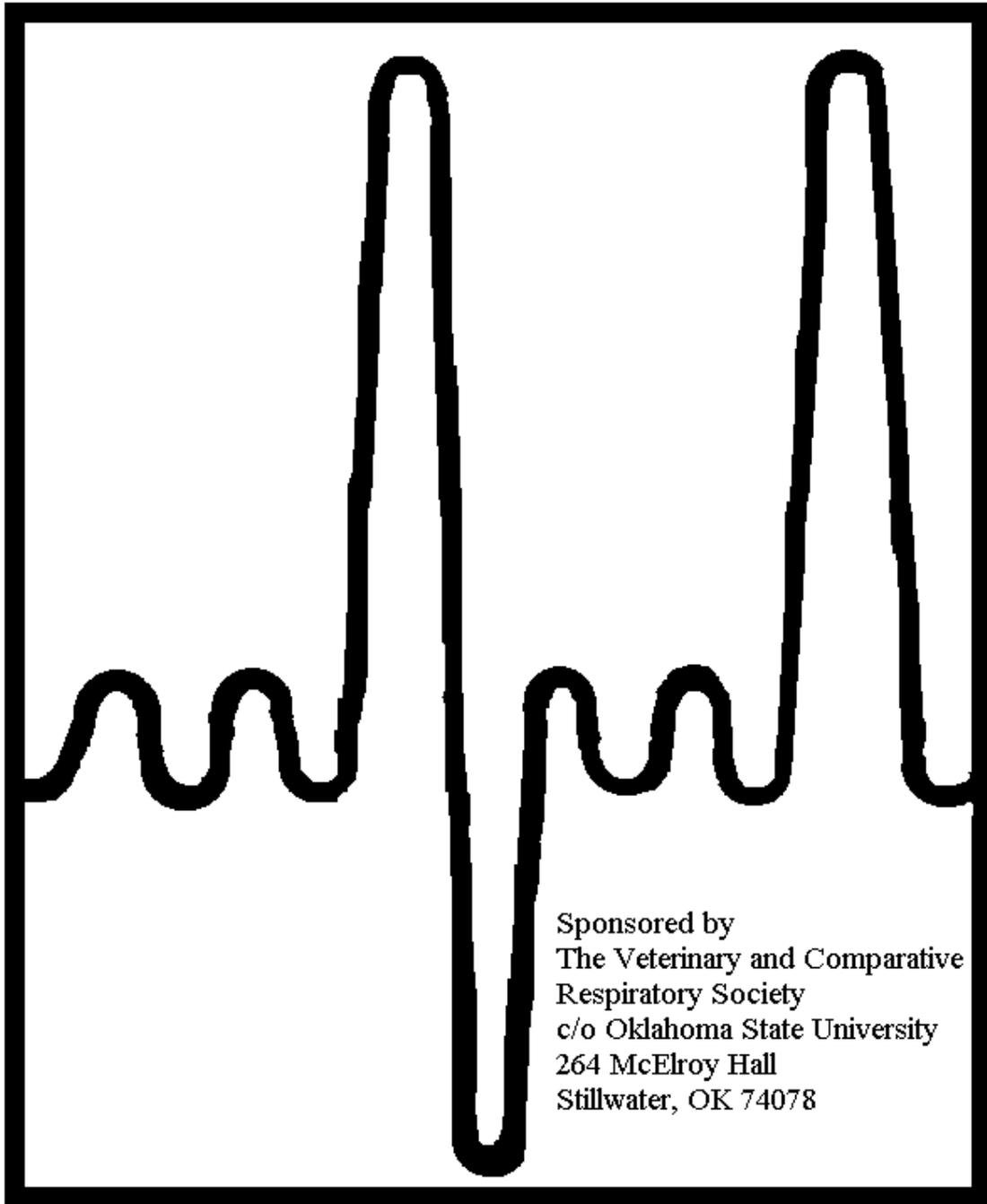


21st Veterinary and Comparative Respiratory Symposium



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21ST SYMPOSIUM OF THE VETERINARY AND
COMPARATIVE RESPIRATORY SOCIETY
SAN ANTONIO, TEXAS
OCTOBER 2 – 5, 2003

Thursday

6:00-7:00 PM Reception

Friday

7:30-8:00AM Breakfast

8:00-8:15 Welcome and Introductory remarks (Dr. Terry Fossum, President, VCRS)

8:15-10:00 “Treatment of Adult Respiratory Distress Syndrome” Dr. Antonio Anzueto, Fellow, UT Health Science Center, San Antonio.

10:00-10:30 Coffee break

10:30-12:00 5 minute introductions to 17 posters (See Pages 5-6 for order of presentation)

12:00-2:00 Lunch and Poster viewing

GRADUATE STUDENT ORAL PRESENTATIONS

2:00-2:20 Subclinical Pyridostigmine Intoxication causes Airway Hyperreactivity to Histamine. KK Williamson, EC McKenzie, CN Pope, MS Davis

2:20-2:40 Pulmonary Antioxidants and Oxidative Stress in Healthy and RAO-Affected Horses following Acute Antigen Exposure. C M Deaton, D J Marlin, N C Smith, P A Harris, R C Schroter and FJ Kelly

2:40-3:00 Mast Cell Protease Concentrations in Equine Bronchoalveolar Lavage Fluid from Control and Heaves Affected Horses. Dacre, K.J., Deaton, C., Marlin, D., Pemberton, A.D., McGorum, B.C.

3:00-3:20 Oxidative DNA Damage in Equine Peripheral Blood Mononuclear Cells (PBMC) Induced by Acute Natural Allergen Challenge. D A Kingston, D J Marlin, N C Smith, C M Deaton, K Saunders, J Kydd, and P A Harris

3:20-3:40 Frequency and Time Domain Analysis of Heart Rate Variability in RAO and Non-RAO Affected Horses S E Norman, L Jeffery and D J Marlin

3:40-4:00 Systemic and Airway Lining Fluid Cortisol Concentrations in Non-RAO And RAO-Affected Horses. J Cartwright, R J Williams, C M Deaton, N C Smith, M C Davies Morel, T L Cuff and D J Marlin

4:00-4:20 Effect of Exercise and Dietary Antioxidant Supplementation on Plasma Cortisol Concentrations in RAO and Non-RAO Affected Horses. T L Cuff, R J Williams, C M Deaton, N C Smith, M C Davies Morel, F J Kelly and D J Marlin

4:20-6:00 POSTER VIEWING (AUTHORS PRESENT)

6:30 Dinner at the Don Strange Ranch

Saturday

7:30-8:00 Breakfast

8:15-10:00 “Acute Respiratory Distress Syndrome (ARDS): Update on pathogenesis and new therapeutic directions” Dr. David Tweardy, Professor of Medicine and Chief, Section of Infectious Diseases, Baylor College of Medicine, Houston, TX

10:00-10:20 Coffee Break

THEMATIC ABSTRACT SESSION: ARDS and INFECTIOUS DISEASE

10:20-10:40 Acute Interstitial Pneumopathy in the Horse – Experimental Induction with Perilla Ketone. Monica Venner, Equine Clinic, School Of Veterinary Medicine Hannover

10:40-11:00 Variable Ventilation Improved Oxygenation and Lung Elastance in Sheep with Adult Respiratory Distress Syndrome (ARDS). C.L. Bellardine, A. Hoffman, L. Tsai, E.P. Ingenito, F. Lopez , W. Sanborn B. Suki, K.R. Lutchen

11:00-11:20 In Vitro Lung Alveolar Epithelial Cell Injury and Inflammatory Response to Particulate Matter-Associated Metals – Modulation by Exposure to TNF α , IL-1 β , or IFN γ . Dye J., Peoples KE, Hayes CL

11:20-11:40 Expression of Bovine Mx1 Protein in Vero Cells Confers Protection against Influenza A Virus. Baise E., Pire G., Leroy M. & Desmecht D

11:40-12:00 Comparison of Azithromycin, Clarithromycin and Erythromycin for the Treatment of Foals with *Rhodococcus Equi* Pneumonia. Steeve Giguère, Stephanie Jacks, Gregory D. Roberts, Jorge Hernandez, Maureen T. Long and Christina Ellis

12:30-6PM Lunch and field trip (Sea World San Antonio)

7:00-8:00 VCRS Banquet & Presentation of the Joan O'Brien Award

8:00-9:00 Evening lecture: Dr. Randy Martin, Associate Dean for Clinical Development, Professor of Medicine (Cardiology), Director, Noninvasive Cardiology, Emory University, Atlanta Georgia

Sunday

8:30-9:00 Breakfast

THEMATIC ABSTRACT SESSION: ADVANCED DIAGNOSTICS AND RAO

9:00-9:20 Lung Biopsy in Horses – Results and Adverse Effects of Two Techniques. M. Venner , S. Schmidtbauer and E. Deegen

- 9:20-9:40 Use of a Halogen Assay for Detection of IgE to Specific Aeroallergens in Horses. J.L. Hodgson, R.F. Kent, D.R. Hodgson, S. McAleese, B.C. McGorum, B.G. Green, T.O'Meara
- 9:40-10:00 Exhaled Breath Condensate (EBC) Collection in Cats – Description of a Non-Invasive Technique to Investigate Airway Disease. C M Deaton, A H Sparkes, E J Mardell, N Kirschvink and D J Marlin
- 10:00-10:20 Attenuation of ^{99m}technetium in the Equine Thorax. D J Marlin, D A Kingston, J Weekes, C M Deaton and R C Schroter
- 10:20-10:40 Bronchiectasis in Three Adult Horses with Heaves. S. Dalle, L. Breton, P. Hélie, J.-P Lavoie
- 10:40-11:00 Investigation of Airway Electrolyte Concentrations in RAO and Non-RAO Affected Horses. V Fowler, D J Marlin, R Williams, J Edwards, & C M Deaton
- 11:00-11:20 The Application of the Comet (Single Cell Gel Electrophoresis) Assay to Study of Oxidative Stress and Genotoxicity in Equine Pulmonary Disease. D J Marlin, D A Kingston, C M Deaton, K Saunders, J Kydd And P A Harris
- 11:20-11:40 Energetic Cost of Breathing and Pulmonary Function in Horses with Recurrent Airway Obstruction and Weight Loss. Melissa R. Mazan, Andrew Hoffman, Shane DeWitt, Edward F. Deveney
- 11:40-12:00 VCRS Business Report, and Introduction of Incoming President

Posters (in order of brief presentation)

NEW INSIGHTS INTO NON-INVASIVE OSCILLATORY MEASUREMENTS OF RESPIRATORY SYSTEM IMPEDANCE (Z_{rs}) IN THE HORSE

Bedenice, D.; Mazan, M.R. and Hoffman, A.M.

CYTOLOGICAL ABNORMALITIES IN BRONCHOALVEOLAR LAVAGE SAMPLES FROM RESTING ALASKAN SLED DOGS

EC McKenzie, KK Williamson, SL Nelson, MS Davis

HYDROGEN PEROXIDE IN BREATH CONDENSATE AS MARKER OF LOWER AIRWAY INFLAMMATION IN AN EXPERIMENTAL MODEL OF FELINE ASTHMA

Kirschvink N, Marlin D., Delvaux F, Leemans J, Vincke G, Clercx C, Snaps F, Gustin P

EFFECTS OF SOMATIC GROWTH AND CIRCADIAN RHYTHM ON RESPIRATORY VARIABLES ASSESSED BY WHOLE BODY BAROMETRIC PLETHYSMOGRAPHY IN HEALTHY CATS

Leemans J, Kirschvink N, Delvaux F, Vincke G, Marlin D., Gustin P

PULMONARY OXIDATIVE STRESS BY CADMIUM INHALATION IN AN ANIMAL MODEL OF BRONCHO-PNEUMOPATHY

Kirschvink N, Martin N, Vincke G, Marlin D., Smith N, Gustin P

PHYSIOLOGICAL VALUES FOR PH AND PCO_2 IN EXHALED BREATH CONDENSATE SAMPLES FROM PIGS AND CALVES

Reinhold, P; Jaeger, J; Langenberg, A.; Foedisch, G.; Marlin, D.

A QUANTITATIVE ANALYSIS OF COLLAGEN DEPOSITION IN THE HEALTHY EQUINE LUNG

C. Barnim, C. Furness and L. Viel

EFFECT OF THE VIRAL LOAD INOCULATED ON THE PATTERN OF PNEUMONIA INDUCED BY SENDAI VIRUS IN THE BALB/C MOUSE

Faisca P., Baise E., Leroy M., & Desmecht D.

VALIDATION OF THE BALB/c MOUSE AS A MODEL OF SWINE INFLUENZA

Flandre T., Leroy M. & Desmecht D.

ALLELIC DIVERSITY AT THE CARBOXY-TERMINAL END OF THE PORCINE MYXOVIRUS RESISTANCE PROTEIN (MX 1)

Thomas A., Palm M., Baise E., Leroy M. & Desmecht D.

Analysis of the CD11a-encoding cDNA in *Bos taurus*

Zecchinon L., Fett T., Baise E., Leroy M. & D. Desmecht D.

ALLELIC DIVERSITY AT THE CARBOXY-TERMINAL END OF THE BOVINE MYXOVIRUS RESISTANCE PROTEIN (MX1)

Gérardin J., Baise, E., Leroy, M., & Desmecht, D.

MODELIZATION OF AN INTERSTITIAL PNEUMONITIS USING THE PNEUMONIA
VIRUS OF MICE IN THE BALB/c MOUSE

Bui Tran Anh D., Baise E., Leroy M. & Desmecht D.

STUDENT: READMINISTRATION OF ADENOVIRUS/CALCIUM PHOSPHATE CO-
PRECIPITATES SEGMENTALLY TO THE LUNG IN THE SHEEP

T. Brown, D. Collie and J-M. Sallenave

STUDENT: EQUINE TRYPTASE AND PUTATIVE EQUINE MAST CELL
PROTEINASE-1: CDNA CLONING AND SEQUENCING

Dacre, K.J., McAleese, S.M., Pemberton, A.D. and McGorum, B.C.

STUDENT: PULMONARY SURFACTANT IN NORMAL FOALS AND FOALS WITH
BACTERIAL PNEUMONIA

K. Uukkivi, W. McDonell, L. Viel, J. Caswell, R. Veldhuizen, J. Lewis, C. Kerr

STUDENT: EFFECT OF ACUTE ANTIGEN EXPOSURE ON FUNCTIONAL RESIDUAL
CAPACITY (FRC) IN HEALTHY HORSES AND HORSES WITH RECURRENT
AIRWAY OBSTRUCTION

C M Deaton, D J Marlin & R C Schroter

STUDENT: INTERFERON ALPHA-INDUCED RESISTANCE TO BOVINE PI-3 VIRUS
IS MEDIATED THROUGH THE MX PATHWAY

Leroy M., Pire G., Gérardin J., Baise E. & Desmecht

Acute Respiratory Distress Syndrome (ARDS): Update on pathogenesis and new therapeutic directions

INTRODUCTION, DEFINITIONS AND PHASES

ARDS has been the subject of a recent review (1), as well as a National Heart Lung and Blood Institute Workshop, which produced a document (2) charting future research directions in ARDS. This State-of-the-Art update on ARDS will focus on recent developments in pathogenesis of ARDS and the implications of these developments on new therapeutic strategies. ARDS was first described in 1967 in 12 patients with acute respiratory distress, cyanosis refractory to oxygen therapy, decreased lung compliance, and diffuse infiltrates evident on the chest radiograph (3) and initially called the adult respiratory distress syndrome (4). An expanded definition was proposed in 1988 that quantified the physiologic respiratory impairment using a four-point lung-injury scoring system (5), then in 1994, a new definition was recommended by the American-European Consensus Conference Committee (6), which, among other things, delineated patients with less severe hypoxemia (as defined by a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of 300 or less, who are considered to have acute lung injury, ALI) from those with more severe hypoxemia (as defined by a ratio of 200 or less who are considered to have the ARDS).

ALI/ARDS has three stages with distinct clinical, histopathological, and radiographic manifestations. The acute, or exudative, phase characterized by the rapid onset of respiratory failure in a patient with a risk factor for ALI/ARDS. Pathologically, one sees diffuse alveolar damage, with neutrophils, macrophages, erythrocytes, protein-rich edema fluid in the alveolar spaces, hyaline membranes, capillary injury, and disruption of the alveolar epithelium. ALI/ARDS may resolve completely in some patients after the acute phase, while in others it progresses to fibrosing alveolitis. Histologically, there is fibrosis along with acute and chronic inflammatory cells and partial resolution of the pulmonary edema. The recovery phase is characterized by the gradual resolution of hypoxemia and improved lung compliance. Typically, the radiographic abnormalities resolve completely and in many patients pulmonary function returns to normal.

EPIDEMIOLOGY AND PROGNOSIS

Clinical disorders that predispose to ALI/ARDS can be divided into those associated with direct injury to the lung (most commonly pneumonia and aspiration of gastric contents) and those that cause indirect lung injury in the setting of a systemic process, most commonly severe sepsis and trauma. Mortality rates from ARDS have ranged from 40 to 60 percent in the past with the majority of deaths attributable to sepsis or multiorgan dysfunction rather than primary respiratory causes. More recent studies indicate a reduced mortality of 36 percent (7) and 34 (8) which likely reflect improvement in treating conditions such as sepsis predisposing to ALI/ARDS and emphasize the importance of using randomized control subjects rather than historical controls in clinical studies.

PATHOGENESIS

Initiation and Perpetuation of the Acute Phase

Information from animal studies and clinical trials targeting disorders that predispose to ARDS, most notably sepsis and trauma, have provided the most insight into the molecular pathogenesis of ALI/ARDS. Direct experimental data on initiation of ALI/ARDS comes from animal studies of sepsis and hemorrhagic shock models. These studies underscore the importance of innate immune system pathways (Toll-like receptors, TLR, and pathogen-associated molecular patterns, PAMPs) and reactive oxygen and nitrogen species that activate pivotal transcription factors notably NF- κ B within cells (9, 10). NF- κ B binds to the promoters of a variety of pro-inflammatory cytokine genes (TNF- α , IL-1 β , IL-6 and G-CSF) and pro-coagulant genes e.g. tissue factor (TF) leading to their upregulation.

Increased pro-inflammatory cytokines result in neutrophil recruitment, which can damage endothelial and epithelial cells, in part, through the action of neutrophil elastase within their granules. In addition, neutrophils themselves can contribute a second wave of pro-inflammatory cytokine production. Recently, the critical importance of epithelial injury to both the development of and recovery from ALI/ARDS has become better recognized and the degree of alveolar epithelial injury is an important predictor of outcome. The normal alveolar epithelium is composed of two types of cells. The alveolar surface consists of flat type I cells, which make up 90 percent of the alveolar surface area cuboidal type II cells, which make up the remaining 10 percent and whose functions include production of surfactant, ion transport, and proliferation and differentiation into type I cells after injury. The loss of epithelial integrity in ALI/ARDS has a number of consequences including contributing to alveolar flooding, disruption of normal epithelial fluid transport (which impairs removal of edema fluid from the alveolar space), reduced production and turnover of surfactant, predisposition to septic shock in patients with bacterial pneumonia and fibrosis.

Increase expression of TF on monocytes and a subset on endothelial cells results in increased generation of thrombin resulting in increased fibrin deposition and fibrinolysis, which has the potential for perpetuation of the inflammatory cascade by generation of innumerable microvascular ischemia/reperfusion injuries.

Fibrosing Alveolitis

After the acute phase, ALI/ARDS in some patients resolves rapidly while in others progresses to fibrotic lung injury. The alveolar space becomes filled with mesenchymal cells and their products and new blood vessels. The finding of fibrosing alveolitis on histological analysis correlates with an increased risk of death and patients who die of the condition have a marked accumulation of collagen and fibronectin in the lung at autopsy. The process of fibrosing alveolitis begins early in the course of ALI/ARDS and may be promoted by early proinflammatory mediators such as interleukin-1 and the early appearance of procollagen III in the alveolar space is associated with an increased risk of death.

Resolution

Resolution of ALI/ARDS involves removal of alveolar water, soluble and insoluble protein and dead cells. While removal of apoptotic cells is mediated by macrophages, removal of water and protein requires type II cell to regenerate type I cells. This proliferation is

controlled by epithelial growth factors, including keratinocyte and hepatocyte growth factors, which have been incorporated into new strategies to hasten resolution as outlined below.

NEW APPROACHES TO TREATMENT

Surfactant Therapy

In one study, treatment with a synthetic surfactant had no effect on oxygenation, the duration of mechanical ventilation, or survival (11). However, newer preparations of surfactant that contain recombinant surfactant proteins and new approaches to their instillation, including tracheal instillation and bronchoalveolar lavage, are being evaluated in clinical trials and will be discussed.

Glucocorticoids and Other Antiinflammatory Agents

Glucocorticoids had no benefit when they were given before the onset of the disease or early in its course. More recently, glucocorticoids have been used to treat the later, fibrosing-alveolitis phase of the disease and a short course of high-dose glucocorticoids could be considered as rescue therapy in patients with severe disease that is not resolving. In addition to glucocorticoids, other antiinflammatory agents designed to interrupt the process of acute lung injury have been investigated but have proved unsuccessful. The failure may reflect the complexity and redundancy of the inflammation in acute lung injury or the inability to deliver these agents early enough in the course of the illness. New anti-inflammatory strategies targeting nitrogen/oxygen reactive species, NF- κ B and neutrophils elastase are being pursued experimentally and will be discussed.

Acceleration of Resolution

Since acute injury to epithelial type I cells causes denudation of the alveolar epithelium an additional approach to hastening the resolution of acute lung injury and the acute respiratory distress syndrome is to accelerate reepithelialization of the alveolar barrier. Administration of keratinocyte growth factor protects against lung injury probably in part by increasing the proliferation of alveolar type II cells and the clearance rate of alveolar fluid and by inducing antioxidant effects and perhaps by reducing lung endothelial injury. Strategies directed at restoring the function of alveolar epithelium will be reviewed.

PREVENTION OF ARDS

Exciting progress has been made recently in the treatment of acutely and severely ill patients with disorders predisposing to ALI/ARDS including activated protein C (12), and early goal-directed therapy for severe sepsis, moderate dose steroids (13) for refractory septic shock and tight control of blood sugar in surgical intensive care unit patients (14) which demonstrate reduced mortality in these patient groups which may be attributable to decreased incidence of ALI/ARDS. In addition, the study in which low tidal volume reduced mortality by 22 percent emphasizes the potential contribution of barotrauma to persistence of ALI/ARDS (15).

CONCLUSIONS

Progress continues to be made in the epidemiology and pathogenesis of ALI/ARDS providing new avenues for therapeutic intervention. The importance of animal

experimentation to gain new insights into disease pathogenesis and potential new therapies cannot be overstated. An important recent development is the formation of the NIH Acute Respiratory Distress Syndrome Network which will provide the infrastructure for large, prospective, randomized trials of new ventilatory and pharmacological strategies developed in animals that will allow rigorous testing of new modalities directed at further reducing mortality from this common clinical syndrome.

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 15. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med*, 342: 1301-1308, 2000.

ORAL PRESENTATIONS

SUBCLINICAL PYRIDOSTIGMINE INTOXICATION CAUSES AIRWAY HYPERREACTIVITY TO HISTAMINE

KK Williamson, EC McKenzie, CN Pope, MS Davis
Oklahoma State University, Stillwater, Oklahoma

Pyridostigmine is a carbamate cholinesterase inhibitor used by the US military for prophylaxis against the lethal effects of nerve agent exposure. The rationale for its use is based on two assumptions: First, a subject can tolerate inhibition of some synaptic acetylcholinesterase (AChE) without displaying clinical signs of toxicity; second, that when synaptic AChE is bound by pyridostigmine, cholinesterase inhibitor nerve agents such as Sarin and Soman cannot bind the enzyme. In contrast to organophosphates and other weaponized cholinesterase inhibitors, the binding of pyridostigmine to AChE is readily reversible. Therefore, the strategy for the successful use of pyridostigmine is to “occupy” a certain fraction of AChE molecules so that upon exposure to irreversibly binding nerve agents, the victim can be removed from the exposure, stop receiving pyridostigmine, and subsequently recover sufficient AChE to lessen the chances of morbidity from the nerve agent.

Routine use of pyridostigmine during the First Gulf War was generally without complication, but there were reports of subjects suffering from acute asthma-like attacks while receiving pyridostigmine. Asthmatic bronchoconstriction is partially mediated by vagal reflexes. Thus, we hypothesize that subclinical intoxication with pyridostigmine increases airway sensitivity to vagally mediated bronchospastic stimuli. To test this hypothesis, we tested airway hyperresponsiveness to aerosol histamine in 5 unsensitized laboratory dogs 3 hr after receiving 0.6 mg/kg pyridostigmine once *per os*. This dose and interval has previously been shown to produce approximately 30% inhibition of AChE in dogs. Dogs were anesthetized with propofol and ventilated at a constant 20 breaths/min and 17 ml/kg tidal volume. Pulmonary resistance and dynamic compliance were measured using a Hans Rudolph pulmonary mechanics computer. Baseline values were the mean of the 20 breaths preceding the histamine challenge. Histamine challenge was delivered as a single 15 s burst of aerosol (5 mg/ml histamine), and the response to histamine (mean of 20 breaths post challenge) was expressed as a net change from the pre-challenge baseline and as a percentage of the baseline. Data were analyzed as paired comparisons (pyridostigmine vs. control), and $p < 0.05$ was considered significant.

There was no significant difference in baseline compliance between control and pyridostigmine administration. However, pretreatment with pyridostigmine caused a significantly greater response to histamine compared to controls (See Table 1). Baseline pulmonary resistance was significantly lower in dogs receiving pyridostigmine, and though pyridostigmine appeared to increase the resistance response to histamine, this effect did not reach statistical significance (See Table 2).

Table 1: Effect of Pyridostigmine on Pulmonary Compliance (ml/cm H₂O). All data are expressed as mean \pm S.D.

Treatment	Baseline	Net Response	% Baseline
Control	48.61 \pm 4.01	6.00 \pm 4.52	87.60 \pm 9.22
Pyridostigmine	50.65 \pm 5.98	10.44 \pm 1.99	79.39 \pm 2.84
T-test	p = 0.09	p = 0.05	p = 0.05

Table 2: Effect of Pyridostigmine on Pulmonary Resistance (cm H₂O/L/S). All data are expressed as mean ± S.D.

Treatment	Baseline	Net Response	% Baseline
Control	11.28 ± 1.41	1.91 ± 1.27	85.84 ± 8.02
Pyridostigmine	10.36 ± 1.58	2.68 ± 0.99	79.66 ± 5.43
T-test	p = 0.04	p = 0.13	p = 0.06

Our data support the hypothesis that subclinical pyridostigmine intoxication can lead to airway hyperresponsiveness to histamine. Further studies are planned to determine the precise mechanism of action of pyridostigmine with regard to changes in pulmonary function, and the relative capacity of other cholinesterase inhibitors to create similar airway hyperresponsiveness.

PULMONARY ANTIOXIDANTS AND OXIDATIVE STRESS IN HEALTHY AND RAO-AFFECTED HORSES FOLLOWING ACUTE ANTIGEN EXPOSURE

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We have previously demonstrated that ascorbic acid is quantitatively the most important antioxidant in equine bronchoalveolar lavage fluid (BALF). In addition, horses affected by recurrent airway obstruction (RAO) with naturally occurring chronic, low-grade airway inflammation, but no signs of dyspnoea, had a reduced concentration of ascorbic acid in BALF and an increase in hydrogen peroxide (H₂O₂) in expired breath condensate (EBC). RAO-affected horses develop neutrophilic airway inflammation and bronchoconstriction when stabled on hay and straw. The aim of the present study was to determine the effects of acute exposure to hay and straw on the concentrations of ascorbic acid in BALF and H₂O₂ in EBC in RAO and non-RAO affected (control) horses. Six RAO-affected and six control horses were stabled for 24 hours with straw bedding and hay. Ascorbic acid and dehydroascorbate (oxidised ascorbic acid) concentrations and neutrophil counts in BALF, H₂O₂ in EBC and airway resistance were determined 7 days prior to stabling and immediately, 3 and 14 days post-exposure. Four out of six RAO-affected horses demonstrated an increase in airway resistance after 24h exposure to hay and straw. No alterations in airway resistance were observed in the control group. Immediately after stabling, RAO-affected horses had an increased number of neutrophils in BALF (186, 113–325/ul; median, 25th–75th percentiles), which returned to pre-exposure levels (10, 7–12/ul) by 14 days post-exposure (P=0.03). Control horses demonstrated a smaller increase in neutrophil number following stabling (21, 17–36/ul post-exposure *versus* 6, 5–7/ul pre-exposure; P=0.046) compared to RAO-affected horses (P=0.004). In RAO-affected horses, BALF ascorbic acid decreased immediately after stabling (4, 3–6 umol/l) compared to prior to stabling (11, 8–14 umol/l; P=0.03). BALF ascorbic acid three days after stabling (9, 5–10 umol/l) was not significantly different from prior to stabling. The decrease in ascorbic acid was not associated with an increase in the concentration of dehydroascorbate. In the control group, BALF ascorbic acid decreased immediately after stabling, but not significantly (11, 10–12 umol/l *versus* 13, 12–16 umol/l). H₂O₂ in EBC did not increase following exposure in either group. When data from the two groups was combined, the percentage decrease in ascorbic acid correlated with the percentage increase in the number of neutrophils (r=0.80, P=0.002) and the percentage increase in airway resistance (r=0.77, P=0.004). In conclusion, ascorbic acid appears to provide the primary defence against neutrophil-derived reactive oxygen species during acute transient neutrophilic inflammation in RAO-affected horses and an increase in H₂O₂ in EBC may only occur if the concentration of ascorbic acid is reduced further.

MAST CELL PROTEASE CONCENTRATIONS IN EQUINE BRONCHOALVEOLAR LAVAGE FLUID FROM CONTROL AND HEAVES AFFECTED HORSES

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Heaves is characterised by reversible pulmonary inflammation induced by inhalation of organic stable dusts and is similar to human organic dust associated asthma (OA). Some studies have supported the role of mast cells (MC) in the pathogenesis of these diseases¹⁻³, and indeed, the action of MC mediators could explain many of the features of heaves. This study was performed to investigate the role of tryptase and equine mast cell protease-1 (eq.MCP-1) in the pathogenesis of heaves.

Equine bronchoalveolar lavage fluid (BALF) was collected from control or heaves affected horses following 6, 12, 24h and chronic natural challenge. Supernatant was isolated (400g, 10min) and concentrated with a centrifugal filter device prior to determination of protease concentration by ELISA using rabbit polyclonal anti-equine tryptase / eq.MCP-1.

Immunofluorescence of BALF cytopins was performed using the above primary antibodies followed by donkey anti-rabbit IgG - Alexa-fluor 488. The number of positive cells per 500 cells was counted.

There was no significant difference in tryptase concentration between control and heaves affected horses. Similarly, there was no significant difference in the number of tryptase positive MC on cytopin preparations from control and heaves affected horses. There was no detectable eq.MCP-1 in equine BALF and eq.MCP-1 positive MC were scarce on cytopin preparations.

OXIDATIVE DNA DAMAGE IN EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) INDUCED BY ACUTE NATURAL ALLERGEN CHALLENGE

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Oxidative stress occurs when antioxidant defence mechanisms are overwhelmed by free radicals and may lead to damage to DNA, which has been implicated in processes such as ageing and cancer. The Comet assay allows detection of oxidative DNA damage in individual cells. As horses with recurrent airway obstruction (RAO) have been shown to demonstrate low antioxidant status and oxidative stress, we hypothesised that peripheral blood mononuclear cells (PBMC) of horses with RAO would demonstrate increases in DNA damage following natural allergen challenge.

Six horses (mean age 15 years, range 8-23 years) diagnosed with RAO (in remission) and 6 healthy breed matched controls (mean age 9 years, range 5-15 years) were studied. Blood samples were collected 7 days prior to challenge and immediately and 3 days after stabling on mouldy hay and straw for 24h. All animals were kept at grass prior to and after the challenge period. Bronchoalveolar lavage (BAL) was performed and neutrophil counts determined. Venous blood samples (15ml) were placed into plain glass tubes containing 0.5ml 15u/ml preservative free heparin in phosphate buffered saline (PBS). PBMC were isolated by gradient centrifugation. Comet assay and analysis by visual scoring were carried out according to Heaton *et al.* (2002). Endogenous damage (endo) and susceptibility to oxidative damage following hydrogen peroxide incubation (exo) were determined in each sample at each time point.

Immediately after stabling, RAO-affected horses had an increased number of neutrophils in BAL fluid (from 10 ± 4 / μ l to 225 ± 136 / μ l; mean \pm sd), which declined to 35 ± 29 / μ l by 3 days post-exposure. Control horses demonstrated a smaller increase in neutrophil number following stabling (28 ± 21 / μ l at 24h from 10 ± 4 / μ l at -7day) compared to RAO-affected horses ($P=0.004$). There were no differences in mean endo or exo oxidative DNA damage between the control (endo= 38 ± 7 arbitrary units, AU; exo= 59 ± 11 AU) and RAO-affected groups (endo= 37 ± 9 AU; exo= 71 ± 21 AU), prior to stabling and neither group demonstrated an increase in endogenous or exogenous DNA damage after challenge.

In conclusion, acute natural allergen challenge does not induce oxidative DNA damage or increase the susceptibility to damage of circulating PBMC in either RAO-affected horses or non-RAO controls.

Heaton, P.R., Ransley, R., Charlton, C., Mann, S.J., Stevenson, J., Smith, B.H., Rawlings, J.M. and Harper, E.J. (2002) Application of single-cell gel electrophoresis (Comet) assay for assessing levels of DNA damage in canine and feline leukocytes. *J. Nutr.* **132**: 1598S-1603S

FREQUENCY AND TIME DOMAIN ANALYSIS OF HEART RATE VARIABILITY IN RAO AND NON-RAO AFFECTED HORSES

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Analysis of heart rate variability (HRV) is a commonly used technique for investigation of autonomic activity. Studies in man have demonstrated reduced HRV in patients with chronic obstructive pulmonary disease, chronic obstructive bronchitis and asthma. HRV has also been applied as an index of autonomic control in the horse, for example in relation to the study of exercise, transport and behaviour. The aim of the present study was to determine if there were differences in autonomic balance in horses affected by recurrent airway obstruction (RAO) compared with non-RAO affected controls using short-term frequency and time domain analysis.

Five non-RAO affected and five RAO affected horses in remission were studied. Horses were matched for age and type. One hour recordings of ECG signal were obtained from each horse using telemetry on two occasions, 24-48h apart, between 2pm and 6pm. Matched RAO and non-RAO horses were always analysed within the same period on each day. The ECG signal was digitised at 1000Hz and RR-intervals calculated online using a Po-Ne-Mah data acquisition and analysis system. RR intervals were verified following recording and errors (<5% of all beats) were identified manually and remeasured using digital callipers. All analysis was performed on blocks of 2048 consecutive beats. For time domain analysis, RMSSD (root mean square differences between consecutive beats) and pRR-50 (% of all consecutive beat differences >50ms) were calculated. For frequency domain analysis blocks of 2048 beats were subjected to the Fast Fourier Transform (FFT) using a sampling interval of 1 and power was estimated using a Hamming window.

There was no significant difference between any indices of HRV between the first and second collection days. There were no differences between non-RAO and RAO affected horses in RMSSD (110 ± 44 and 104 ± 14 , respectively) or pRR-50 (49 ± 10 and 53 ± 6 , respectively). There was also no difference between non-RAO and RAO affected horses for absolute or normalised LO or HI power, LO-HI ratio, PNSI (HI/TOTAL) or SNSI (LO/HI) indicators. RAO affected horses showed a trend (3 out of 5 horses) to lower TOTAL power (4.3 ± 1.3) compared with non-RAO affected controls (7.0 ± 3.6 ; $P=0.07$).

Behavioural studies in horses have demonstrated that individual temperament has a marked influence on HRV indices and no attempt was made in the present investigation match on these criteria. The finding of a trend towards a reduction in TOTAL power is consistent with studies in man on patients with respiratory disease. Further studies are indicated using larger numbers of animals and matching for temperament.

SYSTEMIC AND AIRWAY LINING FLUID CORTISOL CONCENTRATIONS IN NON-RAO AND RAO-AFFECTED HORSES

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During allergen exposure, horses affected by recurrent airway obstruction (RAO) exhibit exaggerated immune mediated responses compared with non-RAO affected horses. A decrease in plasma cortisol concentration is often interpreted to indicate immunosuppression. We therefore hypothesised that RAO affected horses would have higher plasma and or BAL cortisol concentrations compared with non-RAO affected controls. Eight non-RAO affected horses without airway inflammation and 6 RAO-affected horses with and without mild airway inflammation were studied.

Blood samples and bronchoalveolar lavage fluid (BALF) for cortisol analysis were collected between 9 and 11am. Cortisol was analysed in duplicate by RIA. In addition, BALF total protein was determined by a modified Coomassie Blue method. For BALF cytology, a cytopsin preparation was made and stained with haematoxylin and eosin and a minimum of 200 cells were counted to obtain a differential count. Total nucleated cell counts were performed manually using a haemocytometer. Data are presented as mean±sem.

BALF neutrophil percentages were 3±1 (non-RAO), 13±2 (RAO +inflammation) and 3±1 (RAO –inflammation). Plasma cortisol concentration was not different between non-RAO affected animals (140±19 nmol/l) and RAO's with (128±6 nmol/l) or without inflammation (142±15 nmol/l; P>0.05). BALF cortisol was also not different between non-RAO affected animals (12.7±3.0 nmol/g protein; or 1.7±0.4 nmol/l BALF) and RAO's without (7.4±2.1 nmol/g protein; 0.9±0.4 nmol/l BALF) or with inflammation (7.4±0.3 nmol/g protein; 1.0±0.3 nmol/l BALF; P>0.05). There was a weak, but significant, positive correlation between plasma and BALF cortisol concentrations ($r=0.64$, $P<0.02$).

In conclusion, RAO-affected horses do not appear to have evidence of increased plasma or BAL cortisol concentrations either in the presence of mild inflammation or in remission.

EFFECT OF EXERCISE AND DIETARY ANTIOXIDANT SUPPLEMENTATION ON PLASMA CORTISOL CONCENTRATIONS IN RAO AND NON-RAO AFFECTED HORSES

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Exercise has been shown to increase the concentration of plasma ascorbic acid (AA) in both AA synthesising (e.g. the horse) and non-synthesising species (e.g. man). The mechanism for the exercise-induced increase in AA is not clear, but co-release with cortisol from the adrenal cortex or cortisol stimulated release have both been postulated. In view of the fact that recurrent airway obstruction (RAO)-affected horses have low plasma AA, the aim of the present investigation was to determine: 1) the simultaneous AA and cortisol responses to exercise in RAO and non-RAO affected horses; 2) the effect of AA supplementation on these responses to exercise.

Six non-RAO and 5 RAO-affected horses were studied in a 16-week cross-over design. Horses exercised three days a week at the equivalent of 90%VO_{2max}. The study consisted of a 4-week lead-in period, a 4-week supplementation period, a 4-week washout period and a 4-week supplementation period. During each supplementation period the horses were fed a pellet providing 10mg/kg bodyweight of AA. At the end of each period an exercise test was conducted consisting of 2 min exercise at 70, 80 and 90%VO_{2max}. Blood samples were collected before and at 0, 15, 60 min and 24h post-exercise. Plasma cortisol was analysed by RIA and AA by HPLC.

Plasma cortisol was not different prior to exercise between RAO (115±6 nmol/l; mean±sd) and non-RAO horses (132±35 nmol/l; P>0.05). Plasma AA prior to exercise was lower in the RAO group (10.8±3.5 umol/l) compared with the non-RAO group (17.4±4.6 umol/l; P=0.026). Exercise resulted in increases in plasma cortisol in both groups and there was no difference between the responses of RAO and non-RAO horses and no effect of supplementation. Plasma AA was increased following exercise in both RAO and non-RAO groups, but only significantly following supplementation. Plasma AA and cortisol delta increase between pre and end of exercise in lead-in and treatment periods were correlated in non-RAO horses (r=0.92, P=0.01 and r=0.77, P=0.08, respectively) but not in RAO affected horses (r= -0.42, P=0.49 and r= -0.23, P=0.71, respectively).

The association between the change in plasma cortisol and the change in plasma ascorbic acid with exercise in non-RAO horses is in agreement with a previous report in human athletes after a 21km race¹. The explanation for the lack of any similar relationship in RAO-affected horses is at present unclear.

¹Gleeson, M., Robertson, J.D. and Maughan, R.J. (1987) Influence of exercise on ascorbic acid status in man. Clin Sci (Lond) 73, 501-505.

ACUTE INTERSTITIAL PNEUMOPATHY IN THE HORSE – EXPERIMENTAL INDUCTION WITH PERILLA KETONE

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In the present study the chemical substance Perilla ketone (PK) was injected intravenously (PK: 6 mg/kg BW) into ten adult horses to induce an acute interstitial pneumopathy. Repeated blood gas analysis and clinical, cytological, sonographical, radiological and histological examinations made it possible to follow the changes occurring in the respiratory tract.

Before injection of PK, the horses showed a physiological lung parenchyma. Histological examination of ensuing lung biopsy showed that a severe interstitial pulmonary edema developed a few days after administration of PK and was accompanied by a thickening of the alveolar walls. The exudative phase of an acute interstitial pneumopathy (AIP) was diagnosed. After administration of PK this was followed at days 8 to 12 by a proliferation of type-II pneumocytes on the alveolar epithelium, and of fibroblasts in the interstitium. These findings are characteristic of the fibroproliferative phase of an AIP. During the following 50 days of the study, the histological changes resolved progressively until a physiological blood-air-barrier was restored, indicating a nearly complete recovery. However, there was a remarkable increase of mast cells in the lung parenchyma until 60 days after administration of Perilla ketone.

The clinical findings in the exudative phase of an AIP revealed a severe dyspnea without respiratory noises or secretions in the trachea. Severe hypoxemia and mild hypercapnia were also determined. Three of the horses had to be euthanized because of dramatic respiratory distress. During the fibroproliferative phase, the clinical findings returned to normal values by day 15 after administration of PK. During the whole period of the examinations, neither clinical nor laboratory changes were noted in other organs.

Cytological examination after bronchoalveolar lavage (BAL) showed a brief increase of neutrophil granulocytes during the exudative phase of an AIP. The values returned to normal by day 15 after injection of PK. On the other hand, the number of total cells and macrophages constantly increased until day 60. There was also an increase in the number of mast cells similar to but ten days later than in the lung parenchyma. The following biochemical parameters were evaluated in the BAL: urea, protein, LDH and alkaline phosphatase. A brief, significant increase of LDH and alkaline phosphatase during the exudative phase.

Chest radiographs revealed increased interstitial density during the exudative and the fibroproliferative phases. Typical findings were blurred bronchi and vessel walls over the entire lung field.

Sonographical examination of the lungs showed a dramatic increase in comet-tail artefacts on both sides of the thorax and over the lung field. In comparison with the findings of the original examination, there was a significant increase in these artefacts until day 60 after administration of PK.

Perilla ketone induces an acute interstitial pneumopathy that can resolve over 60 days. The present study shows that clinical and imaging diagnostic procedures in combination with laboratory findings correlate with histological changes of lung parenchyma in all phases of an acute interstitial pneumopathy. The sonographic, radiological and cytological findings of this model of AIP in horses could be useful for future diagnosis of clinical cases of AIP in equines.

VARIABLE VENTILATION IMPROVED OXYGENATION AND LUNG ELASTANCE IN SHEEP WITH ADULT RESPIRATORY DISTRESS SYNDROME (ARDS).

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Variable ventilation (VV; syn. 'noisy ventilation') describes a relatively new method of mechanical ventilation to vary inflation pressures randomly over a prescribed range, akin to natural breathing. Improvements in gas exchange and lung mechanics have been observed in a rodent model of acute lung injury (Arold SP et al. *AJRCCM* 165:366-71, 2002). The distribution of pressures, based on the pressure-volume curve of the lung, is compromised of more low than high pressure inflations. The merits of VV are therefore hypothesized to provide a 'protective' strategy for life support, while improving gas exchange and lung mechanics by periodic recruitment. We employed a sheep model of ARDS ($P_{aO_2}/F_{IO_2} < 200$ mm Hg) to test this hypothesis. After saline-lavage, sheep were randomized into one of two groups, and ventilated with equivalent mean airway pressures for 4 hrs: conventional ventilation (CV) (n=6, $V_T=10$ ml/kg, f=16 breaths/min, I:E=1:3, $F_{IO_2}=1$, PEEP=7.5) or VV (n=7, mean $V_T=10$ ml/kg, f and I:E chosen to match CV minute ventilation, $F_{IO_2}=1$, and PEEP=7.5). At 30m intervals, arterial blood gases, and static and dynamic (0.2 to 8 Hz) lung resistance (R) and elastance (E) were measured.

After saline lavage and at 0hr, P_{aO_2} levels were 88.4 ± 29.091 mmHg and 107.417 ± 56.623 mmHg and P_{aCO_2} levels were 57.789 ± 11.332 mmHg and 75.142 ± 28.655 mmHg for the VV and CV groups, respectively. After 4hrs of ventilation, the VV P_{aO_2} levels rose to 171.714 ± 121.094 mmHg whereas the CV P_{aO_2} levels were 146.017 ± 118.445 mmHg. The final P_{aCO_2} levels after 4hr of ventilation were 68.514 ± 18.673 mmHg (VV) and 94.233 ± 37.684 mmHg (CV). Although there was not a significant group x time interaction present for either P_{aO_2} or P_{aCO_2} and the responses among both groups was highly variable, the VV group presented significantly higher ($p < 0.05$) P_{aO_2} levels from 2.5hr to 4hr compared to the initial VV 0hr value whereas CV did not. Additionally, the CV group had significantly higher ($p < 0.05$) P_{aCO_2} values from 2.5hr to 4hr compared to the CV 0hr value whereas VV did not.

The low frequency E value, a measure of non-ventilated lung, at 0 hr was 25.002 ± 19.524 cmH₂O and 34.399 ± 24.29 cmH₂O in the VV and CV groups respectively. The 4hr values were 30.045 ± 16.660 cmH₂O (VV) and 49.875 ± 33.903 cmH₂O (CV). There were no significant changes from 0 hr in either of the groups. However, at the 1, 2, and 3hr mark, the low frequency E value was significantly ($p < 0.05$) higher in the CV group when compared to the VV group. For the CV sheep, the static E and the mechanical heterogeneity increased significantly, in contrast to the VV which exhibited the opposite effect.

In summary, VV sheep showed improvement over the 4hr period in both oxygenation and lung mechanics, but the CV sheep did not. These data suggest that VV can affect gas exchange and lung mechanics despite the introduction of identical mean airway pressures. The strategy of VV requires further testing to discern the optimal distribution of pressures and PEEP, and the effects on lung tissue injury and inflammation.

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IN VITRO LUNG ALVEOLAR EPITHELIAL CELL INJURY AND
INFLAMMATORY RESPONSE TO PARTICULATE MATTER-
ASSOCIATED METALS — MODULATION BY EXPOSURE TO TNF α , IL-
1 β , OR IFN γ .

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Epidemiological studies have linked air pollution exposure to adverse respiratory health effects, especially in individuals with pre-existing inflammatory airways disease. C.A. Pope, for example, reported that increases in particulate matter (PM) levels in Utah Valley were positively associated with hospital admissions for respiratory disease, most notably in children and relating to bronchitis and asthma (1989). Furthermore, asthmatic/symptomatic children appeared to suffer the most acute health effects (Pope 1992). Using PM-derived materials extracted from filters collected in Utah Valley, we have demonstrated that rats intratracheally exposed to samples containing relatively high levels of metals [i.e., copper (Cu) and zinc (Zn)] developed significantly greater lung injury, neutrophilic inflammation, and moderately severe alveolitis (Dye *et al* 2001). In the present studies, we investigated specific Cu- or Zn-induced alveolar epithelial effects using the RLE-6TN cell line derived from rat type II pneumocytes. At Cu or Zn exposures that alone were minimally cytotoxic over a 24h period, data indicated that combined Cu+Zn exposure resulted in minor, but significantly greater epithelial injury. In like fashion, relative to saline-exposed controls, Cu+Zn exposure resulted in a significant (2.0-fold) increase in MIP-2 production; while Zn or Cu+Zn exposure resulted in 1.5- and 2.0-fold increases in RANTES, respectively. Analogous to the inflamed lungs of asthmatics, we next exposed cells under “inflammatory conditions” to determine whether metal-induced effects would be exaggerated. Using rat recombinant cytokines, dose-response studies demonstrated that RLE cell production of MIP-2 and RANTES can be acutely up-regulated by exposure to TNF α , IL-1 β , or IFN γ , with combined cytokine exposure resulting in still greater increases. Data also demonstrated that RLE “pre-inflammation” with either IL-1 β or IFN γ prior to Zn or Cu+Zn exposure resulted in significantly greater RANTES production; with negligible effects on cytotoxicity or MIP-2 release. These studies support the possibility that exposure of asthmatics to emission-source PM containing Zn may result in enhanced chemokine (e.g., RANTES) production thus exacerbating eosinophilic inflammation and potentially contributing to relapse of asthmatic symptoms and disease. (*This abstract does not reflect US EPA policy*).

EXPRESSION OF BOVINE MX1 PROTEIN IN VERO CELLS CONFER PROTECTION AGAINST INFLUENZA A VIRUS

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A murine and a human (huMXA) version of interferon-induced MX proteins have been shown to confer resistance against influenza viruses, whereas MX proteins from other species described so far do not. The present study aimed at establishing whether the bovine MX1 gene recently sequenced (Gérardin et al., VCRS meeting, 2002) yield an antiviral MX protein (boMX1).

Two plasmidic vectors were engineered so that the huMXA and boMX1 cDNAs were placed under the control of the immediate-early cytomegalovirus promoter. In three independent experiments, Vero cells populations were (i) transfected with either plasmids, (ii) then infected with a swine influenza A strain (24h after transfection, m.o.i. = 0.1) and (iii) fixed (7h after infection), permeabilized and double immunostained for huMXA or boMX1 on the one hand and viral hemagglutinin on the other for detection by flow cytometry.

MX-expressing cells, whatever transduced with huMXA or boMX1, exhibited a significantly lower infection rate than nonexpressing cells. Moreover, infection rates were systematically lower in boMX1-expressing cells than in huMXA-expressing cells. These findings yield the first evidence that the bovine MX1 protein displays a strong antiviral activity against influenza A viruses.

COMPARISON OF AZITHROMYCIN, CLARITHROMYCIN AND
ERYTHROMYCIN FOR THE TREATMENT OF FOALS WITH
RHODOCOCCUS EQUI PNEUMONIA

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The objective of this retrospective study was to compare the efficacy and safety of azithromycin-rifampin, clarithromycin-rifampin and erythromycin-rifampin for the treatment of *R. equi* pneumonia in foals. Information on age, gender, breed, physical examination findings, laboratory testing and thoracic radiography was obtained from the medical records of 78 foals with culture-confirmed *R. equi* pneumonia. Foals were divided in 3 groups based on the antimicrobial agent selected for therapy. Short-term (discharge from the hospital) and long-term (healthy as a yearling) success rates, days of hospitalization, days with fever, days with tachypnea and percentage of radiographic improvement were compared between groups.

Foals treated with clarithromycin-rifampin had significantly higher odds of overall short-term (OR = 14.42) and long-term (OR = 23.36) treatment success and significantly less days with fever than foals treated with erythromycin-rifampin. Foals treated with clarithromycin-rifampin had a significantly higher percentage of radiographic improvement and a trend toward higher odds of overall short-term (OR = 8.08) and long-term (OR = 10.45) treatment success compared to foals treated with azithromycin-rifampin. Among foals with severe radiographic lesions, the success rates of foals treated with clarithromycin-rifampin both short-term (90.0%) and long-term (85.7%) were significantly higher than that of foals treated with azithromycin-rifampin (16.7%). For each treatment group, the only reported side effect was diarrhea that was mild and self-limiting in most cases.

The combination clarithromycin-rifampin is superior to both azithromycin-rifampin and erythromycin-rifampin for the treatment of *R. equi* pneumonia in foals.

LUNG BIOPSY IN HORSES – RESULTS AND ADVERSE EFFECTS OF TWO TECHNIQUES

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Percutaneous lung biopsy is performed on the human and equine patients in order to determine a lung tumour, a multifocal nodular disorder or a chronic interstitial lung disease. The technique has been described as a safe procedure in the horse.

The aims of the present study were to compare complications occurring and to compare the quality of the biopsy specimen taken with the Tru-Cut needle (14 G) and with a biopsy device (14 G needle).

Lung biopsies were performed on 59 horses with one instrument on one side of the thorax and with the other instrument on the other side. Clinical respiratory parameters were regularly monitored for two hours after biopsy. Endoscopy of the trachea and of the carina revealed possible bleeding into the airways, and radiography showed a possible pneumothorax. Further sonography and as post-mortem measurement of the surface of the bleeding in the lung parenchyma made it possible to detect a local hematoma around the site of biopsy as well as a possible hemothorax.

The results show that this invasive diagnostic method rarely causes clinical signs such as coughing and bleeding at the nostrils. However endoscopy revealed far more bleeding into the airways than clinical examination. In the horse with a physiological breath rate, the biopsy device induced significantly less bleeding than the Tru-Cut needle. Pneumothorax appears to be an extremely rare complication of lung biopsy. The local traumas induced by the biopsy were evaluated by sonography with confirmation by post-mortem examination. Only small hematomas were detected in the lung. The mean diameter of hematomas on the lung surface was 1.4 cm with the Tru-Cut needle and 0.5 cm with the biopsy device. No bleeding into the interpleural space was detected.

The quality of the lung specimen was good regarding collapse of the lung parenchyma and very good regarding bleeding into the alveoli of the probe. There was no significant difference in the number of erythrocytes found in the alveoli of lung specimens collected with the Tru-Cut needle or with the biopsy device.

This study showed that biopsies obtained with the Tru-Cut needle and with the biopsy device were of similar, good quality. However, there were more complications such as hematoma at the site of biopsy and bleeding into the airways following biopsy with the Tru-Cut needle than with the biopsy device.

USE OF A HALOGEN ASSAY FOR DETECTION OF IGE TO SPECIFIC AEROALLERGENS IN HORSES.

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Type I hypersensitivity reactions are thought to play a role in several syndromes affecting the lower respiratory tract of horses, including heaves, Summer Pasture Associated Obstructive Pulmonary Disease and possibly Inflammatory Airway Disease. Synthesis of IgE after exposure to an allergen is the instigator of type 1 hypersensitivities, and the presence of free IgE in serum or respiratory secretions can be used to quantify and determine the allergen specificity of this response. However, a lack of widely available reagents for detection of equine IgE has limited use of this approach in horses.

The recent development of specific monoclonal antibodies to equine IgE, antisera to epsilon chain antigenic peptides and cloning of the alpha subunit of the equine FcεRI have enabled progress in the detection of serum IgE using ELISA. However, these assays rely on purified antigens to determine specificity of the allergic reaction. Furthermore, no studies have examined the presence of specific IgE in bronchoalveolar lavage fluid (BALF) of horses.

We describe the use of a Halogen assay for detection of specific allergens in serum and BALF from horses with heaves and IAD. The Halogen assay was developed for simultaneous immunodetection of allergens and morphologic identification of individual allergenic particles using human serum. It was subsequently modified to monitor personal exposure to allergens through the development of nasal air samplers. These systems were adapted for use in horses.

Briefly, nasal air samplers were applied to horses. Samplers used a programmable vacuum pump that created a constant vacuum source and an impactor into which a protein binding filter was placed. The impactor was attached to the halter of a horse approximately 15cm from the left nostril and within its breathing zone. Vacuum pumps were programmed to run for 2 hours at a flow rate of 2.0 ± 0.1 L/minute while the horse was in its customary environment. At the end of sampling, the particles attached to the protein binding filters were permanently fixed to the filter with a specialized pressure-sensitive adhesive tape. Alternatively, known allergens such as pollen grains, fungal spores and dust mites were applied to filters and adhered with adhesive tape. Allergens were eluted from the trapped particles onto the filter to create a halo around collected particles. Serum and BALF collected from the same horse were assayed to detect the presence of specific IgE within these samples, with a cloned alpha subunit of the equine FcεRI used for detection of equine IgE. Microscopic examination of the stained filters allowed detection of the eluted antigens (halos) around morphologically identifiable particles. Specific individual particles carrying allergens recognized in serum and BALF of horses with heaves included dust mites, dust mite faeces, pollen grains and paucimicronic particles. No particles were identified in horses with IAD.

EXHALED BREATH CONDENSATE (EBC) COLLECTION IN CATS – DESCRIPTION OF A NON-INVASIVE TECHNIQUE TO INVESTIGATE AIRWAY DISEASE

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In man, and more recently in calves, pigs, dogs and horses, collection of exhaled breath condensate (EBC) has been established as a novel and non-invasive means of assessing lower airway inflammation. Various indicators of airway inflammation have been measured in EBC, including hydrogen peroxide (H₂O₂), pH, leukotrienes, prostaglandins and interleukins. In both man and animals, collection systems have required direct connection to the condensing equipment, most commonly via a closely fitting facemask. From experience, this type of approach would be difficult to apply to cats. The aims of the present study were 1) to investigate the feasibility of collecting EBC from unrestrained cats by condensing the effluent bias flow applied to a sealed chamber containing the animal and 2) to determine if H₂O₂ could be measured in the EBC collected.

The system consisted of a perspex chamber (60 × 30 × 30 cm) with a hinged lid and rubber seal. Inlet and outlet ports (2 cm diameter) were placed on opposite sides of the chamber. The outlet port was connected by polyethylene tubing (3cm x 40cm) to the proximal end of a stainless steel “U” tube (1.5cm x 90cm) in an ice-water bath at ~0°C. The distal end of the “U” tube was connected by a second length of polyethylene tubing to a multistage centrifugal exhaust fan via a flowmeter. The bias flow was set at 900 ml/kg/min to ensure that the flow of air through the box was equal to, or in excess of, three times the animal's estimated minute ventilation. It was found that in order to collect ~1ml of EBC required the cat to be in the chamber for 20-30 minutes. This procedure was performed in 6 healthy adult cats (four females, of which three were entire, and two neutered males). All cats tolerated being in the collection box and showed no signs of distress. A thirty-minute collection period resulted in the recovery of 0.9 to 1.2 ml of EBC. The concentration of H₂O₂ in the EBC ranged from <0.01 to 2.1 µmol/l (median 1.0 µmol/l, mean 0.9 µmol/l). These concentrations are within the range reported in other species. This study demonstrates that, using the described system, EBC can be successfully collected from cats in a non-invasive and well-tolerated manner. However, further work is required to 1) establish the effects of variables such as bias flow rate and collection time 2) establish normal ranges for values of inflammatory markers in larger numbers of cats 3) investigate changes associated with disease.

ATTENUATION OF ^{99m}Tc IN THE EQUINE THORAX

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In large animals, such as the horse, the use of techniques such as nuclear scintigraphy can present technical difficulties due to attenuation of low energy radionuclides, such as ^{99m}Tc and ^{81m}Kr, with increasing distance from the detector. This may be of particular significance when trying to undertake studies to investigate the absolute delivered dose of inhaled radio-labelled compounds or when undertaking ventilation-perfusion imaging studies in large animals. As far as we are aware there are no published data on appropriate attenuation factors for the horse. We therefore undertook an *in vitro* study using a sawdust lung phantom and an *in vivo* study to assess attenuation of ^{99m}Tc and ^{81m}Kr.

A lung phantom was constructed from a cardboard box (38cm x 58cm x 57cm) filled with sawdust. Hollow plastic tubes were placed through the box at an angle of 90° to the field of view of the gamma camera and at distances of 0 to 50cm at 5cm intervals. A line-source of ^{99m}Tc (^{99m}Tc-pertechnetate; 10MBq) was placed in a fine bore catheter which was inserted into the phantom in random order. Acquisitions were made using 30 x 2s frames on a 128 x 128 matrix with a low-energy general purpose collimator. Care was taken to ensure that the source was imaged at the central field of view. The procedure was repeated using ^{81m}Kr eluted in humidified air from an ⁸¹Rb generator. To estimate actual attenuation within the thorax (i.e. combined lung and skin, fat, muscle, etc), a 520 kg mare with a chest diameter of 47cm and with no history of pulmonary disease was used. The horse was sedated and positioned. A point source of ^{99m}Tc with an activity of 34MBq was sealed into a fine bore catheter which was placed within the biopsy channel of a 1.8m videocolonscope. Radioactive markers were placed on the skin surface to calculate the relative position of the source within the chest. Counts were obtained with the endoscope on the camera surface. The endoscope was then advanced so that the source was in the left lung. Left lateral and dorsal images were obtained. The procedure was repeated in the trachea, right lung and on the right external chest wall.

In the sawdust phantom there was an exponential decline in count rate for both ^{81m}Kr and ^{99m}Tc, equivalent to 49% and 44%, respectively at 50 cm from the camera. Within the horse at a distance of 25 cm from the camera (i.e. half-way across the chest) the attenuation of ^{99m}Tc was ~90%. The relationship between distance and count rate was described by an exponential equation ($y = 283539e^{-0.0937x}$; $r^2 = 0.92$, $P < 0.0001$). In conclusion, ^{81m}Kr and ^{99m}Tc appear to have similar attenuation characteristics based on the phantom study. *In vivo*, the attenuation characteristics of ^{99m}Tc indicate that in lateral chest images of the lung, the contribution to the image of the contra-lateral lung is minimal. This has important implications for the expression of lung deposition data.

INVESTIGATION OF AIRWAY ELECTROLYTE CONCENTRATIONS IN RAO AND NON-RAO AFFECTED HORSES

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Recurrent Airway Obstruction (RAO) is a condition of the horse with many similar features to human asthma and represents a major cause of morbidity. Recent human studies have identified a link between the aetiology of certain pulmonary diseases and airway electrolyte concentrations. The aim of this study was to investigate whether RAO-affected horses have altered airway electrolyte concentrations when compared to non RAO-affected controls.

Bronchoalveolar lavage (BAL) samples were taken from 20 non-RAO affected (controls) and 19 RAO-affected horses, with (n=6) and without (n=13) airway inflammation. In addition, exhaled breath condensate (EBC) was collected from 5 RAO-affected horses and 6 controls. BAL was performed using 200ml of 0.9% sterile saline. The concentrations of the electrolytes in BAL fluid (BALF) and EBC were determined using flame photometry (Na, K) and an autoanalyser (Cl). The limit of detection for all electrolyte assays was 0.01 mmol/l.

Na, K and Cl were all detected in EBC. There were no differences between RAO and control horses in EBC K or Cl concentrations. Sodium concentration in EBC samples from controls was 1.0 ± 0.8 mmol/l [mean \pm sd], but was not detected in EBC from RAO-affected animals ($P=0.023$). BALF concentrations for Na, Cl and K are expressed as net BAL uptake/efflux, i.e. after adjustment for the concentrations in the saline used for the BAL. There was no significant effect of age or breed (pony *versus* horse) on BALF adjusted Na, K or Cl concentrations. BALF adjusted Na and Cl concentrations were not different between RAO (in remission) and control animals ($P>0.05$), but BALF K was significantly increased in RAO horses compared with controls ($P=0.008$). RAO horses with inflammation had significantly lower BALF Na than in the absence of inflammation ($P=0.021$).

Mean %BALF return was significantly lower in RAO (55%) *versus* control horses (66%; $P=0.0034$) and this could have contributed to some of the observed differences. However, the electrolyte pattern in BALF was reflected in EBC. The observed differences in Na and K in RAO horses may therefore reflect increased airway permeability or differences in trans-epithelial ion exchange.

ENERGETIC COST OF BREATHING AND PULMONARY FUNCTION IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION AND WEIGHT LOSS

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This study was conducted to determine whether horses with naturally occurring, severe chronic recurrent airway obstruction (RAO) 1) have a greater resting energy expenditure (REE) than control horses, 2) suffer body mass depletion, 3) have significantly decreased REE after bronchodilation and therefore, 4) whether increased work of breathing contributes to the cachexia seen in some horses with RAO. Six RAO horses and six control horses underwent indirect calorimetric measurement of REE and pulmonary function testing using the esophageal balloon/pneumotachograph method before and after treatment with ipratropium bromide at 4-hour intervals for a 24-hour period. Body condition scoring was performed, and an estimate of fat mass was determined using B-mode ultrasonography. O₂ and CO₂ fractions, respiratory airflow, respiratory rate, and pleural pressure changes were recorded, and O₂ consumption, CO₂ production, REE, pulmonary resistance, dynamic compliance, tidal volume, and end expiratory work were calculated. The results showed that RAO horses had significantly lower body condition scores (2.4 ± 0.9 v. 6.3 ± 1.2), and significantly greater REE than controls (42.54 ± 5.36 v. 25.52 ± 2.17 kcal/kg of fat free mass/day). The increased REE was due primarily to higher VO₂ in RAO horses than controls (5.56 ± 0.79 v. 2.88 ± 0.29 mls/kg/min). After bronchodilation, there was no difference in REE between RAO horses and controls, although there remained evidence of residual airway obstruction in RAO horses ($R_L = 2.04 \pm 0.62$ cm H₂O/L/s pre-bronchodilation v. 1.37 ± 0.32 cmH₂O/L/s post-bronchodilation). Unexpectedly, we found that dynamic compliance changed in RAO horses to a greater degree than did pulmonary resistance after 24-hour bronchodilation (85.76 ± 95.73 % v. 29.94 ± 20.32 %). There was less variability in indirect calorimetry than in pulmonary function testing when evaluating the effect of bronchodilation.

BRONCHIECTASIS IN THREE ADULT HORSES WITH HEAVES

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Bronchiectasis is an uncommon condition of people and animals, which is defined as an irreversible dilation of one or multiple bronchi. It usually results from inflammatory-induced airway remodeling. The purpose of this study is to report the clinical, radiographic and pathological features presented in 3 horses with bronchiectasis.

Horses were 11 - 17 years old and included 2 mares and 1 gelding. Two animals were part of a research herd of heaves-affected horses, while the third one was examined because of a chronic and severe respiratory illness. At presentation, horses had clinical signs compatible with severe exacerbation of heaves, including occasional coughing episodes, labored breathing, tachypnea, tachycardia, hypoxemia and hypercapnia. One horse was febrile and had a peripheral blood neutrophilic leukocytosis. Thoracic radiographs in all horses revealed diffusely dilated and thickened bronchial walls visible all the way to the periphery of the lungs. These changes were compatible with bronchiectasis. Bacterial cultures of tracheal washes were positive in 2 horses in which it was performed, while the BAL cytology revealed increased non-degenerated neutrophils in these horses.

The two afebrile horses were treated with corticosteroids and environmental dust control. A rapid improvement in airway function was observed in one horse, while the other horse only partially responded to therapy. In this latter horse, systemic atropine administration resulted in a mild improvement in airway function. This horse was subsequently destroyed for humanitarian reasons. The third horse was administered penicillin and theophylline in addition to corticosteroids and responded favorably to therapy.

Histological evaluation of bronchial biopsies in 2 horses and lung specimens in the other horse was performed. Biopsy samples revealed a moderate lymphoplasmacytic infiltrate with rare eosinophils in the lamina propria in one case. The smooth muscle layer appeared thickened. On gross examination of the necropsied horse's lungs, several emphysematous foci and marked dilation of the bronchi were present. Many small bronchi contained abundant, thick mucopurulent exudate. Histologically, the cartilaginous plates of the dilated bronchus were widely separated from each other and there was multifocally increased matrix eosinophilia. The smooth muscle layer appeared markedly thickened in all bronchi and, to a lesser degree in bronchioles. In the lamina propria, a mild to moderate lymphoplasmacytic infiltrate was present.

In conclusion, bronchiectasis should be considered as a possible sequela of chronic inflammation in heaves and may contribute to the development of irreversible airway obstruction in severely affected horses.

THE APPLICATION OF THE COMET (SINGLE CELL GEL ELECTROPHORESIS) ASSAY TO STUDY OF OXIDATIVE STRESS AND GENOTOXICITY IN EQUINE PULMONARY DISEASE

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Oxidative stress occurs when antioxidant defence mechanisms are overwhelmed by free radicals and may lead to damage to DNA, which has been implicated in processes such as ageing, and diseases such as cancer. The two main techniques currently used to quantify DNA damage are measurement of 8-hydroxydeoxyguanosine (8-OHDG) and the Comet assay (also known as single cell gel electrophoresis). The Comet assay is based on the principle that damage to DNA results in single and double strand breaks. In the simplest form of the assay, cells are embedded in agarose and following lysis, undergo electrophoresis. The greater the number of strand breaks, the greater the migration of DNA across the gel during electrophoresis. The DNA is then stained and visualised under a microscope. The more damaged cells appear as a head with a tail of DNA that has migrated. This gives the appearance of a “comet” and hence the name assigned to this assay. The migration of DNA can be scored based on the visual appearance of the cells or using an image analysis system.

The Comet assay has been most frequently used on PBMC preparations and we have recently validated the Comet assay for use in equine blood. However, whilst cell lines and cell preparations from biopsy material have been studied, only a few studies have attempted to undertake the Comet assay on cells recovered from bronchoalveolar lavage (BAL). In the present study our aim was to harvest cells from BAL, determine their viability prior to and following storage at -80°C and to conduct a modified Comet assay in order to quantify DNA damage.

BAL was performed in 6 healthy horses using 200ml sterile 0.9% saline at 37°C . A manual cell count was performed and samples were slow frozen in a medium of 10% DMSO and 90% foetal calf serum to give $\sim 3 \times 10^6$ cells/ml. For the Comet assay, cells were thawed, viability determined and following lysis, electrophoresis was undertaken at 4°C for 30 min at 0.8v/cm. The appearance of the Comets obtained initially with unfiltered BAL was poor, but when BAL was filtered through a fine mesh to remove mucus, images with low distortion and low background were obtained which were acceptable for analysis. Whilst the horses sampled all had very low numbers of neutrophils in BAL, no attempt was made to separate lymphocytes and macrophages prior to assessment of DNA damage. With further refinement, it is likely that damage in different cell populations and sub-populations could be determined. In conclusion, the Comet assay appears a suitable and novel technique for the investigation of DNA damage in equine airway cells recovered by BAL and provides an additional technique to investigate mechanisms of airway inflammation.

POSTER PRESENTATIONS

NEW INSIGHTS INTO NON-INVASIVE OSCILLATORY MEASUREMENTS OF RESPIRATORY SYSTEM IMPEDANCE (ZRS) IN THE HORSE

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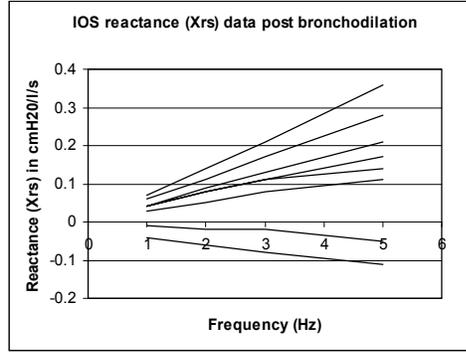
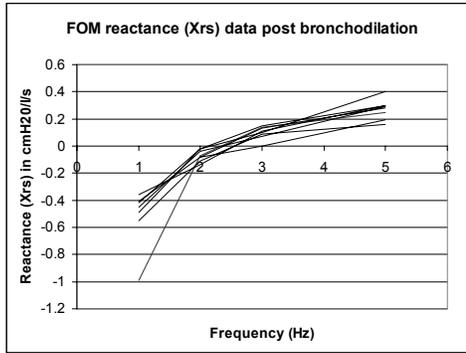
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A comparative analysis of Monofrequency Forced Oscillatory Mechanics (FOM) and Impulse Oscillometry System (IOS) in horses has previously demonstrated that respiratory system resistance (R_{rs}) was well correlated between IOS, FOM and conventional mechanics (esophageal balloon-pneumotachography)¹. However, further studies were required to evaluate the measurement of reactance (X_{rs}) in adult horses by these oscillatory systems. The purpose of this study was, therefore, to compare two techniques of oscillatory mechanics for measurement of respiratory system reactance (X_{rs}) before and after bronchodilation (ipratropium bromide, Atrovent®; 144 mcg every 6 hours for 24 hrs). In 8 horses with varying degrees of airway obstruction, sedated with xylazine (0.2-0.4 mg/kg IV), X_{rs} was measured using FOM and IOS, introduced in random order, at the oscillatory frequencies of 1,2,3 and 5 Hz.

The mean baseline reactance (X_{rs}) was -0.0074 cmH₂O/l/s (SD = 0.27 cmH₂O/l/s) as measured by FOM and 0.06 cmH₂O/l/s (SD = 0.07 cmH₂O/l/s) by IOS. After bronchodilation a mean X_{rs} of -0.041 cmH₂O/l/s (SD = 0.31 cmH₂O/l/s) and 0.08 cmH₂O/l/s (SD = 0.1 cmH₂O/l/s) was measured by FOM and IOS, respectively. Both techniques (FOM vs. IOS) demonstrated that X_{rs} varied significantly ($P<0.05$) as expected, with input frequency (1-5 Hz). However, the correlation of FOM X_{rs} with input frequency ($r_s = 0.9$, $P<0.001$) was far greater than the correlation observed for X_{rs} using IOS ($r_s = 0.36$, $P=0.04$). This was largely due to the fact that low frequency (1-3 Hz) values for IOS X_{rs} were all close to zero. Furthermore, X_{rs} measured with IOS was significantly ($P<0.0001$) different between the two techniques at 1 Hz, 2 Hz and 5 Hz. The mean resonant frequency (F_{res}) calculated by FOM was 2.6 Hz (SD = 0.6 Hz) at baseline and 2.43 Hz (SD = 0.28 Hz) after bronchodilation. A resonant frequency could not be computed through the IOS data analysis due to the observed non-linearity of X_{rs} at low input frequencies.

In order to understand the basis for near-zero values for X_{rs} using IOS, we embarked on two ancillary studies. We first examined the role of impulse interval frequency (1- 5 Hz), and found that this did not improve the acuity of IOS to measure X_{rs} at low frequencies. Secondly, we measured pleural pressure via esophageal balloon in 2 horses during oscillation of the respiratory system using IOS and FOM. We demonstrated that the IOS impulses did not generate detectable pleural pressure excursions. In contrast, the FOM oscillations produced 1.4 cmH₂O changes in pleural pressure at 1 Hz, and 1.5 cmH₂O at 5 Hz.

We propose that the energy generated by the impulse oscillometry system is insufficient for measurement of the out-of-phase component of impedance (X_{rs}) in the horse, which requires adequate energy to alter pleural pressure. The measurements of IOS, including X_{rs} and R_{rs} therefore reflect proximal airway mechanics rather than peripheral airway and tissue mechanics. However, IOS can be used to characterize proximal airway disturbances that may be seen with heaves (RAO), where bronchoconstriction of the central airways is important.



1. Bedenice D, Mazan M, Reinhold P, et al. Non-invasive measurement of respiratory system resistance using two different oscillatory methods in the horse. Proc ACVIM 2003.

CYTOLOGICAL ABNORMALITIES IN BRONCHOALVEOLAR LAVAGE SAMPLES FROM RESTING ALASKAN SLED DOGS

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Racing Alaskan sled dogs have previously been documented to have abnormal bronchoscopic findings and cytological abnormalities in bronchoalveolar lavage (BAL) samples obtained within 48 hours after completing sustained exercise in cold temperatures. Reported cytological abnormalities included increased nucleated cell counts (NCC), and increased macrophage and eosinophil counts compared to trained, rested control dogs. These changes may arise from desiccation of the mucosal lining of the peripheral airways from repeated cold air penetration resulting in airway inflammation. However, the length of persistence of this response is currently not known. The purpose of this study was to determine if sled dogs which had been rested from exercise and cold conditions for 4 months would have abnormal BAL cytological profiles when compared to a population of sedentary control dogs.

Two groups of dogs were studied; 19 racing Alaskan sled dogs that had been rested from training for approximately 4 months throughout the Alaskan Summer, and 5 sedentary mixed-breed control dogs that had never been exposed to sustained exercise or frigid conditions. Dogs were anesthetized and a 5-mm OD fiberoptic bronchoscope was advanced into the lower airways. BAL was performed by infusing and aspirating 3 - 20cc boluses of phosphate buffered saline. Determination of the total NCC in BAL samples was made within 24 hours by use of a hemocytometer. Differential cell counts were determined using cytocentrifuged slide preparations stained with a modified Wright-Giemsa stain. As the data were not normally distributed, NCC and differential cell counts were compared using the Mann-Whitney Rank Sum test. Significance was declared at $p < 0.05$.

Bronchoscopic abnormalities such as intraluminal debris were not observed in any dogs. The average volume of BAL fluid recovered was 30.7 ± 14.6 cc in sled dogs, and 45 ± 15.1 cc in control dogs. Nucleated cell counts were obtained on all dogs, and differential cell counts were obtained on 17 of the 19 sled dogs. There was no significant difference in NCC between sled dogs and control dogs. Sled dogs, however, had a significantly lower percentage of macrophages compared to control dogs although total macrophage numbers were not significantly different between the two groups. Sled dogs also had significantly greater concentrations of lymphocytes and neutrophils in BAL fluid compared to control dogs. There was no significant difference in the percentage or concentrations of eosinophils and basophils in BAL samples from the two groups.

In conclusion, racing Alaskan sled dogs rested for four months in warm temperatures have cytological abnormalities in BAL samples compared to control dogs, including increased lymphocyte and neutrophil numbers. These findings may result from repeated hyperpnea with cold air during training and racing and support the hypothesis that repeated cold air hyperpnea induces persistent airway inflammation and hyperresponsiveness.

	NCC	Mac	Lymph	Neut	Eos	Baso
Sled dogs	397±360	201±190 (50±24.5%)*	68±55* (19±13%)	84±168* (13±13.7%)*	23±39 (7±13%)	3±4.4 (2.9±4.4%)
Controls	230±106	209±106 (90±4.9%)*	14±7.9* (7±5.0%)	1±1.2* (0.5±0.5%)*	5±3.6 (2±1.3%)	2.0±1.5 (1.5±1.5%)

Values presented as Mean ± SD

Cell counts provided in cells/ul (% differential in brackets)

*indicates significant difference between sled dogs and controls

HYDROGEN PEROXIDE IN BREATH CONDENSATE AS MARKER OF LOWER AIRWAY INFLAMMATION IN AN EXPERIMENTAL MODEL OF FELINE ASTHMA

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Analysis of inflammatory markers contained in breath condensate (BC), such as hydrogen peroxide (H₂O₂), isoprostane, is a non-invasive method of lower airway inflammation assessment already described in large animals. The present study aimed at describing a method of BC collection applicable in cats and at testing whether H₂O₂ in BC might be used as marker of lower airway inflammation.

Twelve european shorthair cats (6 neutered males, 6 females, 4.0 ± 1.1 kg aged from 8 to 14 months) were enrolled in the study. Six cats were sensitized to *Ascaris suum* (AS) allergens whereas six other cats serving as controls remained untreated. AS-group and control group were investigated before (PRE) and after (POST) an aerosol challenge of AS-allergen or saline. Cats underwent lung function tests by use of barometric whole body plethysmography (BWBP), BC collection by condensing the outcoming air of the plethysmograph, chest radiography and bronchoscopy followed by bronchoalveolar lavage (BAL). Immediately after collection, BC was analyzed spectrophotometrically for H₂O₂. A scoring system was used for evaluating chest radiographies (min 0–max 9) and bronchoscopies (min 0–max 6) and BAL cytology was performed.

The amount of BC collected within 30 minutes ranged from 500 to 1500 µl. Before being challenged with either AS-allergen or saline, BC H₂O₂ concentrations were similar in AS and control cats, as well as respiratory rate (RR) and enhanced pause (Penh), an index of bronchoconstriction measured by BWBP, radiographic and bronchoscopic scores and BAL cytology. After AS aerosol challenge, BC H₂O₂ concentrations were significantly increased in AS-cats, as well as RR, radiography and bronchoscopy scores (Table 1). BAL cytology of AS-challenged cats showed a significant increase of neutrophil (16±8 versus 27±15, p<0.05) and eosinophil (2±2 versus 53±19, p<0.05) percentage. There was a significant and positive correlation between BAL eosinophil percentage and BC H₂O₂ (r=0.61, p<0.05).

This study describes a method of BC collection and proposes BC H₂O₂ analysis as non-invasive marker of lower airway inflammation in an experimental model of feline asthma.

Group	Treat-ment	BC H ₂ O ₂ (µmol/)	RR	Penh	Radiography score	Bronchoscopy score
Controls	PRE	0.76 ± 0.1	50 ± 9	1.07 ± 0.26	2 (1-2)	0.5 (0-1)
	POST	0.79 ± 0.18	43 ± 12	1.12 ± 0.29	2 (1-3)	0.5 (0-1)
AS-cats	PRE	0.56 ± 0.12	35 ± 13	1.46 ± 0.59	2 (1-4)	1 (0-4)
	POST	1.01 ± 0.35*	54 ± 12*	1.22 ± 0.38	3.5 (2-7) * #	3 (1-7) * #

Table 1: Respiratory variables recorded by BWBP, radiography and bronchoscopy scores of control cats and *Ascaris suum*-sensitized (AS) cats before (PRE) and after (POST) saline or AS aerosol exposure. Data are presented as means ± SD for BWBP variables and as medians (ranges) for radiography and bronchoscopy scores.

* significantly different from respective PRE-exposure value, p<0.05.

significantly different from respective control value, p<0.05.

Supported by: Région Wallonne DGTRE, Belgium.

EFFECTS OF SOMATIC GROWTH AND CIRCADIAN RHYTHM ON RESPIRATORY VARIABLES ASSESSED BY WHOLE BODY BAROMETRIC PLETHYSMOGRAPHY IN HEALTHY CATS

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Many biological variables, such as age, sex, weight or circadian rhythm are reported to influence lung function. Whole body barometric plethysmography (WBBP), a non-invasive method of lung function assessment applicable in cats, which allows repeated and prolonged measurements, appears therefore as an useful tool to investigate physiological variations of lung function. The aim of this study was to determine effects of growth and circadian rhythm on lung function and to yield reference values for respiratory variables measured by WBBP in growing cats. **METHODS. Study 1: Effect of somatic growth:** Eighteen healthy cats (10 MN and 8 FI) were evaluated weekly from the age of 3 to 12 months by 5-min sessions of WBBP. Age-related respiratory parameters are reported in table 1 as mean \pm SEM. **Study 2: Effect of circadian rhythm:** Eighteen healthy cats (10 MN and 8 FI) were evaluated by WBBP during 24 consecutive hours, divided into 12 2-hours sessions. Within each session, all cats underwent successively a 5-min record of WBBP.

The effects of growth and circadian rhythm on tidal volume (TV), respiratory rate (RR), minute volume (MV), inspiratory and expiratory time (Ti, Te), inspiratory and expiratory peak flow (PIF, PEF) and the enhanced pause (Penh), an index of bronchoconstriction, were determined.

Table 1: Respiratory variables recorded by WBBP in growing healthy cats (n=18).

	3-4 months	5-6 months	7-8 months	9-10 months	11-12 months	Growth (p values)	Sex (p values)
Weight (kg)	2.0 \pm 0.5	3.1 \pm 0.7	3.5 \pm 0.9	3.6 \pm 0.1	3.5 \pm 0.1	<0.05	<0.05
Ti (msec)	502 \pm 10	484 \pm 13	525 \pm 15	524 \pm 21	559 \pm 24	<0.05	<0.05
Te (msec)	608 \pm 21	638 \pm 26	648 \pm 27	664 \pm 37	723 \pm 60	NS	NS
TV (ml)	19.7 \pm 0.4	23.1 \pm 0.6	28.4 \pm 1	28.3 \pm 1	28.4 \pm 1.3	<0.05	0.05
RR	59 \pm 1	60 \pm 2	57 \pm 2	57 \pm 2	54 \pm 3	NS	NS
MV (ml)	1164 \pm 32	1359 \pm 31	1693 \pm 81	1554 \pm 69	1529 \pm 75	<0.05	<0.05
PIF (ml/sec)	69.4 \pm 1.8	80.9 \pm 2	96.7 \pm 4.3	93.8 \pm 3.5	88.5 \pm 3.3	<0.05	<0.05
PEF (ml/sec)	74.4 \pm 2.5	81.8 \pm 3	101 \pm 5	98.6 \pm 5.7	93 \pm 5.5	<0.05	<0.05
Penh	1.18 \pm 0.04	1.13 \pm 0.05	1.25 \pm 0.06	1.29 \pm 0.1	1.2 \pm 0.08	NS	NS

RESULTS. Study 1: Effect of growth: Somatic growth had a significant effect on TV, MV, PIF, PEF and Ti. The PIF, PEF, TV, MV and weight were significantly higher in males than in females. Correlation analyses were performed to quantify the relative effect of age and weight. A positive significant correlation was found between weight and PIF ($r_1=0.466$; $p<0.05$), PEF ($r_1=0.286$; $p<0.05$), TV ($r_1=0.462$; $p<0.05$), MV ($r_1=0.437$; $p<0.05$). Ti was significantly correlated with both weight ($r_1=-0.123$; $p<0.05$) and age ($r_2=0.186$; $p<0.05$). **Study 2: Effect of circadian rhythm:** Circadian rhythm had a significant effect on all previous respiratory variables, the most significant variations being observed between 2 and 6

a.m.: P_{enh} , T_i and T_e were higher whereas the opposite behaviour was seen for other variables. CONCLUSIONS. These results show that 1) the recorded modifications followed the same tendency as weight, i.e. that they increased and then stabilised at the age of 7-8 months suggesting that the pulmonary maturity was reached and 2) the amplitude of recorded circadian variations was more pronounced during the second part of the night.

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PULMONARY OXIDATIVE STRESS BY CADMIUM INHALATION IN AN ANIMAL MODEL OF BRONCHO-PNEUMOPATHY

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Oxidative stress is believed to play an important role in the pathogenesis and development of chronic obstructive pulmonary disease (COPD) in man. Cadmium (Cd) being the predominant component of cigarette smoke, which is a predisposing factor for developing COPD, this compound was administrated by nebulization to rats and its repercussions on pulmonary markers of oxidative stress were analyzed.

Forty-five Sprague Dawley rats were divided into 5 experimental groups of 3 placebo-exposed and 6 cadmium-exposed rats. Placebo (saline) and Cd (0.1% in saline) were nebulized for one hour three times weekly. Group 1 underwent a single exposure, whereas group 2 was exposed for 3 weeks and group 3 for 5 weeks. Group 4 and 5 were exposed for 5 weeks and remained untreated for respectively 2 and 4 further weeks. The day after the last exposure, rats were killed and bronchoalveolar lavage (BAL) was performed on the right lung lobe. BAL fluid was analyzed cytologically and the oxidant markers glutathione (reduced :GSH and oxidized: GSSG), ascorbic acid (reduced: AA and oxidized: DHA) and uric acid (UA) were determined. The left lung lobe was fixed for histopathology and alveolar surface quantification. Results are shown in Table 1. Cadmium exposure induced significant changes of all BAL variables over time, the most important inflammatory and oxidative stress response being observed after a single exposure. Alveolar surface significantly increased in Cd-exposed rats of Group 4, suggesting development of pulmonary emphysema as a consequence of long term Cd inhalation.

This rat model of bronchopneumopathy shows that oxidative stress occurs during development of disease by Cd inhalation in rats.

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Table 1: Differential cell count and oxidant marker concentrations of BAL fluid and mean alveolar surface (S²) in placebo (PI)- or cadmium (Cd)-exposed rats. Data are mean ± SD.

Variable (unit)		Group 1 single exposure	Group 2 3 wks	Group 3 5 wks	Group 4 5 wks +2 wks	Group 5 5 wks +4 wks	Treat- ment	Time
Macrophage (cells x10 ³ /ml)	PI	382 ± 116	65 ± 38	112 ± 68	185 ± 26	158 ± 17		
	Cd	716 ± 91	233 ± 43	409 ± 115	239 ± 127	238 ± 90	*	*
Neutrophils (cells x10 ³ /ml)	PI	46 ± 55	0.6 ± 0.4	1.2 ± 2.0	1.2 ± 1.0	0.6 ± 1.0		
	Cd	345 ± 69	466 ± 60	429 ± 91	102 ± 73	39 ± 17	*	*
GSH (µM)	PI	0.5 ± 0.2	3.1 ± 3.4	2.6 ± 3.0	4.0 ± 2.7	1.3 ± 0.9		
	Cd	6.6 ± 2.6	3.2 ± 3.3	1.8 ± 1.6	1.7 ± 1.4	2.8 ± 2.5	*	*
GSSG (µM)	PI	0.1± 0.06	0.29±0.34	0.19±0.26	0.35±0.17	0.51±0.53		
	Cd	0.78±0.31	0.27±0.22	0.14±0.06	0.13±0.08	0.34±0.4	NS	NS
AA (µM)	PI	9.12±1.06	10.3±1.18	9.93±3.50	10.48±1.4	10.56±4.2		
	Cd	8.0±1.34	8.53±2.24	6.63±2.16	6.47±1.28	10.59±8.0	*	NS
DHA (µM)	PI	0.78±0.57	1.23±0.85	0.66±0.56	1.29±1.62	1.03±0.56		
	Cd	0.49±0.31	0.87±0.74	1.04±0.43	0.81±0.51	0.88±0.88	NS	NS
UA (µM)	PI	0.95±0.41	1.43±1.59	0.8±0.57	1.12±0.16	0.61±0.81		
	Cd	3.21±0.96	1.56±1.32	2.75±2.6	0.89±0.72	2.18±0.69	*	*
Alveolar S ² (x10 ³ mm ²)	PI	33.9±3.1	44.3±3.0	33.9±4.5	40.3±4.2	37.8±3.8		
	Cd	32.3±2.6	37.2±6.0	33.6±2.3	42.7±3.0	37.7±5.5	*	*

* significant treatment or time effect (ANOVA-2), p<0.05

PHYSIOLOGICAL VALUES FOR pH AND pCO₂ IN EXHALED BREATH CONDENSATE SAMPLES FROM PIGS AND CALVES

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Aim

To evaluate exhaled breath condensate in animals (either experimental subjects or clinical cases), it is important to compare physiological characteristics between different species. In this study, pH and pCO₂ were analysed in exhaled breath condensate (EBC) samples collected from clinically healthy pigs and calves.

Material & Methods

In 12 pigs (body weight: 15 ± 5 kg) and in 12 calves (body weight: 61 ± 5 kg), EBC was collected from each animal (n = 24 in each species) on two separate occasions using the “ECoScreen” device (VIASYS Healthcare, Germany). The collection period was determined by the length of time necessary to collect 3 - 5 ml of EBC. Measurements of pH and pCO₂ in exhaled breath condensates were made immediately after collection and were repeated after one hour of storage in closed Eppendorf tubes at room temperature (18 - 22 °C) using a commercial blood-gas analyser (ABL 605, Radiometer, Copenhagen). All values were corrected for the actual body temperature of the individual animal measured immediately before each EBC collection. Data were analysed using Statgraphics® *Plus* for Windows (Manugistics, Inc., USA).

Results

The partial pressure of CO₂ (pCO₂) was significantly higher in fresh EBC samples derived from calves compared to the pCO₂ in EBC samples collected in pigs (mean ± SD: 5.38 ± 2.16 kPa *versus* 2.83 ± 1.12 kPa; *P* < 0.001; t-test). Despite these significant inter-species differences in pCO₂, mean pH values were not statistically different between species. Normally distributed pH values ranged between 5.6 - 6.2 in both species immediately after EBC collection. The pCO₂ in native EBC was negatively correlated to the time needed for EBC collection which was significantly longer in pigs (48 ± 12 min) than in calves (22 ± 4 min) due to differences in body weight and minute volume of ventilation. Despite pH and pCO₂ measured in fresh EBC samples were negatively correlated within each species, there was no significant correlation between pH in native EBC and the collection time. Storing EBC samples in closed Eppendorf tubes at room temperature (18 - 22 °C) for one hour lead to a significant decrease in pCO₂ and to a significant increase in pH in both species. In average, pH increased by 0.16 in pigs and by 0.20 in calves, and this increase was clearly correlated with the decrease in pCO₂ (calves: *r* = -0.82; pigs: *r* = -0.70; both *P* < 0.001).

Discussion & Conclusions

Despite a significant difference in pCO₂ (which was caused by a longer collection time in pigs compared to calves), mean pH in native exhaled breath condensate was not different between species. Following this, other significant factors resulting in an acid pH in fresh EBC must be taken into account. In both species (calves and pigs), the mean EBC pH in native samples was lower than the pH reported in degassed samples from man and other species. Despite the current consensus of opinion (that prior to EBC pH measurement, samples should always be degassed) non-degassed samples may more closely reflect the pH on the airway surface and need to be evaluated for their biological information. Losses in pCO₂ due to storage processes are linearly correlated to an increase in pH.

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A QUANTITATIVE ANALYSIS OF COLLAGEN DEPOSITION IN THE HEALTHY EQUINE LUNG

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It has been demonstrated that there is increased deposition of collagen in the airways of human asthmatics (1,2). The condition of heaves in the equine strongly parallels the condition of asthma in human subjects. It is currently unknown if there is an increased deposition of collagen within the airways of equine patients affected with Heaves. The intentions of this particular study were two fold; to develop a practical method to quantitatively analyze the collagen content and to determine the percentage of collagen surrounding the small bronchioles in healthy equine individuals. Pulmonary tissue specimens were collected from 5 horses free of respiratory disease. The horses were euthanized due to the Wobblers neurological syndrome. The ages ranged from 2 years to 14 years. All horses were Thoroughbreds. A complete physical examination was performed as well as a complete blood count, serum biochemistry profile and plasma fibrinogen concentration. Sections of tissue were collected from both the right and left dorsal, ventral, apical and diaphragmatic lung lobes. Tissue specimens were stained for collagen content using Masson's Trichrome. Small bronchioles, appearing to have been cut in 90 degree cross section, were captured using the Motic Images Advanced 3.0 software program and the Moticam 1300. The software program was employed to calculate the percentage of collagen surrounding a small bronchiole by specifically selecting for areas around the bronchiole that stained positively for collagen content. Four bronchioles were selected per slide, and a total of six slides were evaluated per horse. The percentage of collagen found within the dorsal left region of the lung ranged from 20.91% to 30.36%. Within the apical region of the left lung, the percentage of collagen ranged from 17.39% to 25.49%. There was no statistically significant difference in percentage of collagen content between the five horses within each lung segment. In conclusion, it would appear that collagen fibers represent approximately 25% of the lamina propria surrounding the small airway. The study will continue to assess the percentage of collagen deposition throughout the remaining lung lobes. It is the goal of this particular study to characterize the percentage of collagen content within the lung of the "healthy" individual. This will then be compared with the collagen deposition within the small airways of horses with heaves.

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EFFECT OF THE VIRAL LOAD INOCULATED ON THE PATTERN OF PNEUMONIA INDUCED BY SENDAI VIRUS IN THE BALB/c MOUSE

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Sendai Virus, a parainfluenza virus type I, is a natural respiratory pathogen of mice. Different susceptibilities of inbred mouse strains against Sendai virus have been observed in different experiments, with the BALB/c strain being known as the most “resistant”. Designing experiments aimed at quantitating the severity of Sendai virus -induced pneumonia among strains thus requires to know the viral load that is able to cause at least a moderate disease in the most resistant strain.

Different titers of Sendai virus (10^2 , 10^3 , 10^4 and 10^5 PFU/ml) were inoculated intranasally within a volume of 50 μ l. Mice were monitored daily for clinical signs such and were euthanized either at 5 (all dosages), 6 or 7 days (10^4 PFU/ml) after inoculation. Lung lesions were examined by histopathology, virus-containing cells were detected by immunofluorescence and viral replication was evaluated by titrating nasal washes and lung tissue by plaque assay.

It was shown that mild respiratory lesions appear with a viral titer of 10^3 PFU/ml, but that at least 10^4 PFU/ml must be inoculated to cause the lesions classically reported, i.e., interstitial pneumonia, peribronchial and perivascular lymphoid infiltration, and areas of alveolar necrosis. Increasing the inoculation dose up to 10^5 PFU/ml did not cause more marked histopathological lesions, even though it increased the viral titer subsequently found in the lungs. Viral titers in the lungs were the highest at day 5 and decreased progressively until day 7. Histopathological lesions showed increased severity at day 7, with hyperplasia of bronchial epithelium, extensive peribronchial and perivascular lymphoid infiltration and cellular debris and necrosis of adjacent alveoli.

The data collected suggest (i) that a viral titer of 10^4 PFU/ml is needed to induce a moderate disease in the BALB/c mouse and (ii) that killing the mice 6 days after inoculation would simultaneously yield near maximum viral titers and lung lesions.

VALIDATION OF THE BALB/C MOUSE AS A MODEL OF SWINE INFLUENZA

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Influenza A infection is very common in pigs. Although the associated mortality remains low, this disease bears important implications on an economic point of view - because of the high morbidity - as well as in terms of public health, due to the pig role as a "mixing vessel" between avian and human strains. Since years, a mouse model of human influenza has been used for vaccine development, evaluation of antiviral molecules or identification of resistance genes. The purpose of the present work was to validate such a model for swine influenza viruses.

Six healthy 15-wks old female BALB/c mice were inoculated intranasally under anaesthesia with 100 μ l of a viral suspension containing $4.5 \cdot 10^5$ PFU/ml of the A/sw/Belgium/1/98 H1N1 strain diluted in DMEM. The body weight was monitored daily and the mice were euthanized on the 5th day after inoculation. After bleeding, the right lung was processed for histopathology and immunohistology. The left lung was homogenized in a cold mortar and a suspension of 10% was prepared for virus titration by plaque assay.

The body weight significantly decreased, from 24.5 ± 1.2 g before inoculation to 21.8 ± 0.9 g 5 days after. The mean viral titer directly retrieved from the left lung was $1.2 \pm 0.2 \cdot 10^2$ PFU/ml corresponding to $2.1 \cdot 10^4$ PFU per gram of lung. The mean viral titer retrieved after one passage on MDCK cells was $1.5 \pm 0.3 \cdot 10^5$ PFU/ml. Histologically, plugs of neutrophilic exsudate were seen in bronchi, with subacute vasculitis, périfbronchitis and diffuse interstitial pneumonia. Viral particles were identified in airway and pulmonary epithelia by IF.

It is concluded that the procedure described here produces viral titers and respiratory lesions that are comparable to the characteristics of the widely used mouse model of human influenza.

ALLELIC DIVERSITY AT THE CARBOXY-TERMINAL END OF THE PORCINE MYXOVIRUS RESISTANCE PROTEIN (MX 1)

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Several MX proteins among different animal species are associated with an innate resistance against myxoviruses. In laboratory mouse strains, allelic polymorphisms at the *Mx* locus affect the probability of survival after experimental Influenza disease, which underlines the possibility to identify an antiviral MX isoform in pigs and to allow selection programs aimed at improving their innate resistance. An 11-bp deletion was described in Japanese porcine populations (Morozumi *et al.*, 2001), resulting in a frameshift in the exon 14 of the *Mx1* gene and in a significantly different MX1 protein compared to that generated by the nondeleted allele.

The present study aimed at investigating the distribution of the intact and deleted alleles among European wild boar (n = 43), Landrace (n = 78; from Belgium, France, Germany, Britain and Finland) and Piétrain (n = 56; from Belgium and France) pig populations. DNA was extracted from spleen, hair, blood or semen and submitted to a PCR-RFLP according to Morozumi. Sequencing and pyrosequencing were also performed for confirmation.

The deleted allele was found at a relatively high frequency in the Landrace breed, in roughly one fourth of the Piétrain population sampled, but never in wild boars. Sequencing and pyrosequencing confirmed results obtained by PCR-RFLP and the presence of the 11bp deletion at the same position (2064-2074).

Our study succeeded in showing the high frequency of the intact and deleted alleles among European pig populations. The absence vs. high frequency of the deleted allele in wild and domestic pigs, respectively, will provide a basis to investigate the relationship between the *Mx1* genotype and susceptibility to Influenza viruses in the porcine population.

Morozumi *et al.*. Three types of polymorphisms in exon 14 in porcine *Mx1* gene. *Biochem. Genet.*, 2001, **39**, 251-60.

ANALYSIS OF THE CD11A-ENCODING CDNA IN *BOS TAURUS*

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The CD11a/CD18 (LFA-1, $\alpha_L\beta_2$) is a member of the β_2 -integrin family of cell surface receptors. Expressed on all leukocytes, this heterodimer mediates adhesion to a variety of cell types that exhibit one or more of the intercellular adhesion molecules (ICAM-1 to -5). CD11a/CD18 plays a critical role (i) in a wide range of immunological activities such as interaction between lymphocytes, cytolysis of target cells and phagocytosis of complement-coated targets and (ii) in the regulation of leukocyte traffic between the bloodstream and tissues. In this context, an excessive neutrophilic extravasation is known to contribute significantly to the pathogenesis of pneumonia, a leading cause of production losses in the cattle industry throughout the world. Increasing our knowledge about β_2 -integrins is thus of critical relevance. Since the *Bos taurus* CD18 subunit has been well characterized, we have taken up the challenge to clone and sequence the CD11a-encoding cDNA. We report here a comparative analysis with its human and murine homologs. These results give the first opportunity to express *in vitro* the *Bos taurus* CD11a/CD18 as a tool to examine the specificities of pulmonary inflammation in the bovine species.

ALLELIC DIVERSITY AT THE CARBOXY-TERMINAL END OF THE BOVINE MYXOVIRUS RESISTANCE PROTEIN (MX1)

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Mx genes from various species have been shown to confer a resistance against a panel of ssRNA viruses, notably involved in respiratory diseases. In a previous study, we presented the genetical structure of the bovine Mx1 gene and promoter and we showed that the bovine MX1 expression is tightly and strongly induced by type I interferons, some viruses and synthetic dsRNA (VCRS Meeting, 2002). In this study, we report the genetic variability found in the coding sequence underlying the carboxy-terminal end of the protein, i.e., within the domain involved in the antiviral activity.

A pool of 35 DNA sources (semen & cell lines) was first screened for mutations by systematic sequencing of PCR products consisting of the two last exons (14 and 15). Then, the ability of denaturing HPLC (dHPLC) to detect mutation within 2 x 35 fragments was assessed with the aim to check the reliability of such a high throughput technique.

Single nucleotide polymorphisms (SNP) were detected in the intron 13, the intron 14 and the 3' untranslated region. One homozygous or and one heterozygous SNP was also identified in the exon 14, conserving the amino-acid. Beside these silent mutations, an heterozygous SNP was identified in a bull of the French "Blonde d'Aquitaine" breed. This mutation causes the replacement of a valine by an aspartic acid, which potentially leads to a MX protein with modified antiviral activity. DHPLC results tightly matched those yielded by sequencing, pleading for the reliability of the technique for large scale screening.

Implementation of dHPLC on a second pool of 100 other animals yielded 5 and 15% allelic variants in the exons 14 and 15 respectively. It is therefore anticipated that systematic screening for genetic variation at the bovine MX1 locus will produce a spectrum of alleles, raising the possibility that the MX protein may confer an innate resistance to single-stranded RNA viruses in some animals or breed.

MODELIZATION OF AN INTERSTITIAL PNEUMONITIS USING THE *PNEUMONIA VIRUS OF MICE* IN THE BALB/C MOUSE

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The pneumonia virus of mice (pneumovirus of mice – PVM) is a member of the genus pneumovirus, family of Paramyxoviridae. With the aim to identify a possible resistance phenotype among inbred mouse strains, we attempted to develop a model of the infection able to yield significant clinical signs, pulmonary lesions and virus accumulation within the lungs.

Different titers of PVM virus, strain J3666 (500, 750 and 1.000 PFU/ml) were inoculated intranasally within a volume of 50µl. The strain had been passaged several time in mice, then once on BSC-1 cells to prepare the stock solution. Mice were monitored daily for clinical signs and were euthanized at 5 days after inoculation. Lung lesions were examined by histopathology, virus-containing cells were detected by immunofluorescence and viral replication was evaluated by titrating lung tissue by plaque assay.

It was shown that mild diffuse respiratory lesions appeared with the inoculum titrating 500 PFU/ml, but that at least that titrating 10^3 PFU/ml had to be inoculated to cause the interstitial pneumonia classically reported. The presence of the virus was demonstrated, whatever the inoculum, in alveolar and bronchiolar epithelia. Viral titers in the lungs were $1.62 \pm 0.25 \times 10^6$, $3.95 \pm 0.52 \times 10^6$ PFU/g and $6.23 \pm 0.76 \times 10^6$ PFU/g of lung for the inoculums titrating 500, 750 and 1000 PFU/ml respectively.

The data collected suggest that 50 µl of a viral inoculum titrating 10^3 PFU/ml is necessary and sufficient to cause significant viral replication and lung lesions in the BALB/c mouse.

READMINISTRATION OF ADENOVIRUS/CALCIUM PHOSPHATE CO-PRECIPITATES SEGMENTALLY TO THE LUNG IN THE SHEEP

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We are interested in the development of a large animal model of chronic neutrophil mediated lung disease and its use to study the effects of antiprotease gene therapy using adenoviral vectors. To this end we have identified and characterised the gene for the ovine ortholog of elafin (elastase specific inhibitor) and used a FLAG tagged form of the relevant cDNA to construct a replication deficient adenovirus coding for this potentially useful low molecular weight serine antiprotease (Ad-o-elafin-FLAG). Previous work has demonstrated that the incorporation of adenovirus in a calcium phosphate precipitate markedly enhances the uptake of adenovirus into cells deficient in the coxsackie B virus and adenovirus receptor (CAR) including cells of the monocyte/macrophage lineage. We have also shown that the use of calcium phosphate/adenovirus co-precipitates leads to an increase in infection of alveolar epithelial cells and also infection of alveolar macrophages. In order to investigate the time course of events after administration of calcium phosphate/adenovirus co-precipitates and also the effects of a second dose of virus, either Ad-o-elafin-FLAG or Ad-GFP were administered bronchoscopically in conjunction with calcium phosphate to distinct lung segments in the sheep at a dose of 10^8 pfu per segment on two occasions fourteen days apart. These segments were sampled by bronchoalveolar lavage at 3, 7, 10, 17, 21 and 24 days to assess inflammation by total and differential cell counts, and total protein levels. Additionally, alveolar macrophages (AMs) from these segments were cultured at each time point for 20 hours and the supernatant assessed by Western blot for o-elafin-FLAG levels. The cultured AMs from the Ad-GFP exposed segments were assessed visually for GFP expression. Routine haematology was also performed at each time point.

Information from this work is valuable in the field of viral gene therapy for a chronic disease process where repeated administration of a vector may be required.

EQUINE TRYPTASE AND PUTATIVE EQUINE MAST CELL PROTEINASE-1: CDNA CLONING AND SEQUENCING

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Introduction: Mast cell granules contain proteinases, histamine and cytokines, which may contribute to, and modulate, the inflammatory response. It is now recognised that these mediators play an important role in the pathogenesis of human asthma and similarly, their actions could explain many of the pathological features of equine heaves. Examination of equine mastocytoma tissue revealed two mast cell proteinases: the trypsin-like equine tryptase and chymotrypsin-like equine mast cell proteinase-1 (eqMCP-1).

Aim: To clone and sequence the two predominant equine mast cell proteinases, tryptase and eqMCP-1, which will allow generation of molecular tools to probe transcription of these proteinases in control and heaves affected horses.

Materials and Methods: Total RNA was extracted from equine bronchoalveolar lavage fluid (BALF) cell pellets immediately post collection. RT-PCR amplification of cDNA was performed using primers designed from alignment analysis of similar, known proteinase sequences, and taking into account the known N-terminal amino acid sequences of tryptase and eqMCP-1. The tryptase PCR product was sequenced directly, but the eqMCP-1 PCR product required cloning into the vector pCR2.1. The 3' and 5' ends for both cDNA sequences were determined by RACE-PCR. Full-length sequences were then amplified from equine BALF cell pellet mRNA from two other horses.

Tryptase Results: The deduced amino acid sequence of equine tryptase shows good homology with human tryptase- β 1 and sheep tryptase-2 (77% and 71%, respectively). Unusually for trypsin-like proteinases, equine tryptase contains an Ala residue at 216 rather than Gly, which may alter substrate specificity.

EqMCP-1 Results: The uncharged Gln₂₂₆ residue in the deduced amino acid sequence of putative eqMCP-1 is characteristic of chymases, which prefer uncharged, aromatic substrates. The putative eqMCP-1 sequence shows 63% amino acid identity with mouse granzyme B (an aspartase – cleaves after aspartic acid) and 55% homology with sheep MCP-1 (a dual trypsin- and chymotrypsin-like proteinase). Although this sequence has been denoted eqMCP-1, it does not appear to fully correspond to the protein of that name which has been previously purified from equine mastocytoma tissue. Granulocytes of most mammalian species express multiple closely-related proteinases; therefore the cellular origin of this transcript will be defined by *in situ* hybridisation.

MAST CELL PROTEASE CONCENTRATIONS IN EQUINE BRONCHOALVEOLAR LAVAGE FLUID FROM CONTROL AND HEAVES AFFECTED HORSES

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Heaves is characterised by reversible pulmonary inflammation induced by inhalation of organic stable dusts and is similar to human organic dust associated asthma (OA). Some studies have supported the role of mast cells (MC) in the pathogenesis of these diseases¹⁻³, and indeed, the action of MC mediators could explain many of the features of heaves. This study was performed to investigate the role of tryptase and equine mast cell protease-1 (eq.MCP-1) in the pathogenesis of heaves.

Equine bronchoalveolar lavage fluid (BALF) was collected from control or heaves affected horses following 6, 12, 24h and chronic natural challenge. Supernatant was isolated (400g, 10min) and concentrated with a centrifugal filter device prior to determination of protease concentration by ELISA using rabbit polyclonal anti-equine tryptase / eq.MCP-1.

Immunofluorescence of BALF cytopins was performed using the above primary antibodies followed by donkey anti-rabbit IgG - Alexa-fluor 488. The number of positive cells per 500 cells was counted.

There was no significant difference in tryptase concentration between control and heaves affected horses. Similarly, there was no significant difference in the number of tryptase positive MC on cytopin preparations from control and heaves affected horses. There was no detectable eq.MCP-1 in equine BALF and eq.MCP-1 positive MC were scarce on cytopin preparations.

PULMONARY SURFACTANT IN NORMAL FOALS AND FOALS WITH BACTERIAL PNEUMONIA

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Pulmonary surfactant, a mixture of phospholipids and proteins, is responsible for maintaining a low surface tension at the air-liquid interface of the lung. Alterations to pulmonary surfactant contribute to the pathophysiology of bacterial pneumonia in some species. The purpose of this study was to characterize the surfactant system in normal foals, and to determine the effect of bacterial pneumonia, induced with *K pneumoniae*, on the foal surfactant system. Fourteen anesthetized 4-6 month old mixed breed foals underwent static lung compliance (C_L) measurements and a bronchoalveolar lavage (BAL) on Day 0. On Day 1, 10^9 CFU *K. pneumoniae* or phosphate buffered saline (PBS) were instilled into foals randomized to the pneumonia (P) or control (N) groups respectively. Sedated foals underwent a subsequent BAL on Day 3. Foals were anesthetized on Day 5 for C_L measurements. They were then euthanized and their lungs removed to perform a lavage on one side of the lungs. Lavage samples were analyzed for phosphorous levels in the total surfactant (TS), functional large aggregate (LA) and non-functional small aggregate (SA) components; phospholipid composition; total protein levels; and surfactant function.

Static lung compliance on Day 0 in the N and P groups were 2.1 ± 0.4 mL/cmH₂O/kg and 1.8 ± 0.2 mL/cmH₂O/kg respectively. In P foals, compliance was significantly less on Day 5 (0.9 ± 0.2 mL/cmH₂O/kg) compared to Day 0 ($p=0.03$).

The percent LA at baseline in N and P foals was 29.8%. There was no significant difference in this proportion on Day 3 and Day 5 relative to Day 0 in either group of foals. Comparison between the N and P foals showed no significant differences in the %LA.

Total protein levels in TS were significantly higher in P foals on Day 3 compared to N foals and to values obtained on Day 0 ($p=0.009$).

In all foals on Day 0, the % phosphatidylcholine (PC) and % phosphatidylglycerol (PG) in the LA phospholipid fraction were 74.3% and 14.0%. There were no significant differences in %PC on Day 3 or Day 5 relative to Day 0 in the N group. The %PG was significantly lower in the both groups of foals on Day 3 relative to Day 0 ($p=0.01$). On Day 5, %PG was significantly higher in the P group compared to the N group ($p=0.002$).

Functional analysis revealed that LAs of all groups were able to reduce the surface tension to a similar extent. This surface tension was greater in P foals on Day 5 compared to N foals but differences did not reach significance.

The results reveal a similarity of normal foal surfactant to that reported for adult horses and other species. In the model used, pneumonia secondary to *K.pneumoniae* infection decreases lung compliance with only minor effects on surfactant composition and function.

EFFECT OF ACUTE ANTIGEN EXPOSURE ON FUNCTIONAL RESIDUAL CAPACITY (FRC) IN HEALTHY HORSES AND HORSES WITH RECURRENT AIRWAY OBSTRUCTION

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Recurrent airway obstruction (RAO) is characterised by bronchoconstriction, airway inflammation and mucus accumulation. Functional residual capacity (FRC) has previously been demonstrated to show a non-significant trend to be higher in RAO-affected horses. Recently we have presented a refined version of the helium rebreathing technique for obtaining reliable and repeatable measurements of FRC in horses. The aim of the present study was to investigate the effects of stabling with hay and straw on FRC in RAO and non-RAO affected (control) horses. Six RAO-affected horses and six control horses were stabled on hay and straw for 24 hours. Bronchoalveolar lavage (BAL) cytology, airway resistance and FRC were determined seven days prior to challenge and immediately, 3 and 14 days after exposure. Airway resistance was measured using the forced oscillation method at 1, 1.5 and 2 Hz. For measurement of FRC, horses rebreathed a certified gas mixture containing 10% He, 21% O₂, 0.3% CO and balance N₂ from a reservoir bag. The volume of gas in the reservoir bag was equivalent to 60 ml/kg bodyweight. The reservoir bag was connected to a Fleisch No.5 pneumotachometer and facemask by a three-way valve. The horse was allowed to breathe quietly through the system to air for 2-3 minutes. When the pattern of breathing was stable, the horse was switched to breathe from the bag at the end of an expiration. The respired helium percentage was measured continuously with a respiratory mass spectrometer and when the helium percentage had stabilised, the horse was switched back to breathing air. The helium concentration in the bag was measured using a helium analyser.

Prior to exposure there was no difference in airway resistance or the number of neutrophils in BAL fluid (BALF) between RAO-affected horses and controls. No alterations in airway resistance were observed in the control group after hay/straw exposure for 24h. Four out of 6 RAO-affected horses demonstrated an increase in airway resistance after exposure. RAO-affected horses demonstrated a significantly greater increase in the number of neutrophils in BALF immediately after exposure compared to control horses (225±136 cells/ul [mean±sd] and 28±21 cells/ul, respectively; P=0.004). FRC showed a trend to be higher in RAO-affected horses prior to exposure compared to controls (39 ± 14 ml/kg/min and 24 ± 9 ml/kg/min, respectively; P=0.07). FRC was not altered by 24h exposure to allergen in either group (P>0.05). There was no correlation between FRC and absolute or change in airway resistance. These data are consistent with previous reports of a trend towards an increase in FRC in RAO affected horses following allergen challenge. FRC does not appear to be a sensitive index of alterations in lung function following an acute challenge that induced mild-moderate airway inflammation in RAO-affected horses.

INTERFERON ALPHA-INDUCED RESISTANCE TO BOVINE PI-3 VIRUS IS MEDIATED THROUGH THE MX PATHWAY

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The interferon alpha (IFN α) induced MX protein is a GTPase with a potent antiviral activity against several RNA viruses. This study aimed at testing whether the sensitivity to IFN α of a virus from the *Paramyxoviridae* family, bovine parainfluenza type 3 (Pi-3), can be attributed to the implementation of the IFN α - induced Mx pathway.

Vero cells, either stimulated by IFN α or transiently transfected with a plasmid coding for the human MXA protein (huMXA) were infected with Pi-3 at different multiplicity of infection for 24 h. Then, after fixation, permeabilization and labelling with fluorescent antibodies against huMXA and Pi-3, cell phenotypes were analyzed by flow cytometry. Pi-3 proteins expression was significantly inhibited both in cells treated with IFN α and in cells expressing high levels of huMXA protein.

These results suggest that the MX pathway is involved in the resistance conferred to bovine Pi-3 by type I interferons.