

## LACK OF DETECTABLE ALTERATIONS IN BALF CYTOKINE mRNA LEVELS IN EXERCISING HORSES.

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As strenuous exercise predisposes horses to the development of lower respiratory tract infections, we sought to determine if this increased risk was associated with exercise-induced reductions in bronchoalveolar lavage fluid (BALF) cell interferon-gamma (IFN- $\gamma$ ) and interleukin 12 (IL-12) mRNA levels. Two groups of Standardbred horses were studied. Group one (N=5) was stall-rested for 12 weeks; group two (N=6) was exercise-conditioned over a 9 week period prior to completing maximum exercise tests (weeks 10, 11 and 12). Peripheral blood and BALF samples were obtained at the start of the study and at week 12 (24 hours after completion of the third maximum exercise test for group 2 horses). From BALF cells, RNA was isolated and cDNA was synthesized using commercial kits. IFN- $\gamma$  and IL-12 mRNA levels were measured using real-time quantitative PCR and normalized to an endogenous gene (18s rRNA). Time dependent differences within a group were tested using a paired t test; group differences at a given sampling point were tested using a Student's t-test. Significant differences in IFN- $\gamma$  and IL-12 mRNA levels were not detected between the two groups either at the start of the study or as a result of the maximal exercise program. These results do not support the hypothesis that strenuous exercise suppresses T-helper cell 1 function. However, sampling cytokine mRNA levels at an earlier time post exercise and/or measuring the cytokine mRNA levels in segregated lymphocyte and macrophage populations may have yielded a different result. (Funded by the Harry M. Zweig Memorial Fund for Equine Research).

REPEATABILITY OF POST-EXERCISE BRONCHOALVEOLAR LAVAGE ERYTHROCYTE  
COUNTS FOR QUANTIFICATION OF EIPH

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Endoscopic scoring of visible blood in the trachea after exercise has traditionally been used to grade the severity of exercise-induced pulmonary haemorrhage (EIPH) in the horse. Bronchoalveolar lavage (BAL) erythrocyte counts have been advocated as an improved way of quantifying the degree of haemorrhage. To determine the reproducibility of this approach, BAL samples were obtained on 3 separate occasions within a 5 week period (minimum of 7 days interval) from 4 Thoroughbred horses after strenuous treadmill exercise (13-14.5 m/s, 90-120s). Each horse always exercised with the same protocol although protocols varied between horses. BAL was performed 30 min post-exercise, using a fiberoptic endoscope. Horses were sedated (romifidine, 60ug/kg iv) and 20 ml 1% xylocaine was administered locally to reduce coughing. Each lung was lavaged with 2 x 100 ml of 0.9% sterile saline at 37°C. Red blood cell counts were performed on each sample in triplicate using a haemocytometer and expressed as cell number per  $\mu$ l of BAL fluid.

	Left Lung			Right Lung		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Horse B	4090	2310	2330	1740	620	970
Horse E	2100	240	3420	310	570	2260
Horse H	7750	9300	8600	6115	17830	2290
Horse L	510	470	560	250	130	240

BAL return was  $135 \pm 19$ (SD)%. Blood was visible in the trachea only in horse H in tests 1 and 2. In horse L, in which the counts were the lowest, counts were similar over time in both lungs. In horses H and B, BAL RBC were of the same order for each test in the left but not the right lung. Horse E showed marked variation with no obvious pattern. Although each horse followed the same training programme and underwent a constant exercise test protocol on each occasion, BAL RBC counts were variable. This observation is consistent with observations of the variability of the condition in the field.

## MONITORING AIRWAY INFLAMMATION BY EXPIRED BREATH CONDENSATE

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Expired breath condensate has been used to monitor airway inflammation and oxidative stress in man. The concentration of hydrogen peroxide in condensate ( $[H_2O_2]_c$ ) correlates with airway neutrophil activation and is elevated in patients with asthma. To the best of our knowledge the collection of expired breath condensate in the horse has not been reported. A collection method was developed and validated using a 5 litre stainless steel collection tube surrounded by ice and water. For collection, the horse is fitted with a mask and a two-way valve, which caused no signs of distress. A flexible pipe (volume 5l) heated to 30°C connects the expiratory port of the valve to the collection tube. Hydrogen peroxide, hydrogen ions, nitrite and nitrate have been detected in condensate. Vitamin C, glutathione, uric acid and protein have not been detected in any samples. To test the reproducibility of the technique,  $[H_2O_2]_c$  from three consecutive collections repeated on three healthy horses at rest, was measured using a spectrophotometric method:

Horse	Mean ( $\mu\text{M}$ )	Standard deviation ( $\mu\text{M}$ )	Coefficient of variation (%)
A	1.3	0.18	13.6
B	1.2	0.04	3.6
C	0.73	0.02	2.7

Since the technique is non-invasive it can be used repeatedly to follow the time course of an inflammatory response. In two horses following administration of nebulised hay extract, breath condensate was collected hourly for 5 hours.  $[H_2O_2]_c$  increased post-challenge, peaking at four hours; no change was detected using nebulised saline as a control. Collection of equine expired breath condensate appears to be useful for the detection of airway inflammation and permits monitoring of the time course of an inflammatory response.

SPECTROTEMPORAL SIGNATURE FOR IDENTIFYING UPPER AIRWAY ABNORMALITIES  
IN EXERCISING HORSES.

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The study's objective was to map the frequency spectrum and quantified sound level in horses under control, laryngeal hemiplegia and alar fold paralysis conditions during strenuous exercise on a high-speed treadmill. Upper airway sounds were recorded simultaneously with tracheal and pharyngeal pressures in six horses during strenuous exercise at 75%, 90% and 100% of heart rate max exercise intensities. Laryngeal hemiplegia and alar fold paralysis were created by injecting 2% hydrochloride in the left cricoarytenoid dorsalis muscle and around each infraorbital nerve, respectively. A small (1 cm x 0.5 cm) electret condenser microphone (sensitivity of -40 dB and a flat frequency response), attached to a nasopharyngeal pressure catheter placed in the nasopharynx, recorded upper airway sounds. The sounds were amplified using a low-noise preamplifier and passed through a high pass analog filter at 20Hz and an anti-aliasing filter at 2.5 KHz. All signals were acquired at 5 KHz and digitized. Upper airway sounds' digitized data collected at each exercise intensity during 3 inspiratory breaths, 3 expiratory breaths, and three 2-consecutive full inspiratory and expiratory breaths were analyzed as follows: the array was zero-padded and de-noised using a wavelet transform Daubechies 4; and the fundamental frequency and 11 harmonics were determined by an auto power spectrum. A third octave analysis (80 Hz to 2.5 KHz) determined sound intensity using an A-weighting filter; linear equivalent, peak, and mean exponential sound levels and total band power were measured. Airway obstruction effects on the various parameters (fundamental frequency, power, and sound levels) were compared using a three-way ANOVA with horse (the blocking factor), exercise intensity (expressed as percentage of heart rate), and condition (upper airway status), followed by Tukey's multiple comparison procedure. A value of  $P=0.05$  was considered significant. Interaction of treatment and exercise intensity was included in the model. The three-way interaction term (horse, exercise intensity, and condition) was the error term. During expiration, fundamental frequency was significantly ( $p=0.0095$ ) higher for horses with laryngeal hemiplegia (319 Hz) compared to control (255 Hz) and alar fold (255 Hz). During inspiration, fundamental frequency was significantly ( $p=0.0086$ ) higher for horses with alar fold paralysis (307Hz) compared to horses with laryngeal hemiplegia (243 Hz). There was a significant effect of condition on linear equivalent, peak, and mean exponential sound levels and total band power.

## ARTERIAL BLOOD GASES AND CARDIAC DISEASE DURING EXERCISE

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Horses presented between January and December 2000 for evaluation of poor racing performance completed a high-speed treadmill examination which included exercising upper-airway endoscopy and arterial blood gas evaluation, echo-cardiograms, ECG, and post-exercise tracheal wash. The primary goal was to investigate the relationship between occurrences of cardiac abnormalities and exercising arterial blood gases. 87 horses completed the treadmill exam. 48 were Thoroughbreds (TB), 34 were Standardbreds (STB), and 5 had other uses. Mean age of TB was 4.2 years and STB was 4.6 years, with no gender predilection.

34/87 horses had abnormal cardiac findings. 20/34 were TB and 14/34 were STB. Cardiac diagnoses (CD) were divided into arrhythmias (ventricular and/or atrial), and functional abnormalities (valvular regurgitation, decreased post-exercising left ventricular fractional shortening, and/or left ventricular dysfunction). 21/34 were found to have arrhythmias, 7/34 had functional abnormalities, and 6/34 had both. 5/34 had only CD, 3/34 were diagnosed with both CD and upper respiratory tract (URT) disease, 8/34 with concurrent CD and lower respiratory tract (LRT) disease, and 18/34 had abnormalities involving all 3 body systems. Of horses with CD, 17/34 had exercising PaO<sub>2</sub> values lower than those observed in healthy TB. 3/17 had CD only, 2/17 had CD and URT, 3/17 had CD and LRT, and 9/17 had involvement of all 3 systems. 4/17 also had an abnormally elevated PaCO<sub>2</sub>. All had arrhythmias as the only CD. In horses with abnormal exercising arterial blood gases, 10/17 had arrhythmias, 4/17 had functional abnormalities, and 3/17 had a combination. In horses with CD and normal exercising arterial blood gases, 11/17 had arrhythmias, 3/17 had functional abnormalities, 3/17 had both. 29/53 horses without CD had decreased PaO<sub>2</sub>, with 3 also having elevated PaCO<sub>2</sub>.

Arrhythmias were the most common CD with a similar distribution in horses with normal or abnormal blood gases. However, arrhythmias were present in 10/11 cases with the lowest PaO<sub>2</sub>, and were the only CD present in the cases with elevated PaCO<sub>2</sub>. This suggests that arrhythmias may be associated with more severe gas exchange difficulties than are functional abnormalities. Whether they are a result or a cause of diminished gas exchange can not be determined from these data.

LEUKOTRIEN B<sub>4</sub> AND BRADYKININ IN BREATH CONDENSATE OF HORSES WITH  
RECURRENT AIRWAY OBSTRUCTION

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The purpose of the study was to evaluate LTB<sub>4</sub> and bradykinin levels in breath condensate of horses with different degrees of recurrent airway obstruction (RAO).

**Methods:** Clinical and endoscopic findings, arterial blood gas analysis, cytology of bronchoalveolar lavage and ventilatory data were evaluated in 22 horses. Expiratory air was condensed in a refrigerated (-20°C) teflon tube by connecting it with an endotracheal tube in the standing horse. The sampling device was made available by a german lung research society (FILT)<sup>1</sup>. A valve ensured that just expired air passed the tube system. After 10 min. of sampling time, the teflon tube was removed and the frozen breath condensate allowed to thaw. For LTB<sub>4</sub> analysis the fluid immediately was transferred in -80°C, shipped in liquid nitrogen and analysed by using an enzyme immuno assay<sup>1</sup>. For bradykinin measurements, the samples were mixed with protease-inhibitors immediately after thawing. Further preparation steps (i.e. precipitation, centrifugation) had been evaluated in preceding tests and were required for analysis of bradykinin in a radio immuno assay<sup>2</sup>.

**Results:** LTB<sub>4</sub> was detected in all 22 samples in a range of 52 to 330 pg/ml breath condensate with a mean of 163.8 ± 62.0 pg/ml. Bradykinin was detected in 8 of 22 samples (36%) in a range of 3.4 to 38.5 pg/ml. There were no significant correlations found to any of the clinical or laboratory parameters mentioned above.

**Conclusions:** Breath condensate sampling in the horse can be done as a routine diagnostic procedure. However, its usefulness for diagnostic purposes in this species needs further evaluation.

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## SPECTRAL ANALYSIS OF RESPIRATORY NOISE IN HORSES WITH UPPER AIRWAY OBSTRUCTIONS

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**Purpose of the study:** Upper airway obstructions are a significant cause of poor performance in the equine athlete. These disorders are frequently associated with abnormal respiratory sounds during exercise. The aim of this study was to characterise respiratory sounds in horses with upper airway obstructions using Spectral analysis.

**Materials and methods:** Horses referred for investigation of poor performance associated with abnormal respiratory noise were fitted with a facemask containing a miniature condenser microphone. A videoendoscope was passed through the mask and positioned within the nasopharynx. Simultaneous recordings of videoendoscopy, airflow and respiratory sounds were made during a standardised incremental exercise test on a high-speed treadmill. Periods of sound when abnormalities were observed were extracted from the audio signal. These data were partitioned into inspiratory and expiratory time series based on respiratory airflow and characterised using Spectral analysis. Results were compared with data from clinically normal horses.

**Results:** There were characteristic differences in the distribution of spectral energy between normal horses and horses with upper airway obstructions. Normal horses exhibited very little inspiratory noise with peaks at approximately 13Hz and 250Hz. A single low frequency peak at approximately 13Hz was observed during expiration. The presence of dorsal displacement of the soft palate gave rise to additional low frequency peaks in the range 25Hz to 150Hz during expiration and in some cases a small amount of inspiratory noise was observed in the region of 1.6kHz. Horses with dynamic laryngeal collapse had considerably increased inspiratory noise in the range 0 to 600Hz and between 1.2kHz and 2kHz. In these horses, an additional low frequency peak was observed at approximately 25Hz during expiration.

**Conclusions:** Spectral analysis was shown to be a useful technique for characterisation of abnormal respiratory sounds in horses. Changes in spectral energy distribution related to the type of obstruction present. This technique might provide a convenient, non-invasive method for the diagnosis of dynamic upper airway obstructions in exercising horses.

THE EFFECTS OF RESISTIVE BREATHING ON BLOOD FLOW TO THE APICAL LUNG LOBE  
OF EXERCISING HORSES.

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We hypothesized that resistive loading of breathing during exercise would change blood flow through the pulmonary bed.

Six adult Standardbred horses were chronically instrumented with pressure transducers (Konigsburg) in the left atrium and ultrasound transit-time flow probes (Transonic Systems Inc.) around the major artery to the left apical lung lobe. The horses resumed training after recovery from this intervention. Three experiments were carried out in random order on each horse; with the airway unimpeded; with increased inspiratory resistance (temporary laryngeal paresis); with increased expiratory resistance (a one-way valve in the right nostril). Pulmonary artery and wedge pressures were measured with a transducer-tipped catheter (Millar Instruments Inc.) introduced prior to each experiment. Measurements were made on a treadmill with the horse standing, and at speeds predicted to produce 75, 90 and 100% maximal heart rate ( $HR_{max}$ ).

Mean blood flow to the left apical lung lobe quadrupled ( $p<0.05$ ) between standing and 100%  $HR_{max}$ . At exertion, increased inspiratory resistance was associated with a 19% increase ( $p<0.05$ ) in mean blood flow, while increased expiratory resistance had no apparent effect on blood flow. At exertion, increased inspiratory resistance was associated with increased (22%,  $p<0.05$ ) mean wedge pressure; increased expiratory resistance was associated with increased (17%,  $p<0.05$ ) mean pulmonary artery pressure. With expiratory resistance loading, vascular resistance increased 83% ( $p<0.05$ ) in the apical lobe. The difference between mean wedge pressure and mean left atrial pressure increased ( $p<0.05$ ) from 7 mm Hg to 34 mm Hg with exertion.

We conclude that increased vascular resistance during expiratory loading (e.g. soft palate displacement) may prevent an increase in pulmonary blood flow and that pulmonary wedge pressure does not track left atrial pressure during exertion.

## THE EFFECT OF FLAIR™ NASAL STRIPS ON UPPER AIRWAY MECHANICS IN EXERCISING HORSES

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**Purpose:** Nasal resistance accounts for approximately 50% of total airway resistance in exercising horses. The Flair™ external nasal dilator device is meant to support and dilate the nasal valve, increasing the radius of curvature of the nasal passage, thereby reducing airway resistance and work of breathing. Therefore, we hypothesized that the Flair™ external nasal dilator strip would improve upper airway mechanics in exercising horses and decrease the work of breathing.

**Methods:** Six Standardbred horses were used in the study. Tracheal and nasopharyngeal pressures, airflow, and heart rate were measured while horses exercised on a treadmill at speeds corresponding to 75%, 100%, and 120% of maximal heart rate with and without the Flair™ nasal strip. Peak inspiratory and expiratory tracheal and nasopharyngeal pressures, airflows, and tidal volume were obtained from the respiratory function computer. Inspiratory and expiratory tracheal and nasopharyngeal impedances were calculated. Respiratory frequency was determined from the respiratory function computer and minute ventilation was calculated by multiplying respiratory frequency by tidal volume. Heart rate was determined using a telemetric ECG system. Data were analyzed using 2-way ANOVA repeated measures and post hoc comparisons were made, when applicable, using the Student Newman Keul's test, ( $P < 0.05$ ).

**Results:** Peak tracheal inspiratory pressure, inspiratory flow, tidal volume, and minute ventilation were significantly lower in horses wearing the Flair™ than without the nasal strip while exercising at the same speed.

**Conclusion:** The Flair™ nasal strip decreased work of breathing in exercising horses.

## EFFECTS OF INSTILLED AND INHALED HAY DUST ON STORED AND SECRETED MUCOSUBSTANCE IN THE AIRWAYS OF F344 RATS.

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Organic dusts rich in bacterial endotoxins are ubiquitous in barn environments. These dusts are associated with occupational respiratory diseases in humans, and with the asthma-like condition recurrent airway obstruction (RAO) of horses. Previous work in our laboratory demonstrated a dose-dependent increase in levels of the mucin-associated sugar  $\alpha$ -1,2 fucose in the bronchoalveolar fluid (BALF) of F344 rats exposed to endotoxin, and that RAO-affected horses have a persistent elevation of  $\alpha$ -1,2 fucose in BALF. We therefore hypothesized that exposure to hay dust would increase  $\alpha$ -1,2 fucose in airways of F344 rats. Hay representative of that found in barn environments was used as bedding material for F344 rats. Rats were housed for 3 or 10 days. A single dose of dust suspension prepared from barn hay in sterile saline was nasally instilled into a 3<sup>rd</sup> group (200  $\mu$ L/naris, 5 mg dust total exposure). 50,000 endotoxin units/ml of suspension was measured by Limulus amoebocyte assay. BALF was recovered at necropsy, and large diameter airways were collected and stained to highlight stored epithelial mucosubstance. Necropsy of rats housed on hay occurred immediately after end of the housing period, while necropsy of instilled rats took place 72 hours post exposure. Nasally instilled rats and rats housed on hay for 10 days showed a significant increase in stored mucosubstance in 5<sup>th</sup> generation (G5) airways compared to controls (12x and 4x, respectively). Levels of  $\alpha$ -1,2 fucose in BALF of nasally-instilled rats was significantly elevated over controls (P = .002). Numbers of neutrophils and lymphocytes per ml of lavage fluid were significantly increased in nasally-instilled rats as compared to controls. Conclusion: direct effect of hay dust components, such as endotoxin, and/or subsequent inflammatory response increased stored and secreted airway mucins in nasally-instilled rats. These findings indicate that though housing of animals on dusty hay resulted in only a mild inflammatory response, nasal instillation of hay dust in F344 rats can serve as a useful model of dust-induced pathologic airway changes.

## EFFECT OF FUROSEMIDE AND NASAL STRIP ON EXERCISE-INDUCED PULMONARY HEMORRHAGE IN MAXIMALLY EXERCISING HORSES

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The pathogenesis of exercise-induced pulmonary hemorrhage (EIPH) is thought to arise from pulmonary capillary stress failure induced by very positive intravascular and negative extravascular pressures. Recently, our laboratory demonstrated that treatment with either an equine nasal strip (NS; FLAIR™, CNS, Inc.) or furosemide (FUR) significantly reduced EIPH. FUR lowered EIPH (assessed via bronchoalveolar lavage; BAL) more than NS possibly due to a significant reduction in pulmonary artery pressure (Ppa) during severe, but not maximal exercise. The purpose of this investigation was to assess the effectiveness of the NS and FUR to enhance performance and reduce EIPH in maximally exercising horses. To date, 3 Thoroughbred horses have performed graded exercise tests (i.e., start at 7 m/s; increase 1 m/s/min to volitional fatigue) on a motorized treadmill under control, NS, and FUR (i.v., 1 mg/kg, 4 hr prior) conditions (2 weeks between trials; order randomized). Prior to each investigation, a microtipped pressure transducer was placed in the pulmonary artery 8 cm past the pulmonic valve. EIPH severity was assessed via BAL 30 min post-run. NS increased exercise time in 2 of 3 horses (35 and 60 s). FUR increased exercise time in all 3 horses (25, 35 and 60 s). Peak Ppa was similar between groups (CON,  $107.4 \pm 7.2$ ; NS,  $108.6 \pm 5.8$ ; FUR,  $104.1 \pm 7.8$  mmHg). EIPH severity was reduced with NS and FUR treatment in all 3 horses, although FUR appeared more efficacious (CON,  $99.7 \pm 73.5$ ; NS,  $46.1 \pm 23.8$ ; FUR,  $39.4 \pm 32.6 \times 10^6$  RBC/ml BAL fluid). These preliminary findings support the notion that FUR enhances athletic performance and that both FUR and NS are effective in reducing although not abolishing EIPH. Given the similarity of peak Ppa's between trials, these data suggest that either Ppa is not a sensitive indicator of the vascular pressures imparted on the pulmonary capillaries or that FUR reduces EIPH, in part, via extravascular mechanisms.

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## EFFECT OF CHRONIC AIRWAY INFLAMMATION AND EXERCISE ON PULMONARY AND SYSTEMIC ANTIOXIDANT STATUS OF HEALTHY AND HEAVES-AFFECTED HORSES

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In heaves-affected horses the relation between oxidant status and airway inflammation and pulmonary function is unknown. In the present study, the systemic oxidant status (reduced [GSH], oxidised [GSSG] glutathione, glutathione redox ratio [GRR%]; uric acid; 8-epi-PGF<sub>2α</sub>) of healthy (H, n=6) and heaves-affected horses in clinical remission (REM, n=6) and in crisis (CR, n=7) was assessed at rest, at peak-exercise and 15 and 60 minutes after standardised exercise test (SET). The same markers were assessed at rest and 60 minutes after SET in the pulmonary epithelial lining fluid (PELF). The markers were compared between H, REM and CR and were related with pulmonary function parameters (lung resistance [R<sub>L</sub>], dynamic compliance [C<sub>Dyn</sub>], maximal pleural pressure variation [MaxΔppl], arterial partial oxygen pressure [PaO<sub>2</sub>]) and airway inflammation parameters (bronchoalveolar lavage [BAL] neutrophil%, airway inflammation score). Haemolysate GSH was significantly different between groups and was correlated with pulmonary function and airway inflammation; GRR in PELF was increased by CR only and was correlated with with pulmonary function and airway inflammation parameters. SET-induced plasma uric acid increase was significantly higher in REM and CR, whilst PELF uric acid was significantly increased by SET in all groups. Plasma 8-epi-PGF<sub>2α</sub> was significantly higher in H during SET, and PELF 8-epi-PGF<sub>2α</sub> was significantly increased in CR and correlated with pulmonary function and airway inflammation parameters. These results show that oxidative stress occurring in heaves is correlated with pulmonary function and airway inflammation and can be locally assessed by PELF glutathione status, uric acid and 8-epi-PGF<sub>2α</sub>. Systemic repercussions of pulmonary oxidative stress are reflected by haemolysate GSH in resting horses and by uric acid in exercising horses.

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SERUM CORTISOL CONCENTRATIONS IN RESPONSE TO FLUTICASONE PROPIONATE  
INHALATION THERAPY IN HORSES

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Interest in inhaled medications for use in horses has grown considerably. Research executed on corticosteroid inhalation therapy in horses has, so far, focussed mainly on beclomethasone dipropionate (bd). In humans, fluticasone propionate (fp) has been established as a glucocorticoid receptor agonist with an affinity 18-fold greater than dexamethasone, and the amount of systemically bioavailable drug will predominantly depend on absorption from the lungs. The purpose of this study was to determine whether inhaled fp has an effect on the endogenous production of cortisol in horses, as disturbances of the systemic cortisol concentrations can lead to complications such as laminitis.

Eight mares were assigned to 2 treatment groups in a cross over design with a treatment period of 7 days and a 27-day wash out period, during which the horses were kept at pasture. Each group received either placebo (pl) or fp (0,003 g fp = 12 actuations) evenly divided over morning (7.30 am) and evening (7.30 pm) treatment. The aerosol was administered through an Aeromask<sup>TM</sup> with MDI aerosol holding chamber. Blood was taken twice a day immediately prior to treatment on day 0,1,3,5,7 and 8 for serum cortisol determination. On day 1, 3, 5 and 7 morning urine was collected immediately after treatment to determine cortisol/creatinine ratios. On day 8 at 9.00 am an Adreno Cortico Tropic Hormone (ACTH) stimulation test was performed by administering 25 IU of synthetic ACTH (Synacthen®) iv.

Mean morning cortisol concentrations during fp treatment was significantly ( $P = 0,024$ ) lower than during pl treatment. During fp treatment, the diurnal rhythm was depressed. Cortisol/creatinine ratios in urine were lowered during fp treatment. Adrenal response to exogenous ACTH administration was sufficient after both fp and pl treatments.

Fp has similar effects on adrenal function, namely suppression, in horses as is found in equine studies on inhaled bd. Like bd, fp did not reduce adrenal responsiveness to ACTH. One could suggest that, whilst fp is more potent than bd, a similar dosage of fp would result in a greater increase in anti-inflammatory effects with no increased risk of systemic side effects.

LEUKOTRIENE PRODUCTION IN AIRWAY AND PERIPHERAL BLOOD LEUKOCYTES  
FROM HORSES AFFECTED WITH RECURRENT AIRWAY OBSTRUCTION

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In horses affected with recurrent airway obstruction (RAO), dust exposure induces airway obstruction and pulmonary neutrophilia. Neutrophils have the capacity of synthesising leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which is a powerful chemotactic agent and stimulates leukocyte functions. We have studied the *ex vivo* LT biosynthesis in peripheral blood neutrophils and airway inflammatory cells obtained via bronchoalveolar lavage (BAL).

Six RAO-affected, and six control horses, were stabled and exposed to straw and hay for 48 hours. This exposure resulted in a significant increase in dPplmax after six hours and the effect persisted to 48 hours. Before (0) and at 6, 24 and 48 hours post stabling, lung function measurements, withdrawal of peripheral blood, BAL and differential counts of BAL fluid cells were performed. Neutrophils were purified from peripheral blood by Percoll density gradient centrifugation, and neutrophils and BAL cells were stimulated with calcium ionophore A23187 and LT's were measured by HPLC.

Before stabling, there was a significant lower formation of both LTB<sub>4</sub> and LTC<sub>4</sub> (54,8±31,6 and 12,1±3,2 pmol/10<sup>7</sup> cells) in BAL cells from RAO-affected horses, as compared to control horses (159,1±54,1 and 25,7±13,4 pmol/10<sup>7</sup> cells), although there were no differences in BAL differential counts between the groups. After 6 hours, however, a marked increase in LTB<sub>4</sub> synthesis was observed in BAL cells from RAO-affected horses (346±87 pmol/10<sup>7</sup> cells), which correlated to an increased neutrophil content in BAL. In peripheral blood neutrophils, LTB<sub>4</sub> formation was lower in RAO-affected than control horses throughout the study (110±51 and 199±74 pmol/10<sup>7</sup> cells, respectively). BAL cytology revealed an increase at 6 hours and henceforth in neutrophil content and a concomitant decrease in lymphocytes and monocytes/macrophages in RAO-affected horses as compared to control horses. BAL fluid return was less in RAO-affected at 6 hours and throughout the study. Total cells/ml BAL fluid increased in RAO-affected horses after 6 hours, suggesting a considerable increase in potential LTB<sub>4</sub> and LTC<sub>4</sub> formation by airway inflammatory cells in RAO-affected horses as compared to controls (20:1 and 10:1, respectively). The results indicate that increased production of LTB<sub>4</sub> might contribute to the characteristic infiltration of neutrophils into the lungs and the sustained inflammation in RAO.

*CRENOSOMA VULPIS* IN FOXES: AN ANIMAL MODEL FOR BRONCHIOLAR MUCOUS METAPLASIA

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The lungworm *Crenosoma vulpis* (*C. vulpis*) is a broadly distributed lung nematode in wild foxes. Recently, it has been reported as a common cause of persistent cough in dogs in Atlantic Canada. Although the gross pulmonary changes are not outstanding, foxes harboring *C. vulpis* in the lungs have microscopic changes remarkably similar to those lesions described in human beings and horses with chronic obstructive pulmonary disease (COPD). The objectives of this investigation were to microscopically study the bronchial and bronchiolar changes induced by *C. vulpis* and to quantify by morphometric methods the severity of bronchiolar goblet cell metaplasia and bronchial gland hyperplasia, and, to estimate the relationship between these changes and mucous plugs of bronchioles. Studies were conducted in wild foxes naturally infected with *C. vulpis* (n=43) and parasite-free foxes (n= 14) obtained from a commercial ranch. Six anatomical sites systematically sampled from both lungs were fixed by immersion, processed and stained with a combined PAS-Alcian blue stain. The lungs of 79% of the wild foxes had microscopic evidence of *C. vulpis* while none of the controls showed indication of nematodes in the lung. Wild foxes had severe bronchiolar goblet cell metaplasia, abundant mucus in the bronchiolar lumens and hyperplasia of bronchial glands. Some bronchioles were almost exclusively lined with goblet cells and the lumens were plugged with inspissated mucus occasionally containing a few eosinophils, neutrophils and macrophages. The bronchioles of control foxes rarely showed goblet cells or mucus in the lumen. Statistical analyses showed a significant association (Odd ratio 5.48; p< 0.01) between *C. vulpis* and bronchiolar mucus metaplasia. Morphometric analyses revealed that the mucous plugs blocked 63.8 ± 21.9 percent of the bronchiolar lumens. The bronchial glands of wild foxes were significantly (p< 0.01) enlarged and hyperplastic. Bronchial morphometry showed Reid indexes of 44.25% and 19.13% for wild and ranch foxes respectively. Inflation changes like atelectasis or emphysema were not evaluated because of the fixation method. We concluded that *C. vulpis* induces a mild airway inflammation and a severe bronchial gland hyperplasia and bronchiolar goblet cell metaplasia similar to that observed in human or equine COPD. This parasitic disease may provide a reliable laboratory model to study under controlled conditions the chemical mediators involved in small airway disease. Since urbanization has narrowed the interface between wild foxes and domestic dogs, practitioners and laboratory diagnosticians will more frequently encounter chronic respiratory problems associated to *C. vulpis*.

## THORACOSCOPIC PULMONARY WEDGE RESECTION: USEFULNESS AND SAFETY IN HORSES.

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The purpose of this study was to evaluate the efficacy and safety of thoracoscopic guided pulmonary wedge resection in horses.

Eight horses (4 control, 4 heaves-affected in remission) underwent 2 thoracoscopy procedures. During the first procedure, each horse had a thoracoscopic pulmonary wedge resection. At intervals before, during and after surgery, the following variables were measured: heart rate, respiratory rate, arterial pH, PaO<sub>2</sub>, PaCO<sub>2</sub>, systemic and pulmonary arterial pressures. Physical examination, CBC, thoracic radiography and ultrasound were performed 24 hours before, 2 and 48 hours after surgery. Biopsy specimens were assessed by histological examination. A second thoracoscopy 14 days post surgery evaluated the biopsy site and thorax. Statistical analysis was by ANOVA (p<0.05) for repeated measures.

Excellent intraoperative visibility of intrathoracic structures was achieved. Heart and respiratory rate decreased significantly following detomidine (Dormosedan). The procedure had no effect on arterial pH, PaCO<sub>2</sub>, mean arterial and pulmonary arterial pressures. A significant decrease in PaO<sub>2</sub> (baseline mean- 91.65± 5.1 mmHg, post-biopsy mean- 76.9 ± 12 mmHg) and increase in respiration rate (pre-biopsy mean- 13±5, post-biopsy mean- 22±10) occurred immediately after biopsy. There was no difference between normal and heaves horses. None of the animals developed dyspnea and the lowest PaO<sub>2</sub> was 63 mmHg. An excellent biopsy specimen (4X3X2 cm) with sufficient structural elements for histologic evaluation was obtained. All horses except one were clinically normal after surgery. One developed hemothorax due to iatrogenic injury to the diaphragm with a trochar. The second thoracoscopy revealed minimal inflammation at the biopsy site and no adhesions.

Thoracoscopic lung biopsy is well tolerated, and provides a minimally invasive method to obtain lung biopsy specimens in horses. The technique may be applicable for the diagnosis and treatment of disease and to obtain specimens for immuno-histochemistry and in-situ hybridization. Careful technique can prevent complications such as hemothorax. (Supported by Ethicon Endo-Surgery, Inc.).

## EVALUATION OF TRACHEAL WASH AND BRONCHOALVEOLAR LAVAGE FOR DIAGNOSIS OF AIRWAY INFLAMMATION AFTER EXERCISE IN HORSES WITH POOR PERFORMANCE

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Equine practitioners commonly collect tracheal wash (TW) or bronchoalveolar lavage (BAL) samples, but rarely both, from racehorses with poor performance to evaluate the health of the respiratory tract. Usually, samples are collected 1-3 hours after exercise. Many practitioners rely on diagnosis of either presence or absence of inflammatory airway disease (IAD) in these samples and, if absent, conclude that all regions of the respiratory tree are free of inflammation. Therefore, the purposes of this study were to: 1) investigate whether discrepancy occurs between interpretation of results of TW and BAL samples collected after high-speed treadmill exercise in horses performing poorly and 2) whether IAD in these horses could be localised to either the upper or lower airways.

Thirty-one Thoroughbred and Standardbred racehorses presented to the University of Sydney, for not performing as expected were subjected to a standardised high-speed treadmill exercise test. Approximately 30-60 minutes after exercise, a TW sample was collected using a guarded catheter introduced via the biopsy port of an endoscope. A BAL sample also was collected blindly using a BAL<sup>®</sup> tube.

We found no statistical relationship between the relative % neutrophil counts ( $R^2 = 2.3\%$ ) in TW and BAL samples post-exercise. In 16/31 horses (52%) there was discrepancy in interpretation of inflammation between the 2 samples, where a TW in 11/16 horses was interpreted as demonstrating IAD (>20% neutrophils) whereas a BAL did not (<5% neutrophils). In addition, 8/11 horses diagnosed with IAD from a TW sample, but not from BAL, had relative % neutrophil counts > 76%. In contrast, 5/16 horses had BAL samples indicating IAD (>5% neutrophils) whereas a TW did not (<20% neutrophils).

We interpret these results to suggest that the majority of horses diagnosed with IAD using TW after high-speed treadmill exercise would not be diagnosed as having IAD if a BAL were used. This supports the premise of the presence of regional airway inflammation in exercising horses in which high-grade tracheal and/or bronchial inflammation can occur without concomitant small airway inflammation (and vice versa). Equine practitioners therefore should be aware that if either sample is collected alone a conclusion of absence or presence of inflammation in “the lung” may occur, when in fact only one region of the airway is affected.

DEVELOPMENT OF A MODEL OF ACUTE, RESOLVING PULMONARY OXIDATIVE STRESS IN  
THE HORSE BY OZONE EXPOSURE

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Imbalances in pro- and anti-oxidants have been implicated in pulmonary inflammatory conditions in man such as asthma and chronic obstructive pulmonary disease. In order to study the role of reactive oxygen species (ROS) in equine pulmonary disease, we attempted to develop a model for inducing reproducible and mild-moderate pulmonary oxidative stress using ozone exposure. Bronchoalveolar lavage (BAL) was performed in the right lung of 3 (total n=6) healthy horses with no history of respiratory disease before (-7 days) and at 6 and 72 hours after 2h exposure to 800 ppb ozone. All horses had been kept continually at grass for at least 2 months. BAL was performed using two 100 ml aliquots of saline at 37°C. Ascorbic acid (AA), oxidised (GSSG) and total glutathione (TGSH) and uric acid (UA) in BAL were analysed by HPLC. BAL concentrations were corrected to concentrations in epithelial lining fluid (ELF) using plasma and BAL urea concentrations. Glutathione redox ratio (GRR) was calculated as GSSG/TGSH.

	<b>-7 days</b>	<b>+6h</b>	<b>+72h</b>
ELF GSSG (umol/l)	3.7±1.9	37.9±28.1	5.6±2.6
ELF GRR (%)	6.5±6.3	41.2±11.4	13.0±9.8
ELF AA (mmol/l)	0.36±0.32	0.37±0.32	0.52±0.32
ELF UA (umol/l)	2.3±1.1	2.7±0.8	2.2±0.6

The concentration of GSSG and the GRR were increased in all three horses at 6h following ozone exposure, but had decreased markedly by +72h. AA and UA were unchanged by ozone exposure at +6h. None of the horses showed any clinical signs. Exposure of healthy horses with no history of respiratory disease to 800 ppb ozone for 2h induces mild-moderate pulmonary oxidative stress based on the GRR, but without clinical signs of pulmonary dysfunction and which resolves by +72h.

AIRWAY REACTIVITY, INFLAMMATION, IRON AND IRON-BINDING PROTEINS IN HORSES  
FROM URBAN V. RURAL ENVIRONMENTS

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*Purpose:* Inflammatory lung injury due to oxidative stress in humans has been associated with the catalytically active metal, iron, contained in air pollution particles (APP). Subsequently, iron transport and storage proteins may be up-regulated and function to limit oxidative stress. This retrospective study was conducted to determine if horses from urban environments with concomitantly greater exposure to air pollution particles show evidence of airway inflammation, airway hyperreactivity (AWHR), and increased levels of iron and iron transport and storage proteins in BALF, compared to horses from rural environments.

*Methods:* Horses from urban (U; n=13) and rural environments (R; n=11) underwent FOM baseline lung function (1-7 Hz) and bronchoprovocation to establish airway obstruction or AWHR. Airway inflammation was determined by measuring percentages of inflammatory cells in BALF. Total protein, albumin, iron, transferrin, lactoferrin, ferritin, hemoglobin, haptoglobin, and TIBC levels in BALF were also measured. Horses with a recent history of infectious disease, or evidence on BALF of EIPH, were excluded. Data are expressed as mean values  $\pm$  SD. Differences between groups R and U; horses with lower airway inflammation (PMN > 5% or mast cells > 2%, n = 15) v. controls (n = 9); and horses with airway hyperreactivity ( $PC_{100}R_{RS} < 6$  mg/ml histamine, n = 19) v. controls (n = 5), were examined using *t*-tests of independent means. Significance was assumed at  $P < 0.05$ .

*Results:* Urban horses had markedly greater iron levels in BALF ( $210.8 \pm 199.7$  ppb) than rural horses ( $106.6 \pm 28.0$ ), approaching statistical significance ( $P < .08$ ). Urban horses also had greater, but not significant, levels of ferritin in the BALF ( $111.9 \pm 72.7$  ng/ml v.  $82.0 \pm 27.8$ ). Rural horses were markedly more reactive than urban horses ( $PC_{100}R_{RS} = 2.9 \pm 2.0$  mg/ml histamine, v.  $7.1 \pm 5.7$ ,  $P < .05$ ). There was no evidence of airway obstruction in either group (R horses  $R_{RS} = .60 \pm .27$  cm H<sub>2</sub>O/L/s; U horses  $0.64 \pm .21$ ). There were no other significant differences among any groups.

*Conclusions:* Higher iron levels in urban horses may be due to air pollution particles: extravasation of iron from the serum due to airway inflammation is less likely, as U horses had less AWHR, and had no greater evidence of airway inflammation. Higher ferritin levels in U horses may be protective against oxidative stress by sequestering iron. Rural horses had significantly more airway reactivity than U, despite normal baseline RRS in both groups. This finding may reflect an unrelated environmental factor in AWHR in R horses. Increased iron and ferritin in BALF in U horses, despite the lack of statistical significance, supports further study in urban v. rural equines. Horses continually sample the outside air, and may function as a valuable sentinel and model for the role of the environment in airway disease.

## DOES VENTILATION LIMIT MAXIMAL OXYGEN UPTAKE IN THE THOROUGHBRED HORSE?

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Stride and respiratory frequency are tightly linked in the galloping Thoroughbred horse. This leads to very high respiratory rates ( $>120$ ), which likely constrain tidal volume ( $V_T$ ), increase dead space ventilation, and limit alveolar ventilation ( $V_A$ ). To test the hypothesis that stride-related alterations in ventilatory frequency/pattern would increase  $V_A$  and thus  $VO_{2max}$ , 6 Thoroughbred geldings (wt:  $526.3 \pm 18.3$  kg) performed graded exercise tests to fatigue on a flat (F) and inclined (I) treadmill (10%) in random order (two weeks between runs). At maximal exercise on the incline, breathing frequency (I:  $123 \pm 2$  and F:  $128 \pm 3$ ;  $p \leq 0.05$ ) was reduced and  $V_T$  (I:  $13.1 \pm 0.5$  and F:  $11.5 \pm 0.6$  L;  $p \leq 0.05$ ) and ventilation were increased (I:  $1608 \pm 63$  and F:  $1465 \pm 74$  L $\cdot$ min $^{-1}$ ;  $p = 0.09$ ). More importantly, calculated  $V_A$  (I:  $1365 \pm 60$  and F:  $1091 \pm 82$  L $\cdot$ min $^{-1}$ ;  $p \leq 0.05$ ) was significantly increased ( $\sim 25\%$ ) on the incline. These ventilatory adjustments resulted in a significant elevation in arterial  $O_2$  content ( $p \leq 0.05$ ), which increased  $O_2$  delivery to the working musculature, resulting in a significant increase in  $VO_{2max}$  (I:  $80.0 \pm 4.1$  and F:  $63.2 \pm 4.1$  L $\cdot$ min $^{-1}$ ;  $p \leq 0.05$ ). In each condition,  $VO_2$  achieved a clear plateau and end-exercise arterial blood lactate and pH were not different. In conclusion, these data suggest that stride frequency coupled ventilatory patterns ultimately limit  $V_A$  and  $VO_{2max}$  in the Thoroughbred horse. Support: American Quarter Horse Assoc., NIH training grant, NIH-HLBI-50306.

## COMPARISON OF TRACHEAL FLUID CONTAMINATION OBTAINED BY THREE DIFFERENT ENDOSCOPIC TECHNIQUES IN HORSES

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The purpose of this study was to evaluate and compare the degree of oropharyngeal contamination occurring in tracheobronchial fluid samples obtained by three different endoscopic aspiration techniques, with the aim of determining if any technique was reliable enough to replace transtracheal aspiration for bacteriological culture. A flexible fiberoptic endoscope (ETM, Germany) was used to obtain tracheal fluid samples from 18 horses that were under investigation for lower airway inflammatory disease. The horses were randomly divided into three groups. 6 horses were sampled using a 'routine' tracheal wash technique (60 ml sterile saline injected at the level of the carina and sample obtained from the thoracic inlet); 6 using a modified tracheal wash technique (endoscope remaining proximal to the thoracic inlet and only the sterile catheter advanced to the thoracic inlet); and 6 using a guarded aspiration catheter procedure (Darien Microbiology Aspiration Catheter, Mill-Rose Laboratories). The degree of oropharyngeal contamination with each method was compared using quantitative bacteriology and cytology. There was no difference between techniques ( $P < 0.1$ ), with all resulting oropharyngeal contamination. Bacteria characteristic of those found in the oropharynx ( $\alpha$ -haemolytic *Streptococcus* spp, *Staphylococcus* spp, *E. coli* and non-lactose fermenters) were cultured in 17 of the samples and squamous epithelial cells (SEC) found in 15 of the samples. A correlation between SEC/ml and bacterial colony forming units/ml was found ( $P < 0.05$ ). We conclude that oropharyngeal contamination occurs in all 3 techniques, and that none of these techniques are suitable to provide an uncontaminated, tracheobronchial secretion sample for bacteriological culture.

## THE ROLE OF INHALED ENDOTOXIN IN THE AETIOPATHOGENESIS OF HEAVES

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Endotoxins are potent pro-inflammatory substances present in high concentrations in equine environments, and endotoxin inhalation in the horse induces many of the classical features of equine heaves.

Aims: To investigate the relative contribution of inhaled endotoxin and dust particulates (primarily mould spores) in heaves.

Methods: Firstly, the response of 6 heaves horses to inhalation challenge with a hay dust suspension (HDS) before and after lipopolysaccharide (LPS) depletion was measured. Secondly, the response of 6 heaves horses to a 5h housing period in 2 separate hay/straw environments was measured and compared with the level of endotoxin exposure in the horses breathing zones. Thirdly, HDS was fractionated into supernatant (SUP) and washed particulates (WP). The response of 7 heaves horses to inhalation challenge with WP reconstituted in SUP (WP/SUP) was measured and compared with the response to inhalation challenge with WP reconstituted in LPS solution, with an LPS concentration equivalent to that of SUP (WP/LPS).

Results: HDS challenge resulted in a significant ( $P<0.05$ ) increase in bronchoalveolar lavage (BALF) neutrophils at 6h and obstructive lung dysfunction at 5h. When compared with HDS inhalation, challenge with LPS-depleted HDS resulted in a significant reduction in BALF neutrophil count and no detectable lung dysfunction. Add back of LPS to the depleted suspension, at a concentration equivalent to that which was removed, resulted in the re-establishment of the pulmonary inflammatory response, which did not differ significantly from that measured following HDS challenge, in addition to re-establishment of detectable lung dysfunction. Extrapolation from previously reported LPS dose response inhalation studies revealed that the magnitude of this alteration in response could not be solely attributed to the direct activity of the depleted (or added back) LPS. There was a significant difference in the group response to the 2 separate hay/straw exposures with respect to BALF neutrophil count at 6h and arterial oxygen tension at 90min. This response was not however associated with the level of airborne dust or endotoxin exposure. The airway neutrophilic response to inhalation challenge with WP/SUP at 6h was not significantly different from that following challenge with WP/LPS.

Conclusion: Although inhaled endotoxin is not solely responsible in inducing the pulmonary functional and inflammatory responses in heaves-susceptible horses, it does play a significant role in disease aetiopathogenesis (as induced using the HDS model). This may partly be explained by the magnification in the pulmonary response to inhaled endotoxin when co-presented to the lung in association with particulate organic dust components.

EFFECT OF NITRIC OXIDE (NO) SYNTHASE INHIBITION ON OXYGEN UPTAKE KINETICS  
IN THE HORSE

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Across the transition to faster running speeds, the rapidity of increase of oxygen uptake ( $\text{VO}_2$  kinetics) is an important determinant of the aerobic and non-aerobic contributions to ATP turnover. Specifically, faster  $\text{VO}_2$  kinetics reduce the participation of anaerobic glycolysis/glycogenolysis and incurred intracellular perturbation. Muscle mitochondrial inertia is thought to play a deterministic role in limiting  $\text{VO}_2$  kinetics and there is evidence that NO competitively inhibits  $\text{O}_2$  consumption within the electron transport chain. To investigate whether NO is important in setting the dynamic response of  $\text{VO}_2$  at the onset of high intensity (heavy domain) running in horses, 5 geldings were run on a treadmill across speed transitions from 3 m/s to speeds corresponding to 80% of peak  $\text{VO}_2$  with and without L-NAME, an NO synthase inhibitor (20 mg/kg; order randomized). Measurements of  $\text{VO}_2$  were obtained using an open-flow system and data analyzed by means of Kaliedagraph software using one- and two-exponential models. L-NAME did not alter (both  $p > 0.05$ ) baseline (3 m/s; CON,  $15.4 \pm 0.3$ ; L-NAME,  $16.2 \pm 0.5$  L/min) or end-exercise  $\text{VO}_2$  (CON,  $56.9 \pm 5.1$ ; L-NAME,  $55.2 \pm 5.8$  L/min). However, in the L-NAME trial, the primary on-kinetic response was significantly ( $p < 0.05$ ) faster (i.e., reduced time constant; CON,  $27.0 \pm 2.7$ ; L-NAME,  $18.7 \pm 3.0$  s) despite no change in the gain of  $\text{VO}_2$  ( $p > 0.05$ ). The faster on-kinetic response was confirmed independent of modeling via reduced time to 50, 63 and 75% of overall  $\text{VO}_2$  response (all  $p < 0.05$ ). In addition, the onset of the  $\text{VO}_2$  slow component occurred earlier (CON,  $124.6 \pm 11.2$ ; L-NAME,  $65.0 \pm 6.6$  s) and the magnitude of the  $\text{O}_2$  deficit was attenuated (both  $p < 0.05$ ) in the L-NAME compared with the control trial. The speeding of the  $\text{VO}_2$  kinetics by L-NAME suggests that NO inhibition of mitochondrial oxygen consumption may contribute, in part, to the intrinsic metabolic inertia evidenced at the transition to higher metabolic rates in the horse.

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## A COMPARISON OF PHOSPHODIESTERASE ISOENZYME PROFILES IN EQUINE NEUTROPHILS AND LYMPHOCYTES

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Equine COPD is characterised by a peribronchiolar and perivascular lymphocytic infiltrate and neutrophil accumulation within the airways. At present the mechanisms responsible for neutrophil recruitment have not been fully characterised. In human allergic asthma the profile of cytokine expression, which is determined by the lymphocyte population present within the lungs, has been shown to be important in cell recruitment. In equine COPD lymphocytes may therefore play a role in the trafficking of neutrophils to the lungs following antigen challenge. Phosphodiesterase (PDE)4 inhibitors have been shown to attenuate human inflammatory cell activation. Previously we have demonstrated that neutrophils from both normal and COPD susceptible horses contain PDE4 and that the PDE4 inhibitor, rolipram, inhibits neutrophil activation *in vitro*. The purpose of this study was to measure PDE activity, and identify the isoenzymes present, in equine lymphocytes. Neutrophils and mononuclear cells from normal horses were separated by Percoll density gradient centrifugation. The lymphocyte population was enriched by depletion of monocytes through adherence to plastic at 37°C. Non-adherent cells and neutrophils were lysed and assayed for PDE activity using a two-step radioactive assay in the presence of a single high concentration of isoenzyme selective inhibitors. The results obtained suggest that, as in equine neutrophils, the main isoenzyme in equine lymphocytes is PDE4. Thus rolipram caused 69 +/- 3% inhibition (mean +/- SEM; n=4) of total PDE activity. As in equine neutrophils, the mixed PDE3/4 inhibitor, zardavarine, caused a similar amount of inhibition (62 +/- 4%) but a selective PDE3 inhibitor, quazinone, had no significant effect (16 +/- 2% inhibition). This suggests that the inhibitory effect of zardavarine was due to its action on PDE4. However, PDE3 is present in the membrane fraction of lysed lymphocytes from other species and the apparent lack of any significant PDE3 in equine lymphocytes needs to be confirmed using different methods of cell lysis. There was no evidence for the presence of PDE1, 2 or 5 in equine lymphocytes. In view of the presence of PDE4 in these cells, it will be of interest to examine the effects of PDE4 inhibitors on lymphocyte activation.

THE DIAGNOSTIC VALUE OF POST-RACING ENDOSCOPY FOR THE DETECTION OF EIPH  
IN THOROUGHBRED RACEHORSES IN THE U.K.

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Post-race endoscopic examination of poorly performing horses for the detection of EIPH is now available on many racecourses in the United Kingdom. Results of these examinations have been examined to assess the diagnostic value of this procedure. We reported earlier (Roberts 1998, WEAS Research Proceedings, Guelph. p.4) on preliminary results of this study, involving 49 horses, we now report the results of 157 examinations. The severity of EIPH was graded on a scale of 0-5 and the prevalence and severity of the condition in this group of poor performers ('poor performance group') was compared with data from a random sample of 223 racehorses ('control group').

	Control Group		Poor Performance Group	
	Number	% with EIPH	number	% with EIPH
Two year olds	102	40.2	7	0
Three year olds	99	64.6	31	54.8
Older horses	22	81.8	119	60.5
All horses	223	55.6	157	56.7

In both groups of horses the prevalence of EIPH increased with age. When horses of the same age groups were compared, the prevalence of EIPH in the poorly performing horses was not significantly different from that of the control group. Abnormal respiratory noise (12.8%) and/or sudden fading (20.4%) towards the end of the race were reported in 31.9% of the poorly performing horses, raising the possibility of dynamic airway collapse as a possible cause of poor performance.

Since the severity of EIPH necessary to affect performance is uncertain and the prevalence of the condition is no greater in poorly performing horses than the general racehorse population, the results of post-race endoscopy should be interpreted with care if a spurious diagnosis is to be avoided.

ASCORBIC ACID IN EQUINE PLASMA AND EPITHELIAL LINING FLUID IN HEALTHY HORSES  
AND HORSES AFFECTED BY RECURRENT AIRWAY OBSTRUCTION (RAO)

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Unlike man, the horse is able to manufacture ascorbic acid endogenously. Previously in horses, glutathione has been considered as quantitatively the most important lung lining fluid anti-oxidant. Anti-oxidant status has also been implicated as a modulator of inflammation. Therefore we were interested to determine ascorbic acid concentrations in plasma and bronchoalveolar lavage (BAL) of healthy horses and horses affected by recurrent airway obstruction (RAO; previously referred to as equine chronic obstructive pulmonary disease (COPD)). BAL was performed in the right lung of 4 healthy horses with no history of respiratory disease and 10 horses suffering from RAO whilst in clinical remission. All horses had been kept continually at grass for at least 2 months. BAL was performed using two 100 ml aliquots of saline at 37°C. A jugular venous blood sample was obtained immediately prior to BAL. Ascorbic acid (AA) in BAL and plasma were analysed by HPLC using electrochemical detection and UV detection, respectively. BAL concentrations of ascorbic acid were corrected to mmol.l<sup>-1</sup> of epithelial lining fluid (ELF) using plasma and BAL urea concentrations.

	<b>Control (n=4)</b>	<b>RAO (n=10)</b>	
Plasma AA (µmol/l)	11.2 ± 0.7	8.0 ± 2.6	P=0.002
BAL AA (umol/l)	13.1 ± 4.0	2.1 ± 1.4	P=0.013
ELF AA (mmol/l)	3.0 ± 1.9	0.2 ± 0.1	P=0.06

To the best of our knowledge there are no reports of concentrations of AA in BAL of healthy horses or in plasma, BAL or ELF of RAO affected horses. The concentrations of AA in ELF measured in the control horses are 20 to 30 times greater than those of reduced glutathione previously reported in horses. AA is reduced in plasma, BAL and ELF of RAO affected horses. We conclude that AA is the major anti-oxidant in equine lung lining fluid.

## ENDOSCOPICALLY-GUIDED LASER DEBRIDEMENT OF ARYTENOID CHONDRITIS IN FIVE STANDING HORSES.

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**Purpose/Introduction:** Horses affected by arytenoid chondritis display upper airway noise, exercise intolerance or respiratory compromise. Thickened or distorted cartilages are immobile and may produce granulomas. Traditional therapy, arytenoidectomy, decreases athletic function. A previous report describing endoscopic laser removal of laryngeal granulomas makes no mention of intracartilaginous tracts, although not all were resolved. **Methods:** One TB-Percheron and four racing TB horses were referred for exercise intolerance and/or upper airway noise. Endoscopy revealed reduced arytenoid movement, mucosal defects with granulating masses and swelling. One TB diagnosed with left laryngeal hemiplegia some weeks earlier had been referred for prosthetic laryngoplasty. Most had reduced mobility of the affected arytenoid cartilage and contained superficial mucosal defects on the contacting surface of the contralateral arytenoid cartilage. After sedation, the ventral throatlatch regions were surgically prepared, and mepivacaine was infiltrated subcutaneously. Through a 1-cm stab incision, a 5-mm trocar and sleeve was inserted through the cricothyroid membrane using nasopharyngeal endoscopic guidance. A hand-held malleable waveguide containing a 600-micron free beam 980-nm diode laser fiber was inserted through the cannula. Using power settings of 20-40 watts (total joules varied), the granulomas were ablated to the level of the surrounding mucosa. If the tract penetrated the arytenoid cartilage, manual curettage using an angled curette completed the debridement. Two lesions drained purulent exudate. The skin wounds were left open to heal after injecting antiseptic ointment subcutaneously, and broad spectrum antibiotics were administered for 10 days. **Results:** The lesions all contained tracts into the cartilage and resolved in all horses; arytenoid mobility was regained. Three horses have and one is about to return to work. One horse previously diagnosed to have left laryngeal hemiplegia did not regain full function. Corniculate process lesions resulted in noticeable atrophy. **Conclusions:** Laser debridement of focal arytenoid sepsis can restore athletic function in some horses that haven't lost basic arytenoid architecture. The sanitizing effect of the laser may contribute to resolution.

SPIROMETRIC AND ENDOSCOPIC ASSESSMENT OF HORSES WITH LARYNGEAL  
HEMIPLEGIA BEFORE AND AFTER SURGERY

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The outcome of a tie back operation is routinely judged, based on performance history and endoscopic re-evaluation. Exercise spirometry proved to monitor airflow limitations of upper airway diseases reliably. The purpose of this study was to quantify the functional improvement after surgery and to compare it with the increased degree of abduction assessed endoscopically. Nine horses with grade 4/4 laryngeal hemiplegia were assessed spirometrically before and after a combined laryngoplasty and sacculotomy. Respiratory frequency ( $f_R$ ), the inspiratory to total respiratory time ratio ( $T_{in}/T_{tot}$ ), tidal volume ( $V_T$ ), minute ventilation ( $V_E$ ), peak inspiratory (PIF) and expiratory flow (PEF) were measured with an ultrasonic flowmeter during a standard exercise test on a treadmill. After a rehabilitation and retraining period of 5 months, the horses were re-evaluated and cantered at the identical exercise intensity they could sustain before surgery. The difference in arytenoid abduction was surveyed with the help of endoscopic pictures at rest. Pre and post surgery results (mean  $\pm$  sd) are listed in the following table:

	pre surgery	post surgery	difference in %
$f_R$ [1/min]	105 $\pm$ 5.1	106 $\pm$ 4.0	+ 1.2
$T_{in}/T_{tot}$ [%]	57.6 $\pm$ 6.8	52.1 $\pm$ 3.4	- 8.7
$V_T$ [l]	14.5 $\pm$ 2.30	16.4 $\pm$ 2.84	+ 13.1
$V_E$ [l/min]	1318 $\pm$ 285.4	1693 $\pm$ 292.5	+ <b>32.7</b>
PIF [l/s]	48.1 $\pm$ 10.97	67.8 $\pm$ 12.72	+ <b>45.1</b>
PEF [l/s]	68.3 $\pm$ 5.75	73.7 $\pm$ 9.19	+ 8.0
Degree of abduction	16.9 $\pm$ 10.52	22.1 $\pm$ 13.92	+ <b>33.4</b>

Although the main functional parameters as  $V_E$  and PIF and the degree of abduction increased in the same percentile range, no significant correlation between these parameters could be found (Pearson,  $P < 0.05$ ), indicating that moderate surgical correction nevertheless improved respiratory function (prevent dynamic collapse) and wide abduction did not increase function proportionally. In conclusion, sole endoscopic assessment may not reflect in all cases the functional improvement of a tie back.

**POSTER LOCATION FINDER**

<u>Presenting author</u>	<u>Page</u>	<u>Title</u>	<u>Poster Location Grouping</u>
Brown, T		Identification and sequence analysis of the ovine forms of elafin	Comparative Respiratory Biology
Brown, JA		Ventriculocordectomy reduces respiratory noise in horses with laryngeal hemiplegia.	Equine Upper Airway
Charlton		Respiratory air-interface organ culture system	Comparative Respiratory Biology
Cole		The effect of various storage conditions on the stability of gaseous nitric oxide	Comparative Respiratory Biology
Collie		Local lung responses following local lung challenge with recombinant lungworm antigen in systemically sensitised sheep	Comparative Respiratory Biology
Couëtil		Effect of inhaled fluticasone, oral prednisone, and pasture environment on lung function of horses with heaves	Equine Lung Disease (III): Therapy
Dixon		A long-term survey of laryngoplasty in an older, mixed-breed population of 200 horses. 1: maintenance of surgical arytenoid abduction and complications of surgery	Equine Upper Airway
Dixon		A study of an electrodiagnostic technique for the evaluation of equine recurrent laryngeal neuropathy	Equine Upper Airway
Dixon		Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses	Equine Upper Airway
Dixon		Clinical and endoscopic evidence of progression in 52 cases of equine recurrent laryngeal neuropathy (RLN)	Equine Upper Airway
Dixon		A long-term survey of laryngoplasty in an older, mixed-breed population of 200 horses. 2: owners assessment of the value of surgery	Equine Upper Airway
Erickson		Effect of inhaled nitric oxide on pulmonary artery pressure and EIPH in maximally exercising horses	Equine Lung Disease (I): Pathogenesis in profile
Funch-Nielsen		Evaluation of a new spacer device for delivery of drugs into the equine respiratory tract	Equine Lung Disease (III): Therapy
Geering		Tissue glutathione content and redox ratio in different regions of the equine lung	Equine Lung Disease (I): Pathogenesis in profile
Gerber		IgE-protein and IgE-mRNA positive cells in lung tissue samples from RAO-affected and control horses after mouldy hay challenge	Equine Lung Disease (I): Pathogenesis in profile
Heffner		Mucus response of rats to intranasal instillation of hay dust	Comparative Respiratory Biology
Hewson		Efficacy of mometasone furoate in the treatment of heaves in horses	Equine Lung Disease (III): Therapy
Kästner		Can atropine facilitate the detection of bronchoconstriction during lobeline stimulation in mild to moderate RAO affected horses?	Equine Lung Disease (II): Diagnosis
Kirschvink		Comparison of Impulse Oscillometry with the conventional technique for bronchodilation evaluation in horses	Equine Lung Disease (II): Diagnosis
Kirschvink		Adaptation to Multiday Ozone Exposure is Associated with a Sustained Increase of Bronchoalveolar Uric Acid	Comparative Respiratory Biology
Kirschvink		Ozone-induced pulmonary dysfunction and airway inflammation are not improved by antioxidant vitamin cocktail in calves	Comparative Respiratory Biology
Kirschvink		The use of Ecobed® Cardboard bedding material: in vitro assessment of airborne dust and aeroallergen concentration and in vivo effects on lung function of heaves-affected horses	Equine Lung Disease (I): Pathogenesis in profile
Klein		Functional differentiation of obstructive abnormalities in horses by means of impulse oscillo-metry using the low frequency range	Diagnostic techniques
Lekeux		Cyclopentenone prostaglandins inhibit NF-kB activity and inflammatory gene expression in lung epithelial	Equine Lung Disease (I): Pathogenesis in profile
Lekeux		Enhanced survival of lung granulocytes in an animal model of asthma: Evidence for a role of GM-CSF-activated STAT5 signaling pathway	Comparative Respiratory Biology
Lekeux		P65 homodimer activity in BAL cells is correlated to lung dysfunction in equine heaves	Equine Lung Disease (I): Pathogenesis in profile
Lekeux		Ozone adaptation in calves is associated with impaired accumulation of granulocytes in the lung	Comparative Respiratory Biology
Martin		Arterial blood gases in horses examined for poor performance during high-speed treadmill exercise	Diagnostic techniques

Mauchline	Ovine Respiratory Disease-Comparative Cytology	Comparative Respiratory Biology
McAleese	Expression of the equine IgE receptor $\gamma$ - chain and its use for the detection of equine IgE	Equine Lung Disease (I): Pathogenesis in profile
McLachlan	Beta-defensin expression in the ovine respiratory epithelium	Comparative Respiratory Biology
Michelotto	Endoscopic and cytological evaluation of the lower respiratory tract of 129 Thoroughbred yearlings in breaking age.	Equine Lung Disease (II): Diagnosis
Nicholls	Preparation of bronchoalveolar lavage fluid cytology slides by cellular gravitation - method of preparation and comparison with cytopspin preparations	Diagnostic techniques
Nicholls	Filtration of bronchoalveolar lavage fluid results in the selective loss of cells	Diagnostic techniques
Pemberton	Characterisation of tryptase and a granzyme H-like chymase isolated from equine mastocytoma tissue	Comparative Respiratory Biology
Pickles	The effect of time, temperature and fixatives on cytological analysis of equine bronchoalveolar lavage fluid	Diagnostic techniques
Pirie	<i>Aspergillus fumigatus</i> extract dose response inhalation challenges in heaves horses and the contribution of endotoxin contamination to the pulmonary inflammatory and functional response to <i>Aspergillus fumigatus</i> extract inhalation	Equine Lung Disease (I): Pathogenesis in profile
Pirie	Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves	Equine Lung Disease (I): Pathogenesis in profile
Raulo	Inhibition of gelatinases and collagenases by CMTs, bisphosphonates and a CTT-peptide in respiratory secretions of horses with chronic obstructive pulmonary disease (COPD)	Equine Lung Disease (I): Pathogenesis in profile
Rigby	Effect of ozone treatment on airborne bacteria and mould in the stable environment	Equine Lung Disease (III): Therapy
Robinson	Mechanisms of airway obstruction in heaves	Equine Lung Disease (I): Pathogenesis in profile
Schlipf	Comparing in-vitro antimicrobial susceptibility for two common equine respiratory tract isolates	Equine Lung Disease (III): Therapy
Smith	Clinical interpretation of oscillometric impedance measurements in horses	Diagnostic techniques
Tate	Acidification of exhaled breath condensate in patients with cystic fibrosis	Comparative Respiratory Biology
Tremaine	Clinical and ancillary diagnostic findings, treatments and long-term results in 277 cases of equine sinonasal disease	Equine Upper Airway
Walley	Characterisation of equine respiratory tract mucins	Equine Lung Disease (I): Pathogenesis in profile
Wattrang	Cytokines in nasal mucus and serum during experimental equine influenza A2 (H3N8) infections of ponies	Equine Upper Airway
Wilson	The effect of a single acupuncture treatment on pulmonary function in horses with recurrent airway obstruction (heaves)	Equine Lung Disease (III): Therapy

## IDENTIFICATION AND SEQUENCE ANALYSIS OF THE OVINE FORMS OF ELAFIN

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Elafin (elastase-specific inhibitor or skin-derived antileukoprotease) is a low molecular weight, anionic inhibitor of neutrophil elastase which is secreted in the lung. Elafin has been studied and characterised in the human and porcine. We used a process of RACE (rapid amplification of C-terminal ends) PCR using ovine tracheal epithelial RNA extracts with a combination of gene-specific primers (GSP1 and 2) and primers to two RACE oligos attached as part of the RACE procedure (GRP1 and 2) as shown in fig. 1. This was followed by cloning in a topoisomerase vector (pCR<sup>R</sup>4-TOPO<sup>R</sup>). In this way we isolated and sequenced two separate full length ovine orthologs of elafin that were 759bps and 829bps in length. The two forms contained an anti-elastase site and multiple repeats of the transglutaminase substrate domain (GQDPVK). These findings represent the first demonstration of the existence of ovine elafin orthologs. In addition the two orthologs described are shown to be new members of the trappin family, a family of proteins which each contain a transglutaminase substrate domain (cementoin) and a whey acid protein (WAP) domain which here includes the active anti-elastase site. This information is currently being confirmed using a sheep genomic library in EMBL-3 bacteriophage vector.

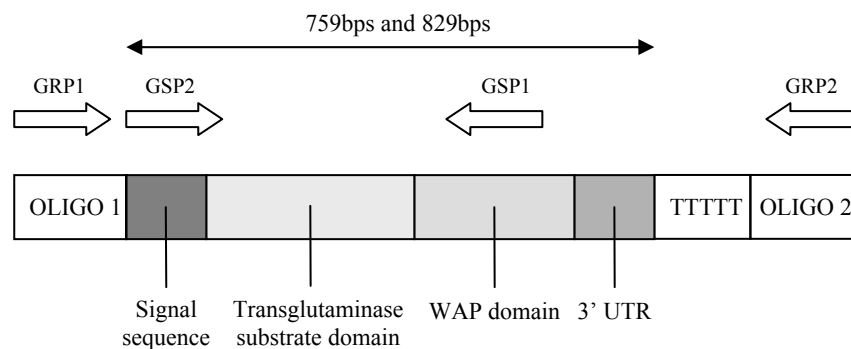


Fig. 1. Showing the structure of the elafin cDNA together with the priming sites used in the RACE procedure. TTTTTT represents the poly T tail after reverse transcription; Oligo 1 and Oligo 2 represent the 2 oligos ligated to the original mRNA as part of the RACE procedure.

VENTRICULOCORDECTOMY REDUCES RESPIRATORY NOISE IN HORSES WITH LARYNGEAL HEMIPLEGIA

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While sport horses are commonly treated with ventriculocordecotomy (VC) to eliminate respiratory noise caused by laryngeal hemiplegia, there is no data to support this treatment. We hypothesized that bilateral VC would reduce the respiratory noise caused by laryngeal hemiplegia.

Materials and Methods: Six Standardbred horses (mean age 6.5 years) were used in this study. Respiratory sounds and inspiratory transupper airway pressure ( $P_{ui}$ ) were measured in all horses before and after induction of left laryngeal hemiplegia (LLH) by left recurrent laryngeal neurectomy, and 30, 90, and 120 days after VC. Respiratory sounds were recorded using a unidirectional microphone and audiocassette recorder while horses were exercising on a treadmill at a speed corresponding to maximum heart rate. In horses with LLH, spectrogram analysis revealed three inspiratory sound formants centered at approximately 400, 1700, and 3700 Hz ( $F_1$ ,  $F_2$ ,  $F_3$ , respectively). The difference between inspiratory and expiratory sound levels (SL (dB)) and the sound intensity of the three inspiratory formants ( $F_1$ ,  $F_2$ ,  $F_3$  (dB)) were measured using a computer-based spectrogram program. All data were analyzed using a 1-way ANOVA and pairwise comparison was made using the Student-Neuman-Keuls test ( $p < 0.05$ ).

Results: In LLH horses,  $P_{ui}$ , SL and the intensity of  $F_2$  and  $F_3$  were significantly increased compared to baseline values. Formant 1 was unchanged. Thirty days following VC,  $P_{UI}$  was significantly decreased, but indices of sound intensity did not change significantly. At 90 and 120 days post-VC,  $P_{UI}$  and SL were significantly decreased compared to LLH values, but remained significantly different from baseline values. The sound intensity of  $F_2$  and  $F_3$  were not significantly different from baseline or LLH values at 90 and 120 days post-VC.

Conclusion: Laryngeal hemiplegia caused inspiratory airway obstruction and a characteristic inspiratory noise. Bilateral ventriculocordecotomy is effective in reducing inspiratory noise in horses with LLH by 90 days after surgery.

	BASELINE	LLH	30 DAYS VC	90 DAYS VC	120 DAYS VC
$P_{ui}$ (cm H <sub>2</sub> O)	31.4 ± 1.75	68.0 ± 6.79*	57.4 ± 6.65*!	46.0 ± 5.21*!	48.0 ± 4.58*!
SL (dB)	-16.91 ± 1.20	-4.98 ± 1.68*	-6.77 ± 2.49*	-11.13 ± 2.50*!	-12.20 ± 3.24*!
F1 (dB)	-52.4 ± 1.89	-47.6 ± 1.67	-44.8 ± 1.49	-47.5 ± 2.21	-48.8 ± 3.31
F2 (dB)	-58.6 ± 2.06	-43.9 ± 3.49*	-46.0 ± 4.04*	-52.5 ± 2.30	-50.9 ± 4.30
F3 (dB)	-61.4 ± 2.06	-50.4 ± 2.64*	-50.5 ± 2.49*	-55.0 ± 1.31	-53.8 ± 3.96

\* Indicates significant difference from baseline values. ! Indicates significant difference from LLH values.

## RESPIRATORY AIR-INTERFACE ORGAN CULTURE SYSTEM

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**Purpose.** Organ cultures of human and rodent respiratory tissues maintained at an air-interface have proved to be more physiological models of infection than submerged organ culture systems for a variety of pathogens. The purpose of this project was to determine whether air-interface culture techniques could be successfully adapted for equine respiratory tissues.

**Methods.** 5mm x 5mm pieces of epithelium were carefully dissected from the nasal turbinates and trachea of seven horses. After washing in cell culture medium supplemented with antibiotics and antifungals, followed by washing in antibiotic-free medium, the epithelial pieces were set up as individual air-interface organ cultures by mounting each piece on a sponge platform in a 5cm petri dish containing antibiotic-free medium and maintained for up to 10 days at 37°C in a humidified incubator containing 5% CO<sub>2</sub>. The viability of the organ cultures was assessed daily by measuring the clearance times of 1mm diameter polystyrene microspheres. At 24 hr intervals cultures were harvested for light microscopy, scanning electron microscopy, bacteriology and cytokine (IL-1, IL-8, TNF $\alpha$ ) mRNA measurement by RT-PCR.

**Results.** Cultures remained viable, assessed by microsphere clearance, up to 10 days in culture. Bacterial contamination was only occasionally encountered. On light microscopy and scanning electron microscopy, the morphology of both nasal and tracheal epithelium did not deteriorate throughout the experiment. IL-1, IL-8 and TNF $\alpha$  mRNA was detectable in all cultures throughout the experiment.

**Conclusions.** Equine respiratory tissues can be successfully maintained in a near-physiological state as air-interface organ cultures. These cultures are likely to provide a more relevant infection model for respiratory pathogens than submerged culture systems.

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## THE EFFECT OF VARIOUS STORAGE CONDITIONS ON THE STABILITY OF GASEOUS NITRIC OXIDE

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**Aim:** To establish whether different storage conditions affect the nitric oxide (NO) concentration measured in gaseous samples contained within sealed bags.

**Methods:** Nitric oxide calibration gas (58ppb in N<sub>2</sub>) and equine exhaled breath were used as a gas source. Variations in storage conditions were made to determine their effect on NO concentration ([NO]) within sealed bags over time. These included (a) storage temperature, (b) storage bag material, (c) gas volume within the storage bag and (d) sterility of the storage bag. [NO] was measured using a chemiluminescence analyser (Seivers NOA 270B) and ten repetitions were performed for each individual alteration in storage conditions.

**Results: *Storage Temperature:*** Storage of 58ppb NO within a PVC Douglas bag at 25°C and 40°C resulted in a time dependant increase in [NO] within the bag. Storage at 25°C resulted in a significant (P<0.01) increase in [NO] from 4h onwards and storage at 40°C resulted in a significant (P<0.01) increase in [NO] from 2h onwards. There was no significant increase in [NO] over 24h when stored at 4°C. At each measured time point (with the exception of baseline) there was a significant (P<0.01) difference between each storage temperature with respect to [NO].

***Storage Bag Material:*** When stored at 25°C [NO] within both PVC Douglas bags and rubber anaesthetic re-breathing bags increase over 24h. The [NO] within the rubber bag was significantly different from baseline from 2h onwards, and at each measured time point from 2h onwards, the [NO] was significantly greater in the rubber bag than in the Douglas bag.

***Initial Gas Volume:*** A time dependant increase in [NO] was noted when 2 initial gas volumes (850ml and 1700ml) were analysed over 24h. The rate of increase in [NO] was greater in the bags containing the lower volume, with a significant (P<0.01) difference between the 2 gas volumes at 8h, with respect to [NO].

***Sterility of Storage Bag:*** Autoclaving of the rubber bag prior to filling with a standard volume of NO failed to prevent a significant increase in [NO] at 6h, however the [NO] at this time point was significantly (P<0.01) lower than in the non-autoclaved bag.

In addition to the above effects of various storage conditions, the increase in [NO] over time is significantly greater from 4h onwards when equine exhale breath was used as a gas source compared with NO calibration gas.

**Conclusions:** The increase in [NO] over time is temperature and volume-dependent. In addition, it is dependent upon the material used for gas storage. The reason for the increase in [NO] over time is unclear, however it may at least be partly due to microbial contamination of the storage bag. In situations whereby gas samples are collected for future [NO] measurement, careful consideration should be given to the storage conditions. Sterile bags should be used for collection, large volume of gas should be collected and bags should be stored at 4°C.

LOCAL LUNG RESPONSES FOLLOWING LOCAL LUNG CHALLENGE WITH  
RECOMBINANT LUNGWORM ANTIGEN IN SYSTEMICALLY SENSITISED SHEEP

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Background: Chronic mast cell-mediated inflammation may contribute significantly towards the extensive tissue remodelling that is a feature of lungworm infection in ruminants. Understanding the factors that control tissue remodelling is a necessary step toward effective management and treatment of conditions that feature such pathology.

Objective: We sought to define in a novel ovine model system, the cellular, immune and mast cell phenotypic events that occur following local lung challenge with a recombinant protein antigen, DvA-1, derived from the ruminant lungworm nematode, *Dictyocaulus viviparus*.

Methods: Two spatially disparate lung segments in systemically sensitised sheep were challenged on three occasions with DvA-1 (3xDVA) and two further segments were challenged with saline (3xSAL). Two months after the third challenge, one of the two segments previously repeatedly challenged with DvA-1 was challenged again with DvA-1 (3xDVA:DVA) whilst the other was challenged with saline (3xDVA:SAL). A similar protocol was followed with the saline challenged segments (3xSAL:SAL & 3xSAL:DVA). Bronchoalveolar lavage fluid (BALF)(n=16) and tissue (n=3) were collected after the last challenge.

Results: Cellular changes 24 h after the fourth challenge were characterised by an increase in the absolute numbers of neutrophils and eosinophils in BALF from 3xDVA:DVA and 3xSAL:DVA segments. Local antibody production was implied through increased levels of antibody in both 3xDVA:DVA and 3xDVA:SAL segments, with the latter being unaffected by inflammation. Levels of active transforming growth factor beta-1 (TGF- $\beta$ 1) were significantly increased in 3xDVA:SAL segments and a trend towards an increase was apparent in 3xDVA:DVA segments. Total TGF- $\beta$ 1 levels were significantly correlated with eosinophil counts in all except the 3xDVA:SAL segments. Such changes in the bronchoalveolar space were complemented by increased ratios of sheep mast cell proteinase-1 expressing cells and tryptase expressing cells, to toluidine blue positive cells in airways from 3xDVA:DVA segments.

Conclusion: Mast cell phenotypic events occurring as a consequence of antigen challenge were limited to segments in which changes in BALF were characterised by neutrophil influx *and* increased local antibody production.

## EFFECT OF INHALED FLUTICASONE, ORAL PREDNISONE, AND PASTURE ENVIRONMENT ON LUNG FUNCTION OF HORSES WITH HEAVES

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The main goal of therapy for horses with heaves is to limit exposure to organic dusts and reduce airway inflammation. This is typically achieved by placing horses on pasture or administering glucocorticoids (GC). This study compared the impact of environmental changes and GC therapy on the lung function of horses with heaves.

Clinical score (CS) and lung function were measured at baseline in 28 horses with heaves. Horses were put into three groups (mild, moderate, or severe degree of airway obstruction) based on mean end-expiratory flow data. Horses were then kept on pasture and treated with inhaled fluticasone propionate (FP), inhaled placebo, or oral prednisone (P). Drugs were administered twice a day (FP = 2 mg; P = 400 mg) for 2 weeks followed by a gradual decrease in dosage over 2 weeks. Clinical scoring and lung function testing were repeated at week 2 and 4.

At baseline, horses with severe airway obstruction had a higher CS ( $13.8 \pm 6.4$ ,  $n=12$ ) than horses with mild obstruction ( $6.3 \pm 5.1$ ,  $n=9$ ,  $P<0.02$ ), but not different than horses with moderate obstruction ( $12.0 \pm 8.0$ ,  $n=7$ ). The CS of horses with severe and moderate airway obstruction decreased significantly 2 and 4 weeks after environmental change, regardless of treatment. Initially, forced expiratory volume at 1.5 s ( $FEV_{1.5}$ ) was lower in horses with severe obstruction ( $24.4 \pm 6.3$  l/s) than in horses with moderate ( $35.1 \pm 3.8$  l/s) and mild ( $40.0 \pm 3.9$  l/s) obstruction ( $P<0.001$ ).  $FEV_{1.5}$  improved only in the severely affected horses during the first 2 weeks of therapy regardless of treatment, and no further benefit was observed at 4 weeks. Maximum change in pleural pressure decreased significantly within 2 weeks of therapy with all 3 treatments. Pulmonary resistance decreased significantly in severely obstructed horses treated with FP as compared to P or placebo.

Clinical score and lung function of horses with severe airway obstruction improved significantly in response to pasture turn out, however treatment with FP or P provided little additional benefit. Horses with mild or moderate airway obstruction experienced marginal changes in clinical score and lung function.

A LONG-TERM SURVEY OF LARYNGOPLASTY IN AN OLDER, MIXED-BREED POPULATION OF 200 HORSES. 1: MAINTENANCE OF SURGICAL ARYTENOID ABDUCTION AND COMPLICATIONS OF SURGERY.

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Following laryngoplasty (LP) procedures (1986-1998) in 200 horses of mixed breed and workload, median age 6-years, the degree of surgical arytenoid abduction achieved was evaluated using a 5-grade system, at 1 day, 7 days and 6 weeks after LP. On the day following LP, most horses had good (median grade-2 overall) arytenoid abduction, with 10% having excessive (grade-1) and 5% having marginal (grade 4) abduction. Due to progressive loss of abduction, moderate (median grade 3, range 1-5) abduction was present overall at 1 and 6 weeks following LP. Further surgery was required to re-tighten prostheses in 10% of cases where excessive loss of abduction occurred, or to loosen prostheses in 7% of horses with significant post-operative dysphagia.

Questionnaires were completed by 198 owners a median of 19 months following surgery. LP wound problems (mainly seromas and suture abscesses) were reported to last <2 weeks in 9% of cases, <4 weeks in 4% >4 weeks in 4%. The partially sutured laryngotomy wounds discharged post-operatively for <2 weeks in 22% of cases, < 4 weeks in 7% and for > 4 weeks in 2%. Coughing occurred at some stage post-operatively in 43% of cases and its presence correlated significantly with the degree of arytenoid abduction. This coughing occurred during eating in 24% of cases and was not associated with eating (or dysphagia) in the other 19% of cases. Chronic (>6 months) coughing remained in 14% of cases, but appeared to be due to intercurrent pulmonary disease in half of these horses.

A STUDY OF AN ELECTRODIAGNOSTIC TECHNIQUE FOR THE EVALUATION OF  
EQUINE RECURRENT LARYNGEAL NEUROPATHY

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The electrodiagnostic measurement of the thoraco-laryngeal reflex (TLR) (“slap test”) latency has been proposed as an accurate and sensitive technique for detection of equine recurrent laryngeal neuropathy (RLN), even in subclinical cases. This technique was compared to five other diagnostic techniques used for evaluation of equine laryngeal function, namely laryngeal muscle palpation, resting and immediately post-exercise endoscopic examinations, and palpable and endoscopic responses to the TLR.

Compared to resting endoscopy, the electrodiagnostic measurement of TLR latency was not found to be an accurate test for the evaluation of recurrent laryngeal neuropathy (RLN), nor was laryngeal muscle palpation or the endoscopic response to the TLR. Twenty five (71%) of 35 Clydesdale horses examined were affected by RLN; 16 (46%) had mild, 5 (14%) had moderate, 3 (9%) had severe hemiparesis and 1 (3%) had total laryngeal paralysis. Within these 35 horses, physical traits such as height or neck length did not correlate with the incidence or severity of RLN. Ten control ponies showed no evidence of abnormal laryngeal function.

LARYNGEAL PARALYSIS: A STUDY OF 375 CASES IN A MIXED-BREED POPULATION  
OF HORSES.

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375 referred cases of laryngeal paralysis (1985-1998) from a mixed-breed equine population, included 351 (94%) cases of recurrent laryngeal neuropathy (RLN) (idiopathic laryngeal hemiplegia) and 24 cases (6%) of laryngeal paralysis from causes other than RLN. Laryngeal movements were endoscopically classified into one of 6 grades, in contrast to the usual 4 grades. The RLN cases had a median grade 4 laryngeal paralysis, which were 96% left-sided, 2% right-sided and 2% bilaterally affected. RLN cases included 204 (58%) Thoroughbred, 96 (27%) Thoroughbred cross, 23 (7%) draught, 16 (5%) Warmbloods and 10 (3%) other breeds, including only 4 (1%) ponies. The median age of RLN cases at referral was 6 years old (range 2-12) and their median height was 16.3 hands (170.1cm).

The work of RLN horses included National Hunt racing 42%, flat racing 1%, hunting 19%, eventing 16% and miscellaneous work 22%. Reported presenting signs in RLN affected horses included abnormal exercise-related respiratory sounds in 90% and reduced exercise tolerance in only 64%. However, many horses were referred before their exercise tolerance could be fully assessed. 40% of the RLN cases had intercurrent disorders, including 10% with additional upper respiratory and 7% with lower respiratory tract diseases.

The 24 non-idiopathic RLN cases of laryngeal paralysis included 12 with bilateral laryngeal paralysis, 11 (92%) of which were ponies. Bilateral laryngeal paralysis occurred with hepatic encephalopathy in 7 cases and following general anaesthesia in two cases. The 12 cases of acquired unilateral laryngeal paralysis included 7 caused by guttural pouch mycosis.

CLINICAL AND ENDOSCOPIC EVIDENCE OF PROGRESSION IN 52 CASES OF EQUINE  
RECURRENT LARYNGEAL NEUROPATHY (RLN).

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The literature indicates that of recurrent laryngeal neuropathy (RLN) is a non-progressive disease primarily affecting younger horses. However, 52 out of 351 cases of RLN (15%) in a mixed breed, older population of horses were found to have evidence of progression of the degree of laryngeal dysfunction over a median period of 12 months (range 1.5-48 months) with the onset of progression occurring at a median of 7 years of age. In 30 cases, there was both endoscopic (median deterioration of 3 endoscopic grades; range 1-5 grades) and clinical evidence - with 29 (97%) of these horses concurrently developing sudden-onset, abnormal exercise-related respiratory noises and 13 (43%) concurrently reporting reduced exercise performance.

In the remaining 22 horses there was solely clinical evidence of RLN progression, including the sudden onset of abnormal exercise-related respiratory sounds in 16 (73%) and the worsening of such sounds in 6 (23%), associated with reduced exercise performance in 13 (59%) of these 22 cases. Endoscopically, 13 (59%) of the latter 22 cases had marked (total or almost total) RLN that did not appear compatible with their previous exercise-performance histories. This evidence of progression of RLN may be of particular significance in disputes concerning horses that are apparently normal at pre-purchase examination but are later shown to have RLN and also in the surgical treatment of less severe cases of RLN.

A LONG-TERM SURVEY OF LARYNGOPLASTY IN AN OLDER, MIXED-BREED POPULATION OF 200 HORSES. 2: OWNERS ASSESSMENT OF THE VALUE OF SURGERY.

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Following laryngoplasty (LP) procedures (1986-1998) in 200 horses of mixed breed and workload, median age-6 years, the degree of laryngoplasty abduction achieved was evaluated using a 5-grade system. A survey of owners, a median of 19 months after surgery showed that 91% of cases had returned to full work and 3% to reduced work. These included 95% of horses with good (grade-2) laryngoplasty abduction at 6 weeks after surgery, 91% with moderate (grade 3) abduction, 88% of cases with minimal (grade-4) abduction and 25% of cases with total loss of surgical abduction (grade-5).

Once back in work, 73% of cases made no abnormal exercise-related “noises” at exercise, with reduced “noises” reported in some of the 21% of horses that still made these “noises”. In 6% of cases, owners were unsure if “noises” were now present. The absence or presence of reported “noises” once horses were back at work correlated significantly with the degree of surgical arytenoid abduction present at 6 weeks after laryngoplasty.

A marked increase in exercise performance following surgery was reported in 64%, a moderate increase in 7% and slight increase in 4% of cases, with 10% showing no difference in exercise performance, 3% showing worse performance and owners unsure of any effect on exercise performance in the remainder. Overall, 86% of owners believed LP was worthwhile, 7% believed it was not worthwhile and 6% were unsure of its value. Surgery was reported to be of most benefit to sports horses (e.g. worthwhile for 100% of showjumpers) and of least benefit to National Hunt (long distance) racehorses where 71% of owners believed it to be worthwhile.

EFFECT OF INHALED NITRIC OXIDE ON PULMONARY ARTERY PRESSURE AND EIPH  
IN MAXIMALLY EXERCISING HORSES

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The exercise-induced elevation of pulmonary artery pressure (Ppa) is thought to play a deterministic role in exercise-induced pulmonary hemorrhage (EIPH) and thus treatment designed to lower Ppa should reduce EIPH accordingly. Five Thoroughbred horses were run on a treadmill to volitional fatigue (incremental step test) under NO (80 ppm) and N<sub>2</sub> (same flow rate as per NO run) conditions (2 weeks between trials; order randomized) to test the hypothesis that NO inhalation would reduce maximal Ppa and cause a concomitant reduction in EIPH. Prior to each investigation, a microtipped pressure transducer was placed in the pulmonary artery 8 cm past the pulmonic valve. EIPH severity was assessed via bronchoalveolar lavage (BAL) 30 min post-run. Exercise time did not differ between the 2 trials ( $p>0.05$ ). NO administration resulted in a small but significant reduction in peak Ppa (N<sub>2</sub>,  $102.3\pm 4.4$ ; NO,  $98.6\pm 4.3$  mmHg,  $p<0.05$ ). In the face of lowered Ppa, EIPH severity was significantly higher in the NO trial (N<sub>2</sub>,  $22.4\pm 6.8$ ; NO,  $42.6\pm 15.4 \times 10^6$  RBC/ml BAL fluid,  $p<0.05$ ). These findings support the notion that elevated pulmonary pressures may reflect, in part, arteriolar vasoconstriction that serves to protect the capillary bed from the extraordinarily high Ppa's evoked during maximal exercise. Furthermore, these data suggest that exogenous NO treatment during exercise in horses may not only be poor prophylaxis but may actually exacerbate the severity of EIPH.

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## EVALUATION OF A NEW SPACER DEVICE FOR DELIVERY OF DRUGS INTO THE EQUINE RESPIRATORY TRACT

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Inflammatory disease is common in the equine respiratory tract. Traditional approaches to treatment have involved bronchodilators, mucolytics and oral corticosteroids. Corticosteroids can have unwanted systemic side effects, particularly immuno-suppression. Delivery of drugs directly into the affected airways may improve the local drug concentrations within the lung combined with reduced systemic uptake. Inhaled corticosteroids are widely used in the treatment of human inflammatory lung conditions, including asthma and chronic obstructive pulmonary disease. Equine recurrent airway obstruction (RAO) is characterised by a marked inflammatory response in the presence of inciting allergens, such as moulds. Nebulisation of liquid corticosteroid preparations has been used, but a number of spacer devices have been developed to allow the use of pharmaceuticals packaged for the human medical market in metered dose inhalers (MDI). In order to study the efficacy of a new device for delivering medications in MDI's to the equine respiratory tract (Equihaler<sup>TM</sup>), 3 healthy horses and 3 horses affected by RAO will be studied. Each horse will be given fluticasone propionate labelled with Tc<sup>99m</sup>. The particle size distribution of both unlabelled and low and high dose Tc<sup>99m</sup> labelled fluticasone propionate is currently being determined. The distribution of high dose labelled Tc<sup>99m</sup> labelled fluticasone propionate in the spacer, face, upper airway, trachea and lung will be determined using scintigraphy.

TISSUE GLUTATHIONE CONTENT AND REDOX RATIO IN DIFFERENT REGIONS OF THE EQUINE LUNG

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Reduced glutathione (GSH) is an important anti-oxidant in biological tissues offering protection from free-radicals and reactive oxygen species produced during oxidative stress. Whilst there are a number of reports of the concentrations of glutathione in equine lung lining fluid, we are not aware of any reports of tissue concentrations. Samples of lung tissue were collected from horses slaughtered by free bullet. None of the animals had overt respiratory disease as determined by a resting clinical examination or by visual examination of the lungs *post mortem*. The animals were hung and exsanguinated and the lungs were removed approximately 10-15 min after death. Sections of lung 1 cm cubed were taken from the right and left apical, ventral and dorso-caudal lung regions. The samples were washed in ice-cold 0.9% saline to remove blood, blotted and frozen in liquid nitrogen. Samples were weighed frozen and freeze-dried at  $-50^{\circ}\text{C}$  without thawing. The dry samples were crushed to a fine powder, de-proteinised with 5% v/v perchloric acid, homogenised for 1 min, centrifuged at 5000g for 10 min, the supernatant neutralised with 10M KOH, centrifuged at 5000g for 2 min. The neutralised samples were diluted in mobile phase (10 mmol/l  $\text{NaH}_2\text{PO}_4$ , 5% v/v methanol, pH 2.7) and the content of GSH and GSSG (oxidised glutathione) determined by HPLC. Results are expressed as  $\mu\text{mol/g}$  of dry tissue (mean $\pm$ SD). (TGSH = GSH+GSSG; GRR = GSSG/TGSH).

	Age (years)	GSH ( $\mu\text{mol/g}$ )	GSSG ( $\mu\text{mol/g}$ )	TGSH ( $\mu\text{mol/g}$ )	<b>GRR (%)</b>
TB (n=10)	9 $\pm$ 3	3.80 $\pm$ 0.7	0.53 $\pm$ 0.15	4.33 $\pm$ 0.90	12.1 $\pm$ 1.8
Pony (n=7)	18 $\pm$ 6	3.04 $\pm$ 0.5	0.28 $\pm$ 0.08	3.32 $\pm$ 0.54	8.6 $\pm$ 2.9
TB <i>versus</i> Pony	P=0.006	P=0.035	P=0.0007	P=0.0141	P=0.01
	8	0			88

There were no significant differences in GSH, GSSG, TGSH and GRR between different lung areas in either TB or Pony (ANOVA,  $P>0.05$ ). Lung tissue GSH, GSSG, TGSH and GRR were all significantly higher in TB compared with Pony. Differences between breeds may be related to a number of factors, including diet, exercise status, environment (e.g. stable *versus* field) and age.

## IgE-PROTEIN AND IgE-mRNA POSITIVE CELLS IN LUNG TISSUE SAMPLES FROM RAO-AFFECTED AND CONTROL HORSES AFTER MOULDY HAY CHALLENGE

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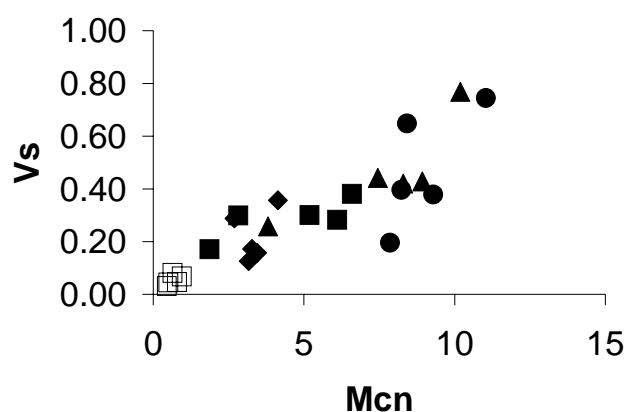
Although it is accepted that moulds in hay and straw trigger a hypersensitivity reaction in Recurrent Airway Obstruction (RAO) it is not clear to which extent IgE-mediated reactions are involved. The aim of the present investigation was to see whether mouldy hay challenge leads to higher numbers of IgE+ cells in lung tissue from RAO-affected horses compared to controls. In a first study, 5 RAO-affected and 5 control horses which had been at pasture and were free of clinical signs were exposed to mouldy hay; RAO-affected horses were slaughtered after onset of symptoms of heaves (1-5 days after stabling; controls slaughtered 1-10 days after stabling). Tissue samples were taken from the periphery of the lung, fixed in 4% paraformaldehyde and embedded in paraffin. Immunohistochemistry was performed following standard protocols and IgE-protein+ cells were detected with a chicken anti-horse IgE antibody, followed by an AP-labeled goat anti-chicken antibody. In situ hybridisation was performed as described in van der Haegen et al. (2001, Equine Vet. J., in press). Positive cells were counted in and around the bronchi, bronchioli and blood vessels and expressed as cells/mm<sup>2</sup>. RAO-affected horses had significantly more IgE-protein+ cells than controls in the bronchioles (median= 158 and 118 cells/mm<sup>2</sup>, respectively) and blood vessels (154 and 100 cells/mm<sup>2</sup>, respectively). These significant differences were mainly due to two RAO-affected horses which had clearly more IgE-protein+ cells than the other RAO-affected and control horses. IgE-mRNA+ cells could be demonstrated but no significant differences were found between the two groups of horses. This study suggests that various immunological reactions are probably involved in RAO and that IgE-mediated reactions are possibly only involved in some cases or in some stages of the disease.

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## MUCUS RESPONSE OF RATS TO INTRANASAL INSTILLATION OF HAY DUST

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The aim of this research was to develop a rat model of hay-dust induced mucus secretion that offers a more economic and practical platform to investigate some of the mechanisms of heaves. Five groups of 5 Fischer 344 rats were intranasally instilled with hay dust suspension. Rats received 400 $\mu$ l of PBS suspension containing 0.0, 0.01, 0.1, 1.0 and 5.0 mg of dust. Hay dust submitted in a 0.025 mg per ml PBS sample contained 412 endotoxin units per ml. Three days post-instillation, cross sections were taken from the fifth (G5) and eleventh (G11) airway generations within the lung and at the level of the incisive papilla in the nasal airway. At all sites, there was a concentration-dependent increase in the amount of stored mucus (Vs) quantified using morphometry. For example, at G5, Vs averaged 0.054, 0.220, 0.287, 0.464 and 0.473 (+/- 0.042) nl/mm<sup>2</sup> basal lamina, for 0.00, 0.01, 0.1, 1.0 and 5.0mg hay dust respectively. Average mucus secreting cell numbers (Mcn) were 0.05, 3.34, 4.51, 7.73 and 8.97 (+/- 0.458) per mm basal lamina respectively. In both bronchial cross sections, the increase Vs and Mcn were directly correlated ( $r = 0.873$  [G5],  $p < 0.01$ ). See fig. At necropsy, total and differential cell counts were taken in broncho-alveolar lavage. Percent neutrophils increased as hay dust dosage increased. Rats offer a good model of hay-dust induced mucus cell metaplasia. A dose of 1.0 mg causes an increase in the amount of stored mucus and the number of mucus secreting cells within the respiratory epithelium of two different levels of the peripheral airways. Future studies will examine the role of endotoxin in the response.



## EFFICACY OF MOMETASONE FUROATE IN THE TREATMENT OF HEAVES IN HORSES.

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Heaves in horses is characterized by hyperresponsiveness and inflammation of the small airways in response to antigenic exposure, causing impairment of pulmonary function. The objective of this study was to evaluate the therapeutic efficacy of a novel inhaled corticosteroid, mometasone furoate, in the treatment of heaves. This study was conducted on 6 mature horses using a double blinded, placebo-controlled, crossover design. Exacerbation of heaves was accomplished by exposure to moldy hay twice daily in an environmental chamber until transpulmonary pressure (P<sub>ppl</sub>) exceeded 20 cm H<sub>2</sub>O. Clinical score, pulmonary function, airway hyperreactivity, and bronchoalveolar lavage fluid (BALF) analysis of cell differential count were evaluated during remission, acute exacerbation of heaves, and after 21 days of treatment with either mometasone furoate (1.6 mg, BID) or placebo administered via Aeromask®. Following treatment, clinical and endoscopic scores for mometasone-treated horses decreased significantly from exacerbation values compared to the placebo group, but did not return to baseline values. As well, significant improvements were observed in the mometasone-treated group compared to the placebo group for P<sub>ppl</sub> (p=0.0196) and dynamic compliance (p=0.0090), but not for lung resistance (p=0.0578). Although treatment had no statistically significant effect on the percentage of BALF neutrophils, mometasone-treated horses showed a 22.1% decrease, whereas a 2.5% increase was seen in placebo-treated horses. This study demonstrates that mometasone furoate caused a functional improvement on the pulmonary system of horses in a state of heaves exacerbation, clearly documented by the quantitative parameters of the lung mechanics test and reduction of the clinical signs, and may have some clinical application for the treatment of heaves.

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CAN ATROPINE FACILITATE THE DETECTION OF BRONCHOCONSTRICTION DURING LOBELINE STIMULATION IN MILD TO MODERATE RAO AFFECTED HORSES?

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Atropine is a potent short acting bronchodilator. The purpose of the present study was to determine whether atropine facilitates the detection of reversible bronchoconstriction by non-invasive spirometry during tidal breathing and chemically stimulated breathing in clinical RAO cases. Two groups of horses were used. Group 1 consisted of five healthy warmblood horses with no history of pulmonary disease (8.8 (1.9) [mean (SE)] years; 524 (18) kg bwt.). Group 2 consisted of 9 mild to moderately RAO affected horses (16 (1.6) years; 526 (15) kg bw). Before bronchoalveolar lavage the animals underwent spirometry during tidal breathing and during lobeline induced hyperventilation (0.25 mg/kg BW Lobelin<sup>®</sup> IV Bolus). Ten minutes after the first lobeline dose atropine sulfate was given IV (0.02 mg/kg BW). Fifteen minutes later a second lobeline challenge was carried out. Spirometry was performed with an ultrasonic flowmeter system (Spiroson scientific<sup>®</sup>).

In group 1 atropine had no effect on respiratory variables. In group 2 responses were variable between horses with no overall statistically significant change. Most horses maintained respiratory frequency ( $f_R$ ), tidal volume ( $V_T$ ) and minute ventilation ( $V_E$ ) during forced ventilation. However, individual animals responded with a large increase in  $V_E$  and  $V_T$  after atropine.

	RAO - before atropine (median [range])	RAO after atropine (median [range])
$f_R$	39 [35 – 45]	41 [36 – 47]
$V_T$ (l)	20.1 [16.5 – 34.2]	20.4 [15 – 31.7]
$V_E$ (l/minute)	830.7 [610.5 – 1197]	850.7 [617 – 1178]
PIF (l/s)	36.0 [27.9 – 42.5]	35.0 [29.2 – 44.6]
PEF (l/s)	37.7 [31.0 – 67.5]	36.3 [29.1 – 56.6]

Atropine may be useful to assess the involvement of immediately reversible bronchoconstriction in more severe cases of RAO.

## COMPARISON OF IMPULSE OSCILLOMETRY WITH THE CONVENTIONAL TECHNIQUE FOR BRONCHODILATION EVALUATION IN HORSES

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Impulse Oscillometry (IOS) is a non-invasive pulmonary function test based on forced oscillations, which measures resistance ( $R_{rs}$ ) and reactance ( $X_{rs}$ ) of the respiratory system from 5 to 35 Hz. The IOS is a quick and comfortable technique which has shown to be reliable, repeatable and sensitive for the monitoring respiratory function in healthy and heaves-affected horses. We wished to apply the IOS in clinical routine to objectively monitor equine patients' response to treatment. The aim of this study was to determine if the IOS could reliably assess bronchodilator therapy in heaves-affected horses. Six heavy horses in clinical exacerbation, unfamiliarised with the IOS technique, were selected for the study. Respiratory mechanics were evaluated before (baseline) and 30 and 60 minutes after an IV injection of 0.8  $\mu\text{g}/\text{kg}$  bwt of clenbuterol hydrochloride (Ventipulmin®). Spectral values of  $R_{rs}$  and  $X_{rs}$  were compared to total lung resistance ( $R_L$ ) and dynamic compliance ( $C_{dyn}$ ) as measured by the conventional reference technique (CRT) based on the oesophageal balloon method. Maximal bronchodilation was observed 30 minutes post-injection.  $R_{5\text{Hz}}$  and  $R_L$  significantly decreased,  $X_{rs}$  from 5 to 15 Hz and  $C_{dyn}$  significantly increased (Wilcoxon signed rank test, p values < 0,05) and frequency dependence of both  $R_{rs}$  and  $X_{rs}$  was altered. Sensitivity of measured variables to bronchodilation ranked as follows:  $X_{5\text{Hz}} > X_{10\text{Hz}} > R_L > C_{dyn} > R_{5\text{Hz}}$ .

The IOS reliably reflected short-term variations in respiratory function. The IOS variable  $X_{5\text{Hz}}$  proved to be significantly more sensitive to changes in bronchial motor tone than all other pulmonary function indices including those measured with the CRT. Thus the IOS is a satisfactory non-invasive alternative pulmonary function test to the CRT that does not depend on active patient co-operation or sedation and therefore is well suited for routine monitoring in diseased horses.

## ADAPTATION TO MULTIDAY OZONE EXPOSURE IS ASSOCIATED WITH A SUSTAINED INCREASE OF BRONCHOALVEOLAR URIC ACID

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The phenomenon of ozone tolerance is described, but the underlying mechanisms remain unknown. We tested whether adaptation to multiday ozone exposure was related to an upregulated pulmonary antioxidant defence. Six calves were exposed to 0.75 ppm ozone, 12hr/day for seven consecutive days. Pulmonary function tests including ventilatory mechanics (dynamic compliance, total pulmonary resistance), arterial blood gas analysis (partial arterial oxygen pressure [PaO<sub>2</sub>]) and plasma lactate and bronchoalveolar lavage (BAL) were performed before, after the first (D<sub>1</sub>), third (D<sub>3</sub>) and seventh (D<sub>7</sub>) exposure. Differential cell count, total proteins, 8-epi-PGF<sub>2α</sub>, glutathione and uric acid were determined in BAL. Dynamic lung compliance and PaO<sub>2</sub> were significantly decreased and lung oedema impaired pulmonary function on D<sub>1</sub>. By repeating ozone exposures, progressive functional adaptation occurred. Ozone induced a significant increase of BAL neutrophil percentage on D<sub>1</sub>. On D<sub>3</sub> and D<sub>7</sub>, neutrophil percentage was progressively decreased, but remained significantly elevated. BAL total proteins were significantly increased on D<sub>1</sub> and decreased progressively until D<sub>7</sub>. 8-Epi-PGF<sub>2α</sub> was significantly increased on D<sub>1</sub> and was returned to baseline on D<sub>3</sub> and D<sub>7</sub>, whilst glutathione significantly increased on D<sub>3</sub> and returned to baseline on D<sub>7</sub>. Uric acid was increased ten fold on D<sub>1</sub>. On D<sub>3</sub>, uric acid was increased six fold and was persistently elevated at D<sub>7</sub>. Our study suggests that ozone adaptation of functional and inflammatory variables is accompanied with sustained BAL uric acid elevation.

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OZONE-INDUCED PULMONARY DYSFUNCTION AND AIRWAY INFLAMMATION ARE NOT IMPROVED BY ANTIOXIDANT VITAMIN COCKTAIL IN CALVES

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Ozone (O<sub>3</sub>) is a powerful oxidant gas which causes acute pulmonary oedema, hypoxemia and neutrophilic lung inflammation in calves. We used this model of oxidant-induced airway disease to assess the effect of antioxidant treatment on calves' lung function. Six calves (5 months, 169 ± 22 kg bw) were used. Seven days before O<sub>3</sub> exposure three calves (principals) received once daily for 7 days vitamin C (30 mg·kg<sup>-1</sup> bw, orally), vitamin E (α-tocopherol, 5 mg·kg<sup>-1</sup> bw IM), and vitamin A (300 IU·kg<sup>-1</sup> bw IM). Control calves were not treated. All animals underwent exposure to 0.75 ppm O<sub>3</sub> during 7 consecutive nights (9h 1<sup>st</sup>, 11h 2<sup>nd</sup> and 12h 3<sup>rd</sup>-7<sup>th</sup> exposure). Prior to the 1<sup>st</sup> exposure (D<sub>0</sub>), plasma analyses of vitamin C, E and A and pulmonary function tests (ventilatory mechanics, arterial blood gas analysis) were performed. Cytology and 8-epi-PGF<sub>2α</sub> concentrations in bronchoalveolar lavage (BAL) fluid were assessed. All investigations were repeated after the 1<sup>st</sup> (D<sub>1</sub>) and 7<sup>th</sup> (D<sub>7</sub>) O<sub>3</sub> exposure. Vitamin supplementation and O<sub>3</sub> exposures had no significant effect on plasma vitamin C and vitamin A levels. Vitamin E levels did not vary throughout O<sub>3</sub> exposure but were significantly lower in controls (1.45 ± 0.33 mg/g cholesterol) than in principals (7.44 ± 0.46 mg/g chol).

Table I : Pulmonary function tests and BAL results

Variable	Control calves (n=3)			Principal calves (n=3)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
C <sub>Dyn</sub> (L/kPa)	4.7±0.1	1.0±0.5*	2.8±0.2	3.7±0.6	1.2±0.4*	2.4±0.3
PaO <sub>2</sub> (mmHg)	94.9±9.9	53.4±9.8*	93.0±10.5	92.6±10.1	56.1±7.3*	98.5±5.5
Neutrophil %	7.0±3.6	93.3±3.8 *	25.0±11.3	13.0±7.36	92.0±3.6 *	47.3±26.6
8-epi-PGF <sub>2α</sub> (pg/ml)	12.9±0.2	13.3±2.4	8.5±0.5 *	12.5±1.64	13.7±1.29	11.6± 0.8♦

Results are presented as mean ± SD.\* significantly different from respective Day0-value, ♦ significantly different from respective control value, P < 0.05.

The results suggest that vitamin supplementation of healthy calves does not modulate O<sub>3</sub>-induced lung dysfunction and inflammation.

This abstract is funded by : FRIA and UCB Pharma, BELGIUM.

THE USE OF ECOBED<sup>®</sup> CARDBOARD BEDDING MATERIAL AS PART OF AN ENVIRONMENTAL CONTROL REGIME FOR HEAVES-AFFECTED HORSES : *IN VITRO* ASSESSMENT OF AIRBORNE DUST AND ALLERGEN CONCENTRATION AND *IN VIVO* EFFECTS ON LUNG FUNCTION

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Environmental control of feeding and stabling conditions is a cornerstone in the long-term management of heaves-affected horses. It was tested in the present study whether shredded cardboard (Ecobed<sup>®</sup>), a novel equine bedding material, is an appropriate minimum-dust bedding material for heaves-affected horses. Standardised *in vitro* measurements were used to assess airborne dust and aeroallergen concentrations of cardboard bedding. Comparison was made with common bedding materials, i.e. straw and wood shavings, previously tested by the same technique. Total airborne dust concentration and concentrations of *Aspergillus fumigatus*, *Faenia rectivirgula* and *Thermoactinomyces vulgaris* in the cardboard bedding were significantly lower than those of straw and wood shavings. Six horses in clinical remission after a three months period on pasture were stabled for two months on cardboard bedding and fed grass silage. Pulmonary function tests (PFTs), including measurement of ventilatory mechanics, arterial blood gas analysis, endoscopic airway inflammation scoring and cytological analysis of bronchoalveolar lavage were performed before, during and after the cardboard bedding period. The PFT results were compared to those obtained from the same horses stabled in poor hygienic conditions and to those of healthy control horses. PFT values measured during and after the stabling period on cardboard bedding were not significantly different from those recorded after the period at those of healthy control horses, whilst being significantly different from values recorded during clinical crisis.

On basis of both the *in vitro* and *in vivo* results it can be concluded that cardboard bedding used in conjunction with low-dust forage, i.e. grass silage, is appropriate in the provision of minimum-dust management of heaves-affected horses.

This abstract is funded by : FRIA, Brussels, Belgium.

## FUNCTIONAL DIFFERENTIATION OF OBSTRUCTIVE ABNORMALITIES IN HORSES BY MEANS OF IMPULSE OSCILLOMETRY USING THE LOW FREQUENCY RANGE

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**Aim:** Different multifrequent methods of forced oscillation technique have been used to analyse respiratory mechanics in horses. Whereas reports based on Impulse Oscillometry (IOS) mostly investigated the frequency range above 5 Hz (Van Erck et al. 1999) applications of pseudo random noise technique already showed results below (Young and Tesarowski 1994, Young et al 1997). The aim of this study was to evaluate IOS impedance measurements in the frequency range between 0.2 and 10 Hz during obstruction at different sites of the respiratory system.

**Methods:** Study 1: Twelve horses (age: 9.3 (3.5) years, body weight: 543.6 (93.7) kg) suffering from middle up to higher degree of recurrent airway obstruction (RAO) were measured before and after treatment using a IOS device specially constructed for horses (IOS MasterScreen, Jaeger GmbH, Höchberg, Germany). Study 2: A different group of five horses without any history or clinical signs of lung disease (age: 2.2 (0.83) years, body weight: 394.0 (51.35) kg) were included in this study. IOS measurements were taken at different head positions, e.g. physiological and vertical flexed head position (artificial extrathoracic obstruction). The following parameters were entered into statistical analysis: Resistance (R) and reactance (X) at 0.2, 0.3, 0.5, 0.7, 1, 2, 3, 5, 7, and 10 Hz ( $R_{0.2}$ ,  $R_{0.3}$ , ...  $R_{10}$ ,  $X_{0.2}$ ,  $X_{0.3}$ , ...,  $X_{10}$  respectively). Results of the first study were analysed by means of Wilcoxon's u-test and of the second study by paired t-test ( $p \leq 0.05$ ).

**Results:** Study 1: Resistance values between 0.2 and 5 Hz showed a significant decrease after therapy. Reactance at all frequency were shifted upwards significantly for all frequencies. Study 2: Resistances values at 5, 7, and 10 Hz were significantly higher at flexed compared to physiological head position. Reactance was not affected.

**Conclusions:** There is a specific behaviour of resistance and reactance between 0.2 and 10 Hz which allows the further differentiation of the horses' respiratory system.

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CYCLOPENTENONE PROSTAGLANDINS INHIBIT NF- $\kappa$ B ACTIVITY AND  
INFLAMMATORY GENE EXPRESSION IN LUNG EPITHELIAL CELLS .

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*Introduction* : Persistent activity of nuclear factor- $\kappa$ B (NF- $\kappa$ B) in the airways is a characteristic feature of equine heaves and is associated with overexpression of many inflammatory proteins, including cytokines. Cyclopentenone prostaglandins have been shown to possess anti-inflammatory properties, partly through inhibition of NF- $\kappa$ B. *Aim* : We investigated the effects of two cyclopentenone prostaglandins, prostaglandin A1 (PGA1) and 15-deoxy- $\Delta^{12-14}$ -prostaglandin J2 (15dPGJ2), on NF- $\kappa$ B activity and NF- $\kappa$ B-dependent cytokine production in A549 pulmonary epithelial cells. *Methods* : Cells were treated with incremental concentrations of cyclopentenone prostaglandins two hours before stimulation with TNF $\alpha$ . NF- $\kappa$ B activity was estimated using electrophoretic mobility shift assays and six hours later, supernatants were assessed for IL-6, IL-8 and GM-CSF concentrations using ELISAs. *Results* : A complete inhibition of NF- $\kappa$ B was obtained for PGA1 and 15dPGJ2 concentrations of 96  $\mu$ M and 36  $\mu$ M, respectively. At these doses, ELISAs revealed a drastic decrease in IL-6, IL-8 and GM-CSF production, as compared with the data obtained with cells exclusively treated with TNF $\alpha$ . *Conclusion* : These results suggest that cyclopentenone prostaglandins may have therapeutic applications in equine heaves through inhibition of the inflammatory activity of lung epithelium.

ENHANCED SURVIVAL OF LUNG GRANULOCYTES IN AN ANIMAL MODEL OF  
ASTHMA: EVIDENCE FOR A ROLE OF GM-CSF-ACTIVATED STAT5 SIGNALING  
PATHWAY

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*Background*-As granulocyte/macrophage colony-stimulating factor (GM-CSF)-mediated delay of granulocyte apoptosis contributes to the accumulation of inflammatory cells at the site of inflammation in many diseases, we sought to determine whether heaves condition is also associated with GM-CSF-dependent increase of lung granulocyte survival. Moreover, because GM-CSF mediates its effects through activation of signal transducer and activator of transcription 5 (STAT5), we also investigated the potential role of STAT5 in heaves inflammation.

*Methods*-Blood granulocytes were recovered from six healthy and six heaves-affected horses. Lung granulocytes were obtained by broncho-alveolar lavage (BAL) from the heaves-affected horses. Granulocytes were cultured in the presence or absence of anti-GM-CSF receptor antibodies for different times and apoptosis was determined using the Annexin-V/propidium iodide detection method. Nuclear protein extracts from cultured granulocytes were analyzed for STAT5 binding activity by electrophoretic mobility shift assay.

*Results*-BAL granulocytes from heaves-affected horses demonstrated a significant delay in apoptosis when compared to blood granulocytes from the same horses. Conversely, the rate of apoptosis in blood granulocytes from healthy and heaves-affected horses was comparable. The enhanced survival of BAL granulocytes from affected horses was suppressed in the presence of antibodies directed against GM-CSF receptors. Increased levels of active STAT5 were found in BAL granulocytes from heaves-affected horses and were markedly reduced after treatment with anti-GM-CSF receptor antibodies.

*Conclusions*-These data demonstrate that granulocyte survival is enhanced in the lung of heaves-affected horses and suggest a role for GM-CSF-activated STAT5 pathway in delaying apoptosis of lung granulocytes in this disease.

p65 HOMODIMER ACTIVITY IN BAL CELLS IS CORRELATED TO LUNG DYSFUNCTION  
IN EQUINE HEAVES

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Nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity, which is a key regulator of inflammatory gene expression, is increased in bronchial epithelial cells from horses suffering from heaves. To determine whether this increased activity extends to distal airways and to other pulmonary cells, cells recovered by broncho-alveolar lavage (BAL) in healthy and heaves-affected horses were assessed for NF- $\kappa$ B activity. NF- $\kappa$ B activity was much higher in BAL cells from heaves-affected horses, especially during crisis (disease exacerbation), than in cells from healthy horses. Moreover, the level of NF- $\kappa$ B activity found in BAL cells was positively correlated to total lung resistance and to the proportion of neutrophils present in BAL fluid. Finally, prototypical p65-p50 NF- $\kappa$ B heterodimers were absent from BAL cells, which mostly contained not classical p65 homodimers. These results 1) show that increased NF- $\kappa$ B activity is a general feature of heaves lung, 2) demonstrate the importance of p65 homodimers in neutrophilic inflammation, and 3) suggest that the use of specific NF- $\kappa$ B inhibitors could improve lung function in heaves-affected horses.

## OZONE ADAPTATION IN CALVES IS ASSOCIATED WITH IMPAIRED ACCUMULATION OF GRANULOCYTES IN THE LUNG

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Lung function decrements induced by an acute exposure to ozone (O<sub>3</sub>) paradoxically resolve during multiday exposure. This phenomena, usually called adaptation, is characteristically accompanied by a gradual attenuation of lung neutrophilia. The maintenance of neutrophilia at the site of inflammation is known to be due to cytokine-mediated delayed neutrophil apoptosis, which is associated with reduced levels of Bax, a pro-apoptotic protein and we sought to determine whether defects in these mechanisms could account for O<sub>3</sub> adaptation. Lung granulocytes obtained at different time points from calves exposed to 0.75 ppm O<sub>3</sub> for 12 h/day for 7 consecutive days neither showed enhancement of survival nor Bax deficiency, when compared to blood granulocytes. To further investigate the effects of an exogenous oxidative stress on neutrophil survival, human granulocytes were treated with hydrogen peroxide alone, or in combination with granulocyte/macrophage colony-stimulating factor, an anti-apoptotic cytokine. Both treatments led to rapid apoptosis associated with downregulation of Bcl-x<sub>L</sub> and Bcl-2, two anti-apoptotic proteins. This study shows that O<sub>3</sub> adaptation is associated with a defect in the mechanisms leading to neutrophils accumulation at the site of inflammation, and suggests that this failure is due to direct pro-apoptotic effects of exogenous oxidative stress on granulocytes.

ARTERIAL BLOOD GASES IN HORSES EXAMINED FOR POOR PERFORMANCE  
DURING HIGH-SPEED TREADMILL EXERCISE.

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Arterial blood gas analysis during high-speed treadmill exercise (HSTM) has demonstrated decreased PaO<sub>2</sub> and increased PaCO<sub>2</sub> in left laryngeal hemiplegia (LLH). We investigated arterial blood gases in horses receiving HSTM evaluation for poor performance.

All horses that received a complete HSTM performance examination and sampling of arterial blood gases before, during and after exercise were included.

Horses were exercised to increase maximum heart rate (MHR) to 200 beats per minute (BPM) or more for 1600 meters. The total test distance was 2800 meters and individualized for the fitness level of each horse.

An intravenous thermistor catheter was placed in the jugular vein to ascertain core body temperature. An arterial catheter was placed in the transverse facial artery to sample arterial blood. Samples taken 1 minute before exercise, immediately after warm-up, at 30-second intervals after the stress test had begun (nine samples) and one minute after exercise ended. Samples were placed on ice then analyzed for temperature corrected arterial PaO<sub>2</sub> and PaCO<sub>2</sub>.

Three hundred and eighty-six horses presented for poor performance were examined using high-speed treadmill videoendoscopy.

Seventy-four of three hundred and eighty-six (19.2%) horses had arterial blood gas samples. Thirty-six of seventy-four (48.6%) horses had significant URT abnormalities. Nineteen horses (52.9%) had PC alone and three (8.3%) horses had PC in combination with other abnormalities. Other abnormalities included ADAF (4), DDSP (5) and LLH (5). Twenty-eight of thirty-six horses (78.5%) had significantly decreased arterial oxygen tensions (PaO<sub>2</sub>).

Several dynamic URT abnormalities including LLH decrease arterial PaO<sub>2</sub> and increase arterial PaCO<sub>2</sub>.

## OVINE RESPIRATORY DISEASE-COMPARATIVE CYTOLOGY

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Examination of bronchoalveolar lavage (BAL) fluid may be used to aid differential diagnosis of chronic respiratory disease in large domestic animals. Although at necropsy gross lesions may appear well demarcated, it is not known whether BAL fluid from apparently unaffected lung will reflect such pathology. The aim of this study was to determine the influence of sampling site (lesional vs non-lesional) on BAL fluid differential cytology.

Two samples of BAL fluid were collected from each ovine lung examined (n=70). The first sample was from a macroscopically obvious lesion. The second was from a macroscopically normal site. 22 lungs had no evidence of respiratory disease and were used as controls.

Fixed tissue from each site was taken to confirm each diagnosis histologically, allowing each case to be assigned to the following chronic disease patterns commonly seen in ovine lungs: maedi visna virus (MVV)-associated lymphoid interstitial pneumonia, sheep pulmonary adenomatosis (SPA), parasitism and nonspecific consolidation. Total and proportional cell counts were calculated.

MVV and SPA tended to elicit a whole-lung response and, as a consequence, there were not enough second samples from “non-lesional” sites in these lung specimens to perform statistical analysis. Parasitised and consolidated lungs were characterised by significantly greater numbers of eosinophils and mast cells in non-lesional areas relative to control lungs. With the exception of parasitised lungs, there were significantly more cells present in BAL fluid from lesional sites of diseased lungs than were present in BAL fluid from control lungs. Although total cell numbers were unaffected in BAL fluid from lesional areas of parasitised lungs, these areas were characterised by significantly more eosinophils and mast cells (accounting for a higher proportion than neutrophils, which were in turn significantly reduced).

Taking into account the extent of histological abnormality in MVV and SPA and the assumption that such pathology would be reflected in BAL cytology, we can conclude that a normal BAL profile indicates the likely absence of the common chronic sheep respiratory diseases.

EXPRESSION OF THE EQUINE IgE RECEPTOR  $\alpha$ - CHAIN AND ITS USE FOR THE  
DETECTION OF EQUINE IgE

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The purpose of this study was to express the extra-cellular part of the alpha chain of the high-affinity receptor for IgE, Fc $\epsilon$ R1 $\alpha$  (EMBL: Y18204), as a recombinant protein and to use it as a reagent for the detection of allergen-specific IgE.

Fc $\epsilon$ R1 is expressed on the surface of mast cells and basophils. It is a tetrameric, transmembrane protein with one alpha, one beta and two gamma subunits. The extra-cellular part of the alpha subunit is thought to be responsible for binding IgE. The cDNA for this region has been cloned and expressed in mammalian cells and in insect cells. The expressed proteins differ in size, presumably due to different degrees of glycosylation. Samples of BALF taken from horses suffering from heaves, after challenge with hay dust extract, were dot-blotted on to nitrocellulose. Recombinant protein obtained from both expression systems bound to the BALF, and binding was reduced when BALF samples were heated at 56°C for 4h to destroy IgE. Western blots of the recombinant protein could be detected using samples of BALF followed by a rabbit anti-equine IgE, followed by an anti-rabbit IgG conjugate.

These results suggest that the expressed protein will bind equine IgE.

This work was supported by the Home of Rest for Horses (grant G.10.00). Horse BALF cells were provided by Professor P. M. Dixon.

$\beta$ -DEFENSIN EXPRESSION IN THE OVINE RESPIRATORY EPITHELIUM

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Defensins are a family of small, cationic peptides with broad-spectrum antimicrobial activity. It is becoming apparent that these peptides play a significant role in innate immunity not only via their direct antimicrobial activities but also through other interactions with components of inflammatory/immune processes. The  $\beta$ -defensins are expressed by epithelial cells are thought to contribute to the defence system of mucosal surfaces. Two  $\beta$ -defensin sequences, SBD-1 and SBD-2, have been isolated in sheep and shown to be expressed in a variety of epithelial tissues. We are interested in the role of  $\beta$ -defensins in the ovine respiratory epithelium in the normal state and during bacterial infections. We have looked at expression of SBD-1 and SBD-2 by RT-PCR in ovine tracheal ring organ cultures and differentiated ovine tracheal epithelial cell cultures grown at an air/liquid interface either untreated or following challenge with bacteria, TNF $\alpha$  or LPS. SBD-1 is expressed all untreated samples whereas SBD-2 is only expressed at very low levels in a small number of untreated samples. Preliminary results from challenged samples suggest no dramatic up-regulation of expression of these peptides although SBD-2 can be detected in a higher proportion of challenged samples. The sequences of the mature active SBD-1 and SBD-2 molecules and their antimicrobial activities have yet to be characterised. We have identified HPLC fractions from extracts of ovine tracheal epithelium, which exhibit antimicrobial activity. Gel electrophoresis of these fractions reveal that there are several bands present in the samples with the strongest staining bands migrating between 3-14 KD. Further purification steps are required to isolate and identify the factor(s) present in these fractions which are responsible for the antimicrobial activity.

## ENDOSCOPIC AND CYTOLOGICAL EVALUATION OF THE LOWER RESPIRATORY TRACT OF 129 THOROUGHBRED YEARLINGS IN BREAKING AGE.

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The respiratory tract of 129 thoroughbred yearlings, 59 male and 70 female, between 18 and 24 months old, from 7 different breeding farms from Paraná State, Brazil, were evaluated in order to see what respiratory diseases could be found in yearlings during braking age, not related to exercise, that could impair a horse future performance. Owners were inquired about barn and food management, sanitary program and previous diseases. A clinical examination was proceeded and all the yearlings had their respiratory tract evaluated by endoscopy. When mucus was present a tracheal aspirate was proceeded and smears prepared for cytological evaluation. The smears were prepared with the Pappenhein's stain and the different cell types were graduated in the 1000 x in the following way: Grade 0 (absence of the cell type), Grade I (one cell or isolated cells or, one to two cells on isolated fields), Grade II (one to two cells in many fields), Grade III (three to five cells in many fields) and Grade IV (more than 5 cells in many fields or diffuse distribution).

The feed management found was pressed oat (47/36,4%), pressed oat with alfalfa hay (26/20,2%) and hole oat with alfalfa hay (56/43,4%). Although the majority of the yearlings (105/83,3%) did not show any respiratory disease during their lifetime, 105/90,8% had tracheal mucus during the endoscopic examination and 22/18,3% had the carina hyperemic and edematous. Curshmann's spirals and Giant cells were present in the mucus from 16 and 4 yearlings respectively. Eosinophils appeared in the tracheal aspirate of 61 individuals showing higher prevalence in the mucus of yearlings that were fed with alfalfa hay; 57 yearlings that had eosinophils in their mucus were being managed in barns and 4 were still in pasture.

The utility of the cytological examination of the tracheal aspirate as a simple and capable technique was demonstrated. It was also possible to evidence the prevalence of lower respiratory tract diseases of variable degrees in thoroughbred yearlings before training and that these findings can be related to stable and feeding management. Assuming that a racehorse spends most of its lifetime stabled, there is a tendency for the worsening of these findings with deleterious impact on their future-racing career.

PREPARATION OF BRONCHOALVEOLAR LAVAGE FLUID CYTOLOGY SLIDES BY  
CELLULAR GRAVITATION – METHOD OF PREPARATION AND COMPARISON WITH  
CYTOSPIN PREPARATIONS.

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**Aim:** To evaluate an alternative method for the preparation of cytology slides in which cells within raw bronchoalveolar lavage fluid (BALF) gravitated onto microscope slides.

**Methods:** The BALF was pipetted into cylindrical drill holes within perspex blocks, which were tightly opposed to microscope slides. The perspex block and the slide were separated by a single layer of paper containing a hole aligned with the drill hole of the block, thus permitting gradual dispersion of fluid (Block Method). Following this procedure, slides were stained with Leishmans stain and a differential cell count was performed, by counting 500 cells on duplicate slides. To optimise the conditions for the Block Method, comparisons were made between different volumes of BALF (200µl vs 300µl; n=17), different time points following pipetting of BALF (1h vs 4h vs 24h; n=9) and different paper types (filter paper vs photocopy paper; n=10). All slides prepared by the Block Method were also compared with a cytopsin preparation (Cytospin Method) made from an identical BALF sample.

**Results:** There was no significant differences between the 200µl and 300µl BALF aliquots with respect to the differential cell counts when cell gravitation was stopped at 4h. The use of filter paper in the Block Method, resulted in a significantly ( $P<0.01$ ) lower lymphocyte ratio and greater macrophage ratio when compared with the Cytospin Method and the Block Method when photocopy paper was used. The Block Method using photocopy paper resulted in a significantly ( $P<0.01$ ) greater lymphocyte ratio and lower macrophage ratio when cell gravitation was stopped at 4h and 24h when compared with 1h, however there was no difference between the 4h and 24h time points. At all time points, the Block Method using photocopy paper resulted in a significantly ( $P<0.01$ ) greater lymphocyte ratio than the Cytospin Method. Also at the 1h and 4h time points the Block Method using photocopy paper resulted in a significantly ( $P<0.01$ ) lower macrophage ratio than the Cytospin Method.

**Conclusion:** The Block Method provided an economical and simple means by which BALF cytology slides can be prepared. The morphology of the cells using this method was comparable to that of the Cytospin Method. In addition, the Block Method using photocopy paper resulted in a greater lymphocyte ratio than the Cytospin Method, which has previously been reported to result in an underestimation of this cell type.

## FILTRATION OF BRONCHOALVEOLAR LAVAGE FLUID RESULTS IN THE SELECTIVE LOSS OF CELLS

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**Aim:** To determine whether filtration of bronchoalveolar lavage fluid (BALF) results in preferential cell loss when compared with non-filtered BALF.

**Methods:** Total cell counts of BALF samples (n=10) were made using a haemocytometer prior to and following filtration through both a double layer of gauze and a 60µm pore size nylon filter. Cytospin preparations were also made before and after filtration. Following Leishmans staining, differential cell counts were made by counting 500 nucleated cells on duplicate slides, and absolute cell counts were calculated from the differential cell counts and the total cell counts. The 3 methods of preparation (non-filtered *vs* gauze *vs* nylon) were compared with respect to differential and absolute cell counts. BALF samples were collected from a random selection of clinical cases, and the median neutrophil ratio calculated from cytospin preparations of unfiltered samples was 15.4% (range 2.5-77.0).

**Results:** Filtration through the 60µm pore size nylon filter resulted in a significantly lower lymphocyte ratio (P<0.05) and count (P<0.01) compared with the gauze filtered samples and a significantly lower (P<0.01) lymphocyte count compared with the non-filtered sample. Filtration through the gauze resulted in a significantly lower mast cell ratio and count (P<0.05) compared with the non-filtered samples, and filtration through the 60µm pore size nylon filter also resulted in a significantly lower mast cell count (P<0.05) compared with the non-filtered samples. Filtration through both the gauze and the 60µm pore size nylon filter resulted in a significantly lower macrophage count (P<0.01) than the unfiltered sample. There was no significant difference between all 3 methods of processing with respect to neutrophil count or ratio.

**Conclusions:** Filtration of BALF results in selective loss of cells, in particular the larger cell types (e.g. mast cells and macrophages). The use of 60µm pore size nylon filters also results in the selective loss of lymphocytes. Filtration did not result in the selective loss of neutrophils, and despite the selective losses of other cell types, filtration had no significant effect on the neutrophil ratio in the 10 samples examined.

## CHARACTERISATION OF TRYPTASE AND A GRANZYME H-LIKE CHYMASE ISOLATED FROM EQUINE MASTOCYTOMA TISSUE

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Mast cell proteinases have been shown to be important mediators of lung inflammation in human and animal models, but until now there has been no investigation of the nature of equine mast cell proteinases. The aim of this study was to identify and characterise equine mast cell proteinases, to facilitate future studies of their role in equine heaves. We describe here the purification and characterisation of two proteolytic components from equine mastocytoma tissue, which were detected using chromogenic substrates for trypsin and chymotrypsin. Following purification by heparin affinity chromatography, the trypsin-like component was found to be equine mast cell trypsin by N-terminal amino acid sequencing, showing a close similarity with human trypsin- $\beta$  (85% identity over 20 residues). It also had similar subunit molecular size (34-36 kDa by SDS-PAGE) and substantially similar cleavage specificity to human trypsin- $\beta$  with the substrates tested. A 32 kDa chymotrypsin-like component was also purified from mastocytoma extract, and termed equine mast cell proteinase-1 (eqMCP-1). The N-terminal amino acid sequence of eqMCP-1 was very similar to human granzyme H (95% over 19 residues). Rabbit antisera directed against trypsin and eqMCP-1 both detected equine mast cells by immunohistochemistry, and will be of use in future clinical studies of the relevance of mast cell proteinases in equine allergic disease.

THE EFFECT OF TIME, TEMPERATURE AND FIXATIVES ON CYTOLOGICAL ANALYSIS  
OF EQUINE BRONCHOALVEOLAR LAVAGE FLUID

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Cytological analysis of bronchoalveolar lavage fluid (BALF) is an accurate and practical diagnostic aid for assessment of equine pulmonary disease. BALF is often subject to time delays, possibly with temperature fluctuations, between sample collection and processing and there are no well-defined guidelines for optimal equine BALF storage prior to processing. The aim of this study therefore was to evaluate the effects of time, temperature and fixatives on equine BALF in order to establish such guidelines.

Method: Equine BALF samples were stored at 4°C, 18°C ( $\pm$  addition of an equal volume of Saccamanno fixative or 100 $\mu$ l 4% formol saline) or 38°C. Total nucleated, differential and absolute cell counts (TCC, DCC and ACC respectively), cell viability, cell morphology and bacterial overgrowth of samples were serially monitored over a 72h time-course.

Results: The time taken for a significant reduction in TCC and cell viability of unfixed BALF samples decreased as the storage temperature increased. There was no diagnostically significant difference in DCC or ACC over the time-course at any temperature. Unfixed BALF samples showed significant bacterial overgrowth and poor morphology at progressively shorter times with increasing storage temperature. Fixed BALF samples showed poor morphology with Leishman's stain compared to unfixed samples.

Conclusions: Equine BALF should be processed within 8h if stored at room temperature. Samples to be processed >8h should be refrigerated or stored on ice. To avoid deleterious changes to cytological parameters, equine BALF should not be exposed to high temperatures. Cell identification on Leishman's stained preparations of fixed equine BALF is difficult. Further work is required to investigate the use of other stains on fixed BALF preparations.

*ASPERGILLUS FUMIGATUS* EXTRACT DOSE RESPONSE INHALATION CHALLENGES IN HEAVES HORSES AND THE CONTRIBUTION OF ENDOTOXIN CONTAMINATION TO THE PULMONARY INFLAMMATORY AND FUNCTIONAL RESPONSE TO *ASPERGILLUS FUMIGATUS* EXTRACT INHALATION.

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Mould extract inhalation challenges have been used extensively in the investigation of heaves. Such challenges have induced pulmonary neutrophilic inflammation and dysfunction, consistent with, but less severe than the natural disease.

**Aims:** To investigate whether insufficient dose delivery was responsible for the shortfall in response to inhaled extract compared with natural disease. In addition to investigate the contributing role of endotoxin contamination in the response to mould extract.

**Methods:** The pulmonary inflammatory and functional response of 6 heaves horses following inhalation of saline (placebo), and 3 doses of *Aspergillus fumigatus* extract was measured. Analysis of the *Aspergillus fumigatus* extract revealed lipopolysaccharide (LPS) contamination, therefore in order to establish the contribution of LPS in the response to mould extract inhalation, the response of the same horses to *A. fumigatus* extract inhalation before and after lipopolysaccharide (LPS) depletion was measured.

**Results:** Inhalation challenge with 0.5, 1.6 and 5mg *A. fumigatus* extract resulted in a significant ( $P < 0.05$ ) increase in bronchoalveolar lavage fluid (BALF) neutrophil ratio when compared with saline inhalation. Only 1.6 and 5mg extract inhalation resulted in significant ( $P < 0.05$ ) lung dysfunction compared with saline. There was no significant difference between 1.6 and 5mg extract inhalation with respect to airway neutrophil numbers or lung function, and good agreement between the responses to these doses, indicating a plateau in both measured responses. LPS depletion of 1.6mg *A. fumigatus* extract resulted in a significant reduction in airway neutrophil numbers and increase in arterial oxygen tension. There was no significant difference between saline and the LPS-depleted *A. fumigatus* extract challenges with respect to neutrophil count and lung function. The reduction in airway neutrophil numbers was greater than would be predicted upon extrapolation from soluble LPS dose response inhalation experiments.

**Conclusions:** The plateau in the dose response curve supports a role for other inhalants, in addition to soluble components of *A. fumigatus*, in the aetiopathogenesis of heaves. In addition the LPS contamination of the *A. fumigatus* extract contributed significantly to the pulmonary inflammatory response following inhalation challenge. Therefore the magnification in response to LPS when inhaled in concert with *A. fumigatus* extract, may further support a role of inhaled endotoxin in the pulmonary inflammation and dysfunction in naturally occurring heaves.

## EVALUATION OF NEBULISED HAY DUST SUSPENSIONS (HDS) FOR THE DIAGNOSIS AND INVESTIGATION OF HEAVES.

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**Aims:** It was hypothesised that the use of a nebulised hay dust suspension (HDS) would reduce the variability of the more commonly used hay/straw challenge method of heaves induction and thus standardise experimental protocols. Furthermore, analysis of HDS would also permit further investigation of the organic dust components responsible for the response.

**Methods:** Three hay dust suspensions (HDS-1, 2 and 3) were prepared from fine hay dust particles for use in the diagnosis and investigation of heaves. HDS-1 and 3 were analysed for endotoxin,  $\beta$ -D-glucan and protein concentrations, general protease activity, and enumeration and size distribution of particulates. Following confirmation that the particulate and soluble components of HDS could be aerosolised by jet nebulisation, the pulmonary inflammatory and functional responses to inhalation challenge with 3 different HDS were determined in 6 control and 7 asymptomatic heaves horses. HDS was also fractionated by centrifugation to yield supernatant (SUP), washed particulate debris which comprised mainly fungal spores (WP) and a wash fraction (WF) used to wash the particulates. Both the heaves and the control groups received inhalation with WP, SUP, WF and a combination of WP and SUP.

**Results:** Heaves horses given HDS challenge developed the characteristic features of heaves, including airway neutrophilia, obstructive airway dysfunction and mucus hyper-secretion. While HDS challenge induced a mild airway neutrophilia in controls, the no response threshold for controls was greater than that of heaves horses, and there was no overlap in BALF neutrophil ratio of controls and heaves horses. Furthermore, HDS challenge did not induce airway dysfunction or mucus hyper-secretion in controls. Thus HDS challenges enabled differentiation of control and heaves horses. Interestingly, in both groups, the airway neutrophilia was a dose dependent response rather than an “all or nothing” response. Inhalation of SUP induced a significant airway neutrophilia in both groups, with the magnitude of the response being significantly greater in heaves horses. SUP induced significantly less airway neutrophilia than HDS in both groups, despite the endotoxin and protease content of HDS and SUP being comparable. WP and WF induced only a slight airway neutrophilia in heaves horses. However, a combined challenge with SUP and WP induced a neutrophilic response approaching the magnitude of that following HDS challenge.

**Conclusions:** This study suggests that HDS challenges are of value in diagnosis and investigation of heaves and that both dust particulates and soluble HDS components contribute to the pulmonary recruitment of neutrophils in the HDS model of heaves.

INHIBITION OF GELATINASES AND COLLAGENASES BY CMTs, BISPHOSPHONATES  
AND A CTT-PEPTIDE IN RESPIRATORY SECRETIONS OF HORSES WITH CHRONIC  
OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Gelatinases and collagenases belong to the matrix metalloproteinase (MMPs) family of over 20 genetically distinct but structurally related Zn-dependent proteinases. Both collagenases (1) and gelatinases (2) have been shown to be elevated in respiratory secretions (RS) of COPD horses.

Collagenases (MMP-8 and MMP-13) and gelatinases (MMP-2 and MMP-9) were identified by Western immunoblotting in equine RS (3). Of these MMP-9, MMP-8 and MMP-13 (4) levels were detected to be clearly elevated in the RS of COPD horses. MMP-9 was further found to be substantially activated. Elevated gelatinase B (MMP-9) levels were also detected in bronchoalveolar lavage fluid of COPD horses after natural allergen challenge (moldy hay challenge or hay extract challenge) (5). Previously, Maisi et al. (6) have shown that gelatinolytic activity in respiratory secretions can be inhibited dose-dependently by CMT-3.

The ability of CMTs (CMT-3, CMT-8), bisphosphonates (pamidronate, zoledronate) and cyclic peptide (CTT-peptide) to inhibit collagenolytic and gelatinolytic activity was tested *in vitro* in respiratory secretions from COPD affected horses. CMT-3 and both bisphosphonates effectively and dose-dependently inhibited gelatinolytic activity. Collagenolytic activity in the samples was inhibited by both CMTs, the bisphosphonates and the CTT-peptide.

These *in vitro* studies give basis for *in vivo* studies of using CMT-3, bisphosphonates and CTT-peptide in COPD horses to test their capacity of inhibiting MMPs *in vivo*.

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## EFFECT OF OZONE TREATMENT ON AIRBORNE BACTERIA AND MOULD IN THE STABLE ENVIRONMENT

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Recurrent airway obstruction (RAO), previously referred to as equine chronic obstructive pulmonary disease (COPD), is a common condition in horses in temperate climates with clinical signs ranging from decreased performance to severe respiratory distress. Since the condition most commonly results from a hypersensitivity to environmental mould spores, reduction of exposure to such potential allergens is a primary concern when treating RAO. Ozone gas has been previously reported to possess bactericidal and fungicidal properties. Whilst viable fungal spores are not necessary to provoke a hypersensitivity response, we hypothesised that ozone exposure may render fungal spores in the stable environment unable to reproduce, thereby reducing fungal burden, decreasing allergen concentration, and reducing the likelihood of a clinical episode of RAO. Sabouraud-dextrose agar plates were exposed to environmental allergens using different protocols. The exposed plates were then subjected to different concentrations of ozone (0 - 800 ppb) for different lengths of time (1 minute - 120 minutes). Plates exposed to the highest concentration of ozone (800 ppb) for the longest duration (2 hours) were found to have significantly lower numbers of bacterial and fungal colony forming units than any of the other ozone-time combination treatments or non-ozone treated controls. Exposure of established fungal colonies to ozone at 800 ppb for 2h caused marked spore formation. This preliminary study suggests that ozone treatment of stables could potentially be used to reduce the viable mould burden. However, it may be unwise to treat established colonies with ozone as this appears to induce spore formation.

## MECHANISMS OF AIRWAY OBSTRUCTION IN HEAVES

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Although it is accepted that airway obstruction of heaves is due to bronchospasm, mucus and airway wall thickening, the magnitude of the non-bronchospastic components is unknown. It is also not known if bronchospasm is mediated totally via cholinergic nerves or by direct action of inflammatory mediators on smooth muscle (ASM). The present study had two objectives 1) to determine the mechanism of bronchospasm, and 2) to quantify the non-bronchospastic component of airway obstruction. We hypothesized that a  $\beta_2$ -agonist, pirbuterol (Pir), would nonspecifically reverse bronchospasm but atropine (Atr) would block only that mediated via cholinergic receptors.

Five heaves-affected horses and 5 controls were studied 1) at pasture ( $\Delta P_{pl_{max}} < 15$  cm H<sub>2</sub>O in the heaves-affected animals); and 2) after 1 and 7 days of stabling. At each time, lung function ( $\Delta P_{pl_{max}}$ ,  $R_L$ ,  $C_{dyn}$ ) was measured at baseline, 10 min. after Atr (0.02 mg/kg IV), and 10 min. after Pir (2 mg via the Equine Aeromask). Pir was repeated until lung function became stable (maximal bronchodilation). Non-bronchospastic obstruction (Nbo) was that remaining in heaves-affected horses after maximal bronchodilation compared to pasture controls.

In control horses, stabling had no effect on lung function. Atr and Pir caused no change in lung function confirming that control horses have no bronchomotor tone. When heaves-affected horses were stabled,  $\Delta P_{pl_{max}}$  and  $R_L$  increased and  $C_{dyn}$  decreased at days 1 and 7. Atr had no effect on lung function of heaves-affected horses in remission but dramatically improved lung function during airway obstruction (days 1 and 7). Pir caused no further improvement in lung function demonstrating lack of a direct effect of inflammatory mediators on ASM. Therefore bronchospasm of heaves is mediated totally via cholinergic mechanisms. Nbo did not differ between horse groups at pasture but increased significantly at day 1 ( $R_L$  &  $C_{dyn}$ ) and further by day 7 ( $R_L$ ) in heaves-affected horses. Nbo in controls was unaffected by stabling. Residual non-bronchospastic obstruction increases during acute exacerbations of heaves presumably due to secretions and airway wall thickening. (Supported by USDA CSREES 99-35204-8366)

COMPARING IN-VITRO ANTIMICROBIAL SUSCEPTIBILITY FOR TWO COMMON EQUINE RESPIRATORY TRACT ISOLATES

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Increasing antibiotic resistance of *Streptococcus pneumoniae* in human patients to frequently used trimethoprim/sulfamethoxazole, extended spectrum cephalosporins and penicillin is well documented and cause for concern among equine veterinarians. The study objective was to identify possible emergence of antibiotic resistance in *Streptococcus zooepidemicus* (34) and *Streptococcus equi* (6) isolates from respiratory tract samples. A retrospective review of diagnostic laboratory records from 1992 and 2000 for equine bacterial culture and sensitivity submissions yielded 40 acceptable isolates. Samples included tracheal aspirates (30), nasal swabs (4), pleural fluid aspirates (3), sinus aspirates (2), and a guttural pouch swab (1). Identified isolates had undergone susceptibility testing by Kirby-Bauer disk diffusion to a variety of commonly used antibiotics (see table).

	92 Strep zoo		00 Strep zoo		92 Strep equi		00 Strep equi	
	S	R	S	R	S	R	S	R
Ampicillin	18/18	0	16/16	0	3/3	0	3/3	0
Cephalothin	18/18	0	15/16	1/16	3/3	0	3/3	0
Chloramphenicol	18/18	0	16/16	0	3/3	0	3/3	0
Erthryomycin	18/18	0	16/16	0	3/3	0	3/3	0
Gentamicin	16/18	2/18	15/16	1/16	3/3	0	3/3	0
Kanamycin	0	18/18	0	16/16	0	3/3	0	3/3
Penicillin	18/18	0	15/16	1/16	3/3	0	3/3	0
Streptomycin	0	18/18	0	16/16	0	3/3	0	3/3
Tetracycline	10/18	8/18	7/16	9/16	3/3	0	3/3	0
Trimethoprim/sulfa	16/18	2/18	14/16	2/16	2/3	1/3	3/3	0
Ceftiofur	18/18	0	15/16	1/16	3/3	0	3/3	0

(92 =1992, 00 =2000, S = sensitive, R = resistant)

Susceptibility results were not statistically different ( $p < 0.01$ ) between 1992 and 2000 for *Streptococcus zooepidemicus* and *Streptococcus equi*. Based on this sample population there is not an identified trend for resistance to popular antibiotic choices of penicillin, trimethoprim/sulfadiazine, ceftiofur and gentamicin. Resistance is always a constant threat and principles for prudent antimicrobial use must be applied to each case.

## CLINICAL INTERPRETATION OF OSCILLOMETRIC IMPEDANCE MEASUREMENTS IN HORSES

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Purpose of the study: Multi-frequent oscillometric techniques for lung function testing apply a broad variety of test-frequencies to the horse's lung which can be used to determine respiratory impedance spectra. Impedance data first of all reflect the resistive and elastic properties of the respiratory system in a clearly differentiated manner. The knowledge of the physical behaviour of frequencies and their relationship to lung physiology of the species can be used to define clinical interpretation models, based on a minimised number of relevant impedance parameters.

Material and methods: In various clinical studies, impedance spectra of horses with known diseases of the respiratory tract were clinically classified. Healthy horses contributed to the reference group. Furthermore, bronchial challenge and dilatation procedures clarified general principles of impedance spectra recognition in relationship to the clinically known changes of lung function.

Results: The resistance spectrum is mainly influenced by the upper airways. Vocal Cord Dysfunction leads to frequency independent elevation of all resistance values.

However, typical lung diseases like Recurrent Airway Obstruction (RAO), Small Airway Inflammatory Disease (SAID) or Heaves lead to frequency dependence of the resistance course and a significant decrease of low frequent reactance, indicating pulmonary obstruction and loss of elasticity. Decreasing reliability of data in the lower frequency range and loss of sensitivity in higher frequencies limit the range for clinically relevant impedance data.

Conclusions: Respiratory impedance spectra which can be measured non-co-operatively and non-invasively in horses, provide valuable information about lung function abnormalities. Characteristic pattern in the spectral impedance are strongly related to pathophysiology.

## ACIDIFICATION OF EXHALED BREATH CONDENSATE IN PATIENTS WITH CYSTIC FIBROSIS

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Although it is established that the cystic fibrosis transmembrane conductance regulator (CFTR) functions as a cyclic-AMP regulated chloride channel, the loss of chloride conductance alone cannot fully explain the severity of lung disease evident in Cystic Fibrosis (CF) patients. Recently bicarbonate secretion has been shown to be impaired in CFTR expressing tissues from CF patients and CFTR is now thought to regulate Cl/ HCO<sub>3</sub> exchange at the apical membrane of epithelial cells. In normal airway epithelial cells it is likely that significant amounts of HCO<sub>3</sub> cross the apical membrane into the airway lumen and this process is facilitated by CFTR . In the trachea, where the apical fluid layer is small it is possible that this disturbance of HCO<sub>3</sub> secretion could have significant effects on the pH of the epithelial lining fluid. We hypothesised that the airway secretions of CF patients would be acidified and that this may be worsened during an infective exacerbation due to increased inflammation burden. We examined the pH ( Corning pH microelectrode , Corning NY, USA) of exhaled breath condensate (EBC) from 10 healthy non smoking controls and 15 patients with CF (8 of whom were in an infective exacerbation). . Nitrite concentration in exhaled condensate was determined by a colorimetric assay based on the Greiss reaction as described previously .

Our results showed that pH of EBC was significantly lower in stable CF patients compared to controls (  $5.5 \pm 0.47SD$  vs  $6.2 \pm 0.15$  ,  $p=0.004$ ). and further reduced in CF patients with an exacerbation ( $5.0 \pm 0.37$ ,  $p=0.001$ ).Nitrite levels in EBC appeared elevated in CF patients compared to control subjects (  $3.8\mu\text{m} \pm 3.7SD$  vs  $0.98\mu\text{m} \pm 0.67$ ,  $p=0.07$ ), but failed to reach statistical significance. No correlation was found between EBC pH and nitrite levels in any group.

We conclude that airway acidification occurs in stable CF and is worsened further during infective exacerbations and may play an important role in the lung pathophysiology of CF patients.

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CLINICAL AND ANCILLARY DIAGNOSTIC FINDINGS, TREATMENTS AND LONG-TERM RESULTS IN 277 CASES OF EQUINE SINONASAL DISEASE.

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The case records of 277 horses referred to the University of Edinburgh large Animal Hospital with diseases affecting the nasal cavities and paranasal sinuses were analysed. Horses were subjected to a standard examination protocol. Ancillary diagnostic techniques included nasal endoscopy, radiography and sinoscopy. The diagnoses included 67 cases (24% of all cases) of primary sinusitis, 61 cases (22%) of dental sinusitis, 37 cases (13%) of sinus cysts, 22 cases (8%) of sinonasal neoplasia, 21 cases (8%) of progressive ethmoid haematoma, 17 cases (6%) of sinonasal trauma, 13 (5%) cases of sinonasal mycosis, 11 (4%) cases of rostral maxillary cheek tooth infection, seven (3%) cases of sinonasal polyps, seven (3%) cases of nasal epidermal inclusion cysts, and 14 (6%) cases with miscellaneous sinonasal disorders. Many disorders showed similar clinical signs including nasal discharge (present in 87% of all cases) and facial swelling (46%). Most disorders were chronic, with a median duration of signs of 12 weeks (range 3 days - 6 years) prior to referral. Sinus cysts and sinonasal neoplasia were significantly more frequently ( $p < 0.05$ ) associated with gross distortion of the nasal passages and facial bones than the other sinonasal disorders. Endoscopic changes were detected *per nasum* in 91% of cases, but contributed to the exact diagnosis in only 20%. Radiography revealed abnormalities in 81% of cases but was diagnostically useful in only 36%. Sinoscopy was diagnostically useful in 70% of the 61 cases where used.

The treatments of these 277 horses with sinonasal disorders were evaluated. The long term (median duration 24 months) outcomes of treatment of the more common disorders were good, with 92% of horses with sinonasal mycosis, 84% with primary sinusitis, 82% with sinus cysts, 78% with dental sinusitis and 75% with sinonasal trauma reported to have complete remission of clinical signs. However, only 33% of horses with progressive ethmoidal haematoma (PEH) and 12% with sinonasal neoplasia reported long term remission of clinical signs.

## CHARACTERISATION OF EQUINE RESPIRATORY TRACT MUCINS

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This research aims to characterize and identify the mucins present in equine respiratory tract mucus. Research in humans has shown that with disease status there is alteration in the type, quality and quantity of mucins produced in the respiratory tract. Preliminary evidence in equine studies has shown an increase in mucin content in response to challenge of the respiratory tract. However, as little is known about equine mucins there is no data on which mucins are involved in the airways response to challenge. Mucins give epithelial mucus most of its properties. Alteration in the mucin content has a large affect on the behaviour of the mucus gel. In order to understand the changes that occur to the epithelial mucus we must first understand the normal character of the mucins therein. Mucus was collected from tracheas and preliminary purification was undertaken using size exclusion chromatography. The mucin containing fractions were further purified by density gradient centrifugation and the resultant samples analysed using biochemical techniques. Two distinct groups of material are present after density gradient centrifugation. Using human anti-MUC5B antibodies it has been shown that the high buoyant density material contains an equine homologue to MUC5B, which is reducible to subunits and is of high charge density. It is assumed that an equine homologue to MUC5AC co-purifies with this material. The low buoyant density material has been shown to be similar to a lipid-mucin complex present in the sputum of cystic fibrosis sufferers and also a complex produced in the feline trachea in response to bacterial endotoxin. The biochemical properties of this mucin-like complex from the horse can be altered with the addition of detergent to the sample. This research has identified equine homologues of human respiratory mucins, including a species produced in disease status, and shown that it is possible to use antibodies raised against human mucins to identify changes in equine respiratory mucus composition.

## CYTOKINES IN NASAL MUCUS AND SERUM DURING EXPERIMENTAL EQUINE INFLUENZA A2 (H3N8) INFECTIONS OF PONIES

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The present study aimed to monitor the kinetics of cytokine production in horses during the course of influenza A2 virus infections. The effects of two “European-type” influenza strains, Newmarket/2/93 and Sussex/89, were compared. Of these strains, Sussex/89 is clinically considered the more pathogenic.

Twenty Welsh mountain ponies, seronegative to influenza by SRH, were infected with equine influenza A2 by nebulised aerosol on day 0. Group A (n=10); were infected with strain Sussex/89 and group B (n=10) with strain Newmarket/2/93. Virus excretion, clinical signs and rectal temperatures were monitored, and serum and nasal mucus samples were collected, daily until day 10 post infection. Interferon (IFN) activity was detected with a VSV-inhibition bioassay on MDBK cells. Interleukin-6 (IL-6) activity was detected with the IL-6 dependent cell-line B9.

The experimental infections induced pyrexia, coughing and nasal discharge in both experimental groups, but ponies in group A showed more pronounced clinical signs. Virus was isolated from all of the infected ponies, with maximum excretion on days 2 and 3. IFN was detected in nasal mucus from all ponies in group A on at least one sampling occasion between days 2 and 6. In group B, only two ponies excreted IFN in nasal mucus, day 3. None of the animals had detectable IFN in serum during the experiment. IL-6 activity was detected in nasal mucus from all of the experimental animals from day 2 and onwards, but in group A the ponies showed markedly higher IL-6 responses compared to in group B (150 vs. 20 U/ml on day 3). In serum, ponies in group A showed a low IL-6 response between days 2 and 9, whereas ponies in group B only had occasional IL-6 positive samples.

Taken together, the equine influenza A 2 infections elicited local, and in some cases systemic, IFN and IL-6 responses in the ponies. Interestingly a strain variation in the cytokine responses was observed which may be correlated to the pathogenicity of the different strains. Analysis for further cytokines are currently under way.

## THE EFFECT OF A SINGLE ACUPUNCTURE TREATMENT ON PULMONARY FUNCTION IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION (HEAVES)

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We aimed to determine by measurement of lung function if a single acupuncture treatment relieves airway obstruction in horses affected by heaves, and to determine the duration of this effect.

Ten heaves-affected horses were used in this blinded crossover trial. Each of the 10 horses received three treatments: 1) acupuncture by an expert; 2) acupuncture by a novice using a recipe treatment; and 3) halter restraint and petting as a placebo treatment. Treatment order was randomized using a Latin square design and each treatment was separated by at least one month. Horses were stabled until maximal change in pleural pressure ( $dP_{pl_{max}}$ ) exceeded 15 cm H<sub>2</sub>O. The treatments were administered in the stable and measurements of pulmonary function were made in the laboratory before treatment, and at 20 minutes and 1, 2, 4, and 24 hours following treatment. Between measurements, horses returned to the stable.

The  $dP_{pl_{max}}$  was measured by use of a balloon sealed over the distal end of a polyethylene catheter and placed in the distal third of the esophagus. A face mask with attached pneumotachograph to measure airflow was placed over the external nares. A lung function computer calculated tidal volume ( $V_T$ ), respiratory rate, dynamic compliance ( $C_{dyn}$ ), pulmonary resistance ( $R_L$ ), and minute ventilation ( $V_E$ ). Data were analyzed by two-way repeated measures analysis of variance and the SNK procedure.

Acupuncture treatment had no effect on any of the measured variables. There was however, a significant time effect on  $dP_{pl_{max}}$ ,  $R_L$ ,  $C_{dyn}$ ,  $V_T$ , and respiratory rate.  $dP_{pl_{max}}$  and  $C_{dyn}$  showed significant ( $P < 0.0001$ ) improvement at the 1- and 2-hour readings;  $R_L$  decreased significantly ( $P < 0.0002$ ) at the 1-, 2-, and 4-hour readings; and respiratory rate decreased ( $P < 0.006$ ) and  $V_T$  increased ( $P < 0.02$ ) at 4 hours. We do not know the reason for these changes but they could have been due to repeatedly moving the horses into the clean laboratory to measure lung function. Our study demonstrates that a single acupuncture treatment has no greater effect on lung function than a placebo treatment. Horses with heaves should receive environmental management and/or pharmacological therapy to relieve their airway obstruction.

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