck-position both persistent and intermittent changes in larynx and pharynx can be seen without any influence on the performance of the horse. Persistent changes in the larynx and pharynx of horses include pharyngeal lymphoid hyperplasia (PLH) of different grades, oedema in the pharynx, constant lateral instability of the pharynx, flaccid epiglottis and recurrent laryngeal neuropathy (RLN). Intermittent change in the larynx and pharynx can be palatal instability (PI), pharyngeal collapse (PC), medial deviation of the aryepiglottic folds (MDAF), dorsal instability of the pharynx and visible cricotracheal ligament.

Still resting endoscopy can be used for some diseases but often the resting endoscopy has to be combined with over ground endoscopy. Many horses tolerate an endoscopic examination without sedation, but some require a sedative. In these cases, it is important to remember that sedation with detomidine or acepromazine changes the endoscopic picture of the upper airways and can lead to incorrect or missed diagnosis, especially of RLN.

Exercise test on high-speed treadmill is still a valuable tool especially for a more standardized performance test regarding speed, distances and inclination.

Conclusion: Every abnormalities in the URT should be correlated with the performance history of the horse, not to interpret clinical healthy horses as diseased horses.

1: Barakzai and Dixon (2011) Eq Vet J 43(1) 18-23
3: Martin et al., (2000) JAVMA 216(4) 554-558
Imaging the upper airway
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Keywords: larynx, ultrasound, RLN, Sinus

Abstract:
Diagnostic imaging of the upper airway adds critical information for diagnosis and management of the diseases of this region. All imaging modalities can be useful for upper airway diagnostics and they are all complementary. The use of radiography, ultrasonography, computed tomography and magnetic resonance imaging for daily clinical work, including the technical aspects has been well described in the literature. The descriptions of advanced tomographic imaging techniques on the standing horse have recently been published. New anatomical features and/or diseases have been described. Sphenopalatine sinus disease is a newly described condition optimally diagnosed using a combination of computed tomography and sinoscopy. Furthermore, it was shown, that computed tomography provides greater information regarding mass extent and features of malignancy for tumors involving the nasal cavity and/or paranasal sinuses. Similarly, magnetic resonance imaging showed the exact localization of the lesions, their size and relation to surrounding structures in a study of 84 cases of head disorders.

Other research groups have focused on ultrasound for the diagnosis of horses with respiratory noise. External Laryngeal Ultrasound can be used for confirmation of recurrent laryngeal neuropathy or its differential diagnoses, arytenoid chondritis and laryngeal dysplasia. Current emphasis is now placed on evaluating the use of ultrasound for early detection of equine upper airway disease.
Room B

July 13\textsuperscript{th} - PM
Obtaining Airway Cytology Samples
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Keywords: Cytology, Mast cell, BAL Storage, observer agreement

Bronchoalveolar lavage (BAL) has been an international standardized procedure for decades. Evidence based guidelines have not been established in relation to laboratory procedures such as storage, staining method, cytospin contra pellet-smear, and skilled versus non-skilled laboratory evaluators.

The storage temperature and from the BAL sampling to laboratory analysis are important factors to consider. For example, the effect of storage temperature and time before analysis of the BAL sample showed a decrease in mast cells when the sample was stored at room temperature for twenty-four hours. BAL samples stored at 6°C showed significant changes in neutrophils and mast cells after twenty-four hours. In conclusion, this study and other studies further elucidate that BAL samples should be cooled immediately after collection and analyzed within twenty-four hours.

The cytospin technique has been the most common method used when BAL fluid is processed. The pellet-smear technique is a cheap and an easy alternative to the cytospin technique. A significantly lower number of lymphocytes and a significantly higher number of macrophages, neutrophils, mast cells and eosinophils were found in cytospin preparations compared to pellet-smears. The cytospin technique is recommended over the pellet-smear technique due to a higher detection of neutrophils and mast cells.

Toluidine blue is the superior cell stain for accurate mast cell identification compared to other staining methods. However, Toluidine blue only stains the metachromatic granules and does not allow further differentiating of nucleated cells. The May-Grünwald Giemsa staining can be used for a full differential cell count as it stains both the metachromatic granules and nucleated cells.

An evaluation of the efficiency of two staining methods, May-Grünwald Giemsa and Toluidine Blue, on the percentage of mast cells showed a significantly higher mean percentage of mast cells in the May-Grünwald Giemsa compared to the Toluidine Blue stain.

Bronchoalveolar lavage cytology observer agreement is important when results from the BAL cytology are interpreted as the level of laboratory experience can affect the final result of the differential cell count. Intra-observer evaluation showed high agreement for experienced observers and adequate to poor intra-observer agreement for inexperienced observers. The inter-observer agreement showed high agreement between experienced observers, except for mast cells, and adequate inter-observer agreement between inexperienced observers. This study shows that laboratory experience is an important factor when the counting is performed.

References
Longitudinal Tracheal aspiration (TA) and Bronchoalveolar Lavage (BAL)

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Keywords: Bronchoalveolar lavage, tracheal aspiration, stabling,

Several factors are known to give variations in TA and BAL cytology; the most important factor is lower airway diseases such as inflammatory airway disease (IAD) and recurrent airway obstruction (RAO). Factors such as age of the horse, the stabling environment and dust exposure, season of the year and the genetic background of the particular horse are also described to affect the TA and BAL cytology results. Furthermore, variations in the endoscopic examination, the TA and BAL sampling and subsequent laboratory procedure also affect the cytology results.

Longitudinal studies on how the stabling environment and management strategies affects the lower airway of horses is lacking.

The objective of this study was to evaluate the inflammatory changes in the airway (evaluated by mucus score, TA and BAL) of conventional stabled horses over a life-long period.

An observational cohort study design was used. Including a homogenous group of healthy riding horses (n=50-75 yearly) over a 12-year period, starting 2005, with sampling once a year (between May and early July). Horses were included in the population at the year of 5-6 and would stay until retirement as a riding horse. During the 12-year period, 27 of the horses were sampled during at least 10 consecutive years. Each year a clinical examination, endoscopy including mucus score, TA and BAL was performed. The results of the TA and BAL has led to improvement of management and daily routines over the years. Between year 2005 and 2006 the bedding material was changed from straw to wood pellets and between year 2008 and 2009 the hours at pasture has been increased. From the start to year 2008 the volume used for BAL was 120 ml, from year 2010 to 2012 a volume of 180ml was used and from year 2014 a volume of 250ml was used.

The laboratory procedures were the same for all years. Total cell count was manually performed and cytospin smears was made and stained with May-Gründwald Giemsa. A microscopic evaluation of five hundred leukocytes including neutrophils, lymphocytes, alveolar macrophages, mast cells and eosinophils.

Results from this study will be presented at WEAS 2017.

Frontiers in central airway remodeling assessment in equine asthma: updates on endobronchial biopsy and ultrasound

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The diagnosis of equine asthma is currently based on the presence of clinical signs indicative of a lower airway disease combined with abnormal bronchoalveolar lavage fluid (BALF) cytology results. However, the type and degree of BALF inflammation does not correlate with the severity of the clinical signs, nor it provides information concerning the prognosis or progression of the disease. Besides inflammation, equine asthma is characterized by structural changes of the airway wall throughout the bronchial tree, commonly defined «remodeling». The observed changes include an increased airway smooth muscle mass, fibrosis, and deposition of elastic fibers. Whether airway remodeling correlates with disease severity or whether it can predict the progression of the disease in the horse is still ill-defined and deserves to be studied more in depth. Endobronchial biopsy and ultrasound are valuable tools to assess airway remodeling and tissue inflammation. Recently, both techniques have been validated for the use in the equine species. Their implementation in clinical practice as well as in research settings should be promoted. The routinary application of these non-invasive techniques could change the clinical approach to equine asthma in favor of an early recognition of the condition. Also, they may provide useful data for the development of targeted therapies to prevent or reverse established tissue remodeling and inflammation.
Quantifying poor performance

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Keywords: Maximal oxygen consumption ($\dot{V}O_{2\text{max}}$), field studies, high speed treadmill, fitness assessment, ergospirometry.

Performance is the result of athletic capacity and skills of a horse. Quantifying performance objectively is a challenge and can be done at a population level (by analyzing race results for example) or at the individual horse level. The presentation will focus on ways to objectively quantify athletic capacity in individual horses.

Objective measurement of fitness and performance should be an accurate and repeatable measure so that data acquired can be compared between- and within- horses, but it is time-consuming and difficult to perform. It can be measured in the field or on a high speed treadmill.

High speed treadmill testing provides a controlled environment and consistency in measuring exercise physiology parameters like maximal oxygen consumption ($\dot{V}O_{2\text{max}}$). However, it is best suited for research where a small number of horses work regularly on the treadmill and are acclimatized to the procedure. The workload and biomechanics on the treadmill are different from field conditions.

Field measurement of athletic performance is traditionally achieved by measuring cardiac and blood parameters to calculate indexes such as $V_{200}$, $V_{180}$ and $V_{La4}$. The field assessment is usually done using an incremental standardized exercise test, where horses work set distance at increasing speed. Data collected includes heart rate, speed, and blood lactate measured immediately at the end of each exercise step. Correlations allow calculating $V_{200}$, $V_{180}$ and $V_{La4}$. These numbers allow some comparison between- or within- horses when they can be repeated in a consistent manner.

The latest development is the availability of a portable ergospirometry system that allows measuring $\dot{V}O_{2\text{max}}$ and spirometry parameters in the field. The horse wears a mask, a bit and the rider wears a backpack with the data acquisition system. Parameters measured over averaged breaths include inspiratory/ expiratory flow and volume, respiratory rate and oxygen consumption. The $\dot{V}O_{2\text{max}}$ measurements allow following and comparing horses' performance in field conditions without incremental exercise tests.

The challenge of these field tests is that conditions are not controlled, speed can be difficult to maintain steady and there is some variability in the data. Another challenge is that many sport horses cannot reach the maximal intensity speed.

The $\dot{V}O_{2\text{max}}$ is a measure of aerobic capacity only and more complicated tests can be used to assess anaerobic capacity of horses.
Stable Dust - What does it consist of?

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Keywords: nuisance dust, particulates, size distribution

Introduction Recurrent airway obstruction (RAO) and inflammatory airway disease (IAD) are equine respiratory syndromes that are characteristically associated with inhalation of organic dust. At present, it is common to advise owners of horses with RAO or IAD to house their horses at pasture. Often, this is not feasible, and in some cases the causative agent is in higher concentration outdoors as compared to within the stable, necessitating the horse be kept indoors in order to effectively manage the disease. In all cases, it is imperative to identify and minimize exposures to indoor air contaminants in order to diminish the resultant inflammatory response.

Objectives Airborne contaminants within stables are often considered to be ‘nuisance dust’ and of relatively low toxicity. Upon closer investigation, size, composition and concentrations of these contaminants are consistent with that which has been associated with development of respiratory disease in humans. Inorganic dusts and irritant gases must also be considered when evaluating the indoor stable environment. Feedstuffs, bedding, and arena footing are readily identifiable sources of particulate matter that, with agitation, have the potential for dispersion into the horse's breathing zone. The origin of airborne contaminants, composition, concentration, size distribution, and common management practices resulting in prolonged suspension and increased exposures will be described. Techniques utilized to identify environmental ‘triggers’ will be presented in the context of developing exposure avoidance plans specific to the individual, an important component of the successful management of horses with severe disease. Tools readily available and commonly utilized to assess the workplace may be easily adapted to evaluate the stable environment. These tools as well as the utilization of visual and other sensory cues to assess stable air quality will be explored.

Conclusions With adequate understanding of the size, composition, and source(s) of indoor air contaminants, practitioners can effectively work with owners to develop focused interventions that will benefit horses with airway disease as well as those that are disease free.
Techniques for reducing the respirable challenge in hay

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Key words: equine asthma, hay, airborne respirable dust, hygienic quality

Introduction: Reducing airborne respirable dust (ARD) from hay fodder is an important factor in the treatment of horses with equine asthma. Initiation of allergenic respiratory disorders in healthy horses could also be reduced by decreasing exposure to ARD in fodder by using suitable pre-feeding treatments.

Objectives: To determine the effect of different wetting treatments on the ARD, microbial contamination, nutrient content and palatability of fodders for horses.

Methods: A variety of fodders, meadow and seed hays and haylage were subjected to the following treatments: dry, soaking in water, steaming in bags (kettle), steaming in bins and steaming in a proprietary hay steamer the Haygain in separate experiments. ARD was measured using a Munro personal sampler (AS 200, Woodford Green, Essex, UK),[1,2] microbial contamination (cfu/g) using culturing techniques with 3M petrifilms, (Carl-Schurz-StraBe 1, Germany), nutrient content [1,3] and palatability [4]. Additionally, different stable management regimes were tested for ARD [5] in American barns and single stables. Data was analysed using ANOVA and Wilcoxon matched-pairs t-test using Genstat, significance level were set at P<0.05.

Results

Table 1. Airborne respirable dust (ARD), mould and bacteria (TVC, cfu/g) in hay when dry, soaked, steamed (ST) in a Haygain (HG), STHG and left for 24 hours, bin (Bin) or pouring a kettle of hot water over hay (K)

<table>
<thead>
<tr>
<th></th>
<th>Dry hay</th>
<th>Soaked hay</th>
<th>ST HG</th>
<th>ST HG + 24hrs</th>
<th>ST Bin</th>
<th>ST Kettle</th>
<th>s.e.d</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARD/l air [3]</td>
<td>1180c</td>
<td>3a</td>
<td>4a</td>
<td>63b</td>
<td>142b</td>
<td>1.81</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ARD/l air [2]</td>
<td>25699a</td>
<td></td>
<td>1586b</td>
<td>5398b</td>
<td></td>
<td>1937</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>TVC cfu/g [3]</td>
<td>23442b</td>
<td>12a</td>
<td></td>
<td>549540b</td>
<td></td>
<td>38.9</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>TVC cfu/g [1]</td>
<td>51286b</td>
<td>186209b</td>
<td>741a</td>
<td></td>
<td></td>
<td>3.21</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Y&amp;M cfu/g [3]</td>
<td>53703b</td>
<td></td>
<td>5a</td>
<td>5012b</td>
<td></td>
<td>16.2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Y&amp;M cfu/g [1]</td>
<td>1047b</td>
<td>380b</td>
<td>2a</td>
<td></td>
<td></td>
<td>3.53</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

abc Values in the same row not sharing common superscripts differ significantly (P<0.001)

Mineral content was unaffected by steaming in the HG steamer (n=30). Water soluble carbohydrate content in dry hay= 126 vs steamed hay 103 (g/kg DM) [3]. Steamed hay was more palatable than dry and soaked hay, with horses eating 0.9 kg/hour compared with 0.2 kg and 0.05 kg respectively [4].

Steam hay and shavings produced the least ARD across both American barns and single stables as detailed in Table 2. When comparing ARD in the breathing zone and general stable zone (Table 3) the dry hay and straw regime produced more ARD in BZ in American barns compared with single stables indicating that neighbouring stables share dust and collectively produce higher levels of ARD compared with when horses are in individual stables with their own air space.
Table 2. Geometric mean ARD/l air in the breathing zone and stable zone from 8 American barns and 8 single stables under 4 different management regimes [5]

<table>
<thead>
<tr>
<th>Management</th>
<th>Shavings &amp; steamed hay</th>
<th>Shavings &amp; dry hay</th>
<th>Straw &amp; haylage</th>
<th>Straw &amp; dry hay</th>
<th>s.e.d</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM ARD/l air</td>
<td>2.474 a</td>
<td>2.775 b</td>
<td>2.928 c</td>
<td>3.351 d</td>
<td>0.0748</td>
<td>0.001</td>
</tr>
<tr>
<td>Numbers(±SD) ARD/l air</td>
<td>313 ± 92.5</td>
<td>623±152</td>
<td>1233±1154</td>
<td>2969±3821</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

abcd Values in the same row not sharing common superscripts differ significantly (P<0.05)

Table 3. Airborne respirable dust / l air in the breathing zone (BZ) and general stable zone (SZ) in American Barn and single stables under four management regimes [5].

<table>
<thead>
<tr>
<th></th>
<th>American Barn</th>
<th>Single stables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SZ</td>
<td>BZ</td>
</tr>
<tr>
<td>Steamed hay + shavings</td>
<td>325</td>
<td>300</td>
</tr>
<tr>
<td>Dry hay + shavings</td>
<td>522</td>
<td>827</td>
</tr>
<tr>
<td>Haylage + straw</td>
<td>972</td>
<td>517</td>
</tr>
<tr>
<td>Dry hay + straw</td>
<td>6250</td>
<td>5079</td>
</tr>
</tbody>
</table>

Conclusions: All experiments showed that when comparing fodders that are dry, soaked, partially steamed and high temperature steamed, the most effective method for reducing ARD while also conserving nutrients and improving palatability and hygienic quality, the Haygain high temperature steamers produce the best and most consistent results.

References:
Getting horse owners to change the environment

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Keywords: Environment, stable hygiene, audit

Environmental factors such as respirable dust, including bio-aerosol, noxious gases and unconditioned air play a determining role in triggering and maintaining recurrent airway obstruction (RAO) and inflammatory airway disease (IAD). The management of airway disease cannot be limited to medical therapy: improving the horse’s direct environment is key to the long-term remission. As healthcare professionals, veterinarians play an essential role in identifying defective environments, however the owners’ perception of risk and implementation of solutions can be complicated. The tools and training for environmental assessment are currently insufficiently accessible to equine veterinarians.

Horses housed indoors are exposed to high levels of dust of various quality and quantity, as well as to other noxious elements such as endotoxins, allergens and ammonia. The concentration and number of inhalable particles varies with stable design as well as with the location of a stall within the building. Variations in particle concentrations depend on management practices, time of day and season of year. Practices such as sweeping, using blowers or entering the stable with an engine–run vehicle should be avoided and horses should be turned-out when boxes are cleaned.

The quality of ventilation, the level of humidity and dust is determined by building configuration. As ventilation is difficult to appreciate subjectively, smoke machines can be used to demonstrate poor ventilation to the owner. Estimating building volume and design, size as well as localization and size of openings is useful to establish the capacity for air renewal and determine if it is sufficient for the number of horses. Poor ventilation creates humidity and pockets of warm air which foster molds and bacteria. Samples can be taken for culture to show level of environmental contamination. They can also serve as reference once disinfection has been done.

Saprophyte microorganisms naturally contaminate hay and straw. The level of microbial development within the bales depends on several factors, which include humidity levels during harvest, duration of drying, length of stem cuts, pressing and storage conditions. The storage of hay and straw within the stable building is detrimental. Dedicated storage areas for bedding and forage should be protected against humidity and soil contaminants, regularly disinfected and distant from manure disposals and tracks or roads. Paper and cardboard beddings yield much lower levels of dust particles and aeroallergens than straw. Large dust-free wood shavings are a good alternative. Soaking hay is a cheap way of reducing inhalable dust, however it promotes bacterial growth and drains sugars. Specially conditioned hay should be considered for RAO or IAD-affected horses, including grass silage, haylage or steamed hay.

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Medical management of equine asthma

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Keywords: horse, airway, inflammation, therapy, aerosol

The goals of medical therapy of equine asthma are to control airway inflammation using mainly glucocorticoids and to relieve airflow obstruction with bronchodilators. Neutrophilic airway inflammation is a common feature in severe asthma (RAO) and in some forms of mild asthma (IAD). There is limited evidence-based data regarding therapy for mild asthma, especially concerning aerosol therapy therefore, most of the drug and dosage recommendations are based on studies conducted in horses with severe asthma.

Both systemic and aerosolized drug administration are effective however, the potential for adverse effects and prolonged elimination times is greater with systemic administration. The advantages of aerosol therapy are ease of administration and safety. The disadvantages are cost and need for delivery devices specifically designed for equine.

A short course of potent, systemic glucocorticoid (dexamethasone, 0.04-0.1 mg/kg q 24h) is recommended to treat acute exacerbation of severe equine asthma. Once clinical signs improve, therapy may be switched to less potent and short-acting glucocorticoids such as prednisolone (0.4-1 mg/kg PO q 24h). Alternatively, inhaled glucocorticoid therapy (beclomethasone, budesonide, fluticasone) can be initiated using metered-dose inhalers or nebulizers. Decreasing exposure to environmental dusts should accompany medical therapy if successful control of airway inflammation is to be achieved.

Diet supplementation with omega-3 fatty acids helps improve clinical signs and decrease neutrophilic airway inflammation in asthmatic horses when combined with reduction in dust exposure.

Systemic or inhaled bronchodilators are valuable, especially in combination with glucocorticoid therapy to help reduce airflow obstruction and airway hyperresponsiveness. Relief of acute, severe asthma exacerbation is achieved rapidly with intravenous N-butylscopolammonium bromide or inhaled albuterol however, the clinical benefits are short-lived (~ 1 h).

References:
Current understanding of EIPH pathogenesis

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Key words: capillary pressure, venous remodeling

Exercise-induced pulmonary hemorrhage (EIPH) is common in racehorses. It is a consequence of the physiological responses to exercise and is unaccompanied by signs of distress and other pulmonary disease.[1] This talk summarizes the physiological changes in breathing and circulation that lead to capillary rupture and EIPH. To deliver the oxygen necessary for running, both alveolar ventilation and cardiac output increase, and, as a consequence, pulmonary capillary transmural pressure (Pcap-tm) exceeds the minimum of 75 mm Hg necessary for capillary stress failure so that blood enters the air spaces.[2] The two determinants of Pcap-tm are pulmonary capillary (Pcap) and alveolar pressure (Palv). To supply the increase in both tidal volume and inspiratory air flow rate during exercise, Palv may decrease to values as low as minus 60 mm Hg during inhalation.[3] Concurrently Pcap may reach 75 mm Hg or greater so that Pcap-tm is 150 mm Hg. Pcap is a function of pulmonary arterial (Ppa) and left atrial (Pla) pressures, cardiac output (CO) and its regional distribution in the lung. Blood flow is greatest in the caudo-dorsal lung, which also is the site of EIPH and its associated lesions. During racing, heart rate increases to 220 bpm. CO, which is 30 L/min at rest, increases 10-fold or more. Even though the pulmonary circulation dilates to accommodate the increased blood flow, Ppa still increases from 25 to 90-plus mm Hg, in part because Pla increases from 10 to almost 50 mmHg. [4] The high Pla assists ventricular filling with about 1.75L of blood during the very short ventricular diastole (0.14 s). The elevated Pla also is, however, most likely the cause of pulmonary small vein remodeling, which by obstructing venous outflow, further increases Pcap. Small vein remodeling is a key EIPH lesion and always accompanies hemosiderin accumulations and interstitial fibrosis.[5] Such lesions are typical of other conditions associated with elevated Pla. Venous remodeling of caudo-dorsal small veins is not restricted to horses with career-ending EIPH because, in comparisons of the mechanical properties of small arteries and veins from raced and non-raced horses, racing is associated with preferential stiffening of caudo-dorsal small veins.[6]

Because of the likely key role of left atrial pressure in EIPH, it would be of interest to determine if individual variations among horses in Pla are correlated with the magnitude of pulmonary hemorrhage.

Heterogeneity of autonomic regulation of small pulmonary vessels in horse lungs

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Keywords: EIPH, adrenergic, cholinergic, caudodorsal, cranioventral

Blood flow distribution and pressure gradients in the pulmonary circulation are determined by vascular anatomy and vascular tone of pulmonary arteries and veins. Little is known about the reactivity of small arteries and veins in the equine pulmonary circulation to autonomic neurotransmitters, and if there is regional heterogeneity in the reactivity of these important resistance vessels that might contribute to preferential distribution of blood flow to the caudodorsal lung. We used wire myography to assess the reactivity of small pulmonary arteries and veins isolated from caudodorsal and cranioventral lung regions from horses with no racing history, to the sympathomimetics, phenylephrine and isoproterenol, and the parasympathomimetic, methacholine. We found that: 1) phenylephrine, was without effect on arteries and veins indicating a lack of functional α-adrenergic receptors in these vessels. 2) In precontracted vessels, the β-receptor agonist, isoproterenol, relaxed caudodorsal and cranioventral arteries, but was more efficacious in caudodorsal arteries (caudodorsal 49% relaxation vs. cranioventral 28% relaxation); isoproterenol was without significant effect on small pulmonary veins from either region. 3) The muscarinic receptor agonist, methacholine, contracted caudodorsal arteries, but relaxed cranioventral arteries, and all veins. Regional heterogeneity in autonomic reactivity in small arteries suggests that during exercise, when sympathetic outflow is increased and parasympathetic outflow is reduced, caudodorsal arteries would preferentially dilate, transmitting a greater proportion of pulmonary artery flow and pressure to the caudodorsal lung, potentially contributing to the pathogenesis of exercise-induced pulmonary hemorrhage. Supported by: Grayson Jockey Club Research Foundation and the Matilda Wilson Endowment Fund at Michigan State University.
Pulmonary arterial calcification in exercising horses

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Keywords: Horse, lungs, arterial calcification, microvasculature.

Calcification of the tunica media of the main pulmonary arteries has been observed in a large proportion of racehorses. In humans, this type of calcification is the most important cause of increased arterial stiffness, and such stiffening has been implicated in the pathogenesis of upstream (cardiac) and downstream (micro-vascular) effects on the cardiovascular system. Increased pulmonary artery stiffness for example has been associated with right ventricular afterload leading to impaired performance and remodelling; and induces inflammatory gene expression, cell proliferation, and leukocyte adhesion in the endothelium of distal vessels. The arterial tree allowed the transport of blood from the heart to peripheral tissues, and simultaneously transform the pulsatile flow into a steady one. The circulation in the brain, kidneys and “lungs” however is unique, as these tissues are continuously and passively perfused during systole and diastole, at high-flow and volume. The vascular resistance in these tissues is very low, and under normal conditions therefore, the vascular tree behaves as the dilated vasculature of other vascular beds in the body with the ability to control flow and pulsatility. The wave reflection from these vascular beds is very low (due to the low impedance), and pressure and flow pulses are able to penetrate into the capillaries with no significant opposition, making them more susceptible to significant fluctuations of flow. Torrential flow and low resistance to flow exposes small arterial vessels to the high-pressure fluctuations that could result in micro-vascular damage. We hypothesized that fibro-calcified pulmonary arteries of racehorses may predispose the microvasculature to repetitive damage, and could play a role in the pathogenesis of exercise-induced pulmonary haemorrhage. Our research group focus on investigating whether pulmonary artery calcification have an effect on the vascular hemodynamic parameters in the lungs.

References
Pulmonary fluid exchange in exercise
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Keywords: Exercise Induced Pulmonary Hemorrhage, Pulmonary Circulation, Transvascular Fluid Fluxes, Starling Forces, Hamburger Shift.

Exercise-induced pulmonary hemorrhage (EIPH) is a common cause of poor performance in horses (1). It seems that the interaction between the mechanics of breathing and the pulmonary blood flow during exertion overcomes the tensile strength of the blood gas barrier. The exact pathophysiology of EIPH, however, is still not known. Depending on the rate of rise of the cardiac output during exertion, pulmonary macro- and microvascular pressures increase accordingly. Pulmonary artery pressure (PPA) in horses during exertion may exceed 80 mmHg (2), which is translated into high transmural hydrostatic (Starling) and net driving pressures (NDP) across the pulmonary circulation (3). Transvascular fluid fluxes across the pulmonary circulation (J) (including edema formation) are traditionally attributed to changes in hydrostatic forces and perfused alveolar capillary surface area (4,5).

Submaximal exercise, acetazolamide and furosemide were used to study J, which provided an indirect insight into the behavior of pressures in the equine lung during exercise (5-8). Surprisingly, these studies showed that J is not coupled to hydrostatic forces (cardiac output) in healthy individuals, but to erythrocyte volume changes across the lung (∆EV). Further evidence indicated that ∆EV is primarily regulated by the Jacob-Stewart cycle/Hamburger shift (Cl- shift) (9). The contribution of the erythrocyte Na⁺K⁺ATPase to ∆EV was also reported to be important. Therefore, dogs were included in J studies because of their very low erythrocyte Na⁺K⁺ATPase activity (10). Despite the unique activity of Na⁺K⁺ATPase in dogs the Jacob-Stewart cycle/Hamburger shift remained the main regulator of ∆EV and J (11). The estimated rate of changes in NDP also indicated that the reduction in pulmonary transmural pressures after treatment with acetazolamide or furosemide is not sufficient to preserve the blood gas barrier from stress failure and EIPH (8,9).

3. Starling (1896) J. Physiol. 19, 312–26
Exercise Induced Pulmonary Hemorrhage in Thoroughbred Horses is a Primary Cardiac Problem

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The equine cardiovascular and pulmonary systems are critical to performance outcome in thoroughbred horse racing. Exercise induced pulmonary hemorrhage is a well recognized problem in these equine athletes. High level exercise performance in these horses can produce capillary hemorrhage which can have both short and long term sequelae for the thoroughbreds who have this issue. Previous research has focused on this being a primary lung issue, thought to be due to pulmonary hypertension from high cardiac output through the pulmonary vascular bed during maximal exercise. Given that this occurs in other mammalian species such as human endurance athletes and triathletes and canine athletes it seems unlikely that there is a different pathophysiologic mechanism of injury in equine athletes than in other mammalian species. In the setting of congenital cardiac surgery and congenital heart disease, supra systemic pulmonary artery pressures can be sustained for extended periods of time, even many months without any evidence of pulmonary hemorrhage, hence the idea that pulmonary hypertension is the cause is unlikely to be correct, essentially eliminating the inflow side of the equation. Pulmonary outflow problems in the operating room due to high left atrial pressures can, and most often do cause acute pulmonary hemorrhage. Increases in left atrial pressure can be caused by anatomic abnormalities but they can also because by left ventricular diastolic dysfunction. Extreme exercise training can result in left ventricular hypertrophy with outstanding systolic function but a decrease in diastolic function, especially at high heart rates. In conclusion, we believe that exercise induced pulmonary hemorrhage in equine athletes is due to left ventricular diastolic dysfunction at high heart rates raising left atrial pressure causing a physiologic pulmonary outflow obstruction that results in the pathologic bleeding. Exercise induced pulmonary hemorrhage in thoroughbred race horses a primary cardiac problem.
Cardiac arrhythmias during exercise are most often incidental findings with no effect on performance. However, some arrhythmias may result in reduced performance or even collapse and death in horses. As it is impossible to auscultate the heart during exercise, the arrhythmias can only be diagnosed by use of telemetric ECG recordings. During exercise the effect of myopotential interference is high and artifacts may make the interpretation of ECGs challenging.

The most common cardiac arrhythmias diagnosed during exercise are supra-ventricular premature complexes (SVPCs). These are characterized by ectopic premature atrial activation often with changes in P wave morphology. During exercise the P wave is often buried in the preceding T wave and therefore the diagnosis is primary based on shortening of the RR interval.

Isolated premature beats originating from the ventricles are termed ventricular premature beats (VPCs) and are characterized by premature ventricular activation without an associated P wave. Both the QRS complex and the T wave often have an abnormal morphology. If more than three VPCs occur repetitively the term ventricular tachycardia is used.

Other arrhythmias such as atrial fibrillation or bradycardic events such as second degree AV blocks may also occur.

The immediate post-exercise period is prone to arrhythmia development during cardiac deceleration. The clinical significance of the post-exercise arrhythmias is unknown but most are considered of no clinical significance.

Defining the safety risks to the horse and rider is challenging, but in general complex ventricular arrhythmias are of concern and further assessment of the cardiovascular system should always be conducted.
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Keywords: EIPH, horse, treatment

EIPH is ubiquitous in Thoroughbred racehorses and also occurs following other types of strenuous exercise. Most episodes of EIPH do not appear to be associated with reduced performance although the slightest evidence of EIPH is used to justify treatment with furosemide (F). Globally, F is the drug most commonly used to attenuate the severity of EIPH, regardless of whether its use is permitted on the day of competition or not.

It is unclear as to the extent of F use during training of racehorses, particularly when its use on raceday is prohibited. While reports are anecdotal, F use is apparently widespread globally in this context, with the frequency of use being highly varied. Little is known about the effects of repeated administration of F under these conditions.

F reduces the severity of EIPH, particularly the more severe episodes (Grades 3 and 4), and horses’ performances are improved following administration of F ~4h beforehand. The mechanism by which F achieves these effects has not been clearly demonstrated although the reduced severity of EIPH is usually attributed decreased pulmonary arterial pressure. Other potentially important extrarenal effects of F have been studied in other species have not horses (eg, effects on pulmonary venous pressure and capacitance).

Personal observation indicates that many horsemen and some veterinarians are unsure of why they are using F to manage EIPH. There is widespread belief that F prevents EIPH rather than attenuates it and that its administration helps performance. It is not uncommon for people with large numbers of horses receiving F before strenuous exercise to believe they have no “bleeders” although they are all getting F. However, if these horses are competing well, no diagnostic test(s) for EIPH have been performed. If they had, it is likely that there would be signs of EIPH.

A minority of horses develop debilitating pulmonary pathologies like veno-occlusive disease and pulmonary fibrosis secondary to the repeated presence of blood in airways and lung tissue, and reducing the likelihood of this may be the best justification for using F.

There are many other practices that are utilized in the cause of managing EIPH. They include pro-coagulants, nitric oxide analogs, phosphodiesterase type 5 inhibitors, antihypertensive agents and other diuretics, bronchodilators, and controlled access to water. None have been shown to be effective in mitigating EIPH severity. However, their use continues in many places.
Room A

July 14\textsuperscript{th} - PM
Airway remodeling in equine asthma.
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Keywords: Severe Equine Asthma,

Severe equine asthma remains an incurable disease although we have effective therapy to control its clinical signs. We postulate that inflammation-induced airway structural changes (remodeling) are responsible for the progression of equine asthma. Recent findings have revealed that remodeling occurs in airways of all sizes in severe equine asthma, but is most marked in the smaller peripheral airways. The airway smooth muscle (ASM) mass, which contributes to the severity of bronchospasm, is 2 to 3 times greater than in age-matched controls. Furthermore, the ASM composition is altered with an increased expression of fast contracting (+) insert smooth muscle myosin isoform (SMMHC) that may increase bronchoconstriction severity (1, 2). We also found that neutrophil-derived exosomes may participate to the progression of asthma by promoting in situ proliferation of smooth muscle and hyperplasia (3). The changes are not limited to the smooth muscle layer, as a thickening of the airway lamina propria also arises due to increased collagen (type 1 and type 3) and elastic fiber deposition (4). These changes correlate with the lung function confirming that airway remodeling contributes to asthma severity.

The airway subepithelial collagen deposition was fully reversed by 12-month treatment with either antigen avoidance or inhaled corticosteroid (ICS) administration. Therapy also reduced ASM mass (30% on average), but it remained greater (2 fold) than in healthy horses (5). Combined ICS and long-acting β2-agonist drugs (ICS/LABA) or ICS monotherapy alone equally induced a 30% decrease of the ASM mass at 3 months (but not after 1 month) (6). However, only ICS/LABA or antigen avoidance alone decreased airway luminal neutrophilia.

These findings reveal that, once established, remodeling of the asthmatic airways is only partially reversible with current therapy. Therefore, efforts should be aimed at the early identification of susceptible horses and the implementation of preventative measures. Studies of the molecular pathways contributing to remodeling are needed for the development of novel therapy in asthma.

References


Epithelial - smooth muscle interactions

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Key words: tissue remodeling, epidermal growth factor receptor, airway tone.

While airway smooth muscle seems to be at the core of the disease, in asthma the epithelium is the first tissue to be exposed to the environmental insults that are responsible for asthma. It may be argued that asthma is an epithelial disease. Epithelial function is complex involving its well-known barrier function but also it has important immune functions and is a source of growth factors that may potentially lead to tissue remodeling. Ligands of the epidermal growth factor receptor are among the potentially important mediators of tissue remodeling. The ligands amphiregulin and heparin-binding epidermal growth factor (HB-EGF) are released by pertinent molecules such as histamine and cysteinyl-leukotrienes as well as the cytokine interleukin-13. These molecules when released may lead to goblet cell differentiation but also conceivably may cause proliferation of the underlying airway smooth muscle. Compressive forces on the epithelium also lead to the release of HB-EGF which has led to the argument that bronchoconstriction per se may lead to airway remodeling. Repeated exposure to cholinergic agonists has been shown to stimulate airway smooth muscle growth in vivo. Organic dust is ingested by airway epithelial cells and results in airway hyperresponsiveness to methacholine and neutrophilic inflammation in the mouse. These responses are related to oxidative stress and are ameliorated by anti-oxidants. In vitro experiments have shown that co-culture of smooth muscle with epithelial cells leads to proliferation of the smooth muscle and is dependent on pro-inflammatory molecules such as interleukin-8. The epidermal growth factor receptor and its ligands have not been implicated in the mechanism of growth although they are released into the medium. However, their highly positively charged state may lead to avid binding to matrix molecules neutralizing their biological effects. Epithelium may produce bronchodilating substances such as nitric oxide and prostaglandin E₂.

In conclusion, airway epithelium has potentially many actions on airway smooth muscle to alter its tone and trigger its proliferation, effects that may have far-reaching consequences for asthma.
Is there an allergic march in horses?

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Keywords: equine asthma, IBH, multiple hypersensitivities

In humans, the development of atopic dermatitis in infancy and subsequent allergic rhinitis and/or asthma in later childhood or adulthood is known as the atopic march.

In certain horse families there is a genetic association between recurrent airway obstruction (RAO; severe equine asthma, EA) and a chromosomal region containing the interleukin-4 receptor α-chain (IL4RA) gene, which is associated with skin allergies and parasite defense in humans and other species. We therefore hypothesized that EA and skin allergies in horses may also have a common immuno-genetic background and/or may be linked to exposure and defense of infectious agents. Indeed, in two independent populations we found that EA is associated with insect bite hypersensitivity (IBH, odds ratio 7.4 and 13.1, respectively). Furthermore, multiple hypersensitivities also included urticaria and were associated with decreased parasite egg shedding. In a subsequent study, horses suffering from IBH were significantly more sensitive to inhaled histamine than healthy controls. Furthermore, horses suffering from IBH had a lower pO2 than healthy controls. These results suggest that IBH is associated with airway hyperreactivity even in the absence of overt respiratory clinical signs. Genetic studies have so far not revealed a common causative variant, preliminary microRNA profiles indicate involvement of a Th17-pathway and investigations of serum IgE against specific allergens and of the microbiome in horses affected with multiple hypersensitivities are underway. Interestingly, in cases of the recently described intestinal gluten hypersensitivity, cutaneous and/or respiratory signs were also observed.

The natural history of IBH and RAO suggests that typically an “allergic march” would lead from the former to the latter. However, further longitudinal studies will have to test such assumptions and may also address other allergic manifestations like urticaria or even food allergies. Presently, we conclude that there is genetic, epidemiological and clinical evidence that in horses, as in other species, different manifestations of hypersensitivity may occur together.

References available upon request
Alveolar macrophage in equine asthma

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Keywords: airway, monocyte, innate immunity

Alveolar macrophages (AMs) are becoming increasingly recognised as key cellular players in human asthma; both in severe, steroid refractory non-Th2 driven asthma [1] and in allergic asthma, where polarisation towards a pro-inflammatory phenotype (from a largely suppressive/homeostatic phenotype) may significantly contribute towards lung pathology, especially following repeated or prolonged allergen exposures [2]. Continued efforts to unveil the mechanisms which determine their relative state of polarisation may inform the development of novel therapeutic approaches which target this “double edged” cell.

Comparative data on the potential role of AMs in equine asthma (EA) is significantly more limited. Disease associated differences (severe EA-susceptible vs healthy) in AM responsiveness (potentially reflecting differences in AM phenotype distribution) have been reported by some [3], but not by others [4]. Furthermore, manipulation of the airway monocyte/macrophage phenotype may partly explain the recently reported therapeutic benefit of airway instillation of bone marrow derived mononuclear cells in severe EA, with an associated increase in bronchoalveolar lavage fluid IL-10 [5]. Despite these proposed interpretations, in light of the sparsity of data and the contradictory results, it remains clear that further research is warranted in this area. Similarly, the role of AMs in the pathogenesis of mild to moderate EA remains undetermined; however, recent studies strongly support a compartmentalised immunosuppressive effect of training at the level of the AM [6] (and Karagianni et al, unpublished data), potentially increasing susceptibility to inhaled infectious agents. Again further work in this area is warranted.

References
Inflamm-aging in the lung

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Keywords: Equine asthma, immunosenescence.

The equine population of geriatric individuals are increasing. It is estimated that about 13 percent of the UK equine population are above 20 years of age. As the population of both elderly humans and geriatric horses are increasing, increased awareness of age-related changes leading to diseases are put into focus.
The phenomenon Inflamm-aging is defined as a chronic low-grade inflammatory status that contributes to age-associated morbidity and mortality. This inflamm-aging is by Franceschi et al. (2000) explained as the result of ‘macroph-aging’ continued antigenic load and immune stress, and with the macrophage being responsible for the age-related increases in pro-inflammatory cytokines.
Immunosenescence opposite inflamm-aging is defined as an age-associated deterioration in immunity characterized by a diminished reaction towards host pathogens, and is thought of as a major contributor of the age-related increased susceptibility and decreased immune response towards infectious diseases.
Overall, no age-related changes in the proportions of BAL cells related to equine asthma have been found. Regarding BAL cytokine levels, an age-related decrease in mRNA expression of IL-1β and IL-8 in LPS-stimulated BAL cells has been reported. Furthermore, the frequency of IFN-γ-producing lymphocytes were found to increase with age, and this increase in IFN-γ was found to be enhanced by exposure to dust in the horse stable environment as compared to horses kept at pasture.
The inflamm-aging characteristics that are well accepted for the peripheral blood, appears to be more controlled within the airspace of the lungs. As example, a decreased mRNA expression of IL-1β and IL-8 with age in stimulated BAL cells from stabled horses are suggestive of a tighter regulation within the lungs. This regulation appears to be more pronounced with age and supported by the relative steady-state of neutrophils within BAL differential cell counts with age. Thus, further studies concerning age-related regulation or natural adaptation within the lungs could improve our understanding of age-related chronic lung diseases.

References
Equine Respiratory Viruses Update

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Respiratory viruses are a major cause of loss of training days in equine athletes but real-time PCR has revolutionised their diagnosis and management. Equine influenza, equine herpesvirus 1 and 4 and equine rhinitis viruses can be identified in nasal swabs or other samples on the day of submission. Furthermore with automated nucleic acid extraction, it is a relatively simple matter to screen all the horses in a yard repeatedly allowing the veterinarian and trainer to monitor both the spread of virus and the extent of virus shedding by infected horses.

Laboratory testing is essential as the clinical signs associated with equine influenza in vaccinated horses are difficult to differentiate from equine herpesvirus infection. However, equine influenza virus does not persist and recovery is less complicated that with equine herpesviruses which establish latent infections and can reactivate under stress. Furthermore, equine herpesvirus 1 and 4 are abortigenic and equine herpesvirus 1 has the potential to cause neurological disease. Testing for the putative neurological marker in the polymerase gene is not a reliable predictor of clinical outcome.

Vaccination against equine influenza is effective and antibodies against the envelope glycoprotein haemagglutinin correlate with protection. Thus, serological assays are extremely useful to monitor vaccine performance and to develop evidence based vaccination programmes. It is important to ensure that all horses in a yard have protective antibody levels as susceptible horses are frequently the index cases and can serve as a focus of infection for their stablemates. Vaccination early in the face of an outbreak reduces the clinical impact.

Vaccination against equine herpesvirus appears to be less effective than equine influenza vaccination but may reduce virus shedding and hence limit the recovery period. Unlike equine influenza there is no correlate of protection that lends itself to routine monitoring of vaccine efficacy. In recent years vaccination regimes have been complicated by interruptions in the supply of some equine herpesvirus vaccines and the use of different products which are often only available on a temporary basis.

The role of equine rhinitis viruses in respiratory disease and loss of performance may be underestimated, particularly in young horses. These viruses establish persistent infections. A recently developed vaccine against equine rhinitis virus A is available in some countries.
**Strangles, diagnosis and carriers**

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Keywords: *Streptococcus equi*, horses, guttural pouches

*Streptococcus equi* subspecies *equi* (*S. equi*) results in considerable suffering for the horses for weeks or even months and some horses develop life threatening complications. Despite quarantine efforts, this disease continues to plague the horse population with outbreaks every year. It is increasingly recognized that a key factor in spread of strangles is the occurrence of horses that have recovered from strangles but continue to carry bacteria in their upper airways, i.e. “silent carriers”.

Recent research has focused on improving strangles diagnostics, and understanding of disease mechanisms, including spread of strangles (1,2). In chronic carriers, bacteria persist in guttural pouches, and are difficult to detect by cultivation (3,4). PCR is currently used to identify chronic carriers. Bacteria cultured from carriers often differ in colony appearance, which may be related to loss of virulence factors (5). Whether these bacteria are attenuated, or if they can revert to their original, highly virulent phenotype is not known. Our earlier studies showed that persistently PCR positive carriers are intermittently culture positive (4).

We have conducted long term follow up studies of strangles outbreaks with the aim to identify silent carriers of *S. equi*. Our overarching goal has been to identify tools that enable a reliable certification of horse establishments free of *S. equi* following an outbreak. Unfortunately, in addition to finding the silent carrier state more common and longer lasting than earlier reported, we found that carriage in the upper airway often occurs in absence of visible abnormalities (3, 4), rather than (6) as linked to empyema or chondroids in the guttural pouch. Equally worrisome, it appears that silent carriers spread *S. equi* bacteria to uninfected stall mates (4), and despite treatment of silent carriers we observed that the *S. equi* remained detectable in a large proportion of treated horses. The majority of clinically healthy silent carriers remain PCR positive but culture negative for *S. equi* months after clinical disease, and their infectious capacity is so far unknown.

5. Harris et al. Genome Res. 2015 Sep; 25(9): 1360–1371
Deciphering the evolution, epidemiology and virulence of *Streptococcus equi* towards the eradication of strangles

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Keywords: Strangles, *Streptococcus equi*, evolution, epidemiology, virulence

Strangles, caused by *Streptococcus equi*, is one of the most frequently diagnosed infectious diseases of horses worldwide. We have defined the global population structure of this important host-restricted pathogen revealing a population replacement in the late 19th or early 20th century. Our data provided new insights into the epidemiology of outbreaks at local, national and international levels. Strangles outbreaks can be complex, involving several different strains of *S. equi*, even within the same horse. We identified the origins of an outbreak of strangles in Norfolk and screened horses using diagnostic tests to minimise its impact. We describe the prevalence of strangles outbreaks throughout the UK and reveal the genetic relationships of *S. equi* strains from around the world. In particular, the genetic diversity of strains recovered from horses in the United Arab Emirates emphasises the benefits of screening for *S. equi* infection prior to the international movement of horses.

The development of new vaccines against strangles would lead to important health benefits for horses. A new experimental technique permits the simultaneous identification of every gene required for *S. equi* to cause disease. Our data are being used to enhance the safety and effectiveness of vaccines against strangles towards the gradual eradication of this disease from horse populations.
Rhodococcus equi pneumonia in foals: Not all infected foals require treatment

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Keyword: pulmonary abscess, reduction of antibiotic use

Respiratory infections are frequent disorders in the growing foal. A common cause is the Gram-positive facultative intracellular bacterium Rhodococcus equi (R. equi), which induces chronic bronchopneumonia with abscess formation. R. equi pneumonia leads to considerable economic losses for breeders because of its high morbidity and treatment costs. In order to minimize foal losses on breeding farms with endemic rhodococcosis, a screening program promoting early detection of pneumonia in the growing foal is recommended. This includes frequent clinical examinations, determination of white blood cell counts and an ultrasonographic examination of the lungs on weekly basis.

Several studies have demonstrated that as many as 2/3 of the foals with pulmonary abscesses (until a score below 10 cm: sum of the diameters of all abscesses seen at sonography) and with mild or no clinical signs resolve spontaneously. Consequently, we should carefully weigh up each decision to administer antibiotics to foals with pneumonia. Each decision of treatment takes into account clinical, haematological and sonographical findings. Some examples of treatment decision and duration of therapy will be presented.

The efficacy of treatment of foals with R. equi pneumonia depends not only on the extension and severity of lung lesions but as well on the choice of antibiotics. Choosing an appropriate antibiotic protocol for the treatment of the R. equi pneumonia is crucial. In order to prevent the emergence of resistant strains the treatment of choice for R. equi pneumonia is therefore rifampin in combination with a macrolide antibiotic (azithromycin, clarithromycin, tulathromycin, gamithromycin ...). Also, the side effects of different antibiotic preparations used are to be taken into account.
Paranasal Sinuses

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Keywords: Paranasal Sinuses; sinoscopy; endoscopy

The horse has seven pairs of paranasal sinuses, namely the rostral maxillary sinus (RMS), ventral conchal sinus (VCS), caudal maxillary sinus (CMS), dorsal conchal sinus (DCS), frontal sinus (FS), sphenopalatine sinus (SPS) and ethmoidal sinus (ES). The FS and DCS are very closely associated and often considered as a single sinus; the conchofrontal sinus (CFS). The position of each of the sinus compartments vary both between individual horses and with age, in particular there is great variation in the position of the maxillary septum and the position of the maxillary sinuses in relation to the cheek teeth apices. With increasing age, the reserve crowns of the cheek teeth become shorter, the maxillary septum enlarges and its rostral limit approaches the infraorbital foramen (Hillmann 1975).

Fluid within the paranasal sinuses drains by ciliary transport toward one of the two drainage ostia. The drainage ostia (NMAs) originate from the CMS and RMS separately, but combine together to form a common nasal exit of the nasomaxillary apertures (NMAs) at the caudal aspect of the middle meatus of the nasal cavity. In terms of drainage the paranasal sinuses can be considered as two functional compartments. One compartment consists of the RMS and VCS, the drainage of which is via the drainage ostia of the RMS. The other compartment is formed by all the other paranasal sinuses, which drain via the drainage ostia of the CMS. Knowledge of the anatomy of the two drainage ostia is vital in understanding the pathophysiology of disease in the paranasal sinuses.

Recently, Sinoscopic techniques have been utilized to diagnose and treat most diseases within the paranasal sinuses. An understanding of the different options for portal location enables easier access into the different sinus compartments.
Problems of the epiglottis

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Introduction: Complications or problems of the epiglottis can be associated with significance primary disease (i.e., epiglottic entrapment, epiglottic condritis, etc.) or from complications associated with their surgical treatments. Although the results of surgery in the literature can be excellent, very difficult problems occur in a small percentage of cases. Yet those problems/ complications can be career ending.\textsuperscript{(1–6)}

Objectives: Review our experiences with the complication epiglottic entrapment performed at either Cornell clinic.

Methods: Cases were reviewed from the respective surgical logs from each electronic medical record (EMR) from 2014-2017 and the complications reviewed. Summary of the handling of the complications were recorded and will be presented.

Results: Diagnosis of persistent epiglottic entrapment (EE) is readily confirmed by endoscopic examination either at rest or during dynamic endoscopy in some cases of intermittent entrapment. Most entrapments are reported to be uncomplicated. However, in our referral situation the majority of horses presented with significant swelling and ulceration. This is likely a referral bias where simple cases are treated locally and only cases thought to be more complicated or have a less favorable prognosis are referred (Figure 1). Uncomplicated cases of EE cause significantly mild airway obstruction. However EE especially ulcerated and thickened membranes may cause DDSP and thus greater airway obstruction. Excessively thickened and inflamed EE induces can be associated with permanent DDSP. All cases were successfully released and no adhesions to the epiglottic cartilage were seen. Problems seen after surgery were persistent DDSP, epiglottic chondritis, excessive swelling and sub-epiglottic ulceration.

These chronic cases can become excessively thickened, ulcerated, with an apparently fibrotic entrapping membrane (Figure 1). The decision tree in the management involves: a) the timing of surgery (very inflamed cases should be treated with local and perhaps systemic anti-inflammatory agents first), b) a release technique which does not risk further trauma to the epiglottic cartilage, 3) weigh the benefit of resection of swollen tissue vs the formation of a sub-epiglottic wound resection.

Prevention of iatrogenic damage/ burn at the tip of the epiglottis when using the hook or laser is important. The use of the hook (guarded or not) should be avoided if the outline of the tip of the epiglottis cannot be identified through the entrapping membrane. If a laser is used the minimal energy level should be used with a target of maximal joules of 500. Use a blunt silicon-covered hook to protect the epiglottis or lift it with forceps. Be very careful to place your cut so that the edge of the epiglottic cartilage is covered dorsally, laterally and ventrally with mucosa.
When performing further sections close to the tip of the epiglottis, the preference is to use endoscopic scissors instead of laser, and forceps to apply rostral traction while cutting.

If persistent DDSP occurs after surgery, it can be due to re-entrapment, epiglottitis, excessive sub-epiglottic swelling, epiglottic abscess/ chondritis, or excessive sub-epiglottic scarring (i.e., epiglottic tie-down). This is managed by proper identification of the cause and targeted treatment. If persistent DDSP is not resolved, then laryngeal tie-forward followed by staphylectomy is used.(7)

Re-entrapment is seen in 5 to 15% using the hook or laser but is more frequent with electro surgery. Recurrence seems to be increased in cases of complicated entrapment with chronic inflammation and ulceration and if some subepiglottic tissue remains after the initial sagittal division. This is managed by Revision surgery, with some excision of the remaining tissue (lateral triangles resection), and prolonged post-operative anti-inflammatory treatment. It is best prevented by using a minimally invasive technique as described earlier. Remove extra tissue according to appearance after swallowing.

**Conclusions:** Epiglottic problems can be one of the most challenging conditions in upper airway surgery. Minimal trauma/energy and time are two of the most efficient methods to manage these occurrences.

**References**

Guttural Pouches: where are we?

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Keywords: Guttural pouch, diseases, evidence

Traditionally, three guttural pouch diseases were recognised (empyema, mycosis & typanmy). Now, guttural pouches are being recognised as associated with a growing list of other disorders, including: Otitis media/interna - Temporohyoid osteoarthropathy; Dorsal displacement of the soft palate; Longus/rectus capitus muscle rupture; Pharyngeal paralysis; Neoplasia; Cystic structures; Atlanto-occipital arthropathy; Mycotic encephalitis; Hypoglossal neuritis/lingual hemiplegia. For guttural pouch mycosis, several surgical advances have been made including fluoroscope guided catheter placement, detachable coil devices, and a standing technique that allow for quicker, safer and accurate placement of occlusion devices at the arterial embolism site. Long-term follow-ups have shown improved survival and return to work.

There is an explosion of interest in guttural pouches associated with the outbreaks and management of strangles (Strep. equi). PCR technology has identified guttural pouches associated with ‘carrier’ states in greater numbers of horses than previously appreciated. Previously, chemicals of mass destruction (e.g. hydrogen peroxide, iodine) were used for the treatment of guttural pouch empyema. These were replaced with repeated saline lavages and evaluations (e.g. endoscopy, culture). Now, instilling penicillin routinely into guttural pouches has gained much acceptance owing to the marked sensitivity of penicillin against Strep. equi and the possibility of distribution of penicillin in the guttural pouch to other sites (e.g. retropharyngeal lymph nodes). However, the popularity of sampling and treatment came before evidence. For example, the contagious risk of relatively ‘normal’ appearing guttural pouches to horse populations was not determined. Also, sampling guttural pouches for PCR commonly includes the use of re-usable medical devices (e.g. endoscope), as reflected in consensus statements which have not been evaluated as free from contamination. Furthermore, penicillin in guttural pouches may not be innocuous. Guttural pouches have complex natural microflora, that differs between horses (e.g. Pseudomonas spp.-including zoonotic types). The instillation of penicillin does impact on this microflora that could lead to other complications (e.g. gram negative empyema).

Temporohyoid osteoarthropathy is associated with headshaking, ear flopping and rubbing, vestibular disease, facial paralysis and exposure corneal keratitis. This may be the result of trauma to the petrous temporal area, ascending respiratory tract infection, or extension of otitis externa to the middle and inner ears. The result is inflammation and fusion of the stylohyoid bone to the petrous temporal bones. Using brainstem auditory evoked response (BAER) studies has revealed that auditory dysfunction and/or loss appears to be a common manifestation that appears to be permanent regardless of medical or surgical intervention, or overall neurologic improvement. Endoscopy of the guttural pouch is a commonly performed as well as magnetic resonant imaging or computed tomography. Cytological and microbiological diagnosis of otitis media/interna can be achieved through tympanocentesis and lavage. Endoscopic evaluation should include: Evaluation of the stylohyoid bone for the presence of a fracture; Evaluation of the head of the stylohyoid bone for the presence of osteopathy, including bony enlargement and fusion with either the tympanic bulla or petrous temporal bones; Evaluation of the guttural pouch mucosa in the temporohyoid region (e.g. hyperemia/thickening, oedema, purulent debris); Evaluation of the internal carotid artery as it enters the base of the skull (e.g. internal carotid artery can appear compressed or out of position due to bony enlargement from the temporohyoid region). Since temporohyoid osteoarthritis is typically unilateral, then endoscopic evaluation of the both guttural pouches (temporohyoid regions) is very important to rule out common anatomic variations. The final diagnosis is a balance of the functional neurologic deficits with changes in the temporohyoid region. The goal of treatment, includes: Resolving pain and inflammation at the temporohyoid joint (± fracture site); Antimicrobial treatment for confirmed bacterial otitis media-interna and/or meningitis; Treatment of exposure keratitis; Stabilization of the temporohyoid articulation to prevent fracture or ongoing trauma at a fractured.
Surgical procedures (partial stylohyoidectomy, ceratohyoidectomy) have been described that aim to decrease movement and abnormal biomechanical forces on the temporohyoid joint. Currently, the surgical treatment considered most effective is ceratohyoidectomy, with good outcomes reported.

Rupture of the longus/rectus capitus muscle/s can be a cause of guttural pouch haemorrhage and epistaxis. This is associated with acute trauma and may also be a sequel to guttural pouch mycosis. Distinction between this condition and guttural pouch mycosis alone requires endoscopic examination of the entire guttural pouch, particularly at the site of insertion of the strap muscles on the base of the skull. Treatment involves conservative management, stall rest for 4-6 weeks, antibiotics, and analgesics.

Standing laryngoplasty

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Introduction: Laryngoplasty is the treatment of choice for many horses with arytenoid cartilage collapses. The procedure was first used for routine laryngoplasty in draft horses to minimize the anesthetic risk. Early results revealed that in addition to the avoidance of general anesthesia, the procedure yields greater mobilization of the larynx and permits a more accurate degree of abduction tailored to the horse’s anticipated function. It has since expanded to right side laryngoplasty and revision laryngoplasty.

Objectives: Review of our experiences with the procedure

Methods:
The ventriculocordectomy is first performed under the following sedation protocol:
- Detomidine 6mg IV & Butorphanol 4mg IV prior to entering stocks. Bring in once a light sedation is exhibited.
- Top off with 2mg Detomidine IV during procedure if extra sedation is needed.
- After VC send back to the stall to drain for at least 30 minutes.

Bring back to the surgery area and sedate with 200mg Xylazine IV. Proceed to clip the area outside of the stocks after the sedation has taken effect. Dress the horse with the “slinky” which has a cut out for the surgical area on the appropriate site (to prevent hair particles from falling near the incision site). Move the horse into the stocks and position the head stand to the appropriate height. The head stand is needed to maintain the position of the extended head and neck. Maximal neck extension is detrimental to the procedure. A relatively neutral position is needed and facilitates movement of the larynx within the incision. Sedate with 2-3mg of Detomidine IV if needed. The halter is then replaced by a bioplastic head halter without a throat latch. Alternatively use a rope halter and tie the throat latch piece back onto itself so that the throat latch is exposed. For left laryngoplasty, place a full cup left eye blinker and half cup on the right eye of the horse and tuck the sides into the rope halter and secure with white tape. Insert flexible ear plugs into both ears.

With the horse secured in the stocks and fully dressed attached the Detomidine CRI (1mg/100mL). Proceed to scrub the surgical site. Twenty cc of local anesthesia (2\% Carbocaine hydrochloride) is used ventral to the linguofacial vein and immediately medial to the sternocephalic tendon junction to the linguofacial nerve to desensitize the second cervical nerve.

During aseptic preparation of the surgical site, we run CRI initially at a medium drip rate (3-4gtts/1sec) until the surgery starts and after the first incision is made. The approach and the procedure are routine. At that point slow the drip rate down to 1-2gtt/sec and then drip to effect. For most of the horses this provides a nice stable plain of sedation. If the horse gets responsive either adjust the fluid rate accordingly or top off with 2mg Detomidine IV.
The main advantages of the procedure are ease of surgery, avoidance of general anesthesia and ability to accurately assess degree of laryngeal abduction.

**Results:** The procedure was complete in all but one horse whom would not stand quietly. The ease of surgery and complications rate was as reported below.

**Conclusions:** The procedure is safe and effective for performance of the laryngoplasty procedure and presents a valid alternative to the standard approach.

**References**

Biomechanics of Laryngoplasty and Ventriculocordectomy

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Keywords:laryngoplasty; ventriculocordectomy; biomechanics

The Laryngoplasty procedure has a number of potential complications which can result in long-term morbidity for the horse. The presence of a maximal degree of arytenoid abduction postoperatively is associated with coughing and dysphagia. Some aspiration of feed material is likely the result of dysphagia caused by the surgically abducted arytenoid failing to protect the airway during swallowing, however dysphagia may also be the result of pharyngeal muscle dysfunction following surgery secondary to inadvertent damage to the cranial laryngeal nerve. In severe cases of post-operative coughing and dysphagia a second surgery is indicated to loosen the prosthesis. Post-operative coughing and dysphagia may resolve spontaneously within several weeks following surgery as the prosthesis loosens or the swallowing reflex adapts. In those horses with persistent low grade dysphagia, it will result in chronic lower airway inflammation and poor performance. The mechanism of abduction loss and ultimate failure of the prosthesis is not fully understood, however, several recent in-vivo and ex-vivo studies have helped in understanding this mechanism and in developing new devices to prevent these problems.
Treatment of acute laryngoplasty complications

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Introduction

Prosthetic laryngoplasty (PL) is the technique most commonly used to treat recurrent laryngeal neuropathy. It uses a synthetic prosthesis to ‘tie back’ the affected arytenoid cartilage in a permanent state of partial abduction to increase rima glottides and prevent dynamic collapse. Despite significant surgical successes, the undesirable prevalence of surgical failure and the high frequency of postoperative complications, outcome is considered moderate, at best, particularly in horses used for high intensity exercise. The prognosis in sport horses is usually better. Post-operative complications include acute or progressive loss of PL abduction and various degrees of dysphagia and coughing. Other complications such as seroma and infection are not uncommon and can have devastating effects.

Wound infection can be a complication of large seroma (or large seroma can be the first signs of infection). It can be consecutive to prosthesis penetration through the laryngeal mucosa, nasopharyngeal mucosa, or through the vestibulum esophagi at the rostral part of the MP.

Vestibulum oesophagi at the rostral part of the muscular process. Voe: Vestibulum oesophagi; Th: Thyroid cartilage; MP: Muscular process; Oe: oesophagus

Isolation of the adventitia of the vestibulum oesophagi during a laryngoplasty

Mucosal perforation at the cricoid

Isolation of the cricoid cartilage from the underlying mucosa using a towel clamp

Most laryngoplasty infections, when detected early in the postoperative period, can be effectively treated by drainage, lavage and appropriate antimicrobials based on culture and sensitivity patterns, and without suture removal. In the case of penetration, remove the prosthesis immediately after its detection to prevent chronic inflammation of the area. As early removal of the suture often results in failure of the arytenoid abduction, a partial arytenoidectomy can be performed during prosthesis removal.

Marked or complete acute loss of LP abduction occurs usually in the first 7 days following surgery. LP failures can occur both from pullout at the cricoid or at the muscular process.

In our experience, acute failure of the laryngoplasty construct is most likely related to inappropriate placement...
of the sutures.
Acute prosthesis failure is uncommon but requires immediate surgical revision.
Two sutures should be replaced, using strategies to reinforce anchorage of the sutures within the cartilages, such as metallic suture buttons at the cricoid cartilage. Antibiotics are prolonged for a minimum of 5 days, as well as hospitalization.

Some degree of dysphagia and coughing, are inevitable consequences of laryngoplasty due to the concept of the technique. Coughing during the immediate post-operative period is usually associated with a high level (Dixon grade 1) of arytenoid abduction and food and saliva aspiration into the trachea is usually the most common cause of this problem.

Bilateral VC also leads to an increased risk of dysphagia by decreasing the seal and ability of the rima glottis to close at its ventral part during swallowing.

Repeat laryngoplasty with replacement of the sutures is indicated when postoperative overabduction of the left arytenoid abduction.

References can be asked for.
Laryngeal reinnervation-update

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Currently, the standard treatment for Recurrent laryngeal neuropathy (RLN) is prosthetic laryngoplasty, with the goal of permanently abducting the left arytenoid. A more physiologically viable treatment option for RLN is the reinnervation of the CAD with transplantation of the first (C1) or second (C2) cervical nerve. The omohyoideus muscle is innervated by a nerve formed by the first and second cervical nerve branches, hereinto referred to as the C1/C2 nerve. The junction of branches of this nerve to a small (5mm) block of muscles are used for the neuromuscular pedicle nerve graft (NMP) graft because the omohyoideus muscle (OH) is an accessory muscle of respiration (active on inspiration) and the C1/C2 nerve is in close proximity to the larynx. The recently described nerve-implantation technique is replacing the nerve-pedicle procedure. Both procedures are very similar except for the procuring the CAD muscle pedicles.

Although any horse with RLN is a candidate for laryngeal reinnervation, younger horses and those with grade 3 laryngeal movements are the ideal candidates for this surgical procedure. Patient preparation and the initial surgical approach are similar to laryngoplasty. The procedure is usually performed on general anesthesia but can be performed in the standing patient in selected cases.

The main body of the C1/C2 nerve is identified with the help of a single pulse nerve stimulator (1mA). The nerve branch that produces the strongest contraction of the OH is selected and gently dissected proximally, as well as one or two other smaller branches. Once judged to be sufficiently freed, the distal end of the nerves are cut.

The thyropharyngeus and cricopharyngeus muscles are bluntly separated. The rostral 2/3rds of the cricopharyngeus muscle are sectioned transversally to expose the CAD. The larynx is subsequently rotated laterally. The selected main branch of the nerve is implanted within the CAD tunnel using a Reverdin needle from medial to lateral and secured to the lateral belly of the CAD muscle. Any excess nerve branch is implanted directly in a small slit in the lateral muscle belly of the CAD as described.

Dissection oft he C1 nerve. OH: Omohyoideus muscle
The partial myotomy of the cricopharyngeus and the surgical incision are closed. The horses resume training at 6 to 12 weeks postop according to the remaining postoperative function of the CAD, and the expected improvement after ventriculocordecomy. Exercising endoscopy and ultrasound-guided percutaneous stimulation of the first cervical nerve at the level of the alar foramen are used to confirm successful reinnervation postoperatively.

The current success rate of this new procedure is comparable to that of prosthetic laryngoplasty. For young horses that have never been raced and horses of high value because of their breeding potential, reinnervation can be offered because restoration of a functional CAD is a more physiologically efficacious method of treating RLN. If the result of laryngeal reinervation is unsatisfactory, a laryngoplasty may still be performed.

References


Dynamic Laryngeal Collapse Associated With Poll Flexion: diagnosis, pathophysiology and treatment

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Dynamic laryngeal collapse (DLC) associated with poll flexion, defined as bilateral vocal fold collapse with some degree of concurrent bilateral arytenoid cartilage collapse, is a newly described dynamic upper respiratory tract (URT) obstructive disorder diagnosed most frequently to date in Norwegian Coldblooded Trotters (NCT).1,2 The disorder has also been diagnosed in Standardbreds, Icelandic horses and other breeds of gaited horses3 which perform in poll flexion with a “high” head carriage. The disorder is often accompanied by other dynamic URT abnormalities including dorsomedial deviation of the epiglottic margins and/or aryepiglottic folds.1,2 Complaints of abnormal URT noise and poor performance are associated with periods of enforced poll flexion during training and racing. Diagnosis can only be established by high-speed treadmill videoendoscopy (HSTV) or overground endoscopy with periods of induced poll flexion. This is achieved by examining horses with full headgear and driving or riding them into the bit.4 Resting endoscopy and HSTV without enforced poll flexion are within normal limits.

DLC associated with poll flexion can vary in severity among affected individuals, but in certain racehorses can cause a moderate to marked inspiratory obstruction. Petsche et al. demonstrated that induced poll flexion results in mild inspiratory obstruction in normal Standardbred (STB) horses exercised at maximum heart rates.5 Another study compared tracheal pressure measurements in a group of 5 elite (E) NCT racehorses to 6 affected (A) individuals during alternating phases of free head carriage and poll flexion. Both groups of horses had similar baseline inspiratory pressures during the initial phase of free head carriage (E -30.66 cm H₂O; A -31.28 cm H₂O). Both groups had significantly greater negative inspiratory pressures during poll flexion relative to respective free head carriage measurements; however, the affected group had much greater negative inspiratory pressures (-58.4 cmH₂O) relative to the elite group (-39.4 cm H₂O).6

The cause of DLC associated with poll flexion appears to be a more rostral positioned larynx, which becomes externally compressed in a “narrow” intermandibular space, during periods of enforced poll flexion with the head held high.7 In NCT racehorses only 12 degrees of poll flexion is necessary to induce DLC in affected individuals.8 Other breeds may require more restraint into the bit (poll flexion angle) to induce the disorder. A recently published histopathology study did not support a neuromuscular cause of the disorder.9 The author has unintentionally induced this disorder in several NCT racehorses treated with Tie-forward surgery for DDSP.

There is no satisfactory treatment to date for the disorder. A modified checkrein to limit poll flexion in racehorses has shown promise,8 but is not in practical use in Scandinavian racing. Bilateral ventriculocordectomy2 and unilateral prosthetic laryngoplasty3 have been attempted to salvage horses for athletic use.

References:

Outbreaks of bilateral laryngeal collapse with asphyxiation

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Keywords: bilateral recurrent laryngeal neuropathy; asphyxiation; polyneuropathy, ataxia

Horse vets are very familiar with left sided recurrent laryngeal neuropathy: indeed, our evidence suggests that most if not all large breed horses have some degree of left sided neuropathy. Further, the most severely affected horses, have histological evidence of right sided disease. Despite this, clinically-relevant right sided laryngeal neuropathy that presents in combination with left sided signs is rare. In this presentation, I will present the clinical data and investigation of 2 outbreaks of bilateral recurrent laryngeal neuropathy that occurred in 2016 in the UK, involving 10s to 100s of horses.

In the spring of 2016, 2 groups of horses in the south of England presented with varying degrees of bilateral laryngeal neuropathy. Signs of severe inspiratory stridor, pulmonary oedema, epistaxis, collapse and death occurred and signs were exacerbated by exercise, travel or anxiety. Endoscopy revealed partial or complete left and right sided arytenoid stasis, with variable pharyngeal collapse. Ultrasound revealed widespread intrinsic laryngeal muscle involvement, though not to the extent of the most severe RLN cases. Routine blood work ruled out hepatic encephalopathy and there was no history of organophosphate use, or toxic plant exposure. Clinical investigations and post mortems revealed in both outbreaks a motor and sensory polyneuropathy with evidence of central involvement. Some horses had generalised moderate neurogenic muscle atrophy, ataxia and CSF analysis revealed macrophagic degenerative or inflammatory central changes with a normal total protein concentration. Long tract degeneration and cerebellar involvement was apparent (Purkinje cells) in some horses as well as an axonopathy detected by semithin resin embedded sections and electron microscopy.

Horses were removed from the pasture in one location; in the other, signs appeared to resolve with no new cases later that summer. However, signs have returned this spring in this latter case (2017). Ongoing investigations includes a search for heavy metals toxicosis and mycotoxins and B vitamin deficiencies. The approach to the cases with differential diagnosis will be discussed. The clinical findings in these horses might provide clues as to the pathogenesis of the much more common recurrent laryngeal neuropathy of horses.
Farm dust and endotoxin suppress development of allergic asthma by altering epithelial and dendritic cell communication


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Keywords: Allergy, Epithelium, TNFAIP3, Endotoxin, Asthma

Growing up on a dairy farm protects children from allergy, hay fever and asthma. A mechanism linking exposure to this endotoxin-rich environment with protection has remained elusive. We could show that chronic exposure to low dose endotoxin or farm dust extract before allergen sensitization protects mice from developing asthma to house dust mite (HDM). Endotoxin exposure reduced allergen-induced epithelial cell cytokines that recruit and activate dendritic cells (DCs). Furthermore endotoxin treatment suppressed the proliferation of, newly developed, HDM-specific 1-DER T cells in the MLN and their production of IL-5 and IL-13, thereby suppressing type 2 immunity to HDM. Selective loss of the ubiquitin modifying enzyme TNFAIP3 in lung epithelium abolished the protective effect on asthma development. A single nucleotide polymorphism in the TNFAIP3 gene was associated with allergy and asthma risk in children living on farms, while asthmatics expressed lower levels of TNFAIP3 in barrier epithelial cells. Thus the farming environment protects from allergy development by modifying the communication between barrier epithelial cells and DCs, via induction of TNFAIP3.
Recurrent airway obstruction, Th17 and IL-26

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Recurrent airway obstruction occurs in several mammals including humans and horses. Evolving evidence suggests a number of different endotypes of this condition, frequently referred to as asthma in humans. These endotypes are signified by different types of inflammatory events associated with more or less specific clinical phenotypes. The most well-characterized of these endotypes in humans displays T helper cell (Th) 2 activity associated with eosinophilic inflammation and a clinical phenotype that frequently responds well to inhaled glucocorticoids. A less well-characterized endotype in humans displays Th17 activity associated with neutrophilic inflammation and a clinical phenotype with highly variable airway obstruction and relatively poor sensitivity to inhaled glucocorticoids. So far, the latter endotype has been assessed mainly as cytokine signaling via the archetype Th17 cytokine interleukin (IL)-17A and there is data from horses along these lines as well. Given that IL-26, although in a different manner compared with IL-17A, exerts effects on the mobilization of neutrophils, it can be anticipated that airway disorders displaying an excess of these innate effector cells may also involve IL-26. In line with this, recently published evidence suggests that the Th17 cytokine IL-26 is involved in pulmonary host defence as well as in asthma among humans. However, one published report suggests that the IL-26 gene is inactivated in certain mammals including horses. Collectively, the reviewed evidence forwards a rationale for targeting IL-17A in reversible airway obstruction of both humans and horses. In contrast, the corresponding rationale for targeting IL-26 may be restricted to humans. Further study of these therapeutic approaches is warranted.

interleukin, neutrophil, glucocorticoid

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Effects of stables on human health

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Keywords: horse, environment, dust

Many people are highly engaged in the equine industry around the world. Consequently, they spend a considerable portion of their day in stable environments, either as workers in the care and training of horses, or in their leisure time. Exposure to organic dust, microorganisms and endotoxins from different farm animal stabling systems can cause respiratory disease in humans as rhinitis, asthma, bronchitis, or intrinsic lung disorders such as farmer’s lung/allergic alveolitis. Studies of conventional stables have shown that recommended levels of endotoxin, which may cause inflammation in human airways, are often exceeded (1). The type of building, type of feed, bedding and sanitary procedures, can have a complex influence on stable-air quality (1).

For those working and living on the horse farm, whether the farm environment is a risk factor for developing allergic disease and asthma is under debate. There are several studies now showing that working in a stable can have adverse health effects on the respiratory function similar to those experienced by farmers (2, 3, 4).

Primary prevention in the stable should involve reduced exposure to organic dust, irritating gases, and microbial agents. Feeding and cleaning activities can be done in a way that creates less dust in stable air. Also ventilation is important, where the installation of mechanical ventilation can increase the air exchange rate and reduce particles and gaseous substances in stable air (3).

Secondary preventive measures of stable-related airway disease could involve pre-employment testing of stable workers followed by medical advice for those with a risk of developing airway-related diseases, such as hay fever and asthma. The examination could include a medical history (allergy and airway disease), physical examination of the airways and skin disorders (eczema), a pulmonary function test and an allergen test.

Feasibility of particulate reduction in animal housing

1 Hedelin A, Sundblad B-M, Sahlander K, Wilkinson K, Seisenbaeva G, Kessler V, Larsson K, Palmberg L. Lena Palmberg MD, PhD and associate professor Karolinska Institutet Sweden Lena.palmberg@ki.se Keywords: Innate immunity, Particle separator, Inflammation

Farmers have an increased prevalence of chronic bronchitis and chronic obstructive pulmonary disease. Also, we have shown that pig farmers have altered innate immune responses1. Additionally, the effect of using respiratory personal protective equipment when exposed in farming environment was able to reduce the inflammatory response in the respiratory tract2. This indicates that the adverse effects seen in humans exposed to pig farm environments is likely caused by exposure to environmentally related particulate matter.

The aim of the study was to evaluate the health effects from exposure measurements performed in stables both with and without the use of particle separator. If the use of a particle separator both reduces the exposure to particles and have health impacts, the work environment for farmers will be improved.

Eleven healthy participants were exposed in a cross-over design including 2–3 weeks wash-out between the 2 exposures in 2 different facilities, with and without installed particle separators,

The number of fine particles sized 0.3–0.5 µm, was reduced in the particle separated building environment (PSE) compared with that in the conventional building (CE). In CE both headache (p=0.03) and increased body temperature (p=0.016) were more pronounced than in the PSE. Compared with PSE, exposure in conventional stable yielded higher interleukin (IL)-6 (p=0.02) and IL-8 (p=0.04) levels in the upper respiratory tract, as assessed by nasal lavage.

After installing particle separators in swine stables the number of fine particles were reduced. In naïve never exposed volunteers, this reduction resulted in reduced adverse health effects caused by acute exposure in swine buildings with particle separators compared with exposure to the conventional swine building environment.

**Airway Microbiome in human disease**
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Keywords: Microbiome, Bacterial communities, Microbiota

The respiratory microbiota is less well studied compared microorganisms in other human microbiomes, this is partly due to accessibility of samples and partly due to the mistaken belief that the healthy lungs are a sterile environment. This myth has been conclusively debunked over the last decade with the increased availability and decrease in costs associated with a range of culture independent techniques. It is now clear that the respiratory tract contains a diverse and dynamic microbial community in both health and disease.

Understanding the nature of the microbiota within the healthy respiratory tract has allowed investigations into how disease and other community perturbations, such as smoking or antibiotics, effect microbial communities. Large amounts of variation are observed in lung microbial communities between individuals, however research has revealed alterations in bacterial communities associated with specific disease. These alterations include, changes in bacterial diversity, community composition and dominance of specific genus.

This talk will introduce and discuss the current understanding of the human respiratory microbiome with reference to both healthy and diseased populations. I will present methodologies and experimental design of microbiome studies using both published and newly generated data, alongside the results of new research on children with persistent bacterial bronchitis and adults with chronic obstructive pulmonary disorder.
The lung microbiome in equine asthma

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Keywords: bacterial microbiota, pulmonary

There is evidence that lung microbiome differs between asthmatic patients and healthy subjects (1), and that microbial dysbiosis could play a role in asthma by contributing to the perpetuation of inflammation and altering response to therapy. However, the effect of environmental conditions and medication is unknown and difficult to control (1, 2). We hypothesized that A) the environment influences lung, nasal and oral bacterial microbiome and B) the microbiome in the equine asthmatic lung is primarily affected by disease status.

Six asthmatic horses and 6 controls receiving no medication were kept on pasture (“Low antigen exposure”), then exposed to indoor allergens by being housed in a barn and fed good quality hay (“Moderate exposure”) and then poor quality hay (“High exposure”), in a cross-over design. Lung function was recorded and bronchoalveolar lavage, as well as oral and nasal rinses were collected. 16S rRNA gene sequencing was performed with the Illumina MiSeq platform. Sequences were analyzed using mothur (3) and the vegan package in R.

Horses with asthma developed airway obstruction and inflammation with moderate and high antigen exposure, while controls showed only mild inflammation in the high exposure environment. Lung, oral and nasal communities clustered strongly by environmental conditions (PERMANOVA, P=0.004, <0.001, 0.02). Lung communities were composed of a large number of low-abundance sequences and were not dominated by any specific operational taxonomic unit. Their composition however was significantly different between healthy and asthmatic horses at the family level of taxonomic designation (P=0.006), which was not the case for oral and nasal communities. Oral and nasal communities were distinct from the lung communities, and had greater richness and diversity.

This study shows that in these untreated animals housed together, pulmonary, oral and nasal microbiome is influenced by environmental conditions but that only pulmonary microbiome differs between horses with and without asthma. The difference between asthmatic and healthy horses was mainly present when airway inflammation was present in horses with asthma but not in controls, suggesting that the altered lung microbiome observed in asthma is not inherent but coincident with inflammation.

Funding: Natural Sciences and Engineering Research Council of Canada (#06090)

Keywords: respiratory viruses; airway inflammation; risk-factors

Mild Equine Asthma (MEA), which definition is largely debated during this symposium, is currently defined as a non-infectious disease (1). The potential role of viruses in either initiation or persistence of lower airway inflammation is however still uncertain and under epidemiological/experimental investigation.

Large epidemiological studies have been performed, based either on serological analyses or more recently on direct detection of viral genome by qPCR in various airway samples. Apart from γ-herpesviruses (EHV-2, EHV-5) which detection is ubiquitous in horse population; the somehow high (sero)prevalence of equine rhinitis-A virus (ERAV) and ERBV in athletic horses, and their significant association between viral detection and (clinical signs of) MEA are worthy of importance. Severity of the syndrome seems however not to be associated with the level of virus quantification in airway samples (2), and no cut-off values for viral loads have been validated to date, unlike bacterial counts in tracheal wash.

Alternatively, several experimental infections/reactivations on a limited number of horses have been performed with either equine influenza, ERAV or EHV-2; leading to the observation of respiratory clinical signs and/or abnormal cytological profiles persisting for up to 3 weeks post-challenge, as well as significant associations between genome virus detection and increased neutrophil proportions in the airways.

To date, very limited data are available on the underlying immune mechanisms of MEA, and most of Koch postulates are still to be fulfilled for defining infectious causality. The multiplicities of concomitant viral genome detection in the airways, as well as the coexistence of multiple strains of a single virus, are further confounding factors for the characterization of MEA physiopathology. There is though growing evidence that a number of respiratory viruses represent major extrinsic risk-factors of MEA, along with other environmental triggers. Since the etiology of MEA is yet very likely to multifactorial, the “whole picture” is still remaining to be characterized ...

Deciphering the evolution, epidemiology and virulence of *Streptococcus equi* towards the eradication of strangles

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Keywords: Strangles, *Streptococcus equi*, evolution, epidemiology, virulence

Strangles, caused by *Streptococcus equi*, is one of the most frequently diagnosed infectious diseases of horses worldwide. We have defined the global population structure of this important host-restricted pathogen revealing a population replacement in the late 19th or early 20th century. Our data provided new insights into the epidemiology of outbreaks at local, national and international levels. Strangles outbreaks can be complex, involving several different strains of *S. equi*, even within the same horse. We identified the origins of an outbreak of strangles in Norfolk and screened horses using diagnostic tests to minimise its impact. We describe the prevalence of strangles outbreaks throughout the UK and reveal the genetic relationships of *S. equi* strains from around the world. In particular, the genetic diversity of strains recovered from horses in the United Arab Emirates emphasises the benefits of screening for *S. equi* infection prior to the international movement of horses.

The development of new vaccines against strangles would lead to important health benefits for horses. A new experimental technique permits the simultaneous identification of every gene required for *S. equi* to cause disease. Our data are being used to enhance the safety and effectiveness of vaccines against strangles towards the gradual eradication of this disease from horse populations.
Room A
July 15\textsuperscript{th} - PM
Abstracts presentations
An overview of Exercise Induced Inspiratory Symptoms (EIIS) in humans, and a comparative approach to future understanding of upper respiratory tract collapse.

Zoe Fretheim-Kelly, Eric Strand, Ola Roksund, Thomas Halvorsen, John-Helge Heimdal BSc (hons) Physiotherapy, BVSc, MSc Veterinary Science Norwegian University of Life Sciences. Haukeland University Hospital, Norway zofr@nmbu.no  zoe.louise.kelly@helse-bergen.no Keywords: EILO, CLE- test, comparative medicine

EIIS are a relatively newly described set of pathologies causing breathing difficulties at exercise in otherwise healthy individuals. The majority of these pathologies are associated with transient, reversible laryngeal narrowing. Consequently these conditions are collectively labelled Exercise Induced Laryngeal Obstruction (EILO) (1). Human and equine patients present in a similar way: reduced athletic performance with dyspnea, frequently with stridor. In humans panic reactions can occur. Symptoms improve as soon as exercise is ceased (1). Prevalence in the general population is 5-7 % (2). Gold standard for diagnosis is treadmill endoscopy (1). The exact setup and monitoring varies between clinics, but most include real-time endoscopy and ECG. In humans respiratory flow loops, respiratory gas analysis, sound and video recordings are also used as well as alternative forms of exercise such as swimming, rowing or riding a bike (1, 3). As with equines, some forms of EILO appear to be positional or associated with a particular activity, hence the importance of recreating the situation that predisposes to collapse, as veterinarians do with tack and poll flexion in the treadmill test.

EILO is classified as supraglottic; involving the corniculate, cuneiform cartilages and aryepiglottic folds collapsing over the laryngeal vestibule, and glottic pertaining to inappropriate adduction of the vocal folds (4). There is no such anatomical division in equines but the same anatomical structures are involved. The underlying pathophysiology is considered multifactorial; most likely a predisposed phenotype being unable to support the soft tissue structures of an otherwise normal airway during the increased pressure gradients of exercise. With the most severe symptoms occurring at maximal exertion (1). In equines dynamic respiratory tract collapse is a well-documented group of pathologies, in humans, however they are relatively newly described and the significance of the collapse of certain structures has not been established. EILO has until recently been misdiagnosed as Exercise Induced Asthma, hysteria and a stress reaction (1, 4). A failure to establish precise nomenclature, diagnostic criteria and outcome measures has hampered current understanding of EILO (4). It is therefore hoped that by working collaboratively a better understanding of pathogenesis and treatment can be achieved for both equine and human. Current research in humans is focused on determining an outcome measure and more robust information about the severity of collapse. From experience in the equine world tracheal pressure measurements are now being introduced into EILO investigation. It is hoped that they will give an objective measure of collapse and an objective outcome measure. Other areas of research interest are in development of computer models and automated visual analysis techniques to improve diagnostics.

Treatment of EILO is dependent on the structures involved with supraglottic collapse being treated both conservatively and surgically, while glottic collapse is treated conservatively (1). Conservative treatments include both systemic and local medication, respiratory muscle training, breathing techniques, biofeedback and psychotherapy. Surgical techniques include laser supraglottoplasty and epiglottic augmentation (1).

Diagnosis and Surgery of Alar Fold Collapse in Horses

Eric Strand, Professor of Equine Surgery, Norwegian School of Veterinary Medicine, Norwegian University of Life Sciences.

From 1998 to 2013, 478 harness racehorses, 12 Thoroughbred racehorses and 2 Icelandic horses were referred to the Norwegian School of Veterinary Medicine and underwent a comprehensive, standardized dynamic evaluation of nasopharyngeal, laryngeal, and nostril regions on a high-speed treadmill (1). Twenty-five horses from this population were diagnosed with bilateral AFC and received alar fold (AF) resection surgery. AFC was diagnosed when a continuous abnormal expiratory vibrating flutter noise coincided with visible inspiratory filling of the nasal diverticulum throughout the exercise test, and when temporarily suturing the AFs in a dorsal position alleviated these signs. Surgery involved complete resection of both AFs, including three to five cm. of the ventral conchae to avoid any pocket formation at the bottom of the diverticulum nasi (2). The owners and/or trainers of all of the included horses were contacted by telephone, and racing records were obtained from the national official race statistics.

Presenting complaints according to the owners/trainers were poor performance (#7), abnormal respiratory noise (#10), or both (#8). Three of the 25 horses had previously undergone partial AF surgery, but the owners/trainers were not satisfied as the horses still had abnormal respiratory noise and perceived poor performance. The 25 AFC affected horses were from two to eight years of age at the time of surgery (mean 4.3 years). Seventeen horses (68 %) were male, nine stallions and eight geldings, and eight (32%) horses were female. Twenty-one horses (84%) were Standardbred horses, two were Coldblooded trotters, one was a Thoroughbred racehorse and one an Icelandic stallion used in high-speed gaited competitions. Ten of the Standardbreds, or 40 % of all horses in the study, were also diagnosed with intermittent dorsal displacement of the soft palate (iDDSP).

Eighteen of the 25 owners/trainers stated that the abnormal respiratory noise from the nostrils improved after resecting the AFs and that twenty of 25 horses had excellent cosmetic results. Twenty of 25 horses continued racing or competing after surgery. Fifteen horses (75 %) of these 20 horses improved their mean kilometre racing time performance demonstrating a beneficial effect of AF resection. Five horses did not return to racing after AF surgery for reasons not related to AFC.

Nasopharyngeal pressure was measured preoperatively (baseline and after tying open the alar folds) in five of the horses, and when racing again post-operatively in three of these horses. Our data demonstrates that AFC creates a dynamic expiratory obstruction (mean +33.5 cm H₂O). Tying open the AFs returns expiratory pharyngeal pressures to the normal range (mean +19.6 cm H₂O) and complete surgical resection seemingly improves the expiratory nasopharyngeal pressures further (mean +15.0 cm H₂O). Since iDDSP also creates a dynamic expiratory obstruction, and was diagnosed in 40% of the horses in this study, it is interesting to speculate whether there is a physiologic association between these disorders due to the restriction of airflow in the region of the nares. Nasopharyngeal pressure measurement shows promise as an objective test to screen horses for AFC.

Nasopharyngeal collapse - clinical presentation

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Keywords: Upper airway obstruction, nasopharynx, overground endoscopy

The nasopharynx (NP) is a sleeve of soft tissue that is maintained open only through the action of its intrinsic muscles and dependent on the indirect positioning of mobile cartilages. This particular configuration makes them highly susceptible to dynamic collapse when submitted to important transmural pressures, such as those physiologically encountered during exercise. Any local inflammation or infection of the mucosa, muscles or nerves of the pharynx or the guttural pouches, can affect NP stability as they disrupt NP function. Maintenance of patency can be further challenged by an abnormal position of adjacent anatomical structures such as tongue, hyoid bone, larynx, etc.... The biomechanics of the upper airways are inherently dependent on head and neck position and riding can significantly alter NP stability. For these reasons, resting upper airway endoscopy is not suitable to diagnose a nasopharyngeal collapse (NPC) and overground endoscopy during ridden exercise is considered to be the “gold standard” for making a definitive diagnosis.

A number of forms of NPC have been described in exercising horses. The most common is dorsal displacement of the soft palate (DDSP). Palatal instability is thought to be a precursor to DDSP. The pathophysiology of all forms of NPC, although not fully elucidated, generally involves neuromuscular dysfunction. NPC induces significant alterations in upper airway pressures, a decrease in airflow and an increase resistance. It has been associated to poor performance both in racehorses and sport horses.

NPC can also cause respiratory noise, but it is not a constant clinical sign, especially in sport horses that work at submaximal levels. NPC can be exacerbated by lower airway inflammation and thus be associated to cough. Vibration of the NP walls or of the soft palate with or without inflammation can trigger the cough and a motive of referral can be cough during exercise. NPC can also be associated to local pain or high mucosal sensitivity. In these circumstances, normal ridden work can become challenging: horses can display anxious behavior or defenses during work, seeking to open head and neck angle, pull on the reins or avoid the bit. Symptoms can be worsened when riders use more coercive tack or equitation. Alleviating rider intervention, changing tack or local anesthesia can help reduce or resolve NPC. On the opposite side of the scale, some horses have NPC as a consequence of a local proprioceptive deficit; these horses will display more stability when the NP is stimulated.

As the use of overground endoscopy expands, we will understand more of the complexity of NP biomechanics, its clinical translation and applicable therapeutic possibilities, including physical rehabilitation.
2017 Non-RLN Collapse - Recognition and Treatment
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Introduction: There are many laryngeal collapses whom by the noise they created or their endoscopic appearance at exercise resemble RLN prompting our human counterparts to suggest the term “laryngeal movement disorder”. (1) The underlying pathophysiology of these laryngeal collapses is poorly understood in some cases and are the subject of hypothesis and not evidence-based data above level 4.

Objectives: Review our experiences on horses with dynamic laryngeal collapse at exercise with no significant evidence of recurrent laryngeal neuropathy at either Cornell clinic.

Methods: Cases were reviewed from the dynamic (treadmill or overground) from each electronic medical record (EMR) from 2010-2017 and videoendoscopic images retrieved.

Results and discussion: Five presentations of laryngeal collapse were observed. Type I referred to horses where the corniculate process displaces medially into the airway because (hypothesis) of an apparent structural weakness at the junction of the corniculate and the body of the arytenoid cartilage. Indeed, we propose that a greater percentage of elastic content is present at the junction between the body and corniculate cartilage leading to the collapse at exercise. Although we have limited experience, these horses do not respond to a laryngoplasty as the same degree and location of collapse is observed after surgery since the collapse it is not due to a failure of abduction by the CAD muscle. Treatment at this time is still experimental.

Type II refers to bilateral laryngeal collapse has been reported in the Norwegian cold blood trotters, (2) and we have observed it in saddlebred, Morgan, Hackney pony, warmblood and Thoroughbred racehorses. Dr. Catherine Fjorbakk’s PhD thesis is an exhaustive explanation of the pathophysiology of the condition. (3,4) It is associated with poll flexion leading to an external compression of the larynx. This occurs because poll flexion leads to a more cephalic position of the larynx and narrowing of the laryngeal diameter. The source of the intra-mandibular external compression of the larynx is not identified but is obvious on the CT exam. Although some histopathological evidence of RLN is present, the prevalence of this histopathological lesion is similar to those of non-affected controls. These animals also do not respond well to ventriculocordectomy with or without laryngoplasty. A device that restricts poll flexion has been shown to resolve this problem. (5)

A more recently described form of laryngeal collapse is recognized with a ventro-medial luxation of the apex of the corniculate process of the arytenoid cartilage (VMAD). (6) The condition may be unilateral or bilateral, may be obstructive or not and can be observed in horses that also have RLN although this is rare in the author’s experience. In humans a similar endoscopic appearance is observed associated with a posterior (i.e., dorsal) cleft of the cricoid cartilage. We have not performed a post mortem exam in any affected horses but have performed esophageal ultrasound in one case and failed to detect a cricoid cleft. Barakzai has reported the post mortem exam of an affected Clydesdale horse and has suggested that the condition is due to an abnormally wide transverse arytenoid ligament. Treatment at this time is still experimental.

Horses affected with this condition present with various degrees of restriction or inability to abduct their affected arytenoid cartilage(s). The condition can be unilateral or bilateral. Current evidence is this condition is a result of a progressive infection following mucosal injury to the body of the arytenoid cartilages. The cause of the direct mucosal trauma has been associated with direct trauma from endoscopy, attempts at nasogastric intubation, inhaled foreign body (from kickback on dirt track?), or more violent evidence of coughing when both vocal processes contact each other. Excessive vocalization has been hypothesized as a cause. The author has observed an association between sub-epiglottic lesions and these mucosal lesions: by inspection it appears that the ventral-lateral edge of the epiglottis can contact the medial surface of the arytenoid cartilage and creates a mucosal ulceration.
Type IV is arytenoid chondritis of the arytenoid cartilage is believed to start with a mucosal ulcer which extends through the basal membrane into the arytenoid cartilage. The etiopathogenesis was pursued in an experimental study where mucosal ulcers were created by nasotracheal intubation in 21 horses.(7) In 52% of these horses, the lesions were healed within one week and another in three weeks. None of the horses developed granuloma nor clinically evident chondritis. This is consistent with the clinical findings that these mucosal lesions are rare and only a small percentage of horses with mucosal injury progress to intraluminal granuloma or chondritis. Smith et al., 2006 reported in a large survey of 2317 New Zealand Thoroughbred racehorses during sales (range 15-24 months of age) only 33 horses (1.2%) had arytenoid mucosal lesions including five with overt chondritis. Horses with mucosal lesions were not statically different than control in terms of number of horses that raced. Kelly et al., reported on 3312 post sales endoscopies in Australian Thoroughbreds and 21 horses (0.63%) had arytenoid mucosal lesions.(8) Five of the 21 were known to develop intraluminal granulomas and only one of them developed chondropathy. The low percentage of mucosal ulcers progressing to arytenoid chondritis is also consistent with the low prevalence of chondritis in Thoroughbred racehorses ranging from 0.21 to 0.22%. Management was either by removal of granuloma or by a partial arytenoidectomy.

The last type (Type V) of laryngeal collapse is laryngeal dysplasia- this is well described elsewhere.

References

Room B

July 15th - PM
DDSP - expanding etiology
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Introduction: There are two forms of DDSP: intermittent (iDDSP) associated with airway obstruction and persistent (pDDSP) associated with airway obstruction and often dysphagia. DDSP results in a marked airway obstruction during exhalation and therefore, performance impairment occurs quickly after the occurrence of palate displacement which is referred to using terms such as “choking down” or “hitting a wall” by trainers and/or riders, The dysphagia may be associated with a neurological dysfunction of the soft palate or it may be a result of tracheal aspiration leading to tracheitis.

Objectives: Review of the literature on etiopathological causes of DDSP and contrast those with our experiences in clinical patients at either Cornell clinic.

Methods: Cases tabulated from electronic medical records (EMR).

Results and discussion:
There are multiple etiopathogenesis to this condition. The evidence-based data that we have are from experimental studies in experimental horses where three reproducible models of this disease have been reported: 1) desensitization of the pharyngeal branch of the vagus(1,2) 2) bilateral resection of the thyrohyoideus muscle(3), and 3) bilateral desensitization of the hypoglossal nerves at the level of the ceratohyoid bones.(4) The first model introduced by Holcombe et al., 1998 hypothesized that DDSP was secondary to a dysfunction of the palatinus and palatopharyngeus muscle. The model did reproduce DDSP at rest and at exercise and therefore was a more severe form of palate displacement than most naturally-occurring cases. However this model also resulted in dysphagia which is not a feature of iDDSP. In this model the cause of DDSP is a neuromuscular dysfunction of the nasopharynx. In their study glossopharyngeal and hypoglossal desensitization at the level of the gullet pouch did not result in DDSP.(5) The latter two models were able to recreate iDDSP and do so only at exercise and without dysphagia. These latter two models of DDSP indicated that factors other than the intrinsic neuromuscular structure of the nasopharynx were implicated in iDDSP namely the thyrohyoideus muscle and/or one or more of the following muscles: genioglossus, styloglossus, geniohyoideus and hyoglossus. More recent observations supported that early muscle fatigue may be at the root cause of some iDDSP.(6) The current working hypothesis is that cervical descent of the larynx is the underlying cause of iDDSP. Strap muscle resection and laryngeal tie-forward appears to be effective in preventing caudal descent of the larynx.

The non evidence-based data are centered on observation of intermittent or persistent DDSP associated with structural abnormalities surrounding the soft palate and/or epiglottic areas: palatal cysts, sub-epiglottic masses, granuloma and epiglottic chondritis. These structural abnormalities as a cause for DDSP are anecdotal, yet frequently observed. The mechanisms by which they are associated with displacement was thought to be structural: interfering with laryngo-palatal seals. However, we have observed recently that sensory input may play a role because blocking the sensory afferent results in near immediate resolution of the palate displacement on endoscopy.

In summary, at the level of the nasopharynx, neuromuscular deficits, sensory abnormalities, and structures that interfere with the laryngo-palatal seal represent the intrinsic pathway to DDSP. Extrinsinc pathway appears to be tied to early fatigue of the external musculature that controls the position of the larynx.
References

**Longitudinal overground endoscopy findings for conservative management of IDDSP**

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Key words: overground endoscopy, palate, horse, longitudinal, conservative

**Introduction and background**

Intermittent dorsal displacement of the soft palate (IDDSP) is the most commonly observed abnormality of the upper portion of the respiratory tract (URT) in Thoroughbred racehorses and has been estimated to affect between 10-20% of two to three year olds. Despite this high prevalence, the aetiology of the condition is poorly understood.

Multiple conservative treatment approaches for IDDSP are advocated by trainers and veterinary surgeons. Studies investigating the effectiveness of conservative treatments have been performed. However, these studies have relied on measures of athletic performance after treatment, rather than endoscopic diagnosis during exercise. With the introduction of overground endoscopy, it is now possible to easily examine the URT of horses, during exercise in their normal training environment.

**Materials and Methods**

The study was conducted on a Thoroughbred training yard in the UK, training horses predominantly for the flat racing industry, over a period of 5 seasons.

Palate displacement was categorised as:

1. Sustained IDDSP for more than 8 seconds, or horse repeatedly displaced, with any swallowing of limited effect.
2. Any displacement – short periods of displacement (>8 seconds), easily replaced and maintained in correct position.
3. Displacement at pull up only (as horse slowed down).
4. Palatal instability.

The study was divided into two phases with the first assessing the prevalence and progression of IDDSP, in a group of young horses, over time, and the second, a crossover randomised-factorial study design, assessing the effect of several conservative treatments on IDDSP.

**Summary of results**

Overall 73 of 104 horses (70.2%), with an overground endoscopic diagnosis of IDDSP, either stopped displacing or displaced at pull up only (including horses with either no evidence of IDDSP and those that displaced their palates as they pulled up). In this group of horses, individuals with evidence of widespread upper airway instability, such as vocal cord prolapse (VCP) and medial deviation of the aryepiglottic folds (MDAF) during the initial examination were more likely to have complete resolution of IDDSP by the final examination.

Eighty-three horses have been recruited to phase two of the study to date, the findings suggest that in the short-term, the use of a grackle and Cornell collar prevent IDDSP during exercise but that a tongue tie is not effective.

**Discussion/Conclusions**

In conclusion, the findings reported here suggest that improvement or resolution of IDDSP can be expected in a significant number of horses with an overground endoscopic diagnosis of the condition, and that conservative methods of treatment for IDDSP are probably no more effective than further training and time. Consequently surgical treatment for IDDSP may not be necessary in the majority of young horses affected by this condition.
Nasal Obstruction

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Key Words: Nasal, Obstruction, horse, airway, performance

Diseases that result in nasal obstruction in the horse are perhaps of greater clinical significance compared to other species due to the anatomical arrangement of the upper portion of the equine airway and the fact that the horse is an obligate nasal breather. Diseases affecting this region may result in impairment of nasal airflow and may ultimately lead to loss of performance.

The requirement for rapid delivery of large volumes of air, at the correct temperature and humidity to facilitate maximal athletic performance in horses necessitates an increase in airflow of up to 60 times resting values. Much of the upper portion of the equine airway is narrow and unsupported by cartilage or bone. This leads to significant airflow resistance and 80 - 90% of this resistance is thought to occur in the nasal cavity. Nasal airway diameter is increased during exercise by dilation of the nostrils, retraction of the false nostrils and vasoconstriction of the venous plexi covering the nasal conchae. The nasal cavity is divided in two by the nasal septum. Composed of hyaline cartilage and resting on the incisive bones rostrally and the maxilla and vomer bones caudally, this structure may be deviated or become thickened in disease resulting in obstruction. The dorsal and ventral conchae divide the nasal cavity longitudinally into the dorsal, middle and ventral meati and the nasal cavity communicates with the pharynx via the choanae. The two nasal conchae or turbinates of the horse consist of single scrolls of thin bone of which the dorsal concha is the largest.

Several diseases of the nasal cavities lead to significant stenosis and therefore obstruction to airflow. Clinical signs of nasal obstruction include abnormal respiratory noise, occurring both during inspiration and expiration, loss of performance, facial distortion, nasal discharge and epistaxis. Traumatic injuries, congenital and acquired diseases have all been implicated as have a few conditions which are only apparent during high speed exercise. Diagnosis of diseases of the nasal passages requires careful clinical examination, endoscopy, both at rest and during exercise and imaging. Plain radiographs, while often effective have been greatly enhanced in recent times by the increasing availability of computed tomography, commonly performed in the standing horse.

Treatment of disease of the nasal passages can be challenging, complex three-dimensional anatomy, the narrow nature of the structures concerned and limited access for standard instrumentation can make surgical manipulation difficult. In recent times innovations such as the introduction of surgery performed with the patient standing and sedated, advanced imaging modalities and the use of LASER surgery, has greatly enhanced the ability of veterinary surgeons to obtain positive outcomes when treating problems in this region.
Abstracts and Posters in Alphabetic Order after Title
Title: A hydrosoluble form of curcumin (NDS27) limits morphological changes in equine neutrophils induced by ex vivo exposure to lipopolysaccharides

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Introduction:
NDS27 is a soluble curcumin derivate that can be administered by inhalation. It is able to reduce neutrophil numbers in broncho-alveolar lavage fluids (BALF) from horses naturally affected by heaves or experimentally exposed to lipopolysaccharides. Further, it reduces the myelo-peroxidase concentration in BALF. Delayed apoptosis is the hallmark of heaves and although effective to reduce clinical signs and neutrophil counts in affected horses, the possibility that NDS27 induces neutrophil apoptosis still needs to be proven.

Objectives:
The aim of the study is to document the effect of NDS27 on the morphology of neutrophils exposed to lipopolysaccharides (LPS) ex vivo.

Methods:
BALFs were recovered from three different horses admitted to Equine Hospital for chronic inflammation of the lower airways. Cells (10^7) were placed in culture dishes filled with 10 mL of PBS and incubated for 1 hour at 37°C, either with NDS27 120 µg (Bioptis, Belgium), or with LPS 25µg (Sigma Alderich, Belgium) or both agents together. Controls cells stayed in PBS solution only. After incubation, cells were centrifuged onto microscope slides using Cytospin 4 (Thermo Electron, UK). Slides were fixed in fresh methanol for 10 s and stained with a fast May-Grunwald-Giemsa stain kit (RAL555, MSN, Belgium). Stained cells were imaged using a light microscope Microscope (Eclipse E800, Nicon Belux, Belgium) and characterized into cells with typical lobulated morphology of the nucleus and neutrophils with clumped, rounded and lightened nucleus.

Results:
Cells exposed to NDS27 only and controls cells showed 100% of neutrophils having a nucleus with typical lobulated morphology. Neutrophils exposed to LPS only showed 100 % modified nucleus morphology, while in NDS27 treated and LPS exposed neutrophils neutrophil morphology was heterogonous, with 18% maintaining their initial morphology.

Conclusions:
Further studies should investigate whether the process initiated is apoptosis, necrosis or NETosis and at what concentration NDS27 is effective to favour neutral morphology of neutrophils exposed to LPS.
Title: A Novel Adaptation of a Monopolar Electrosurgical Triangle Tip Knife for Transendoscopic Ventriculocordectomy in Horses

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Introduction:
Ventriculocordectomy (VC) is an efficacious technique for the supportive treatment of recurrent laryngeal neuropathy. Traditionally, VC is performed via diode laser or sharp dissection through laryngotomy incision. Adaptation of other minimally invasive transendoscopic devices to perform VC would allow a cost-effective alternative to increase availability of transendoscopic VC in practice.

Objectives:
Describe and evaluate the safety and feasibility of a transendoscopic electrocautery triangular-tip knife for performing VC.

Methods:
The triangle tip knife was applied in contact fashion to perform VC in all horses (Figure 1). The technique was performed in two-cadaver heads prior to use in live animals. Left VC was then performed under standing sedation in 9 donated horses (Figure 1) and in 3 clinical cases (1 thoroughbred racehorse and 2 draft show horses) with recurrent laryngeal neuropathy along with prosthetic laryngoplasty. Endoscopic images of the upper airways of the donated horses were blindly graded for inflammation by three board-certified surgeons at pre-op, immediately post-op, 24-hours post-op, and in two cases at 7 and 14 days post-op. Histopathology of four larynxes was performed on two 24-hour and two 14-day specimens with Hematoxylin & Eosin, and Masson’s Trichome stain to evaluate for inflammation and collagen as suggestion of fibrosis (Figure 2).

Results:
VC was successfully performed in all horses. Endoscopic grading revealed a median score of normal (pre-op), mild (immediately post-op), mild (24-hours post-op), mild (7-days post-op) and normal (14-days post-op), respectively. Histopathological grading revealed a median of commonly seen inflammation at 24-hours and 14-days post-op (Figure 2B). Commonly increase in collagen deposition (fibrosis) was noted at 14-days, post-op (Figure 2C). The clinical cases returned to training successfully.

Conclusions:
The novel adaptation of an electrocautery triangle tip knife appears to be a safe and feasible technique to effectively perform VC in horses. Further work comparing efficacy of electrocautery VC versus diode laser VC is warranted.

Figure 1. The triangle-tip knife passed throughout the biopsy channel of the endoscope (A). Endoscopic images of the initial proximal and distal transsections of the left vocal fold using the triangle-tip knife in contact fashion (B) and left ventriculocordectomy immediately post-operatively (C) in one of the healthy horses.
Title: A Novel and Cost Effective Dynamic Endoscopy Unit for Evaluation of Upper Airway Abnormalities in Horses

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Introduction:
Dynamic endoscopy is a useful diagnostic technique for evaluation of multiple upper airway disorders not apparent at rest in horses. However, dynamic endoscopy equipment is not readily available to many practitioners in the field due to expense.

Objectives:
To describe the efficacy of a custom made and cost-effective dynamic endoscopic unit for the evaluation of the upper airway in horses.

Methods:
The endoscopic unit was built using a custom made design connected to a digital recording device that allowed visualization and noise recording of the upper airway while the horses were resting and exercising (Figure 1). The dynamic endoscopic examination was performed at the racetrack for 7 horses (Figure 1B), in a swimming pool in 2 horses (Figure 1C) and in a sand arena for 1 horse. Only first try placement, single evaluation attempts were performed, and compared to current gold standard dynamic endoscopic unit using similar criteria. Quality was graded by 3 ACVS surgeons using a 4-point scale to assess quality of the video in the non-clinical cases (n=9). Statistical analysis was performed comparing individual groups and between groups with significance established if p<0.05.

Results:
The custom dynamic endoscopy unit was successfully used in all scenarios to perform upper airway endoscopy at exercise and it was well tolerated in all horses. Overall quality of visualization had a median of 1 (=good) in both groups. One of the healthy horses that was examined had dorsal displacement of the soft palate (Figure 1B insert) visualized at rest and during exercise, although no noise or history of poor performance was present. The clinical case examined was diagnosed with pharyngeal wall collapse and respiratory noise was evident while swimming and was identified by the trainer to be the same noise reported while the horse is routinely working and racing. There was no statistical significance in any correlations evaluated (p>0.05).

Conclusions:
This novel dynamic endoscopic unit can be used successfully in horses to evaluate the upper airway at rest and during exercise in a variety of scenarios. Additionally, the visualization and quality of the video images are comparable to the current gold standard unit during first placement/attempt. Further work comparing both endoscopic units in clinical cases is warranted.
**Title:** Efficacy studies of Strangvac®, a vaccine against strangles

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**Introduction**

Strangles, caused by *Streptococcus equi*, is a serious and contagious infection of the horse. It causes severe suffering and the disease has great economic importance as a single index case can subsequently affect a whole riding facility. Vaccines, based on attenuated strains, have shown occasional serious side effects. A novel vaccine, Strangvac®, composed of recombinant proteins derived from *S. equi* has been developed and subjected to clinical trials.

**Objectives**

The objectives of these studies were to 1) quantify the efficacy of Strangvac® against experimentally induced strangles, 2) confirm the lack of adverse events and 3) measure long-term immunologic memory.

**Methods**

Vaccination: Ponies were vaccinated with 2 ml Strangvac® intramuscularly on two occasions, 4 weeks apart. One group was given a re-vaccination three months later. Ponies in control groups were given placebo, containing only adjuvans (MatrixC, Novavax). Challenge: ponies were experimentally infected by application of $10^8$ CFU *S. equi* intranasally two weeks after last vaccination. In one group challenge was given two months after basic vaccination. Injection site score, pyrexia, clinical signs of disease and inflammatory markers were followed for 21 days or until euthanasia. Post-mortem examinations were performed to measure the severity of disease. The incidence of strangles was defined as pyrexia ($≥39°C$) for two days over a three-day period.

**Results**

The studies showed that Strangvac significantly protected ponies against experimentally induced strangles. The onset of immunity of two weeks after re-vaccination study found that Strangvac conferred 94% ($p<0.0001$) protection. When challenged two months after basic vaccination, without a booster vaccination, the level of protection was approximately 60%. Clinical observations and post-mortem scoring supported the observations based on pyrexia. All placebo ponies were pyretic after challenge. Ponies which had been vaccinated, but where Strangvac did not completely protect them from strangles, showed significantly reduced clinical signs of disease ($p=0.002$), lower inflammatory marker levels ($p<0.001$) and lower post-mortem scores ($p=0.006$). An additional study demonstrated the presence of immunological memory for at least six months; where a booster effect was observed upon revaccination.

**Conclusions**

These studies show that Strangvac is safe and efficacious and could play a significant role in the prevention of strangles in horses.
Title: An Investigation of Neonatal Dysphagic Foals—Association with Environmental Chemical Exposure

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Introduction:
We investigated a farm clustering of neonatal dysphagia (increasing prevalence from 25% to 92% over 5 years) at a broodmare farm located near an active unconventional natural gas development (UNGD) site in PA. At a separate broodmare facility in NY, owned by the same individual and not situated near UNGD activities, similar health problems had not occurred in foals leading us to hypothesize that environmental chemical exposure was associated with the neurological deficits in foals.

Objectives:
We sought (1) to quantify air- and water-borne environmental chemicals and (2) to determine if alterations in physical exam and clinical laboratory parameters of newborn foals reflected exposure to environmental chemical stressors. For the latter, we sought evidence of systemic inflammation, hepatopathy or renal dysfunction, oxidative stress, disruption of thyroid hormone biosynthesis or alterations in steroid hormone biosynthesis.

Methods:
Over the study period (12/2014-7/2016), environmental and biological testing was performed every 6 weeks on both farms: We obtained grab samples of water, feed and soil and deployed passive sampling devices (PSDs) in ambient air, well-water and attached to mares’ halters to detect bioavailable organic contaminants, including polycyclic aromatic hydrocarbons, in the environment. From the mares, blood and milk samples were obtained at foaling and during pregnancy. From the neonates, samples of blood, hair and urine were obtained; thereafter, blood samples were taken every 6 weeks until foals were weaned. Biological data were screened by Mann-Whitney tests to detect differences between dysphagic and non-dysphagic foals.

Results:
Dams of affected foals spent a greater percentage of their total pregnancy, their 2nd and 3rd trimesters at the PA farm. No differences between the two groups of foals were detected in gestational age, birth weight, biochemistry data, thyroid hormones, selenium and vitamin E concentrations or in blood glutathione peroxidase activity. Serum barium and strontium concentrations in PA newborn foals exceeded those in control foals. Polycyclic aromatic hydrocarbon concentrations in well-water-PSDs were 2-20X greater at the PA farm.

Conclusions:
Analysis of serum samples for polycyclic aromatic hydrocarbons is underway to detect an association with dysphagia. Our preliminary results suggest that there may be adverse health risks to horses residing near UNGD facilities during last two trimesters of gestation. (Funded by NIEHS).
Title: Anti-microbial and additional anti-inflammatory effects of inhaled essential oils in horses treated for IAD

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Introduction:
Inflammatory airway disease (IAD) is highly prevalent in the working equine population. Because travelling, exercise and competition have been shown to alter the immune system; equine athletes are more vulnerable to infection. The use of medication, including antibiotics, is strictly regulated and there is a need to find alternatives for preventing and managing risks to these horses’ respiratory system.

Objectives:
The aim of this controlled clinical study was to evaluate the antimicrobial and anti-inflammatory effect of an inhalable solution (IS) used in adjunction to inhaled IAD therapy, in horses with IAD. The IS contained essential oils (Eucalyptus globulus leaf oil, Eucalyptus radiate, Melaleuca alternifolia leaf oil, Mentha arvensis, Lavandula hybrida, Rosmarinus officinalis, 1,8-cineole, Cinnamomum camphora) selected for their antiseptic and immuno-stimulant properties.

Methods:
Thirty-nine competitive sport horses diagnosed with IAD and bacterial and/or fungal infection were included in the study. Horses were randomly assigned to three groups: all three received inhaled corticosteroids (C) for 3 weeks and a specific antimicrobial (AM) for a maximum of 10 days based on tracheal wash (TW) culture results. For group CAM, this was the only treatment (CAM group, n=15). Groups EO and IS received two different essential oils in addition to this therapy: group EO received a commercially available essential oil (n=8) and group IS received the combination of essential oils described above (n=16). Respiratory endoscopy, TW cytology, bacteriology and mycology and broncho-alveolar lavage (BAL) cytology were done before and after therapy.

Results:
All horses from the CAM and the EO group had a positive post-treatment bacterial or fungal culture. The isolated pathogenic strains differed from the ones targeted by the initial antimicrobial therapy. Only 1/16 horse from the IS group had positive TW bacteriology (Pseudomonas sp.). The TW neutrophil count was significantly decreased only in horses receiving IS (26% to 17%) in comparison to the other groups (CAM, 31% to 23%; EO, 36%to 26%). BAL neutrophils decreased similarly in all groups, as expected with the corticosteroid treatment.

Discussion:
This study shows that despite initial antimicrobial therapy, inhaled corticotherapy during a 3-week period in sport horses with IAD increases the risk of secondary microbial infection in the airways. The use of IS reduced that risk whereas a solution with a single EO was insufficient.

Conclusions:
The use of a IS containing a combination of essential oils has a preventative anti-microbial and additional anti-inflammatory effect in competitive horses treated for IAD.
Title: Arytenoid cartilage abduction in sports horses undergoing prosthetic laryngoplasty and its relation to post-operative inspiratory noise and performance

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Introduction:
Prosthetic laryngoplasty (LP) is a frequent treatment in horses with recurrent laryngeal neuropathy. Studies suggest that maximal arytenoid cartilage abduction is not required to restore ventilatory function to normal; this may be particularly true for sports horses, which perform less high-speed exercise than racehorses.

Objectives:
To 1) assess degree of arytenoid cartilage abduction loss after LP in sports horses, and 2) to correlate post-operative performance and inspiratory noise with degree of arytenoid abduction.

Methods:
Non-racehorses undergoing left-sided laryngoplasty and ipsilateral vetriculectomy were recruited retrospectively. Horses were included, if the following information was available: pre-operative Havemeyer endoscopic laryngeal function grade (I-IV, subgrades not used), and post-operative arytenoid cartilage abduction grade (1-5) obtained before discharge (normally 6 days after surgery). For a subset of horses, resting endoscopy was also performed 1-9 years after LP and arytenoid abduction graded. Owners subjectively assessed post-operative performance (better, worse, maintained) and inspiratory noise (unchanged, reduced, absent, worse). Standard non-parametric statistical analyses were performed.

Results:
A total of 94 horses were included (mean age at LP = 6.4 years). Of these, 51 had long-term endoscopic follow-up (performed after a mean of 3.1 years). The mean Havemeyer grade was 3.4. Mean arytenoid cartilage abduction grade assessed before discharge was 2.4; mean grade at long-term follow-up was 3.3. The Havemeyer laryngeal function grade was significantly correlated with the arytenoid cartilage abduction grade assessed before discharge (p = 0.0041, Spearman r = 0.3). The arytenoid cartilage abduction grades assessed before discharge and long-term correlated significantly (p = 0.0023, Spearman r = 0.45). Post-operative performance was assessed to be maintained (n = 19) or improved (n = 50) in all but one horse. Performance was not associated with Havemeyer grade or short- or long-term cartilage abduction grades (P > 0.05), nor was inspiratory noise.

Conclusions:
The arytenoid cartilage abduction grade after LP in sport horses decreased almost a full grade 1 to 9 years after surgery. The Havemeyer laryngeal function grade and the arytenoid cartilage abduction grade were not associated with post-operative performance. Achieving maximal arytenoid cartilage abduction thus seems to not be necessary in sports horses when performing LP.
Title: Asthmatic diseases as an underlying cause of dorsal displacement of the soft palate in horses

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Introduction:
Dorsal displacement of the soft palate (DDSP) is a common upper respiratory tract (URT) functional disorder. As it has been described in previous studies, DDSP in sport and race horses can be attributed to different causes, and its treatment has to be adjusted accordingly. In the literature, these causes are often divided into two groups depending on whether they are related to the intrinsic muscles or to the extrinsic palatal musculature.

Objectives:
The aim of the current report is to discuss asthmatic diseases as a possible aetiology of DDSP in horses.

Methods:
13 pleasure horses with a history of asthmatic diseases were incorporated in the study. All horses were examined in the exacerbation phase of the asthmatic disease. In all cases we performed a bronchoalveolar lavage (BAL) cytology and tracheal lavage (TL) bacteriology. The URT was evaluated during rest and during overground endoscopic examination. As a preliminary study we had re-examined 7/13 horses in the remission phase of the asthmatic disease.

Results:
Inflammatory airway disease (IAD) in 2 horses and recurrent airway obstruction (RAO) in 11 horses were confirmed on the basis of BAL cytology. The TL bacteriology of all horses were negative. DDSP was detected in both resting and exercising conditions in all RAO cases. In the 2 IAD cases, DDSP was visible during resting endoscopy, however during exercise only severe palatal instability (PI) could be detected. All the horses were coughing simultaneously with the displacement during exercise. When we re-examined the horses in the remission phase of RAO we found no functional problems or just light PI in 5/7 cases, and in 2/7 cases DDSP was visible occasionally, but less frequently and for shorter periods compared to the original examination.

Discussion:
All DDSP cases were suspected to be of inflammatory or obstructive origin which developed secondary to asthmatic diseases in the examined horses. Accordingly, we suggest a primary treatment of the lower airways in such cases of DDSP. Resting endoscopic examination is sensitive in the cases where RAO is the suspected as the origin of DDSP. This is in contrast with studied cases of racehorses with DDSP of different origins where resting endoscopy was found to be insensitive. We suspect that there might be a correlation of the severity of the asthmatic disease and the development of the functional problem, as DDSP was observed in all the cases of RAO, while only PI was found in the cases of IAD during exercise.

Conclusions:
We suspect that there might be a correlation of the severity of the asthmatic disease and the development of the functional problem, as DDSP was observed in all the cases of RAO, while only PI was found in the cases of IAD during exercise.
Title: At what speed barrel Racing horses bleed (EIPH)?

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Introduction:
High speed exercises, as performed in the Three barrel modality, are conditions for the appearance of Exercise Induced Pulmonary Hemorrhage (EIPH) in horses. However, there is no published data for this sports modality.

Objectives:
The objective of this study was to evaluate if an association exists between endoscopic bleeding scores and speed of Quarter Horses before and after barrel racing exercise.

Methods:
Sixteen horses were divided into two groups of eight animals each: regular training (RT - five times per week) and sporadic training (ST - twice a week). Animals were warmed up and made one run on the three barrel course with measure distances similar to those of official events. The maximum speed (m/s) was determined with the use of the Polar G1® GPS sensor placed on the right forearm of the rider and the data receiver clock on his right wrist. The animals were trained by the same rider. ANOVA was used for comparisons of groups and moments. The Spearman correlation test was used for the EIPH and maximum speed variables, in addition to the Mann-Whitney test for HPIE (P <0.05).

Results:
EIPH affected animals of both groups, with 4/8 (50%) in the RT group and 3/8 (37.5%) in the ST group. The maximum speed reached by the animals was 12.2 m/s (mean±SD: 11.08 m/ 3.10 (RT) and 10.52 m/s ± 2.16 (ST), respectively). The animals that presented EIPH reached an average speed greater than 10.5 m/s. A strong correlation (r = 0.75; P=0.0342) was observed in horses from ST group.

Discussion:
There was a great number of horses with EIPH in barrel horses, some people suspect these can occur because demands high force in a very small time to finish the race. The speed necessary to occur de EIHP is very similar to thoroughbreds in races. In barrel races it is expected that the animals will make bigger efforts in comparison with training which may have as a consequence more horses with EIPH

Conclusions:
The results suggest that three barrel horses are subjected to EIPH and that at high speeds animals that are not adapted (ST) to exercise can develop significant damage to the lungs, leading to HPIE.
Title: Autologous Conditioned Serum therapy in racehorses with IAD: A clinical prospective randomized blinded study

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Introduction:
Autologous Conditioned Serum (ACS) therapy is based on the use of sterile incubated serum, harvested from venous blood. Based on its anti-inflammatory properties, involving the blockage of IL-1, ACS has been extensively used in horses with osteo-arthritic diseases. In equine athletes, respiratory diseases are the most frequent cause of loss of performance and days in training after osteo-articular conditions. The involvement of IL-1 has been demonstrated in several respiratory inflammatory processes and blocking this pathway could reduce respiratory inflammatory in horses at risk.

Objectives:
The aim of this randomized blinded control study was to assess the effect of ACS for the treatment of horses with naturally occurring inflammatory airway disease (IAD). Our hypothesis was that horses treated with ACS would show reduced airway inflammation and improved respiratory function in comparison to horses receiving no treatment.

Methods:
We initially evaluated the administration of ACS either by inhalation, intra-tracheal or intra-muscular injection (IM). The IM route was found to be most efficient and easiest to apply. Twenty-two flat-race Thoroughbreds diagnosed with IAD, in active training and from the same training yard, were randomly divided into a control group (P) receiving no treatment and a treated group (ACS). Blood drawn from the jugular vein was incubated for 72h at 37°C in special containers then centrifuged to obtain serum. Ten ml of ACS was administered every third day for 6 weeks (T6).

Results:
In the P group, a progressive increase in TW and BAL inflammatory neutrophils occurred from T0 to T6, whereas they were significantly lower at T6 in the ACS group. The reactance at 5Hz (X5Hz) improved with training in all horses, however it was significantly higher at T6 in the ACS group (see Table).

Discussion:
This study shows that in intensively working racehorses, a 6-week IM ACS treatment is effective in both preventing progression of IAD and in improving lung function parameters such as lower airway reactance (compliance) in horses in active training. The underlying mechanisms of action warrant further investigation.

Conclusions:
Autologous conditioned serum is effective in controlling IAD in horses during intensive training.
Title: Clinical outcome after surgical correction of cleft palate by laryngeal tie-forward in 2 horses.

Authors: Irene Tosi, Jose Arevalo, Alexandra Salciccia, Sigrid Gruke, Tatiana Art, Geoffroy de la Rebière

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Introduction:
Cleft palate is a rare congenital defect in horses. Its description in literature is generally limited to cases of young foals referred for milk regurgitation from nostrils, coughing and dysphagia, with a guarded to poor prognosis if not treated. Surgical intervention consists mainly in palatoplasty that is considered a salvage procedure and complications are frequent. Only few cases of cleft palate in adult horses are described in literature. Laryngeal tie-forward, a surgical technique generally used to treat dorsal displacement of the soft palate, has been described only in one 4-year-old pony with cleft palate but clinical improvement after surgery was partial and temporary.

Objectives:
To describe laryngeal tie-forward as a valid option for the treatment of cleft palate in two adult horses not suitable for palatoplasty.

Methods:
Clinical records of two 8 y.o. jumping horses performing respectively at a low and intermediate intensity of exercise and competition. Both horses were referred for respiratory noise, exercise intolerance, cough and alimentary nasal discharge; they were diagnosed with a moderate soft palate defect and surgically treated by laryngeal tie-forward. A clinical examination and control endoscopy were realized at 4 months for one horse and at 3 years after surgery for the other.

Results:
In both horses postoperative endoscopy showed a visible reduction of the gap between soft palate and the larynx so that the epiglottis was covering the soft palate defect. One horse was examined 4 months after surgery, a residual respiratory noise during exercise was still present but lighter than prior to intervention, its performances had remarkably improved. The second horse was controlled 3 years after surgery, some cough was still observed but limited to the onset of exercise, performances were satisfying and the horse was working at the same intensity level. Globally, in both horses, initial symptoms had disappeared or were significantly reduced after surgery and owners were satisfied. Reduction of the soft palate defect was still effective, even several months after surgery.

Conclusions:
The description of these two clinical cases shows that some horses can grow up to adult age with moderate defects of the soft palate; they are likely to show clinical signs of variable severity. In such cases laryngeal tie-forward should be considered as an option if the defect is too large or asymmetrical to be corrected with palatoplasty.
Title: Clinical outcome after tracheal resection and anastomosis for correction of a tracheal stenosis in a sport pony.

Authors: Alexandra Salciccia, Charlotte Roose, Zoë Joostens, Alexandra Gougnard, Geoffroy de la Rebière, Irene Tosi, Sigrid Grulke

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Introduction:
Tracheal stenosis, which refers to a narrowing of the tracheal lumen, is infrequent in horses. The literature is scarce about outcome after treatment of this pathology.

Objectives:
To report our experience using tracheal resection and anastomosis to treat a severe trauma-induced tracheal stenosis (< 1 cm diameter of lumen) involving 2 tracheal rings causing a loud respiratory noise at rest in a 14 y.o. event pony.

Methods:
The pony was trained to wear a martingale preoperatively. Before the surgery, a distal tracheotomy was performed for placement of the tracheal tube for maintenance of gaseous anaesthesia. Through a 35 cm ventral midline cervical incision, the trachea was exposed and separated from adjacent tissues. Stay sutures were placed in tracheal cartilage adjacent to the segment to be removed. The 2 tracheal rings involved in the stricture were subsequently removed. On both remaining segments of the trachea, the mucosa was turned back over the open end and sutured to the adventitia. The head was then flexed and tracheal ends were apposed using 5 stainless steel wires placed equidistantly around the trachea without mucosal penetration. The anastomosis was completed by a simple continuous suture of resorbable material on the adventitia. A closed suction drain was applied in the soft tissue before routine closure of the subcutaneous and cutaneous layers. The martingale was applied before the recovery from anaesthesia, which was uneventful. The tracheotomy tube and the suction drain were left in place for 48h. The martingale was removed after 3 weeks. The pony was discharged 1 month after surgery. Control endoscopies were performed during hospitalisation and at 7, 10, 17, 21 and 30 weeks and 1 year after surgery.

Results:
A mild to moderate cicatrix recurred. The excessive tissue was injected with triamcinolone and then with 4% formaldehyde during the first endoscopies and remained stable thereafter. Some stainless steel broke and displaced slightly. This caused the formation of a self-resolving seroma caudally to the trachea. A moderate respiratory noise was still audible at low-intensity work, which was resumed 4 months postoperatively. However, work was stopped before achieving the preoperative level because the pony developed a lameness.

Conclusions: Although a mild to moderate tracheal cicatrix recurred after surgery, tracheal resection and anastomosis clearly improved the quality of life of this pony.
Introduction:
The Vaquejada is a cultural equestrian modality commonly performed in the Northern and Northeast regions of Brazil. During the race, two horses and riders run close to a bull, seeking to catch its tail and knock it down within a predetermined zone. For a top performance, the horse must have agility and develops high speed in a short period of time, which could increase the risk for development of exercise-induced pulmonary hemorrhage (EIPH).

Objectives:
The aim of this study was to evaluate the cytology of bronchoalveolar lavage fluid (BALF) in horses after a Vaquejada race.

Methods:
Fifteen Quarter Horses living in barns, with no history of respiratory disease or poor performance were included in the study. The horses completed a day of Vaquejada competition (8 to 13 sprints of ±12 seconds) and BALF collection was performed 48 hours later.

Results:
All horses presented alveolar macrophages containing hemosiderin, showing that an active EIPH occurred during race. Eight horses showed neutrophil count greater than 5% and eosinophils count greater than 1%, which characterized the cytological profile of pulmonary inflammation.

Discussion:
The results of BALF suggest that all Vaquejada racing horses may exhibit some degree of EIPH followed by a neutrophilic inflammation. Therefore, preventative methods should be taken to control exposure to airborne dust or allergen, once these horses may be susceptible to the development of heaves.
Title: Cytological profiles of equine bronchoalveolar lavage fluid: a retrospective study (2013-2015)

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Introduction:
In addition to clinical signs and functional investigation, the definitive diagnosis of moderate to severe equine asthma relies on cytological profiles of bronchoalveolar lavage fluid (BALF). Among others, controversial results have been found concerning season as a risk factor of airway inflammation.

Objectives:
To determine the prevalence of cytological abnormalities in a large cohort of BALF sampled by practitioners and their association with extrinsic (environment) and intrinsic (clinical signs) features.

Methods:
Medical records of 2762 horses for which BALF has been harvested and sent to the laboratory for cytological investigation between 2013 and 2015 were reviewed retrospectively. Association between categorised variables were determined using Chi-square tests and risk-factors for clinical signs were assessed by multivariate logistic regression.

Results:
The quality of 1876 samples did not comply with the required inclusion criteria (volume, time ...) and were excluded from the study. A total of 886 horses were then finally investigated, which were mostly adults (>5 years; 76.4%) kept in stalls either permanently (25.2%) or with limited access to pasture (36.2%). The main clinical signs were spontaneous coughing (53.2%), nasal discharge (28.8%) and dyspnoea (25.5%). Increased tracheal mucus score (>2) was found in 47.7% of investigated horses. Based on the less severe consensual criteria for BALF cytology, 21.5% of horses had normal profiles, 33.5% and 45.0% were respectively compatible with moderate and severe equine asthma. Season was significantly associated with coughing (decreased in fall; p=0.01), tracheal mucus ≥2 (increased in spring; p=0.0003). Severe neutrophilia (>25%) was less prevalent in fall (p<0.0001) while increased proportions of eosinophils (>5%) and metachromatic cells (>2%) were significantly (p=0.007) increased in summer, as was yeast detection in BALF (p=0.0004). Coughing was significantly associated with tracheal mucus (OR=2.34, p=0.0003) and BALF neutrophil proportions (OR=1.55, p<0.0001). Dyspnoea was significantly associated with tracheal mucus (OR=1.86, p=0.04), BALF Curschmann spirals (OR=1.96, p=0.002), and negatively associated with fungi detection (OR=0.72, p=0.02).

Conclusions:
This large retrospective study confirms the seasonal influence on BALF cytological profiles and the relevance of scoring tracheal mucus when investigating airway inflammation. Further studies are required to specifically determine the potential influence of yeast/fungi detection in BALF on equine asthma.
Title: Cytology of bilateral bronchoalveolar lavage fluids: diagnostic reliability of pooled samples for exploring both lungs

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Introduction:
It has recently been demonstrated that bronchoalveolar lavage fluid (BALF) cytology from one lung does not forecast the other lung in the same horse, leading to the necessity of performing two BALF cytologies per horse.

Objectives:
The aim of the study was to determine if pooled BALF from both lungs was representative of individual samples and lead to the correct diagnosis, once established the best cutoff values for this method.

Methods:
Fifty-one horses referred to ONIRIS equine clinic (CISCO) for respiratory function evaluation were included in the study. BALF was collected from each lung using 250ml of warmed isotonic saline. An isovolumetric pool was then carried out with the remaining collected fluid from each lung. Cytological analyses of each individual BALF and pools were carried out in a blinded manner. Cell proportions were log-transformed and compared using ANOVA, with Dunnett’s post-hoc test. For each cell type, correlations between pooled and individual BALF were assessed by Pearson coefficient, numerical agreement by intraclass correlation coefficient (ICC), cutoff values determined through ROC curves, and categorical agreement by Cohen’s kappa coefficient. P< 0.05 was considered significant.

Results:
No significant difference was observed between pooled and individual BALF for the different cell types. Correlations between pooled and both right and left BALF were excellent for neutrophils (R= 0.90 and 0.91 respectively) and haemosiderophage/macrophage ratio (R= 0.89 and 0.81 respectively), and poor for mast cells (R= 0.20 and 0.19 respectively) and eosinophils (R= 0.09 and 0.24 respectively). Numerical agreement was moderate for mast cells (ICC= 0.71 and 0.68) and good for the other cell types (ICC>0.90). Thresholds for pooled BALF were set at 3, 9% and 22% neutrophils compared to 5, 10% and 25% respectively with individual samples. Considering 3 groups of horses (RAO, IAD, control) only 3.9% (2/51) of divergent diagnosis were detected, either with restrictive (kappa= 0.86) or alternative (kappa= 0.78) thresholds. In comparison, sampling one lung only led to respectively 11.8% (6/51) and 13.7% (7/51) of divergent diagnosis.

Conclusions:
This new methodology allows to investigate both lungs with a single cytology and to minimize the price for the owner. Cytology from pooled samples appears to be very reliable for cytological diagnosis of equine asthma and decreases the number of wrong diagnosis compared to individual samples.
Title: Development of a Multiple Protein Extract Microarray for Profiling Allergen-Specific Immunoglobulin E in Horses with Severe Asthma

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Introduction:
Severe equine asthma is a prevalent, performance limiting, hypersensitivity to inhaled allergen in genetically predisposed horses. Previous work has demonstrated increased allergen-specific immunoglobulin E (IgE) against a range of fungi, bacteria, pollen and mites in affected horses, however, a limited number of allergen have been screened to date. Protein microarrays enable the evaluation of several hundred proteins with specific antibodies on a miniaturised scale, using techniques such as fluorescence for image processing. The diagnostic value of these tests can be similar to standard laboratory tests, such as ELISA and UniCAP, and has successfully been utilised for insect bite hypersensitivity in the horse.

Objectives:
The objectives of this study were to (1) develop a comprehensive protein microarray to profile IgE and (2) identify which allergens were immunogenic in horses with severe asthma.

Methods:
Six horses with severe asthma and six control horses were included in the study, and classified according to clinical assessment, including physical examination, pulmonary function test and bronchoalveolar lavage fluid (BALF) cytology. Protein extracts and pure proteins from pollen, bacteria, fungi and arthropods were purchased (134), or produced in-house (10), and spotted onto 16 pad nitrocellulose FAST slides. Fifty microliters of serum were used as a source of primary antibodies, and IgE quantified using anti-horse IgE (3H10) and rabbit anti-mouse IgG1 Dylight 649. Slides were scanned in a GenePix 4000B with GenePix Pro 6 software and results compared using two-sample-for-means Z tests.

Results:
A total of 40/144 proteins screened were associated with severe asthma (table 1). The most significant allergens were Dermatophagoides farinae (Der f 2), Blattella germanica (Bla g 5) and Aspergillus restrictus (Asp r 1). The fungal group showed the most significant difference between severe asthma and control horses.

Discussion:
Most major allergens identified were associated with the stabling environment. The comprehensive profiling of IgE with a complex protein microarray system uses a very small volume of blood, is specific, sensitive and may assist in the diagnosis and tailored treatment of horses with severe asthma.

Conclusions:
Further work identifying common causative allergens throughout the equine severe asthma population could enable diagnosis via serological methods.
Title: Development of an inspiratory muscle testing and training device in the horse: a feasibility trial

Authors: Kate Allen, Alison McConnell, Sam Franklin, Laura Fitzharris  Mail: kate.allen@bristol.ac.uk
Affiliation: Equine Sports Medicine Centre, University of Bristol.

Introduction:
Respiratory muscle training has been subject to extensive investigation in humans with studies demonstrating significant improvements in athletic performance. However, its use has not been investigated in equine athletes.
Functional testing of the respiratory system is also extremely limited in horses in comparison to humans, due to the inability to undertake maximal volitional efforts. In humans, several indices of respiratory strength are available; the most common method is by measuring the maximal pressure at the mouth during a quasi-static inspiratory effort against an occluded airway.

Objectives:
To develop equipment and assess the feasibility of measuring respiratory strength and undertaking inspiratory muscle training in horses.

Methods:
For both testing and training, a bespoke airtight mask covering the entire muzzle was produced. For inspiratory muscle training, one-way inspiratory loaded valves were evaluated. For the strength assessment, commercially available electronic equipment for measuring respiratory muscle performance in humans was used. As maximal inspiratory pressure (obtained during a maximal voluntary effort) cannot be obtained in the horse, incremental threshold loading protocols from the human literature were adapted. Adaptations ensured inspiratory muscle strength was preferentially tested over endurance. Repeatability of the initial incremental loading protocol and the resulting strength index was tested in 10 horses. Subsequently feasibility of respiratory muscle training and respiratory strength testing was evaluated in 10 thoroughbred racehorses. Respiratory strength was assessed before and after an 8-week inspiratory muscle training program.

Results:
The tolerance to inspiratory muscle training and the incremental loading test were good. The results of the initial testing suggested that higher values were obtained when horses were familiarised with the procedure. Following the 8-week training program the mean inspiratory strength index increased from 27 cmH2O to 41 cmH2O.

Conclusions:
This study demonstrates the feasibility of undertaking respiratory muscle training and respiratory muscle testing in horses. Further research is required to understand whether the respiratory strength index correlates with other physiological and/or performance variables and to elucidate whether the responses to training result from respiratory muscle hypertrophy, improved neuromuscular activation, or improved ability to undertake the test.

<table>
<thead>
<tr>
<th>Table 1. Calculation of PRSS</th>
<th>Table 2. PRSS and Average earnings before and after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finishing place</td>
<td>Control (n = 120)</td>
</tr>
<tr>
<td>1 (won race)</td>
<td>Before</td>
</tr>
<tr>
<td>PRSS score</td>
<td>6</td>
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Average earnings (£)

<table>
<thead>
<tr>
<th>PRSS</th>
<th>Before</th>
<th>After</th>
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<tr>
<td></td>
<td>2.77</td>
<td>2.52</td>
<td>2.50</td>
<td>2.84</td>
</tr>
<tr>
<td>Average earnings (£)</td>
<td>1,663.30</td>
<td>2,048.18</td>
<td>1,279.23</td>
<td>1,947.23</td>
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</table>
Title: Dynamic respiratory endoscopy in the Thoroughbred racehorse. Does a more specific diagnosis lead to improved racing performance following tailored surgery?

Authors: Marc Collas, Henry O’Neill, N. Edward Robinson, Bruce Bladon
Affiliation: Donnington Grove Veterinary Group, Oxford Road, Newbury, RG14 2JB, UK
Mail: marc_collas@hotmail.com

Introduction:
Dynamic respiratory endoscopy (DRE) is considered the ‘gold standard’ imaging modality when assessing functional obstruction of the upper respiratory tract (URT). To date, no studies have validated if surgery based on DRE findings improves racing performance.

Objectives:
To compare the effects on performance and earnings of URT surgery based on DRE, with those based on static-endoscopy +/- abnormal respiratory noise.

Methods:
Case records from all racehorses subject to URT surgery between 2010-2016 at our practice were reviewed. Exclusion criteria included: 1. an obvious anatomical abnormality on resting examination 2. prior surgical intervention 3. those without at least one race prior to and following surgery. Horses were grouped into those operated on following the findings of DRE (Group DRE), or the findings of static endoscopy +/- audible respiratory noise during exercise (Control). Racing performance was evaluated using race ‘earnings’ and a point ranking system score (PRSS), in which points were allocated according to race participation and position Table.1. Data were collected for the five races (where available) before and after surgery, and analysed using non-paired and paired t-tests, as appropriate, with significance set at P ≤ 0.05.

Results:
Table 2. summarises the results of 335 racehorses admitted for URT surgery, 128 controls and 120 DRE fulfilled the inclusion criteria. Horses averaged 4.2 (Control) and 4.1 (DRE) races before surgery, and 4.5 races following surgery (both groups). Males were over-represented (Control = 105 male: 22 female, DRE = 105 male: 15 female). The average PRSS before surgery did not differ between groups. Following surgery, there was a significant deterioration in Control PRSS (P=0.04) and an improvement in DRE PRSS (P=0.05) so that the two groups differed significantly after surgery. The average earnings pre-surgery did not differ between groups (P=0.56). Following surgery, there were no significant changes in either group although median earnings for Control deteriorated and DRE improved.

Conclusions:
Horses were likely to place higher in races following DRE-dictated surgery than horses receiving surgery based on static endoscopy/noise; however this was not reflected when earnings were compared.

<table>
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<tr>
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<tr>
<td>Finishing place</td>
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<tr>
<td>PRSS score</td>
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<table>
<thead>
<tr>
<th>Table 2. PRSS and Average earnings before and after surgery</th>
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<tr>
<td>Control (n = 128)</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Before</td>
</tr>
<tr>
<td>PRSS</td>
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<tr>
<td>Average earnings (E)</td>
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</table>
Title: Effect of Prostaglandin D2 in granulocytes of allergic horses

Authors: Georg M. Racic, Birgit Brodacz, Eva M. Sturm, Akos Heinemann

Affiliation: Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

Mail: georgvet@gmx.net

Introduction:
Like humans, horses are prone to develop allergic skin and respiratory symptoms with similar immune response mechanisms. The prevalence of equine allergies like recurrent airway obstruction (RAO) or insect-bite hypersensitivity (IBH) has distinctly increased in the last decades, thus there is a growing need for additional treatment options. Cyclooxygenase (COX) -derived lipids such as prostaglandins play a crucial role in the pathogenesis of human allergic diseases and antagonism of prostaglandin D2 (PGD2) receptors has been shown to diminish allergic symptoms in humans.

Objectives:
Our main goal is to unravel the role of lipidmediator PGD2 and its receptors DP1 and CRTH2 in leukocyte function of allergic (RAO and IBH) and healthy horses.

Methods:
Experiments are performed using whole blood or isolated polymorphonuclear cell preparations. The impact of PGD2 on eosinophil and neutrophil responsiveness is evaluated by means of shape change and chemotaxis assays. Expression pattern of DP1 and CRTH2 are characterized by flow cytometry.

Results:
Similar to humans, eosinophils from allergic and healthy horses show a distinct shape change and migratory response towards PGD2 and its CRTH2-selective metabolite, DK-PGD2. Chemotactic responses are increased in eosinophils and neutrophils from allergic horses. In presence of the selective CRTH2 antagonist OC000459 and the DP1 antagonist MK-0524 chemotaxis is significantly attenuated in eosinophil migration. PGD2 did not induce neutrophil chemotaxis, but showed a priming effect on neutrophil migration towards interleukin-8. DP1 receptor expression is attenuated on eosinophils and neutrophils from allergic horses, whereas CRTH2 expression is enhanced.

Conclusions:
PGD2 might play an important role in equine inflammation and allergic diseases. Inhibition of PGD2 induced effects by antagonism of its two receptors (DP1 and CRTH2), might represent a potential novel therapeutic approach for equine allergic diseases. Research of this topic is of high interest in veterinary medicine and might serve as a study model for human allergic diseases, mainly asthma.
Title: Epidemiological study of respiratory conditions affecting racehorses in the southeast of Brazil

Authors: Maria Verônica Da C. Ferreira, Fernanda Ribeiro, Amanda L. Da Silva, Mateus S. Ribeiro, Mariana de S. e Silva, Marcia T. Ramos, Juliana V.B. Inglez, Maria Fernanda de M. Costa

Affiliation: Brazilian Jockey Club, Hospital Octávio Dupont, Rio de Janeiro, RJ, Brazil,

Mail: equipecentaurovet@hotmail.com

Introduction:
Detection and treatment of pathologies affecting the respiratory system of horses play a major role in guaranteeing adequate performance in sport horses, second only to prevention of musculoskeletal conditions. Undiagnosed respiratory diseases have the potential to cause loss of training days, a decline in performance, and economic losses to the equine industry in general. Knowledge of the epidemiology of respiratory diseases is desirable, especially when considering management changes and strategies to reduce the incidence of respiratory problems in horses during athletic training and competition.

Objectives:
To conduct an epidemiological investigation of respiratory pathologies affecting racing Thoroughbreds competing at a racetrack using post-race endoscopic examination.

Methods:
Retrospective evaluation of all the results from post-race endoscopies conducted from the 25th of May 2015 to 20th of February 2017 was conducted. All endoscopies were treated as single events.

Results:
Three thousand and forty-one (3,041) endoscopies were included in this study. The population studied included males, females and geldings from 2 to 8 years of age. Most common findings included presence of tracheal mucous secretions (51.7%) and exercise induced pulmonary haemorrhage (39.2%). The most common upper airway condition observed was left laryngeal neuropathy (3.6%). Males (54.3%) and four year-olds (36.9%) were most affected. Exercise induced pulmonary haemorrhage grades 1 (585/1197; 48.9%) and 2 (223/1197; 18.6%) were most prevalent. Mucous secretions were observed 1591 times, with 57.9% of these being grade 1 and 30.7%, grade 2. Mucopurulent secretions were observed in only 17 instances. Eighty percent of the observations came from horses not medicated with furosemide and this majority repeated itself across all individual pathologies. When each animal was included once in the tally, 1357 individuals were represented. Males accounted for 52.8% of the total, but females were the majority of individuals with epiglottic entrapment (11/20), lymphoid follicular hyperplasia (19/39), and purulent secretions (10/17).

Discussion:
Despite examining racehorses postrace, considered to be fit and well to race, an elevated number of respiratory pathologies were observed, indicating a prevalence of sub-clinical disease. These findings highlight the importance of endoscopic examination in apparently healthy horses.

Conclusions:
Post race endoscopic examination revealed a high incidence of respiratory pathologies, mainly in inflammatory origin as evidenced by tracheal secretions. Females had a higher incidence of upper airway conditions and purulent secretions when compared to males.
**Title:** Equine asthma in pasture horses in Brazil southeastern

**Authors:** Eliene P. Sad, Katia M. Silva, Luís Felipe A. Toledo, Daniel A. B. Lessa, Paulo de Tarso L. Botteon

**Affiliation:** Department of Veterinary Medicine and Surgery (DMCV) Universidade Federal Rural do Rio de Janeiro Seropédica, Rio de Janeiro, Brazil **Mail:** elienesad@gmail.com

**Introduction:**
Inflammatory Airway Disease (IAD) and Recurrent Airway Obstruction (RAO), also known as mild and severe equine asthma respectively, are causes pulmonary dysfunction in horses worldwide, severely limiting equine health and performance. Airway hyper-responsiveness is a defining characteristic for the pathophysiology of both respiratory disorders, characterized by increased airway sensitivity to inhaled aeroallergens.

**Objectives:**
Describe the presence airway inflammation in pasture horses in southeastern Brazil during summer.

**Methods:**
Endoscopy to assess tracheal mucus score and bronchoalveolar lavage (BAL) were performed during summer in 40 pasture horses (4-21 years). Animals were classified based on the cytology analysis as normal (group I) or inflammatory airway group (group II). BAL was obtained by infusing 500mL of saline solution. Slides were prepared by cytocentrifugation and two observers performed the cytology analysis by differential counting of 500 cells. In the current study, BAL neutrophils values >10% were considered positive for airway inflammation.

**Results:**
The mean percentage of differential nucleated cell count of groups and mucus is presented in table 1. Neutrophil differential count cell ranged from 3 to 32%. Overall there were in group I and II, 13 (32,5%) and 27 (67,5%) animals respectively. Data were analyzed for normality by the shapiro-wills test, the normal ones and coxbox transformation passages were analyzed by the Student’s t test and those that could not be normalized were for the Wilcoxon test.

**Discussion:**
As summer-pasture-associated obstructive pulmonary disease, this animals shows a neutrophilic response and mucus accumulation, however not all horse showed increased respiration effort at rest or caught, most just demonstrated mild signs of respiratory distress. Severe asthma affected horses had more mucus accumulation compared with others as in barn confinement. Changes in BALF cytology and mucus accumulation by natural environmental exposure in pasture horses seems quite the same as in indoor horses.

**Conclusions:**
Equine Asthma also occurs in outdoor horses during summer. January climatic conditions (raining and warm season) and/or inhalation of allergens presents in pasture (Andropogon bicornis L.), are probably involved in pathophysiology.
Title: Evaluation of metalloproteinases and their inhibitors in equine asthma under the course of cpg-odn inhalation therapy

Authors: Ann Kristin Barton, Tarek Shety, Sabine Geis, John Klier, Ralf Einspanier, Heidrun Gehlen
Affiliation: Equine Clinic, Freie Universitaet Berlin, Germany
Mail: Ann-Kristin.Barton@fu-berlin.de

Introduction:
In equine asthma, overexpression of matrix-metalloproteinases (MMPs) contributes to pathological tissue destruction, while tissue inhibitors of metalloproteinases (TIMPs) counteract MMPs with overexpression leading to fibrosis formation. Inhalation of immunostimulatory bacterial DNA segments (cytosine-phosphate-guanosine-oligodeoxynucleotides, CpG-ODN) has been shown to normalize clinical and cytologic parameters in severe equine asthma (recurrent airway obstruction, RAO).

Objectives:
We hypothesized that CpG-ODN inhalation reduces the misbalance of elastinolytic activity in horses affected by RAO, immediately and 6 weeks after the end of therapy.

Methods:
20 horses diagnosed as RAO by clinical examinations using a scoring system were included. All horses were treated with inhalative CpG-ODN for 14 days in 2-day intervals. MMP and TIMP concentrations were measured in tracheal aspirates using equine ELISA kits (USCN Life Science Inc.).

Results:
MMP and TIMP concentrations correlated with the results of clinical scoring in all stages of equine asthma. Inhalation therapy led to significant reductions in clinical scores. MMP and TIMP concentrations were also reduced significantly (table 1).

Conclusions:
Misbalance of elastinolytic activity was positively influenced by CpG-ODN inhalation for at least 6 post therapy, which may reduce the remodeling of the extracellular matrix in equine asthma.

Table 1: Concentration of MMP-2/9, TIMP-1/2 in tracheal aspirates from horses affected by equine asthma before (t0), immediately (t1) and 6 weeks (t2) after CpG-ODN inhalation. The results are expressed as mean ± SE. * marks significant improvement (P < 0.05) compared to prior therapy.

<table>
<thead>
<tr>
<th></th>
<th>t0</th>
<th>t1</th>
<th>t2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>15.9 ± 0.5</td>
<td>14.6 ± 0.3*</td>
<td>13.0 ± 0.3*</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>606.9 ± 85.5</td>
<td>324.8 ± 81.7</td>
<td>210.6 ± 38.4*</td>
</tr>
<tr>
<td>TIMP-1 (pg/ml)</td>
<td>139483.1 ± 11325.7</td>
<td>115455.1 ± 15955.8</td>
<td>88426.2 ± 16975.9*</td>
</tr>
<tr>
<td>TIMP-2 (ng/ml)</td>
<td>124.6 ± 10.6</td>
<td>77.6 ± 5.3*</td>
<td>70.1 ± 6.3*</td>
</tr>
</tbody>
</table>
**Title:** Evaluation of the correlation between laryngohyoid conformation and electromyographic activity of thyrohyoideus muscle in horses with intermittent dorsal displacement of the soft palate (DDSPi)

**Authors:** Giovanni Stancari, Enrica Zucca, Norm G. Ducharme, Luca Stucchi, Bianca Conturba, Serena Ceriotti, Elisabetta Ferro, Francesco Ferrucci

**Affiliation:** Department of Health, Animal Science and Food Safety (VESPA), Università degli Studi di Milano, Milano, Italy

**Mail:** giovanni.stancari@unimi.it

**Introduction:**
DDSPi seems to be related with dysfunction of Thyrohyoideus (TH) muscle possibly caused by incorrect laryngohyoid conformation (LC). Evaluation of TH muscle activity at rest and LC by means of surface electromyography (sEMG) and ultrasonography (US) respectively, could give more information concerning DDSPi pathogenesis.

**Objectives:**
To evaluate the electromyographic activity of TH muscle and its possible correlation with LC in both normal and DDSPi affected horses

**Methods:**
12 racehorses that underwent treadmill endoscopy were selected. In 5 horses DDSPi was detected (Group A) while normal pharyngo-laryngeal function was observed in the other 7 horses (group B). For each patient sEMG of TH muscles at rest during deglutition and US of the laryngohyoid region were performed. A Shapiro-Wilk test was used to assess the normal distribution of the data followed by a t-test to compare data between group A and B. Pearson correlation coefficients (r) were used to establish a possible correlation between US measurements and EMG activity of the TH muscles in the two groups. Multiple linear regression analysis was used to investigate the effect of US measurements, age, weight, sex and attitude on the EMG activity of TH muscles. The effect of the DDSPi status (group A vs B) on US variables was investigated with a different linear regression analysis. Significant values were set at P < 0.05

**Results:**
sEMG did not show any significant differences of TH muscle activity in DDSPi horses vs controls. US showed a significant correlation among several laryngohyoid measurements in DDSPi horses only. Concerning the correlation between EMG activity and LC, a significant effect (p = 0.037) of the US depth of the thyroid cartilage on the mean of EMG activity of TH muscle was found in both group of horses

**Discussion:**
Functional abnormalities of TH muscles activity in DDSPi affected horses at rest was substantially ruled out. The correlation in the laryngohyoid measurements found in the DDSPi horses suggested in this group an incorrect LC. The correlation between US measure and EMG found in both group, suggested that the different LC does not result in alteration of EMG at rest. It seems more plausible that the abnormal conformation leads to TH fatigue during exercise with subsequent onset of DDSPi

**Conclusions:**
The results obtained showed that LC is not secondary to TH muscle activity at rest. Further research concerning the EMG activity of TH muscles during strenuous exercise may provide more information on DDSPi pathogenesis.
Title: Evaluation of topical medication methods for the pharynx and larynx of the horse

Authors: Britta S. Leise, Aimee C. Colbath, Eileen S. Hackett
Affiliation: Department of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA USA
Mail: bleise@lsu.edu

Introduction:
Topical administration of medication delivered by pharyngeal or ‘throat’ sprays, is routinely recommended post-surgically for conditions of the equine larynx and pharynx. Though commonly administered, the efficacy and distribution of pharyngeal medications is unknown.

Objectives:
To determine the pharyngeal and laryngeal distribution of radiopaque contrast media administered orally or via nasopharyngeal catheter in standing horses using radiographic assessment.

Methods:
A blinded crossover study was performed in 5 healthy horses. Twelve milliliters of radiopaque contrast media was administered orally or via nasopharyngeal catheter. Pharyngeal and laryngeal distribution of contrast media was determined from radiographs taken immediately following administration. Upper airway endoscopic examination performed following radiographic examination was used to confirm radiographic distribution.

Results:
Radiographs performed after nasopharyngeal administration revealed contrast in the nasopharynx 5/5(100%), oropharynx 2/5(40%), laryngopharynx 3/5(60%), and larynx 5/5(100%) of horses. Radiographs performed after oral administration identified contrast in the oropharynx 4/5(80%) and larynx 1/5(20%) of horses. Endoscopic examination of the nasopharynx, laryngopharynx, and larynx confirmed radiographic findings, and was determined to be sensitive for contrast detection in the laryngopharynx where detection rates were 40% higher with both administration methods.

Discussion:
Results of the present study suggest that medication administered using a nasopharyngeal catheter will result in topical distribution within the nasopharynx, including the dorsal soft palate, and larynx. Oral administration distributes primarily in the oropharynx.

Conclusions:
Distribution of topical pharyngeal and laryngeal medications should be confirmed in horses with clinical airway disease to confirm if these findings are consistent.
Title: Exercising upper respiratory videoendoscopic evaluation of 42 competition draft horses with abnormal respiratory noise and/or poor performance

Authors: Eileen S. Hackett, Britta S. Leise
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Mail: Eileen.Hackett@colostate.edu

Introduction:
Upper respiratory endoscopy at rest has been the most common diagnostic method utilized in competition draft horses with upper respiratory tract conditions. Overground respiratory endoscopy is well-suited for draft horse respiratory evaluation, as it allows the horses to exercise driven with harness, overcheck, and cart load under similar conditions to those experienced in the show ring.

Objectives:
To describe the upper respiratory function of competition draft horses with abnormal respiratory noise and/or poor performance.

Methods:
Medical records of competition draft horses undergoing exercising videoendoscopic evaluation between January 2013 and January 2017 with a chief complaint of abnormal respiratory noise and/or poor performance were reviewed. All horses had video recordings of overground exercising respiratory endoscopy evaluated.

Results:
Forty-two competition draft horses were examined. The median age was 6 years, range 3-12 years. There were 37 Percherons, 2 Clydesdales, 2 Belgians, and 1 Shire, with 7 mares and 35 geldings. Thirteen had previously undergone upper respiratory surgery. Abnormalities were detected in 40 horses and included recurrent laryngeal neuropathy and left arytenoid cartilage collapse (n=28), intermittent dorsal displacement of the soft palate (n=1), palatal instability (n=9), vocal fold collapse (n=18), medial deviation of the aryepiglottic fold (n=10), epiglottic deviation (n=5), epiglottic retroversion (n=1), and dynamic laryngeal collapse (n=1). The majority of horses had complex abnormalities (n=33).

Discussion:
Exercising upper respiratory videoendoscopic evaluation was a useful technique to identify upper respiratory disorders in competition draft horses. The distribution of conditions identified in draft horses may be unique to this performance breed and warrants further study.

Conclusions:
The distribution of conditions identified in draft horses may be unique to this performance breed and warrants further study.
Title: Exhaled breath analysis in equine medicine using Gas Chromatography-Ion Mobility Spectrometry (GC-IMS): Detection of parenteral Marbofloxacin administration

Authors: Carmen C. Klein, Antje Genzel, Sven Wietstock, Ralf Regenthal, Getu Abraham
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Mail: gabraham@vetmed.uni-leipzig.de

Introduction:
Analysis of drugs and their metabolites in breath samples by GC-IMS has the potential to become a novel non-invasive and quick method in the equine medicine.

Objectives:
Aim of the study was to investigate the detectability of drugs e.g. marbofloxacin in exhaled breath after parenteral administration.

Methods:
The alveolar fraction of exhaled breath and blood serum samples (16 sampling points within six days, thereof 11 points in the first 24 hours) were collected from eight adult healthy female horses prior and after intravenous administration of a single dose of marbofloxacin (2.0 mg/ kg BW).

Results:
Blood serum level of marbofloxacin decreased continuously starting immediately after application. The limit of quantification (LOQ marbofloxacin 0.05 µg/ml) was reached between 12 and 24 hours. A total of 222 clusters of peaks representing volatile organic compounds (VOCs) were found in the exhaled breath samples. Peak volumes of one cluster (c89) exhibited significant differences (p < 0.001) between the different points in time: Maximum peak areas in this cluster were found between 30 and 90 minutes after medication (Figure 1). This VOC occurred with a delay of approximately 30 minutes after maximum marbofloxacin plasma concentration.

Discussion:
Marbofloxacin seems to influence the pattern of VOCs in exhaled breath. The next step is to identify the VOC represented by the peaks of this cluster by means of Gas Chromatography-Mass Spectrometry.

Conclusions:
Detection of pharmacological substances or associated molecules by breath analysis could be used to develop new diagnostic procedures for medication control.

Granted by the German Federal Ministry of Economics, Reg.-No.VF130004.
Title: GoPro: The Future for Training Technicians, Students, House Officers and Clinicians for Many Upper and Lower Airway Diagnostic and Therapeutic Procedures in Horses

Authors: David G. Suarez-Fuentes, DVM, Dane M. Tatarniuk, DVM, MS, DACVS
Affiliation: Veterinary Clinical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA
Mail: dsuarez@iastate.edu

Introduction:
GoPro cameras have been widely used in many industries since their development in 2001. The high definition of the image provided by the camera provides a unique perspective for recording in many environments. Recently, this camera has been used in human medical and surgical fields to facilitate visual training and education.

Objectives:
The objective of this report is to describe to clinicians how to properly use the camera in medical and surgical airway settings, and describe the future impact that could develop in the training of veterinary students, interns, residents, clients and fellow clinicians in our field.

Methods:
This report will describe the proper usage of the GoPro camera, including discussion of various attachments, disinfection, and durability. Visual examples of the diagnosis and treatment of common equine airway conditions, such as endoscopic procedures and minimally invasive surgery, will be demonstrated.

Results:
Successful recording of videos for various procedures involved in upper and lower airway diagnostic and therapeutic procedures in the equine patient can be performed using the GoPro camera system, including the newer 360 degree video technology.

Conclusions:
GoPro cameras provide value for visual demonstration of airway procedures during examination and surgery. This technology has the potential to contribute dramatically to the learning experience when training and educating of students, technicians, interns, residents, clients, and clinicians in our field.

Figure 1. GoPro camera at a 360-degree video setting. This set up can provide a 3D video appearance of in this case of a horse that a respiratory endoscopic procedure has been performed. Note how clinicians, resident, students, technicians and owner are present in the image. This setting can provide complete view of entire examination or surgical procedure with the ability to zoom in the video.
Title: Hay dust particles measured by flow cytometry in steamed versus dry hay

Authors: Marie Orard, Erika Hue, Marilyne Guillamin, Meriel Moore Colyer, Anne Couroucé-Malblanc, Laurent Couëtil, Magali Demoor & Eric A. Richard

Affiliation: LABÉO Frank Duncombe / Normandie Université, UNICAEN, EA7450 BIOTARGEN, Caen, France

Mail: marie.orard@laboratoire-labeo.fr

Introduction:
Hay contains many particles contributing to exposure of horses to high levels of inhalable dust (< 100 µm). Among these, the respirable fraction (particles < 5 µm) is likely to be central in the pathophysiology of equine asthma. Immersing or soaking dry hay significantly decreased respirable particulate exposure, while limited data are currently available concerning steamed hay. Flow cytometry is a robust method used to quantify suspended particles < 50 µm, and might easily and accurately quantify particles suspended from hay samples. Our hypothesis is that steaming hay is responsible for a significant while only transient decrease of respirable particulate concentrations.

Objectives:
To sequentially compare the concentrations of total and respirable hay dust fractions suspended from dry and steamed hay over a 48h period, using flow cytometry.

Methods:
Hay samples (1 kg) were collected from 14 square bales, before and 24 hours after steaming by HG-600 (Haygain) and systematically kept in a storage bag. Among these bales, 6 were also harvested immediately (T0), 6h and 48h after steaming. Each sample was shaken manually 30 minutes and the resulting dust sieved through a 2 mm grid then on a 1000-100 µm column. For ‘dry’ samples, hay dust suspension (HDS) was obtained by adding 10 ml sterile saline per gram of dust collected. After steaming, hay dust was suspended in the same volume of saline used for corresponding dry samples. HDS was filtered through 48 µm pores and diluted in PBS with a known number of 5 µm beads as standard. Samples were analysed by flow cytometry, providing absolute particle counts and relative concentrations (particles/µl). Data were compared by paired Student t test and repeated (one-way) ANOVA, and expressed as mean ± SD; p<0.05 was considered significant.

Results:
Steaming hay resulted at 24h in lower levels of both respirable (29805 ± 31198 vs. 576449 ± 316162 particles/µl; p<0.001) and inhalable (419 ± 365 vs. 4895 ± 2200; p<0.001) dust fractions. Moreover, there was a significant (p<0.01) reduction of particles at T0, 6h, and 48h after steaming when compared to dry hay; while no significant difference was observed between post-steaming time points.

Conclusions:
Flow cytometry showed that steaming hay significantly and sustainably decreased both inhalable and respirable particles generated by hay shaking. Further investigations are warranted, to characterise size distribution of particles < 5 µm and their implication in initiation/maintenance of equine asthma.
Title: How effective is a ten-day inhalation therapy with fluticasone and salmeterol via a metered dose inhaler in improving clinical signs of horses suffering from equine asthma?

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Introduction:
Equine asthma is a chronic progressive disease of stabled horses and exacerbations are observed in the vast majority of the patients. The administration of medication by inhalation, due to its local effectiveness, enables the successful treatment of equine asthma while simultaneously reducing the dose, whereby the risk of undesired side effects can be minimized. The repeated and long-term administration of systemic corticosteroids in the horse is often related to undesired side effects. Where a resistance to systemically administered beta-2 sympathomimetics exists, the inhalation route of administration often leads to an improvement of the clinical symptoms.

Objectives:
The purpose of this study was to examine the effectiveness of a combination product administered via a metered dose inhaler using the Equine Haler inhalation device.

Methods:
Using a scoring system, 10 horses with a history of equine asthma were examined (clinical examination, arterial blood gas analysis, bronchoscopy and bronchoalveolar lavage cytology) to confirm the diagnosis and classify respiratory disease as mild to moderate (RAO in remission) or severe (RAO in exacerbation). Horses received aerosol therapy with fluticasone (4mcg/kg BDW twice daily) and salmeterol (25 mcg/actuation) over ten days. Afterwards, the clinical examination was repeated and differences in clinical scores and single parameters before and after therapy compared between the two groups.

Results:
Aerosol therapy led to clinical improvement in all 10 horses. The clinical score was significantly reduced in the overall population, as well as the score of the auscultation findings and the score of the bronchoscopy findings. In addition, a significant improvement of the oxygen partial pressure, the arterial alveolar gradient and the percentage of neutrophils of the bronchoalveolar fluid could be determined after the inhalation therapy.

Discussion:
The use of a combination preparation may also ease the handling of affected horses, since the inhalation time could be reduced. Further studies should compare the effectiveness of the combination product to that of two single compounds. The result of the present study should be an incentive to promote the regulatory drug approval of such a combination preparation for horses.

Conclusions:
In conclusion, the therapeutic regimen investigated here can be recommended even in severe cases of equine asthma.
Title: i15 versus epoc for blood gas and chemistry analysis in horses

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Introduction:
For the last 10 years, miniaturization has allowed portability of numerous tools to be used in veterinary medicine. The well known epoc analyser is a good example of those for blood gas and chemistry analysis. Recently arose the i15 (Edan), a brand new POCT device designed for critical uses in human and veterinary medicine. Portable and easy to use, blood gases and many chemistry parameters can be analysed everywhere in field clinical routine and emergency situations with those machines working on battery.

Objectives:
In order to test reliability, easiness to work, price and time to complete analysis in field equine practice, both analysers were used during clinical routine examinations and general anesthesia.

Methods:
30 same intravenous or arterial blood samples were performed and analysed by pair at the same time on the epoc and i15 machine. pH, PCO2, pO2, HCO3-, ABE, SO2, Na+, K+, Ca++, tCO2, Ht, Hb, BE, glucose and lactate values were statistically compared with a Student t-test (p<0,05 significant). Time for measurements were evaluated, as well as easiness to use and number of analysis failures.

Results:
Also results for all parameters were were higher with epoc, there was no statistical difference except for K+ and Ca++ values (4 vs 3,9 and 1,43 vs 1,54 for i15 and epoc respectively). i15 allowed to measure and calculate more parameters, such as Anion Gap, H+, osmolarity, pO2/FiO2, RI, pO2(A-a) and pO2(a/A).
Both were easy to use, but i15 didn’t need a previous waiting time before injecting the sample into the cartridge. Total analysis time was shorter with i15. Printer and big screen are included in i15, which makes it nice to read the results. Sample carttridge are a little bit cheepper and do not need to be stored in the fridge.

Discussion:
Both i15 and epoc are portable ans easy blood machines for veterinary use.

Conclusions:
i15 offers more parameters and reliable results for routine and anesthesia blood gases and chemistry analysis.
Title: Identification of genetic risk loci for Recurrent Laryngeal Neuropathy in the Thoroughbred horse

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Introduction:
Recurrent Laryngeal Neuropathy (RLN) is an upper respiratory tract disease causing poor performance with economic significance in the Thoroughbred racing industry. This disease is considered to be a complex genetic trait, with previous genome-wide association studies (GWAS) investigating RLN reported. One study identified an association with RLN and the LCORL gene in Thoroughbreds; however, the association did not remain significant when height was included as a covariate in the analysis. Another report of a GWAS in mixed-breed horses identified significant SNPs in haplotypes on chromosomes 21 and 31, although these results could not be replicated when a GWAS was performed in individual breeds. Both studies used phenotypes based on resting upper airway endoscopic examination which is known to result in false positives for RLN.

Objectives:
The aim of this study was to test the hypothesis that use of strictly-defined RLN and control phenotypes based on resting and exercising endoscopic and laryngeal ultrasound examinations would result in the identification of genetic variants associated with RLN.

Methods:
A GWAS was conducted using n=171 Thoroughbreds (n=111 males, n=60 females) with a mean age of 40±16 months. Cases were defined as grade B or C at exercise (Havemeyer scale) with an abnormal left cricoarytenoideus lateralis (CAL) echogenicity on laryngeal ultrasound. Controls were defined as grade 1.1/2.1 at rest and grade A at exercise, with similar left and right CAL echogenicity.

Results:
Using the above-defined phenotypes, n=46 cases and n=125 controls were identified. Using approximately 49,000 equine SNPs, a significant heritable contribution to the trait was identified \( h_m^2=0.49, P<1.21\times10^{-4} \) when wither height, sex and age were included as covariates and adjustment for an 8.3% disease prevalence was applied. Five significant SNPs defined three QTLs on chromosomes 1, 14 and 25 which contained compelling candidate genes with functions related to neuronal development. Using a Benjamini-Hochberg correction, 272 significant SNPs were identified on 26 chromosomes.

Discussion:
This study identified previously unreported SNPs associated with RLN that may lead towards the development of a predictive test for genetic risk for RLN. Although stringent inclusion and exclusion criteria were used, control animals may have been incorrectly phenotyped if they developed RLN at an older age.

Conclusions:
Knowledge of genetic risk for RLN may help improve management of horses in-training and inform on breeding strategies.
Title: Inhalation provocation with specific allergens in horses affected with recurrent airway obstruction (RAO)

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Introduction:
Equine asthma is a chronic disease of stabled horses and exacerbations are observed in the vast majority of the patients. The immunopathological aspects of Equine asthma remain unclear.

Objectives:
The aim of this study was to compare the results of four different allergy tests from healthy and RAO-affected horses and to evaluate the clinical relevance of positive tested allergens via a specific inhalation provocation test.

Methods:
Nine RAO-affected horses and 6 healthy horses underwent a complete lung examination including clinical parameters, arterial blood gas analysis, and bronchoscopy with bronchoalveolar lavage cytology. For allergy testing, venous blood samples were collected for the functional in vitro test and serum samples for the Fc-Epsilon receptor test and IgE ELISA, and were submitted to specialized laboratories. An intradermal skin test (IDT) with 38 different allergens was performed in all 15 horses. Subsequently, a histamine inhalation provocation test was performed in all horses and the intrapleural pressure was measured indirectly via an esophageal probe. For each horse, two individual allergens were chosen for the allergen inhalation provocation test (AIPT) based on IDT results and were inhaled in increasing concentrations (one allergen per day).

Results:
None of the four allergy tests revealed a significant difference between healthy and RAO-affected horses. There was also no correlation between the results of the allergy tests. The intrapleural pressure results showed a large individual variability. A significantly positive reaction within the AIPT was only detectable in two RAO-affected horses 6 hours after allergen inhalation with Aspergillus fumigatus and Cladosporium herbarum, with an increase of intrapleural pressure close to 300% in comparison to the baseline (PBS inhalation). During inhalation, no changes of pressure were detectable in these two horses.

Discussion:
Since all of the allergy tests used in this study can only detect type I immune reactions, these tests are probably not suitable for an etiological diagnosis of RAO according to these results. It is plausible that low intensity immune reactions against the allergen challenge remained undetected because of the great individual variations of the baseline parameters. A larger sample size and more sensitive evaluation parameters are needed in order to elucidate the complex and important immunopathogenesis of RAO.

Conclusions:
Therefore, a pure type I immediate hypersensitivity reaction is unlikely to be involved in causing the symptoms of RAO in this group of horses. Because of the delayed reaction after allergen provocation, other immune mechanisms (e.g. type IV hypersensitivity) could be plausible.
Title: Interactions of Streptococcus equi and mast cells

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Introduction:
Streptococcus equi subsp. equi (S. equi) causes a disease called strangles, a highly contagious upper respiratory tract infection in horses. S. equi produces several virulence factors (e.g. enzymes, adherins and toxins) important for the initiation of the disease. However, little is known about the underlying mechanisms for how these factors influence streptococcal virulence.

Objectives:
The aim of this project was to understand mechanisms of infection and identify virulence factors expressed by S. equi by studying interactions with an innate immune cell, i.e. bone marrow-derived mast cells (BMMCs).

Methods:
For the above described purpose, we used a number of unique S. equi mutants lacking one or several virulence factors. The mutants included S. equi variants 1) lacking all four superantigens (ΔSAg), 2) deficient in both phospholipase A2 toxins (ΔFL) and 3) a multi-knockout strain (ΔSHMAPR2), which lacks both superantigens and phospholipase A2 toxins and is deficient in hyaluronan synthase, M-protein, hemolysin, 3-dehydroquinate synthase, dihydroorotase and recombination protein RecA.

Results:
After 4 hours of coculture of S. equi with BMMCs, the cells and supernatants were collected, followed by RNA extraction and ELISA analysis of released protein. As shown in Fig. 1, IL-6, TNF-alpha and Nr4a3 gene expression were significantly upregulated in BMMCs incubated with wild-type S. equi and to a similar extent after incubation of BMMCs with superantigen-deficient (ΔSAg) or phospholipase A2 toxin-deficient (ΔFL) S. equi. In contrast, when BMMCs were challenged with the S. equi multiple mutant lacking superantigens, phospholipase A2 toxins, hyaluronan synthase, M-protein, hemolysin, 3-dehydroquinate synthase, dihydroorotase and recombination protein recA (strain ΔSHMARP2), no detectable upregulation of these genes was seen (Fig 1). These results were confirmed at the protein level, by showing that S. equi wild-type strain 4047, superantigen-deficient strain (ΔSAg) as well as the phospholipase-deficient strain (ΔFL) were capable of inducing a robust cytokine response in mast cells whereas, in contrast, the multi-knockout strain (ΔSHMARP2) was completely without effect (Fig 1.).

Discussion:
These results reveal that factors lacking in the multi-knockout S. equi strain, most probably a combination of superantigens and phospholipase A2 toxins are essential for the induction of pro-inflammatory responses in mast cells.

Conclusions:
These results and future studies have the potential to identify novel virulence factors of S. equi with ability to activate the immune system as represented by mast cells.
Title: Intra-articular triamcinolone effects on lung function of severe equine asthma horses

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Introduction:
Mild equine asthma (IAD) is common, but underdiagnosed in high-performance horses. It is often present without overt clinical signs indicative of pulmonary disease. We hypothesize that intra-articular therapy with corticosteroids improves the performance of horses with subclinical lung diseases.

Objectives:
Determine whether an intra-articular injection of triamcinolone acetonide (TA) improves the lung function in horses with severe equine asthma.

Methods:
Severely asthmatic horses, during disease exacerbation, were administered either 20 mg of TA in both tarsocrural joints (n = 6; 40 mg/horse) or 40 mg of TA intramuscularly (n = 5). The therapeutic response was evaluated by daily clinical examinations and weekly lung function measurements.

Results:
The pulmonary resistance (RL) and pulmonary elastance (EL) values were similar in both groups of horses prior to TA administration. RL and EL decreased starting at day 7 and at day 14, in the intra-articular (p = 0.0001 and p = 0.003, respectively) and intramuscular groups (p = 0.004 and 0.03, respectively), respectively. The lung function was improved compared with baseline up day 35. The area under the curve (AUC) for RL (p = 0.61) and EL (p = 0.62) were similar in both groups of horses with therapy. While the overall serum triamcinolone acetonide concentrations were similar in the two groups (AUC p = 0.47), the kinetic differed. Serum concentration was significantly higher in the intra-articular group than in the intramuscular group at day 3 (p < 0.0001). However, serum TA was significantly higher in the intramuscular group than in the intra-articular group from day 14 to day 35 (p = 0.0003 – 0.0150).

Discussion:
The pulmonary effects exceeded the TA withdrawal period permitted by most sports regulatory bodies. Furthermore, when improvement in performance following intra articular injections of TA is solely imputed to joint disease, it may delay the diagnosis of equine asthma and the implementation of preventive treatments.

Conclusions:
Results from this study indicate that intra-articular and intramuscular TA injections are equipotent at improving the lung function in asthmatic horses for 4 weeks.

Even if the global effect is similar between the two groups, the pic serum concentrations of triamcinolone acetonide with intra-articular injections were greater and of a shorter duration when compared to an intramuscular administration. The therapeutic response followed this kinetic.
Title: Investigating the Effects of Different Potential Prophylactic Treatments on EIPH

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Introduction:
There is growing global pressure to eliminate race-day furosemide administration despite demonstrated efficacy in reducing EIPH severity.

Objectives:
Identify a prophylactic treatment that mitigates EIPH severity without compromising patient health when initiated 24h before maximal exercise.

Methods:
6 horses underwent maximal treadmill exercise 24h after initiating 7 treatment protocols – 0.5mg/kg (LD) and 1.0mg/kg (HD) furosemide iv with free or controlled access to water (H2O; 6ml/kg q4h until 8h pre-exercise; LDW, HDW); 24h controlled access to H2O (W); 4h after 0.5mg/kg furosemide iv (F), or placebo (C). Bronchoalveolar lavage fluid red cell count (BALF#) was determined before and 45-60min post-exercise (after endoscopy to assign an EIPH score). Body weight, urine volumes and ion excretions, H2O intake and fecal H2O were recorded for 24h. Data were analyzed using linear mixed effects models, and the most promising treatment evaluated further using endoscopy and BALF# following 1100m simulated races.

Results:
BALF# and differences between pre- and post-exercise BALF# (ΔBAL#) underwent log10 transformations to satisfy requirements for normality. F decreased post-exercise BALF# (p=0.04), but not ΔBAL#. For ΔBAL#, LDW was less than C (p=0.04) and approached significance for post-exercise BALF# (p=0.052; Table 1). Horses incurred no ill effects from treatment. Consequently, LDW was evaluated in the simulated races the findings being that BALF# was not affected by LDW (p =0.17), while EIPH endoscopic score was (p =0.03). When H2O access was controlled, 24h net H2O loss was greater than for C when access to H2O was controlled; ie, with LDW, HDW and W. H2O consumptions and urine volumes with LD and HD were both greater than for C, resulting in no difference in net H2O loss. Body weight loss was greatest with HDW and LDW, and W was also greater than for HD and LD. Furosemide administration greatly increased excretion of Ca, Na, Mg and Cl, and K and PO4 to a lesser degree. 24h excretion of these ions was greater than that after 4h with F (Fig 1).

Conclusions:
Effects of furosemide on H2O and ion excretion were evident for 24h, but no adverse effects were detected. LDW has potential for reducing severity of EIPH when initiated 24h before strenuous exercise. Compared to results with F, the effect of LDW on EIPH score and BALF# was no different. However, because n=6, results lack the desired statistical power. A larger prospective study is needed to further evaluate the ability of LDW to mitigate EIPH.

Table 1. Effects of treatments on body weight and fluid balance in horses

Table 2. Effects of treatments on body weight and fluid balance in horses
Title: Is ace activity post-race in eiph positive horses influenced by furosemide administration?

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Introduction:
Exercise Induced Pulmonary Haemorrhage (EIPH) affecting racehorses continues to raise questions regarding animal welfare and to-date no effective treatment has been identified. The mode of action of Furosemide on EIPH, the only medication for the condition accepted in some racing jurisdictions, has not been completely elucidated.

Objectives:
To investigate the interaction between Furosemide on Angiotensin Converting Enzyme (ACE) as a potential pathway for future investigation of EIPH treatment options.

Methods:
Horses racing on 8 official race days at Gávea Racetrack had respiratory endoscopy data and blood samples collected after the race and were grouped into Furosemide medicated and Non-Furosemide medicated horses. ACE measurement was conducted using fluorescence in a previously validated method. Environmental, race and haematological data were also recorded. A Multiple Regression Model was used to analyse the data collected, with further analysis including Fisher's Exact test and Pearson's Chi-squared test.

Results:
Seventy-three horses were included in the study. Collections were not conducted in every race since in some instances there were no furosemide medicated horses in a race, due to age or grade of the race. ACE activity between horses not medicated and medicated with furosemide was significantly different (p = 0.006). Multiple regression analysis demonstrated that pre-race furosemide significantly influenced ACE activity post-race (p = 0.03), while distance raced (p = 0.06), temperature (p = 0.25), humidity (p = 0.21), and haematocrit (p = 0.80) did not. Horses medicated with pre-race furosemide still demonstrated some degree of bleeding after the race and were at higher risk of presenting EIPH than non-medicated horses.

Discussion:
Animals medicated with furosemide had lower circulating ACE activity when compared to non-medicated horses, which might indicate a protective effect of furosemide, reducing endothelial damage, therefore reducing the amount of ACE released to the systemic circulation from damaged endothelial cells during EIPH. Alternatively, it could be hypothesized that furosemide increased activity of ACE 2, promoting vasodilation and reducing the availability of Ang I for conversion by ACE. In order to definitively test these hypotheses, activity of ACE isoforms should be measured with specific assays, and pharmacological properties of furosemide directly on the pulmonary endothelium explored further.

Conclusions:
Although the study investigated ACE activity, it is not possible to discard the effect of other Renin-Angiotensin Aldosterone System components, such as other ACE isoforms in the interaction observed. Although the effects of exercise on ACE are known, this study did not measure ACE activity before and after Furosemide in horses at rest. Horses medicated with Furosemide had lower circulating ACE activity than horses not medicated. Furosemide reduced EIPH severity after a single bout of exercise, but it did not abolish or reduce its occurrence. To further investigate the effect of Furosemide on ACE the authors are currently collecting samples for further analysis.
Title: Mast cells in the bronchoalveolar lavage fluid of 152 horses with and without respiratory signs

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Introduction:
Mast cells are present in the equine lower airways and can be detected in the bronchoalveolar lavage fluid (BALF). Mast cells contain granules of histamine and heparin, and their presence and activity have been associated with allergy and potential respiratory disease in horses. However, clinically normal horses have shown variable amounts of mast cells in BALF, and therefore the clinical significance has not been fully elucidated.

Objectives:
To study the percentage of mast cells in BALF collected from horses presenting with or without respiratory signs.

Methods:
152 privately owned horses were subject to BAL during 2009–2015 in the Helsinki University Equine Teaching Hospital. Of these horses, 33 were asymptomatic and were sampled for research purposes with owner consent (Group 1), 77 horses presented with either cough, nasal discharge or exercise intolerance (Group 2), and 42 horses presented with both cough and nasal discharge or marked tracheal mucus accumulation confirming clinical respiratory disease (Group 3). BALF was obtained from the right lung with 240–360 mL of sterile 0.9% saline and cytology analyzed by counting 300 cells. The statistical analysis was performed with Kruskal-Wallis one-way analysis of variance for group comparisons and Spearman's correlation coefficient.

Results:
The median [IQR] of BALF mast cell percentage was 2.4 [1.4–3.7] for Group 1, 2.3 [1.4–3.7] for Group 2 and 1.6 [1.0–2.7] for Group 3. The median (range) age was 8 (3–19) for Group 1, 10 (1–21) for Group 2 and 13 (8–26) for Group 3. There was no difference in the BALF mast cell percentage among the three groups. BALF mast cell percentage did not correlate with age. A very weak negative correlation was detected with mast cell percentage and the amount of mucus in the trachea (p=0.008, ρ=−0.22) and BALF neutrophil percentage (p=0.006, ρ=−0.22).

Conclusions:
In this patient population with naturally occurring disease the percentage of mast cells did not differ among asymptomatic horses and horses with mild or severe respiratory signs. This finding supports the conclusion that BALF mast cell percentage may not relate to the respiratory signs. However, further research is warranted to study if horses with specific signs or disease show increase in BALF mast cell percentage.

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Proceedings of the World Equine Airway Symposium, Copenhagen, Denmark - 2017
Title: Measurement of IL-17 in horses at pasture with and without subclinical airway inflammation

Authors: Katia M. da Silva, Maria F. de M. Costa, Johnatas D. Silva, Luis F. A. Toledo, Anna C. P. Caruso, José G. de Almeida, Andreza A. da Silva, Eliene P. Sad, Pedro L. Silva, Daniel A. B. Lessa

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Introduction:
Usage of inflammatory cytokines as a pathway to differentiate between certain equine respiratory conditions or disease stages has not been fully explored. IL-17 is a potent proinflammatory cytokine which mediates attraction of granulocytes to tissues undergoing inflammation.

Objectives:
Investigate a commercial ELISA assay for measurement of IL-17 in horses at pasture, with and without subclinical airway inflammation as evidenced by abnormal cell cytology in bronchoalveolar lavage (BAL) fluid.

Methods:
39 clinically healthy mixed breed horses kept at pasture were used. BAL samples were collected from all animals followed by slide preparation and examination of 500 cells, including epithelial cells. Animals were separated into two groups according to BAL fluid cytology: group A with normal airway cytology (neutrophils < 5%) and group B with neutrophilia (> 10%). IL-17 concentrations were measured using a commercial ELISA kit (Nori Equine IL-17C Elisa Kit - BAL, Genorise, USA) as per manufacturer’s instructions.

Results:
33 horses (84.6%) had IL-17 readings within the detection range for the ELISA kit used, with concentrations ranging from 12.2 ng/ml to 238.8 ng/ml (average 126.6 ± 60.3 ng/ml). Twelve horses (30.77%) had BAL fluid neutrophilia and 10 (25.64%) had normal cytology. Group A IL-17 values (130.2 ± 65.8) were not significantly different (p = 0.35) from Group B (140.5 ± 57.1). Post hoc stratified analysis of horses considered healthy (neutrophils below 5%, n = 10), equivocal (5% < neutrophils < 10%, n = 11), and with airway inflammation (neutrophils > 10%, n = 12) with ANOVA showed no significant difference in IL-17 in each of the three categories (p = 0.44; Pooled SD = 60.6).

Discussion:
Most samples assayed fell within the detection range for the ELISA kit used but the large variation found in IL-17 concentration could not be explained by cytology of BAL fluid in the animals studied. Protocol variations and changes in sample dilution rates might optimize results, although other reasons for the variation in results, both physiologic and pathologic, should be sought. A decrease in the power of the study mandates larger samples to be obtained and reassessed.

Conclusions:
Measurement of IL-17 concentration in BAL fluid of horses using a commercial ELISA kit was successfully achieved without protocol complications. No significant difference in IL-17 between horses at pasture with and without BAL fluid neutrophilia was detected.
Title: Nerve stimulation-guided injection to the recurrent nerve – a potential technique for the use of stem cells in the treatment of laryngeal hemiplegia

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Introduction:
Laryngeal hemiplegia is a common disease of which the pathogenesis is not completely understood. Affected horses show varying degrees of denervation atrophy of the muscle innervated by the recurrent nerve. The latter generally shows lesions of demyelination but also of remyelination, which is not related to clinical remission. Animal models have shown an interest in applying stem cells to demyelinated nerves.

Objectives:
The aim of the study is test feasibility and safety of perineural injection of muscle-derived mesenchymal stem cells to the recurrent nerve in healthy horses.

Methods:
Five horses were used for this protocol. Muscle-derived stem cell were obtained from 4 Standardbred horses as previously described (Ceusters et al., 2017). Horses were sedated with 10 µg/kg detomidine and movements of the larynx were recorded via upper airway video endoscopy. The left recurrent nerve was approached with an insulated nerve block needle under ultrasound control. Nerve stimulation was applied starting at 2mA and successful abduction of the left arythenoid was monitored and recorded endoscopically. Stimulation intensity was progressively reduced and when motor response was lost at 0.5mA, 10*106 autologous muscle-derived stem cells were injected. An injection of 1 ml of 2% lidocaine was performed in the first horse. The four other horses were treated with autologous stem cells and had their laryngeal function scored by two examiners blinded to the treatment before and at day 1, 7, and 28 after stem cell injection.

Results:
In the first horse correct positioning of the needle was further confirmed by injection of 1 mL of 2% lidocaine, which resulted in loss of motor response and temporary paralysis of the left arytenoid cartilage. Full recovery of laryngeal function was confirmed endoscopically the day after the injection. All horses tolerated the nerve stimulation of the recurrent nerve very well. There was no difference between pre-injection scores of laryngeal function and scores obtained day 1, 7 and 28 after stem cell injection.

Discussion:

Conclusions:
This study shows that approaching the recurrent nerve with nerve stimulator guidance is possible and that nerve stimulation is well tolerated by the horses. None of the horses showed modification of their laryngeal function after injection of stem cells. Further studies should demonstrate the effects of peri-neural injection of autologous muscle-derived mesenchymal stem cells in horses affected by laryngeal hemiplegia.

References
NEUROLOGICAL SIGNS IN RELATION TO PROGRESSIVE ETHMOIDAL HEMATOMA

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Introduction: Progressive ethmoidal hematomas (PEHs) are non-neoplastic expanding masses in the sinonasal areas. Reports on neurological signs in relation to PEH are sparse and all 3 reported cases were fatal.1,2,3

Objectives: Describe two horses with neurological signs in relation to PEH

Results:

Case 1 – 13 year old Warmblood gelding, diagnosed with a left-sided PEH by endoscopy. Computed tomography (CT) showed involvement of sphenopalatine sinus. Treatment with formalin injection was initially successful, but regrowth occurred after a year necessitating new injections. Another year later, the horse was reported by the owner to have episodes with impaired vision and mental distance. A new CT showed expansion of the PEH into the nasal cavity, invasion into the cribiform plate and into the caudal sphenopalatine sinus in the area of the optic canal and the orbital fissure that contains CN III, IV, V and VI.4 The horse was euthanized.

Case 2 – 10 year old Warmblood gelding, diagnosed with a right-sided PEH by endoscopy. X-ray evaluation did not reveal involvement of the sinus. The horse was treated with a series of formalin injections. The PEH showed good regression, and a third injection was performed under sedation six weeks after the first. Within minutes following intrallesional injection, the horse became agitated and compulsively trotted in circles to the left. Eventually he calmed down and got fairly normal again. Approximately 10 minutes later he became obtundated, ataxic in all 4 legs, showed ptosis and a maximally mydriatic pupil with no direct or indirect light reflex in the right eye but with menace response. Following metacam treatment, he improved over a couple of hours, but remained slightly ataxic for 7 days. The only remaining sign after 3 months was right-sided mydriasis. The horse returned to full training. Chemical injury was suspected to CN III (possibly at the level of the sphenopalatine sinus)4 and to the brain.

Conclusions: Neurological signs can develop as a result of the expansion and pressure of the mass, but also as a result of treatment of PEH. Partial recovery is possible following chemical neuronal injury but prognosis for full recovery is guarded.

References
Title: Occurrence of epiglottic retroversion in one family of silesian draft horses.

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Introduction:
The epiglottic retroversion is poorly understud upper airways dynamic disorder in horses. To this day there is no sucessful treatment, to cure affected horses, for this reason horses with epiglottic retroversion have poor prognosis to perform at the level of expectation. There is no proof that this disease is heritable.

Objectives:
Establishing genetic predisposition of epiglottic retroversion in horse.

Methods:
Three horses from the same family of silesian draft horses where admitted for examination of upper airways, reason for examination was loud inspiratory noise and poor performance. Horses where from the same mother, and two of them where from the same stallion. Third horse was from the same mother but his father was grandfather of other two, the family relationship was very close. In all horses dynamic respiratory endoscopy was performed with use of Optomed DRS system. Horses were examined during casual training under skilled rider.

Results:
In all three horses epiglottic retroversion was found as the reason of poor performance and loud respiratory noise.

Discussion:
There is no data about genetic predisposition for epiglottic retroversion in horse. Disorder might be correlated with anatomical conformation of these horses and this might be inherited.
One gelding had a very intensified disorder and was retired, two other horses where able to compete at lower level as dressage and draft horse. It might be interesting to establish factors witch might influence intensification of this disorder.

Conclusions:
Those cases indicate that there might be genetic predisposition of epiglottic retroversion in horses. Occurrence of epiglottic retroversion as a genetic disorder need a further investigation.
Title: Overground endoscopy in thoroughbreds and standardbreds to evaluate the effect of tongue ties

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Introduction:
There is contradictory published evidence on the potential efficacy of ‘tongue ties’ (TTs) on upper airway function and for treatment of intermittent dorsal displacement of the soft palate (DDSP) in racehorses.

Objectives:
To evaluate the effect of TTs on pharyngeal and laryngeal diameters as well as occurrence of DDSP in 30 Thoroughbred and Standardbred racehorses in Germany using a prospective, cross-over blinded clinical study.

Methods:
22 Thoroughbred and 8 Standardbred racehorses were examined using overground endoscopy under full-intensity exercise on their training race tracks with and without fixation of the tongue by use of TTs. Equivalent exercise intensity was ensured by measuring heart rate (bpm), speed (GPS) and venous lactate. Pharyngeal diameter was expressed as pharyngeal-epiglottis-ratios as described in former studies and laryngeal abduction accordingly as laryngeal-median-ratios.

Results:
The pharyngeal diameter increased significantly in all horses between rest and full-intensity exercise (P<0.01). Multi-variable-analysis revealed that this effect was significantly decreased by the application of tongue ties (P<0.01). No significant effects of TTs on laryngeal parameters were found. DDSP was found in 4/30 examinations with TT (13.3%) and in 1/30 examinations without TT (3.3%).

Conclusions:
The results of this study do not support the use of TTs to support upper airway function and to prevent the occurrence of DDSP.
**Title:** Pharmacological manipulation of Conventional Primary Equine Bronchial Epithelial Cells

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**Introduction:**
The airway epithelium plays an important role in physiological and pathological processes including inflammation, innate immunity and regenerative responses in the respiratory tract with regard to airway diseases such as human asthma, COPD and Equine Recurrent Airway Obstruction (RAO). Well-differentiated primary equine bronchial epithelial (EBEC) cell cultures are pivotal for airway disease research, particularly for development of drugs. Horses often suffer from an asthma-like airway disease and can be used as large animal models.

**Objectives:**
We initially determined the electrophysiological and morphological characteristics of EBEC, cultured under conventional and air liquid interface (ALI) conditions and here, the influence of the RhoA kinase (ROCK) inhibitor Y-27632 (Y). This drug is used to establish long-term equine bronchial epithelial cell cultures.

**Methods:**
Fresh isolated EBECs were cultured in the presence and absence of Y-27632 under conventional and ALI conditions in supplemented airway epithelial cell growth medium. The effects of Y-27632 on cell viability, morphology, proliferation and differentiation were examined.

**Results:**
When treating EBEC cultures with the inhibitor, cells grew exponentially for a number of passages (>4) with less contamination of the EBEC with fibroblasts. Passaged EBEC formed confluent mucociliary ALI cultures. There were differences in cell morphology, tight junction formation and current magnitude as a function of extended passages between Y-27632-treated and not treated EBEC.

**Discussion:**

**Conclusions:**
The ability to rapidly generate many primary EBE cells from bronchial specimens provides significant opportunities for cell-based diagnostics and therapeutics. This study confirms the benefits of Y-27632 to provide the opportunity to culture primary EBEC for extended periods of culture.
Presence and genetic variation of Streptococcus zooepidemicus in Finnish weanling horses

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Introduction:
Streptococcus equi ssp. zooepidemicus (SEZ) is part of normal upper airway microbiota of horses and an opportunistic pathogen associated with equine respiratory infections. SEZ is also capable of causing variety of pyogenic, potentially severe infections in horses, other animal species and humans.

Objectives:
To evaluate the presence and genetic variation of SEZ in weanling foals in Finland in 2013-2015.

Methods:
The study population included 83 weanling foals from six loose housing (LH) and five stable (SF) farms. Nasal swabs were acquired from 70 of these. Bacterial isolates were identified using conventional methods. The percentage of foals from which SEZ was isolated on the first sampling was calculated. To get an overview of circulating SEZ strains, isolates were typed with pulsed-field gel electrophoresis (PFGE) by using Smal restriction enzyme. Similarity level ≥ 85% (1.5% tolerance and optimization) was used to assign the isolates into clusters. Representatives of clusters were further typed with multi-locus sequencing (MLST) (https://pubmlst.org/szooepidemicus/).

Results:
Out of 70 foals, 31 foals (44%) from five LHs and two SFs were positive for SEZ in their first sampling. Of LH foals 53% carried SEZ (29/55) while the respective proportion for SF foals was 13% (2/15) (p=0.008, Fisher’s exact test). PFGE was performed for the 29 and MLST for 18 isolates. SEZ isolates were distributed into 11 PFGE clusters. One isolate was not typeable. The largest cluster (E) had six isolates followed by cluster A with five isolates, and clusters I and J with three isolates. Of the six cluster E isolates, five were from the same farm (LH5) and of a new sequence type (ST). The remaining isolate, ST65, was from another farm and was a triple locus variant of the former. The isolates of Cluster A were of ST5, and were from two different farms (LH5 and LH3). Cluster J, from LH3, had a new proS allele. The following STs were from seven different farms: ST15, ST113, ST138, ST174, ST330, ST331, and ST340. In addition, two more new types were observed: a strain with non-amplifiable proS gene, and the other with a novel allele profile.

Discussion:

Conclusions:
SEZ was isolated in 44% of the weanling horses. However, the presence of SEZ was four times higher in LH foals compared to SF foals. The genetic variation of SEZ was wide.
Title: Prevalence of Exercise-Induced Pulmonary Hemorrhage in Barrel Racing Horses in the Pacific Northwest

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Introduction:
Exercise-induced pulmonary hemorrhage (EIPH) refers to presence of blood in the airways caused by strenuous exercise. It has been documented in racing, polo and eventing horses, but little is known about EIPH in barrel racing horses.

Objectives:
The objective of this study was to test for the presence of EIPH in barrel racing horses, estimate its prevalence in the Pacific Northwest and determine whether a relationship between EIPH and performance exists.

Methods:
This study enrolled 158 barrel racing horses competing at events in WA., ID., and MT., after obtained owner consent. Data collected included signalment, illness/respiratory history, race division, and race day medications. Tracheobronchoscopy was scored based on quantity of blood in the trachea (0=no blood to 4=large amounts throughout the trachea). Erythrocyte counts were obtained from bronchoalveolar lavage fluid. Statistical analysis included logistical regression, Chi squared, Fisher’s Exact T-test, linear regression and calculation of correlation coefficient. Significance was set at P < 0.05.

Results:
The prevalence of EIPH in our study was 54%. Horses that ran faster had a significantly greater likelihood of bleeding P < 0.014. Bleeding did not significantly affect performance, although horses that bled finished lower than horses that didn’t bleed. Significant (P < 0.001) positive linear relationship between the tracheal score and BAL erythrocyte count was shown. Correlation coefficient between these 2 tests was poor (r2 = 0.14).

Conclusions:
This study shows EIPH is present in barrel racing horses in the Pacific Northwest and may impact performance. The long term effects of EIPH and other variables requires further study.
Title: Prevalence of fungi in respiratory samples of horses with inflammatory airway disease

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Introduction:
Fungi have been shown to contribute to the inflammatory response of lungs in horses with recurrent airway obstruction (RAO) and in some forms of asthma in humans. The role of fungi in IAD (inflammatory airway disease) has not yet been assessed to the knowledge of the authors.

Objectives:
The purpose of this prospective clinical study was (1) to evaluate the prevalence of fungal isolates in the respiratory samples of horses diagnosed with IAD (2) to describe clinical signs associated with the presence of fungi in respiratory samples (3) to assess the risk factors associated with IAD and with the presence of fungal elements in the airways. The study was performed in a population of working sport, race and leisure horses based in Europe.

Methods:
A total of 482 horses, referred to a specialized ambulatory practice, for respiratory problems or loss of performance, entered the study. For each case, an environmental evaluation, a clinical examination, an airway endoscopy, a tracheal wash (TW) and a bronchoalveolar lavage (BAL) were performed. The TW and the BAL underwent cytologic evaluation and the TW was submitted for bacteriology and mycology. Diagnosis of IAD was established based on BAL fluid cytology. On both cytologic examinations, the presence of fungal elements (spores, conidiophores, hyphae) and signs of active proliferation were recorded.

Results:
IAD was diagnosed in 84% of cases. The fungal culture was positive in 49% of IAD+ cases and in 44% of IAD- cases. The most commonly isolated fungus were Aspergillus sp, Penicillium and Rhizomucor. No relationship could be established between positive mycology and cytologic evidence of fungal elements or active proliferation of fungi in the airways. However, horses with fungal elements on the TW cytology had 3.8 more chances of having IAD than horses with no fungi (OR=3.8; 95% CI 1.8-7.8; p=0.0003). No clinical sign was sensitive or specific for the presence of fungal elements in the TW. Risks of fungal elements in TW were higher when horses were bedded on straw vs. shavings or fed dry hay vs. steamed hay.

Discussion:
In the current study, mycology culture seems to lack sensitivity in the detection of fungi in respiratory samples.

Conclusions:
Horses inhaling aerosolized fungal particles are at a significantly higher risk of developing IAD. The type of bedding and forage represent significant risk factors for IAD and fungal contamination of equine airways.
Title: Relationship between dynamic upper airway disorders of pharynx or larynx and inflammatory airway disease in horses of different use.

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Introduction:
Respiratory diseases are a common cause of poor performance in equine athlete. Diagnosis of poor performance in horse often demand an investigation both of upper and lower respiratory tract. There is limited data describing correlation between dynamic upper airway disorders and inflammation of lower airways in horses of different use (pleasure, racing, sport).

Objectives:
Purpose of this study was establishing relationship between occurrence of dynamic upper airway disorders of pharynx or larynx and inflammatory airways disease.

Methods:
Study was conducted on 29 horses patients of Warsaw Equine Clinic University of Life Sciences. Horses were examined with use of high speed Haico 4000 treadmill. Horses was running at different speed, slope and time witch were adjusted to the use of the horse. Resting endoscopy and was performed before and after exercise test. Cytology and bacteriology of tracheal and bronchoalveolar lavage fluid was done in every horse. According to cytology results and endoscopic appearance horses where divided in two groups: horses with IAD and horses with no lower airways abnormality.

Results:
From IAD group only 2 had structural disorders of pharynx and larynx in rest. In 8 horses structural disorders was diagnosed in pharynx and in one horse in pharynx and larynx.
In non IAD group, 11 horses had no structural disorders during resting endoscopy. In exercise endoscopy 10 horses showed disorders of larynx in 3 horses disorders were found in pharynx and in last 3 horses disorders were both in pharynx and larynx.

Discussion:
Despite continuous improvement and implementation of new diagnostic methods, including portable dynamic endoscopes, it is still regarded that exercise tests on a high-speed treadmill are highly efficient for diagnosing dynamic disorders in the pharynx and larynx.
It might be possible that horses with lower airways inflammatory disease are more prone to dynamic disorders of the pharynx as there is no rigid cartilage skeleton supporting pharynx and the negative pressure in inflamed airways is much higher in affected horse. The similar negative phenomenon can be observed in horses with asthma and higher occurance of EIPH.

Conclusions:
In horses which are diagnosed as IAD, disorders of pharynx during treadmill exercise test are much more frequent then in horses undiagnosed as IAD, where more often disorders of larynx were diagnosed.
Title: Relationship between Tracheobronchoscopic Score and Bronchoalveolar Lavage Fluid Erythrocyte Count in Horses with EIPH

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Introduction:
EIPH is diagnosed and its severity assessed by post-exercise tracheobronchial endoscopic score (TBE), and/or enumeration of erythrocytes (RBCs) in bronchoalveolar lavage fluid (BALF). However, the correlation of the result of one to the other is unclear. TBE is easier to perform than BAL and is the diagnostic method of choice for most equine practitioners, with a score of 0-4 assigned according to a single observation 30mins to 2hr post-exercise. Although sensitivity and specificity of TBE are presumed to be high (low rate of false negatives and false positives), this has not been confirmed.

Objectives:
Based on clinical observations (see Fig 1), we hypothesized that TBE score has a low sensitivity but high specificity for diagnosis of EIPH, and is weakly correlated to BALF RBC count.

Methods:
Two data sets were analyzed: 1) Sensitivity and specificity of TBE scores were calculated using TBE scores and pre- and post-supramaximal exercise BALF RBC counts from 102 Thoroughbreds. The EIPH+ gold standard was an exercise-induced BALF RBC increase ≥500/μl and a post-exercise RBC number ≥1000/μl. RBC counts not meeting these criteria were regarded as EIPH-. 6 horses also had BALs performed twice 24hrs apart to evaluate the effects of BAL on RBC count. 2) Correlation of TBE score to RBC number was determined for 151 horses undergoing 223 exercise events (treadmill or racetrack exercise to fatigue or barrel racing) with a Spearman rank order test. Significance was set at p<0.05.

Results:
BAL itself had no effect on RBC count (Table 1). TBE scores had a low sensitivity (0.63) and good specificity (0.88) for diagnosis of EIPH. The correlation between TBE scores and BALF RBC counts was weak but significant (r²=0.18; p<0.001) (Table 2).

Conclusions:
Because of its low sensitivity and poor correlation with BALF RBCs, reliance on TBE for diagnosis of EIPH or estimation of its severity is not ideal. This high potential for false negatives has important clinical implications for detecting and assessing severity of EIPH. A TBE score of 0 does not rule out the occurrence of EIPH and it might be advisable for practitioners to perform a BAL on poorly performing horses with this score before ruling out EIPH as a cause of the poor performance. Specificity of <1.0 was unexpected, as the presence of blood in the trachea cannot be a false positive. Therefore, the calculated specificity likely reflects limitations with BAL as a diagnostic method for EIPH.
Introduction:
Chronic respiratory diseases in horses develop as a consequence of infectious, allergic or parasitic causes. Horses are often empirically treated with antimicrobials, However, due to increasing antimicrobial resistance problem, their use should be justified. Antimicrobial therapy is indicated in lower respiratory tract (LRT) bacterial infection but its recognition based on clinical presentation may not always be straightforward.

Objectives:
The objective of this retrospective study was to analyse history and clinical data from horses with chronic respiratory disease and assess the relationship between the incidence of the particular history and clinical parameters and the isolation of bacteria from tracheal wash.

Methods:
This study includes data from 42 cases of chronic respiratory disease in adult horses. History data, clinical signs, endoscopy findings and results of tracheal wash culture were analysed. Horses were divided into two groups: those with positive and those with negative tracheal wash cultures. The incidence of the particular history and clinical data were investigated within each group. Statistical significance was assessed by using chi-square and Fisher's exact test.

Results:
The most frequently recorded history data were coughing (95.2%), previous antimicrobial therapy (62.0%) and breathing difficulty (52.4%), while the most frequently observed clinical signs were cough provocation (83.3%), breathing difficulty (54.8%), increased respiration frequency (54.8%) and abnormal lung sounds (50.0%). Bacteria were isolated from a tracheal wash of 19/42 (45.2 %) horses. In 11/19 (57.9%) cases gram-negative (Actinobacillus equuli, Pasteurella caballi, Klebsiella sp., Acinetobacter sp.), while in 8/19 (42.1%) cases gram-positive bacteria (Streptococcus sp., Staphylococcus sp.) were isolated. In the group of horses with positive tracheal wash culture higher incidence of previous antimicrobial therapy, abnormal lung sounds and a greater amount of tracheal mucus were recorded (p<0.05).

Conclusions:
There was a greater likelihood of isolation of bacteria from tracheal wash in horses previously treated with antibiotics, in horses with abnormal lung sounds and those with increased amount of tracheal mucus. Therefore, the presence of these parameters emphasises the need for tracheal wash bacteriological examination and adequate antimicrobial therapy.
Title: Respirable particulate size and concentrations in five Newmarket (UK) training yards

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Introduction:
Airborne particulate matter is implicated in the pathogenesis of Inflammatory Airway Disease. There is a need to investigate variations in air hygiene under normal stabling conditions, to inform recommendations for optimal racehorse husbandry.

Objectives:
To determine particulate size concentration ranges in Newmarket training yards during summer and winter.

Methods:
Airborne particulate mass concentration (mg/m3) and size were recorded by a direct reading instrument (DustTrak™). Five yards were examined for one day each in summer (July–August 2016) and winter (December 2016–February 2017). Representative boxes were assessed (i) early morning (ii) midday and (iii) evening. Statistically significant differences between the summer and winter were analysed with the Wilcoxon signed rank test (P<0.05).

Results:
Data on particles sized ≤2.5μm and ≤10μm were obtained from 116 boxes.

Minimum summer particulate concentration range 0.007–0.195 mg/m3 was similar to the winter range 0.010–0.125 mg/m3. In the morning, summer values for both particle sizes were statistically significantly greater than in winter (P= 0.006 for ≤2.5μm and P = 0.003 for ≤10μm). During winter, midday (P = 0.039 for ≤2.5μm and P = 0.008 for ≤10μm) and evening (P = <0.01 for ≤2.5μm and P = 0.021 for ≤10μm) values were statistically significantly greater than the summer.

Maximum winter particulate concentration range 0.027 – 2.192 mg/m3 was significantly greater than the summer range 0.020 – 9.505 mg/m3 (P = 0.015 ≤2.5μm and P = 0.022 for ≤10μm). There was no consistent pattern when comparing the summer and winter three time points.

Mean summer particulate concentration range 0.019 – 0.392 was similar to winter range 0.015 – 0.597 mg/m3. At all time points, summer average values were greater than in winter; only during the midday measurement was this statistically significant (P = 0.031 for ≤2.5μm and 0.017 for ≤10μm).

Conclusions:
Statistically significant differences were found between summer and winter sampling, however the clinical relevance of this remains unknown. Particulate concentration ranges for ≤2.5 and ≤10μm were established for Newmarket training yards that are similar to other studies using racehorse populations. Further investigation is required to understand how further yard-level variables influence respirable airborne particulates.
Title: Respiratory Responses to Tethered Swimming Exercise in Horses

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Introduction:
Swimming is commonly used as a method of training and rehabilitation in both Thoroughbred and Standardbred racehorses. However, there is limited knowledge of the breathing strategy used by horses when swimming and the impact of swimming on the patency of the upper respiratory tract (URT).

Objectives:
To describe the respiratory responses and endoscopic appearance of the URT during tethered swimming in horses.

Methods:
Eight race fit horses (3 Thoroughbreds and 5 Standardbreds), with no history of URT obstruction and were trained to swim tethered, were used. Endoscopic examination, sound recordings, and above and below water video recordings were obtained during a 2 minute swim. Sound recordings were analysed by measuring 6 breaths for each horse using Sony Sound Organizer program. Mean (± SD) inspiratory (TI), expiratory (TE) and breath holding (apnoea) times were recorded. Plasma lactate concentration was measured before and 5 mins after exercise. Tracheal endoscopy was performed 30 minutes after swimming to assess for presence of blood or mucus.

Results:
All horses had complete URT collapse on each breath during swimming. This included closure of the external nares, complete constriction of the nasopharynx and closure of the rima glottidis due to bilateral adduction of the arytenoid cartilages and vocal folds. Elevation of the epiglottis was also observed in 2 horses.
Mean (± SD) breathing frequency was 28 ± 5 bpm during swimming, with a brief inspiration (mean ± SD TI= 510 ± 80ms), followed by a period of apnoea (1590 ± 530ms) when the airways were closed and then a short forced expiration (TE =420 ±50ms). Locomotor-respiratory coupling was not observed. Mean (± SD) plasma lactate post-swim was 6.9± 7.91mmol/L versus 0.73± 0.20mmol/L pre-swim, indicating an anaerobic component to the exercise. Post-exercise endoscopy revealed grade 1 EIPH in 2 horses. Median mucus grade was 1 (range 0-3).

Conclusions:
Horses experienced complete collapse of the URT and post-inspiratory apnoea when swimming. This may occur as a result of the mammalian diving response – a survival reflex to preserve oxygen stores and prevent water entering the lungs. It has been suggested that the resultant increase in lung volume may also aid buoyancy. The triggers for this phenomenon during surface swimming and whether or not central control plays a role are uncertain and warrant further research.
Title: Respiratory workload and capacity at submaximal activity in caspian miniature horses

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Introduction:
Respiratory and cardiovascular examination have essential role in the evaluation of exercise tolerance in equine sport medicine. Standardized exercise testing (SET) is used by researchers to judge the fitness level of horses. Field training is often favored as it produces results similar to that which a horse would achieve in competition or racing. Horses are generally exercised and competed in the field or an open space, introducing other factors such as the rider, weather and terrain that are not present in treadmill testing (Serrano et al. 2001).

Increase in respiratory rate, changes in its quality and presence of abnormal sound during exercise along with alterations in blood lactate concentration are used to respiratory evaluation of horses.

Objectives:
Respiratory examination analysis and proportion of sub maximal effort of Caspian miniature horses to blood lactate concentration was objected in this study.

Methods:
Twenty Caspian miniature horses (12 mare and 8 stallion) aged 4-13 years and 200-250 kg weight were selected and examined carefully for respiratory characteristics such as rate, type and symmetry and a blood sample was taken before the exercise. Plasma harvested immediately from the blood for lactate measurements. Horses were exercised in 1000 meters sand track and respiratory examinations were repeated after the exercise along with lactate measurements.

Results:
The results of the mean respiratory rate and lactate concentration are shown in table 1. All measurements had significant increase after exercise (p<0.05). Horses that had more speed, showed less lactate concentration. There was no obvious respiratory insufficiency among horses. Although horses ran at the fastest speed as they can, their speed did not reach to more than 6.6 m/s.

Table 1. Mean values of the mean respiratory rate and lactate concentration the Caspian miniature horses
<table>
<thead>
<tr>
<th>RR(min)</th>
<th>Lactate mmol/l</th>
</tr>
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<tbody>
<tr>
<td>Before exercise</td>
<td>23.83±7.4</td>
</tr>
<tr>
<td>After exercise</td>
<td>92.53±15.6</td>
</tr>
</tbody>
</table>

Discussion:
Post-exercise blood lactate concentration can also be used to indicate the fitness of the horse (Serrano et al. 2001). As a horse's fitness increases, post-exercise blood lactate concentrations should decrease (Seren et al. 1977; Art et al. 1990). Courouce et al. (2002) found that training affected blood lactate concentration during a SET, where blood lactate concentration decreased after exercise as training duration increased. However, Hamlin et al. (2002) found that acute overtraining was related to an increase in post-exercise blood lactate concentration.

Conclusions:
The mean, maximum respiratory rate and mean/maximum lactate concentrations in different horse were measured for the first time in caspian miniature horse and for future analysis of SET examinations. These horses are used to horse matches in special miniature class.
Title: Reversibility of pulmonary artery remodeling in severe equine asthma

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Introduction:
Recurrent episodes of airway obstruction, hypoxemia and pulmonary hypertension (PH) are present during exacerbations of severe equine asthma (SEA). Pulmonary hypoxic vasoconstriction is known to contribute to the development of PH, which may lead to cor pulmonale. However, as PH is only partially reversible by oxygen administration, other etiological factors are likely to be involved. In human chronic obstructive pulmonary disease, pulmonary artery (PA) remodeling contributes to the development of PH. Furthermore, allergic airway inflammation results in remodeling of pulmonary vasculature in mouse models, suggesting that similar findings may be present in asthma. We therefore postulated that PA remodeling is present in SEA and contributes to PH.

Objectives:
To investigate 1) the presence of PA remodeling in severe equine asthma and its distribution throughout the lungs, 2) the involvement of vascular smooth muscle (VSM) alterations, and 3) their reversibility following long-term antigen avoidance strategies or inhaled corticosteroids administration.

Methods:
Using histomorphometry and tissue bank (ERTB) lung samples, the PA wall was measured on sections stained with hematoxylin-eosin saffron, collected post-mortem from different lung regions of 12 asthmatic horses and 6 age-matched controls. Pulmonary vascular smooth muscle (VSM) mass was also measured on sections stained for α-smooth muscle actin collected with in vivo thoracoscopy or post-mortem peripheral lung biopsy from 5 controls, 6 asthmatic horses in remission, and 11 asthmatic horses while exacerbation and after 1 year of antigen avoidance alone (5 horses) or treatments with fluticasone (6 horses). Data were compared using one tailed unpaired t tests with Welch correction or paired t tests (p<0.05).

Results:
Increased PA wall surface was detected in apical (p=0.002) and caudodorsal (p=0.03) lung regions of asthmatic horses in both exacerbation and remission, when compared to controls. The VSM mass was similarly increased (p=0.03) when compared to controls. A tendency for a normalization of the VSM mass was observed after treatment with antigen avoidance (p=0.05), but not with fluticasone (p=0.27).

Conclusions:
Remodeling of the PA develops in SEA and is associated with an increase in VSM. The resulting narrowing of the lumen of the PA could enhance hypoxic vasoconstriction, contributing to PH during exacerbation of SEA. VSM mass normalization is better achieved by antigen avoidance than by corticosteroids.
Title: Role of particulate exposure and airway inflammation in racing performance

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Introduction:
Mild equine asthma is a well recognized cause of poor performance and is presumed to arise in response to environmental exposures. Based upon bronchoalveolar lavage (BAL) cytology, different phenotypes have been identified. Little information exists regarding the relative importance of differing inflammatory phenotypes upon performance and whether differing etiologies exist.

Objectives:
The study examined the relative impact of BAL neutrophils, mast cells, and eosinophils upon performance in racing Thoroughbreds and the relationship between these inflammatory cells and environmental exposures.

Methods:
Horses were recruited at an Indiana racetrack 24-48 hours prior to the race. Within one hour of race completion, physical examination, respiratory endoscopy, and BAL were performed. Manual differential cell counts were performed on BAL fluid. Inhalable and respirable dust exposures were measured at the breathing zone within one week of racing. Controlling for age, training stable, and pulmonary hemorrhage, generalized estimating equations were used to model the relationship between performance and BAL inflammatory cell proportions. Mixed models were used to model the relationship between BAL cytology and measures of dust exposure.

Results:
Performance and BAL data were collected on 62 individual horses from 6 stables for a total of 92 race performances and 72 dust exposure measurements. BAL mast cell proportions negatively affected performance (p=0.012), as did neutrophil proportions (p=0.046). Respirable dust was significantly associated with BAL neutrophil proportions (p=0.0066), but inhalable dust was not, (p=0.53). BAL mast cell proportions were not associated with exposure measurements (p>0.5).

Discussion:
Mast cell, and to a lesser extent, neutrophilic airway inflammation negatively impact racing performance. Respirable dust exposure is associated with neutrophilic airway inflammation. This study provides no evidence that inhalable dust impacts respiratory health. Causes for mast cell airway inflammation require further investigation.

Conclusions:
This study confirms the effect of airway inflammation on racing performance and suggests a greater relative impact of mastocytic inflammation. Respirable dust appears to be the more pertinent measure of particulate exposure in the study of equine respiratory health.
Title: Seasonal effect on tracheal secretions of thoroughbred racehorses competing in hot and humid climates

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Introduction:
Tracheal secretions are a macroscopic indicator of inflammation of the airways and the characterization of the appearance of the secretion, as well as its subjective quantification, allows for practical follow up of respiratory conditions. Although serous secretions can be seen in healthy animals after exercise, more copious or thicker secretions point toward a respiratory pathology. Much has been investigated regarding seasonality of respiratory diseases in the Northern Hemisphere, although little is known in regards to countries where the climate is hot and humid with mild winters.

Objectives:
This study examined the occurrence of tracheal secretions in Thoroughbred racehorses in competition over the course of 21 months to investigate the possible effect of a hot and humid climate with mild seasonal variation.

Methods:
Tracheal secretions were observed during endoscopic examinations after each race. Inclusion criterion was the presence of tracheal secretions other than blood. Tracheal secretions were classified according to their viscosity and their subjective quantities in the trachea.

Results:
1,628 endoscopies from 1,048 horses demonstrating tracheal secretions were recorded. Mucous secretions were the overwhelming majority with 97.9% (1026/1048) of the observations, while purulent (1.1%) and serous (1.0%) secretions completed the observations. In regards to overall grading, grade 1 secretions were the most common (608/1048; 57.9%), followed by grades 2 (30.2%), 3 (10.2%) and 4 (1.6%). When time analysis was conducted, a linear trend could be detected with winter having the most (504/1628; 29.6%) and autumn the least (16.5%) occurrences.

Discussion:
In countries with clear seasonal changes, weather features such as cold spells in winter and production of spores in spring have been implicated with inducing respiratory inflammation in horses leading to accumulation of tracheal secretions. This study found that in a hot and humid climate there is an increase in tracheal secretions during the winter months, despite low temperature averages rarely exceeding 19 degrees.

Conclusions:
Despite the lack of harsh winters, horses stabled in hot and humid climates still present a higher incidence of tracheal secretions during the winter months.
Title: Seasonal variation in bronchoalveolar lavage cytology for adult clinically healthy stabled horses

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Introduction:
Stabling is known to induce clinical symptoms in horses diagnosed with Inflammatory Airway Disease (IAD) and Recurrent Airway Obstruction (RAO), and stabling has been associated with an increase in either neutrophils, eosinophils or mastcells in BAL cytology. Dust particles and aeroallergens are causative factors in the induction of IAD and RAO and their concentration is affected by season.

Objectives:
The objective of the study was that TA and BAL cytology were unaffected by seasonal changes in adult clinically healthy stabled horses.

Methods:
63 adult clinically healthy stabled Danish Warmblood horses were included in June 2015 and November 2014. An endoscopic examination including tracheal mucus score, TA and BAL samples were performed. TA and BAL cytology was evaluated via cytocentrifugation and May-Grünwald-Giemsa stain. 500 cells were microscopically identified as macrophages, lymphocytes, neutrophils, eosinophils, or mast cells.

Results:
Significantly different climatic conditions were found in November compared to May, significant lower temperature (p<0.001), fewer hours of sun (p=0.004), less rain (p=0.006) and more humidity (p<0.001). A significant higher percentage of BAL neutrophils (p<0.001) and higher mucus score (p=0.008) were found during winter compared to summer.

Conclusions:
Mild winter weather conditions with lower temperatures, fewer minutes of sun and a higher degree of humidity resulted in a significantly higher neutrophil percentage and significantly higher mucus score in stabled clinical healthy horses. In concordance with this study, the prevalence of lower airway inflammation and the percentage of BAL neutrophils has previously been found to increase during winter.

References
Introduction:
Lower airway inflammation and obstruction are commonly observed in stable horses due to continuous exposure to airborne dust or allergen. The disease could be mild to severe depending on the degree of neutrophilic inflammation, bronchial hyperresponsiveness and bronchospasm, which lead to accumulation of mucus in the airways, frequent coughing, increased respiratory effort and poor performance. The disease can be controlled by the administration of corticosteroids and bronchodilators. However, management is the most important strategy to avoid environmental triggers.

Objectives:
The present report describes the outcome of four horses treated with parenteral betamethasone for prevention of inflammation and severe obstruction in lower airways.

Methods:
The horses were medium level show jumpers, aging from 14 to 17 years old. History included occasional coughing and decreased performance of 2 to 3 weeks duration. During a random episode of an acute respiratory distress with dyspnea and severe coughing, a single dose of 0.04 mg/kg of betamethasone (0.5% as dipropionate and 0.2% as sodium phosphate) was administered intramuscularly providing rapid relief. Environment was investigated and Aspergillus spp. was identified on hay. Exercise activity was gradually resumed and horses achieved the same level of performance within 2 weeks.

Discussion:
The most common corticosteroids administered to horses with lower inflammatory airway diseases are dexamethasone, prednisolone, beclomethasone and fluticasone. Betamethasone is a long acting corticosteroid usually administered intra-articularly for osteoarthritis. For the present cases, a single dose of betamethasone was efficient to rapidly resolve dyspnea and cough, and its long action controlled inflammation and obstruction until management measures were taken to reduce allergens exposure.
Title: Subclinical respiratory disorders in Thoroughbred racehorses training at the Brazilian Jockey Club

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Introduction:
Despite several studies described horses with airway conditions, to the authors’ knowledge none reported the incidence of subclinical airway disease in racehorses at the Brazilian Jockey Club (JCB), which is notably one of the most important racetracks in Latin America.

Objectives:
To investigate the occurrence of airway disorders in asymptomatic Thoroughbred racehorses training at the JCB, while describing the most common abnormalities and the association between them.

Methods:
72 clinically sound and actively racing Thoroughbreds, aged 2–6 years, were examined at the JCB after morning exercises. A Ventigraph® was used to investigate interpleural pressure differences. ΔPplmax ≤ 4 cm H2O was considered as physiological, 5–8 as mildly, 9–14 as moderately, and ≥15 cm H2O as severely increased. Endoscopic exams were performed with a Pentax flexible fiberscope, model 38LX. The results were subjected to statistical analysis with Minitab®17.3 software using the Chi-square test (p ≤ 0.05), to investigate the correlation among ΔPplmax, tracheal mucus ≥ 2 and carina edema.

Results:
47% of the horses presented some degree of bronchospasm evidenced by ΔPplmax > 4 cm H2O. When only those horses were considered, 94% had pressure variations between 5 and 8 cm H2O, while only 3% reached 11 cm H2O. 50% of the horses had at least one airway abnormality on endoscopy. The presence of tracheal mucus was the main finding (16/72), followed by pharyngeal lymphoid hyperplasia (14), carina edema (13), EIPH (9), dorsal displacement of soft palate (3), recurrent laryngeal neuropathy (1), and epiglottic entrapment (1). Statistical analysis demonstrated that there was a significant association (p = 0.002, χ² = 10.01) between elevated ΔPplmax and a mucus score (MS) ≥ 2. The mean ΔPplmax for horses diagnosed with MS ≥ 2 was 7.00 ± 1.93, while it was 4.88 ± 1.32 for horses with MS < 2. Using a two-sample t test, this difference was found to be significant (p = 0.019, 95% CI, −3.78—−0.47). When ΔPplmax values from horses with and without carina edema were compared, the difference was also statistically significant (p = 0.033, 95% CI, −1.93—−0.01).

Conclusions:
There is a high incidence of subclinical respiratory disorders in Thoroughbred horses trained at the JCB, and also a significant association between a tracheal mucus score ≥ 2, carina edema, and elevated ΔPplmax.
Introduction:
Current descriptions of the tie-forward procedure utilize a skin incision extending from the basihyoid to the cricoid cartilage. However, in an attempt to minimize convalescence, the procedure was modified and performed through two shorter skin incisions.

Objectives:
To describe the technique of minimally invasive tie-forward (MITF), and report the complications encountered with the procedure.

Methods:
A short ventral mid-line incision is made over the cricoid cartilage. A suture of 5 metric Fibre Wire® is then anchored in each thyroid cartilage at the level of the insertion of the sternothyroid tendon by passing three times through the cartilage. A bilateral sternothyroid tenotomy is then performed. A second ventral midline incision is made, centered over the basihyoid bone. Using a pair of curved Rochester-Pean forceps passed from the cranial to caudal incision, the Fibre Wire® sutures are drawn through and exited through the cranial incision. Using a wire passer from retrograde the left sutures of Fibre Wire® are drawn around the rostro-left aspect of the basihyoid bone and likewise, the right sutures are drawn around the rostro-right aspect. With the head then flexed approximately 90°, the sutures are tightened and knotted together in one single knot. This results in rostral displacement of the larynx, such that the apex of the thyroid cartilages is now completely underneath the basihyoid bone, and the knot is lying over the lingual process of the basihyoid bone, with the sutures in a continuous loop. The wounds are repaired with simple continuous sutures of three metric poliglecaprone 25 in the sternohyoid muscle and simple continuous sutures of three metric poliglecaprone 25 in each skin incision. Horses are discharged with instructions for five days box rest followed by five days horse walker exercise followed by resumption of full training.

Results:
A total of 132 horses underwent MITF. Post-operative radiography was used in the initial stages to confirm successful advancement of the larynx. No major intraoperative complications were encountered, with only minor haemorrhage occurring at both surgical sites. Ten horses had complications following the procedure. Of these, three had mild postoperative colic that responded to medical management; all three had undergone a concurrent castration procedure. A total of three horses required repeat MITF at later dates for perceived implant failure. One horse did race three times before examination revealed a fracture of the stylohyoid bone. Two horses experienced mild incisional swelling and pyrexia in the immediate 48 hours following surgery. Additional minor complications encountered included pyrexia and nasal discharge (1) and superficial corneal ulceration (1) following general anaesthesia.

Conclusions:
A MITF can be used to advance the larynx into a tied-forward position, allowing for shorter convalescence. The authors acknowledge Bryan O’Meara for the development of the technique.
Title: Survey of Horse Owners on Equine Asthma in Warmblood Horses

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Introduction:
Equine asthma is a chronic progressive disease of stabled horses and exacerbations are observed in the vast majority of the patients.

Objectives:
The aim of the descriptive study was to investigate information about individual trigger factors, performance level, diagnostic strategies, therapeutic approaches and their effectiveness in horses with Equine Asthma in Germany.

Methods:
The data were collected by a questionnaire homepage and analyzed using the SPSS program. Overall, 266 completed questionnaires were evaluable, of which 129 horses were warmbloods.

Results:
Overall, 266 completed questionnaires were evaluable, of which 129 horses were warmbloods. Due to the high prevalence in warmblood horses (48%), this population was analyzed separately. The chosen medium has been well accepted by the horse owners, thus data on veterinary issues can be processed very easily by this mean. The owner reported a decrease in the performance-level of their horses in 69% of the cases, the overall success at horse shows was diminished in 22% of the cases. The RAO disease of their horse appears to be a great emotional burden on the affected horse owners and was associated with significant limitations in use, wherefore 64% of owners exclude the reacquisition of a COB patients categorically. The financial burden of the horse owners due to the disease was 600 euros per year on average. According to the survey results RAO is a chronic progressive disease and exacerbations could be seen in 91% of the patients even with an environmental control regime. Exacerbations occurred every 6 months and patients showed symptoms for the duration of 8 weeks then.

Discussion:
The homepage was well accepted by the horse owners. Auscultation as a sole diagnostic tool is insufficient for the recognition of the initial stage of the disease. A cytologic examination should be part of every endoscopy. Long-term environmental control should be the highest priority for the horse owner. Frequent sources of error of environmental control should be avoided (f.e. feeding straw, stable beside storage areas). “Enforced abdominal pattern” was the symptom with the greatest increase during exacerbations. Poor performance was correlated with the increase of breaths per minute at rest.

Conclusions:
Due to the severity and long persistence of symptoms with every exacerbation, research on causal therapies, such as immunotherapy, should be given highest priority.
Title: The effect of clenbuterol on airway inflammation in healthy South African thoroughbred race horses

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Introduction:
Poor performance is commonly attributed to airway inflammation in apparently healthy race horses. Clenbuterol has been shown to improve the tracheal mucociliary clearance rate, which may impact airway inflammation.

Objectives:
Determine the prevalence and severity of airway inflammation in a population of apparently healthy thoroughbred race horses in training in South Africa and determine whether airway inflammation is decreased by the administration of clenbuterol.

Methods:
A convenience sample of 2-year old horses in training was selected at a well-managed training barn. Horses with clinical abnormalities (e.g. fever, coughing) and poor performers were excluded. Broncho-alveolar lavage (BAL) and airway endoscopy were performed on 11 horses. Horses were randomly assigned to treatment (n=6, 0.8 g/kg clenbuterol PO q12h) or control (n=5, no treatment) groups. All horses remained in training. BAL cytology and endoscopic tracheal mucous scores were assessed at 0 and 21 days of treatment by blinded evaluators. BAL neutrophil percentage was compared between groups using the Wilcoxon rank sum test. There was insufficient mucous score data for meaningful statistical analysis. The trial was performed in winter (July-Aug).

Results:
Neutrophilic inflammation (BAL neutrophils >15%) was detected in all horses at all sampling times, with an average of 50.6% neutrophils (range 29-74%, median 46%) and 47% (range 25.7-60.9%, median 48%) at 0 and 21 days respectively. Clenbuterol treatment did not decrease BAL neutrophils (p=0.089) or mucous scores significantly.

Discussion:
Moderate to severe lower airway inflammation is ubiquitous in this population of apparently healthy young thoroughbred race horses in winter. Improved screening and management strategies to monitor and combat airway inflammation are needed.

Conclusions:
Clenbuterol did not decrease airway inflammation significantly. Further studies at different times of the year and in different locations should be performed to determine the relevance of findings for the local and global equine population.
Title: The effect of exercise induced pulmonary hemorrhage on some performance indices in standardbred racehorses: preliminary results

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Introduction:
Exercise-induced pulmonary hemorrhage (EIPH) is an important disease of horses, characterized by bleeding from the pulmonary capillaries during or after exercise. EIPH is mostly identified in racehorses, affecting over 80% of Thoroughbreds and Standardbreds performing high-speed exercise.

Objectives:
There is a moderate evidence that EIPH can have a negative impact on horses performance. However, there is very low quality evidence that horses affected by EIPH have impaired fitness parameters such as blood lactate. Aim of the present work is to evaluate some performance indices in a population of Standardbred racehorses with EIPH by means of treadmill exercise testing.

Methods:
Sixteen Italian Standardbred racehorses (average age 3.1±1.0 y.o., 10 males, 6 females), at the same level of performance and at the same stage of their training, were selected. All horses underwent an accurate clinical examination, that allowed to rule out any overt disorder. After two days of familiarization, they underwent a treadmill endoscopy, which ruled out the presence of any dynamic upper airway obstruction, and an endoscopy after the exercise. The latter allowed the categorization of the horses in two groups: 8 horses with no evidence of EIPH, and 8 horses with grade ≥1 of EIPH. Then the horses performed an incremental exercise test on a high speed treadmill. During the test heart rate was monitored with a pulsometer. Blood samples were taken with a 14G teflon venous catheter placed in the jugular vein, and plasma lactate was measured with an enzymatic colorimetric method. Data were analyzed by a specific software and the speed at 4 mmol/L (VLa4), the speed at 200 bpm of heart rate (V200), the maximum heart rate (HRmax), the peak of lactate (Lamax), and the lactate and heart rate at 1, 5 and 30 minutes after exercise were calculated. Data for both groups were statistically compared by T-student test for unpaired sample, and statistical significance was set at p<0,05.

Results:
Concerning VLa4, HRmax, Lamax and lactate after exercise, no differences were observed between the two groups. There was a significant (p<0,05) lower V200 (Fig. 1) and a highly significant lower (p<0,01) heart rate at 30 minutes after exercise (Fig. 2) in EIPH group.

Discussion:
The lower V200 in EIPH horses may suggest a negative impact of the disease on performance. The significance of the lower HR after exercise will be further investigated.

Conclusions:
As already described, very few parameters differ between EIPH and non EIPH horses.
Title: The effect of tongue-tie application on stress responses in resting horses

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Introduction:
Tongue-ties (TT) are devices used to hold the tongue in a fixed position during exercise, with the goal of facilitating control of the horse and as a conservative treatment for dorsal displacement of the soft palate (DDSP). The conflicting evidence for its efficacy at preventing DDSP, combined with public concerns of potential welfare implications, have led to the banning of TT by the Fédération Equestre Internationale (FEI). However, TT are still approved for use in racing.

Objectives:
To investigate behavioural and physiological markers of stress in response to TT application in resting horses.

Methods:
Using a randomised crossover design, Standardbred horses (8 mares, 4 geldings; mean (± sd) age = 11.5 ± 3.0 years) were assigned to two treatments: tongue-tie application (TTA), and tongue manipulation (TM). TM involved grasping of tongue to mimic the application of the TT and was conducted for 30 seconds at the start of Phase 2. For each treatment, horses were observed for a total of 80 mins, comprising three phases (Phase 1: 30-min baseline; Phase 2: 20-min treatment and Phase 3: 30-min recovery). During TTA, a commercially available elastic TT was looped twice around the tongue and secured to the mandible. Behavioural data (head tossing/shaking, ear position, gaping and lip-licking) were videoed for analysis using behaviour analysis software. Heart rate was recorded using a telemetric ECG and eye temperatures were measured at 5 minute intervals using an infrared thermographic camera. Saliva samples were taken at the end of each phase for cortisol assays.

Results:
Compared to TM there was increased headshaking/tossing (p<0.001), gaping (p<0.001) and time spent with the ears pointing backwards (p<0.001), during TTA in Phase 2. Similarly, compared to TM, lip-licking frequency increased during Phase 3, after TTA (p<0.001). Horses with previous experience of TT use (n=6) showed more headshaking (p=0.04) and gaping (p=0.03) than naive horses (n=6). Salivary cortisol increased following TTA compared to TM (p=0.047). There was a trend for HR to be higher during the last 10 minutes of TTA compared with TM (p=0.08). Mean eye temperatures were not significantly different between treatments at any time point.

Conclusions:
Tongue-tie application results in changes in behavioural and physiological parameters that suggest a stress response.
Title: The effects of WF10 in horses with recurrent airway obstruction (RAO)

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Introduction:
RAO is an equine respiratory disease which is among others characterised by hypersensitivity in response to inhaled antigens. Several symptomatic treatments of RAO have been described but proved to be unsatisfactory whereas validated alternative treatment options are lacking.

WF10 is a 10% dilution of OXO-K993 containing chlorite, chlorate and other components. Its pharmacological profile is best described as immunomodulatory featuring anti-inflammatory properties. The effect of WF10 has been studied in many diseases including allergic rhinitis (AR) in humans with encouraging outcomes. Due to the similarity of the symptoms of RAO and AR -both characterized by an inappropriate immune response to airborne allergens- it was of high interest to evaluate the effects of WF10 as a new therapy option for the treatment of RAO.

Objectives:
The aim of this study was to evaluate the clinical effects and possible side effects of WF10 in horses with RAO.

Methods:
The study was designed as a randomized, double blind and placebo controlled trial. Forty-two prediagnosed horses with history of RAO-like symptoms were recruited and randomly assigned to either a treatment or control group. All horses received a 5 day treatment cycle with daily 1-hour intravenous infusions of 0.5 ml/kg WF10 diluted 1:10 in 0.9% NaCl or the equivalent volume of 0.9% NaCl (placebo).

Physical examinations and blood samples were taken before and after each treatment. Evaluations included hematology, blood chemistry, arterial and venous blood gas analysis, biomarkers of inflammation (serum amyloid A, haptoglobin, IgG and IgE), thorax radiographs, bronchoscopy, bronchioalveolar lavage fluid and parasitological examinations. All examinations were repeated three weeks after treatment.

Results:
RAO specific read outs did not show significant differences between the treatment and the control group at any time point. Slightly lower values of IgG and IgE were observed in horse receiving WF10 three weeks after the treatment.

Non RAO-related effects of WF10 comprised reduced hematocrit and haptoglobin and higher methemoglobin and bilirubin concentrations in treated horses.

Conclusions:
The treatment of five WF10 infusions was tolerated well by all horses and no clinical side effects were observed.

Related to placebo, WF10-treated horses did not show any significant improvement of RAO-specific symptoms.
Title: THS from TEW, not BAL – Preliminary Study

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Introduction:
Diagnosis of Exercise Induced Pulmonary Haemorrhage (EIPH) is accomplished through tracheobronchoscopy after intense exercise or through observation of haemosiderophages in the bronchoalveolar fluid of horses and calculation of what is called THS or total hemosiderin score. Bronchoalveolar lavage (BAL) is widespread in most equine practices, although it is not well accepted by racehorse trainers in Brazil. Due to its high incidence it is relevant to the veterinary community to study means of diagnosing and following up the course of the condition.

Objectives:
This study evaluated the use of THS obtained from transendoscopic tracheal lavage (TEW) as a way of quantifying EIPH in horses.

Methods:
Tracheobronchoscopy was conducted 60 minutes after races and 8 horses diagnosed with EIPH by visualization of blood in the trachea were included in this study. Forty-eight hours after the race a second tracheobronchoscopy was conducted, during which TEW was obtained. Slides obtained by TEW centrifugation were stained with Prussian Blue. Macrophages with stained nuclei were classified using a score from 0 to 4, per the intensity of the colour, thus obtaining a THS score. EIPH scores (1 to 4) and THS scores were evaluated by statistical trend analysis. Correlation between THS score and volume recovered during TEW was investigated through ANOVA. Significance level was set at 0.05.

Results:
Average THS score was 121.86 ± 70.65. Statistical trend analysis and regression demonstrated a positive relationship between EIPH and THS score obtained from TEW (Accuracy measurements MAPE = 1.86; MAD = 2.3; MSD = 5.85). ANOVA determined there was no significant variation of THS in relation to TEW volume recovered ($r^2 = 0.12; p = 0.45$).

Discussion:
THS from BAL samples have been successfully used for evaluation and diagnosis of EIPH in horses but there are no previous descriptions of the use of tracheal samples for THS calculation for diagnosis of EIPH. This preliminary study has demonstrated that an association exists between THS scores from TEW obtained forty eight hours post-race and EIPH grade observed up to 60 minutes post exercise, thus allowing diagnosis of EIPH. Analysis of results showed that THS score from TEW samples increased with current EIPH grade in the horses studied.

Conclusions:
THS obtained from TEW samples can be used as an indicator of EIPH severity and as a diagnostic tool for detection of EIPH.
Title: Tongue-tie use in Australian Thoroughbred horses over a 5 year period (2009-2013)

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Introduction:
Tongue-ties (TT) have been used in horses for over 100 years. The primary indications for their use are to aid control and as a conservative treatment for breathing disorders. A study of Thoroughbred (TB) racehorses in the UK reported that TT were used in approximately 5% of starts. However, there is no data on the prevalence of TT use in Australia.

Objectives:
To estimate the prevalence of TT use within the Australian TB racing industry and to identify potential factors associated with their use.

Methods:
A retrospective single cohort study was performed. Data from all TB horse races from 2009 to 2013 calendar years were sourced from Racing Information Services Australia (RISA) and formatted for analysis. Prevalence of TT use was estimated at three levels: start, horse and trainer. Patterns of TT use were explored at the horse level using a smaller dataset restricted to 2010-2013 due to the large number of horses. Prevalence estimates were stratified by age, sex, race type, ground condition, track surface, race grade, race distance and number of career starts.

Results:
During the five-year period, there were a total of 899,460 starts completed by 72,206 horses and 5,260 trainers. The prevalence of TT use at the start level was 21.3% over the period and was stable from year to year. Prevalence of starts with TT was highest in the Northern Territory (26.5%) and lowest in Western Australia (11.8%). At the horse level, 32.9% wore a TT in at least one start, whilst 71.9% of trainers had applied a TT to at least one horse over the five years. TT use was higher in geldings (35.4%) than entire males (24.2%) or females (28.4%). TT prevalence was higher in horses competing in unlisted races (32.1%) compared with listed (21.5%) and Group (23.1%) races and was higher in jump (44.6%) versus flat races (32.2%). The median age at which a horse first wore a TT was 3 years (range 2-11), with a median of 4 races completed before a TT was first applied (range 1-60). Subsequently, a TT was applied in 84% of races after their first use. Horses that had worn a TT competed in more races over their careers (median 11, range 1-71) than horses that never wore a TT (median 6; range 1-70).

Conclusions:
The use of TT is common in Australian TB racehorses and considerably higher than previously reported in the UK. Further research is required to understand the rationale for TT use in these horses.
Title: Transoral thermal cautery of the soft palate in the standing, sedated horse

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Introduction:
Despite the paucity of evidence supporting thermal cautery of the oral surface of the soft palate (TCOSP) as an effective treatment for palatal instability/displacement, it remains a commonly requested procedure among the UK and Irish Thoroughbred community. Traditionally the technique has been performed under general anaesthesia and has yet to be described in the standing patient.

Objectives:
To describe the technique of TCOSP in the standing sedated horse, and report the complications encountered with the procedure.

Methods:
Prior to surgery, horses receive standard antibiotic and anti-inflammatory medication. Following positioning in stocks, the horse is brought to a deep plane of sedation. The mouth is opened using a Millennium® speculum and the tongue, soft palate and caudal oral mucosa anaesthetised using topical lidocaine. The soft palate is additionally injected with ~30mls of lidocaine at the junction of the hard and soft palates. The speculum is then removed and a suitable time delay is allowed for anaesthesia to occur. After repositioning and full opening of the speculum, the rostral aspect of the soft palate is cauterized with the use of pre-heated, “L” shaped irons. Rotation of three irons minimizes surgical time, and the use of a vacuum and head torch provide constant visibility of the oral cavity. A customized tongue depressor/palate elevator is then used to access the caudal aspect of the soft palate. Upon completion, horses are returned to the stable and food withheld for a total of four hours following the procedure. Once re-introduced, soaked hay and soft feed are provided from the floor. Horses are routinely hospitalized overnight and monitored for clinical parameters and appetite before permitting discharge.

Results:
A total of 151 horses have been treated with TCOSP as a standing procedure either alone or in combination with additional upper airway surgery. Complications have occurred in four horses, including abandonment of the procedure due to unpredictable patient head movement (1), ptyalism and inappetence with (1) and without (2) lingual ulceration. Intraoperative interference of the tongue has not been found to be an issue.

Conclusions:
Thermal cautery of the oral surface of the soft palate can be performed safely as a standing procedure, thus avoiding the necessity for general anaesthesia.
Title: Treatment of DDSP and effect of the laryngeal tie-forward procedure in a group of Danish trotting horses.

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Introduction:
Laryngeal tie-forward (LTF) is the most common surgical procedure used to treat dorsal displacement of the soft palate (DDSP), also it can be applied as a treatment option in horses with palatal instability (PI). Both DDSP and PI are frequently diagnosed in racehorses. Few studies have investigated the success rate of the LTF procedure and to the authors’ knowledge no studies have investigated the postoperative conditions in the pharynx and larynx using overground endoscopy (OE).

Objectives:
1) To investigate the conditions in the pharynx and larynx using OE before and after the LTF procedure and to evaluate the success rate based on postoperative OE and owner questionnaire. 2) To determine the effect of the LTF procedure on racing speed in Standardbred trotters.

Methods:
Seventeen Standardbred trotters that had the LTF procedure performed at the Large Animal Teaching Hospital, Copenhagen, between 2005 and 2016 were included in the study. Fifteen owners answered the questionnaire regarding symptoms, performance, and complications. Pre- and postoperative OE was available for 6 horses. Race results were available for 14 Standardbred trotters and were collected from www.trav.dk and www.travsport.se. Racing speed from 4 races both pre- and postoperatively was obtained for 11 horses, whereas the last 3 had 1-4 races pre- and postoperatively. The mean racing speed pre- and postoperatively was calculated for each horse, and a paired t-test was performed. The statistical significance was set at P<0.05.

Results:
Based on the owner questionnaire, 47% (7/15 horses) had improved performance after the LTF procedure. On preoperative OE, all 6 horses showed DDSP and in addition 5 also had PI. Based on follow-up OE, 2 horses had PI in spite of improvement of symptoms, 1 horse had extensive pharyngeal collapse and axial deviation of the left aryepiglottic fold but no DDSP, and 3 horses showed no improvement (still DDSP and symptoms). The success rate of the LTF procedure based on its ability to prevent DDSP was 50% (3/6 horses). No significant difference in racing speed pre- and postoperatively was found (P=0,575) for the included horses. Six horses improved their racing speed (0,36–3,2 seconds faster).

Conclusions:
The success rate for the LTF procedure, based on owner questionnaire and pre- and postsurgical OE found in this study was markedly lower than the 80% reported by Woodie et al. (2005). No significant change in racing speeds pre- and postoperatively was found.
Title: Upper and lower respiratory tract microbiota in horses: bacterial communities associated with health or mild asthma (Inflammatory Airway Disease [IAD]) and effects of dexamethasone

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Introduction:
The microbial composition of the equine respiratory tract, and differences due to mild equine asthma (Inflammatory Airway Disease [IAD]) have not been reported. The primary treatment for IAD are corticosteroids.

Objectives:
The objectives were to characterize the upper and lower respiratory tract microbiota associated with health and IAD, and to investigate the effects of dexamethasone on these bacterial communities.

Methods:
See Fig. 1 for study design. High throughput sequencing was performed. Code for the OTU table construction pipeline is available (https://github.com/ucvm/vmmp). Diversity analysis was performed in R.

Results:
The respiratory microbiome of horses was dominated by four major phyla, Proteobacteria (43.85%), Firmicutes (16.82%), Bacteroidetes (13.24%) and Actinobacteria (21.63%). Fifty genera had a relative abundance >0.1% (Table 1). The upper and lower respiratory tract microbiota differed in healthy horses, with a decrease in richness in the lower airways, and 2 OTUs that differed in abundance. There was a clear separation between bacterial communities in the lower respiratory tract of healthy and IAD horses; 6 OTUs in the tracheal community had different abundance with disease status, with Streptococcus being increased in IAD horses. Treatment with dexamethasone had an effect on the lower respiratory tract microbiota (Fig. 2), with 8 OTUs increasing in abundance, including Streptococcus, and 1 OTU decreasing.

Conclusions:
The lower respiratory tract microbiota may contribute to the pathogenesis of IAD, and further research on the role of Streptococcus is warranted. Perhaps the control of bacterial overgrowth in IAD horses treated with dexamethasone could be part of the treatment strategy.
Title: Use of methacholine bronchoprovocation test for the diagnosis of asymptomatic severe equine asthma

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Introduction:
A group of experts in the field of equine respiratory disorders has recently proposed to rename Inflammatory Airway Disease and Recurrent Airway Obstruction as mild to moderate and severe equine asthma (SEA) respectively, in reference of the human asthma. One of the characteristics of asthma, in equine and human patients, is the absence of clinical signs during clinical remission. Methacholine bronchoprovocation test (BPT), which is a standard diagnostic test for detection of asymptomatic human asthma, has been used in some research protocols in horses but no study has examined its potential value for clinical diagnostic.

Objectives:
To assess whether the methacholine bronchoprovocation test is an applicable and repeatable clinical test to distinguish control horses and SEA horses in clinical remission.

Methods:
Twelve horses (6 with SEA history and 6 controls). Seven of them (3 SEA and 4 control horses) underwent BPT twice at a 24-hour-interval to determine the repeatability of the test. Then all horses were tested before and after a 7-days period in a stall environment. Simultaneously, usual ancillary pulmonary examinations were performed.

Results:
The BPT had a good feasibility and a significant repeatability. Before the stay in stalls, SEA and controls horses could not be differentiated by the BPT. After a 7-days period of straw and hay exposure, the bronchial hyperresponsiveness was increased in both SEA and control horses, while the other clinical or functional parameters were not significantly affected. The 7-days period in stalls resulted in a significant difference in bronchoreactivity between SEA and control horses.

Conclusions:
Methacholine BPT does not permit to differentiate control and asymptomatic SEA horses in clinical remission, unless the horses have been kept in stalls. These results suggest that the mechanisms underlying the bronchial hyperresponsiveness in asthma differ between human and equine patients. Nevertheless, the BPT may be used to detect asymptomatic severe asthmatic horses after a light environmental challenge. Further researches are needed to assess the clinical interest of BPT for subclinical mild-moderate equine asthma patients.