VCRS 2004

The 22nd Symposium of the Veterinary and Comparative Respiratory Society

Marriott SpringHill Suites
Old Montreal
Montreal, Quebec
October 1st-3rd, 2004

PROCEEDINGS

of the

22nd Veterinary and Comparative Respiratory Society Symposium

October 1st – 3rd, 2004

Marriott SpringHill Suites
Old Montreal
Montreal, Quebec

Major sponsor of the VCRS:

Boehringer Ingelheim

Boehringer-Ingelheim
Animal Health

Veterinary and Comparative Respiratory Society
2004

Officers

President : Jean-Pierre Lavoie, Saint-Hyacinthe, Quebec
Executive Secretaries : Mike Davis, Stillwater, OK
Past President : Theresa Fossum, College Station, TX
President-Elect : Petra Reinhold, Jena, Germany
European Secretary : Petra Reinhold, Jena, Germany
Treasurer : Mike Davis, Stillwater, OK

Board Members

Virginia Beuchner-Maxwell, Blacksburg, VA
David Collie, Edinburgh, Scotland
Laurent Couëtil, West Lafayette, IN
Juergen Daemmgen, Ingelheim, Germany
Mike Davis, Stillwater, OK
Jan Dye, Research, Triangle Park, NC
Theresa Fossum, College Station, TX
Andrew Hoffman, Grafton, MA
Lynelle Johnson, Davis, CA
Jean-Pierre Lavoie, Saint-Hyacinthe, Quebec
David Marlin, Kentford, UK
Brendan C. McKiernan, Wheat Ridge, CO
Petra Reinhold, Jena, Germany
Ronald Slocombe, Victoria, Australia
Laurent Viel, Guelph, Ontario

SPONSORS

The Veterinary and Comparative Respiratory Society wishes to thank the following sponsors. Without their generous support, a meeting of this caliber would not be possible.

Développement économique et régional et Recherche
Québec

MERCK

Merck Research Laboratories

Wyeth
Animal Health

22nd SYMPOSIUM OF THE VETERINARY AND COMPARATIVE RESPIRATORY SOCIETY
MONTREAL, QUEBEC, CANADA

PROGRAM

Thursday, September 30th, 2004

18:00-19:00 Welcome reception (wine and cheese)
Dean Raymond S. Roy
Faculté de médecine vétérinaire, Université de Montréal

Friday, October 1st, 2004

8:00-8:15 Introductory remarks (Jean-Pierre Lavoie, President, VCRS)

8:15-9:15 **"Airway Remodeling in Asthma"**
Jim Martin, Professor, Dept. of Medicine, Director, Meakins-Christie Laboratories, McGill University, **pg 9**

9:15-10:15 **"Tissue injury and remodeling due to inflammation"**
Qutayba Hamid, James McGill Professor, Depts. of Medicine and Pathology, Associate Director, Meakins-Christie Laboratories, McGill University

9:45-10:15 Coffee break

10:15-12:00 **5 MINUTE INTRODUCTION TO THE POSTERS**

"Effect of repeated cadmium inhalation on MMP-2 and MMP-9 activity in bronchoalveolar lavage fluid in rats", G Vincke, N Kirschvink, M Belleflamme, C Desmet, M Peck, P Gustin, **pg. 58**

"Development and application of an ovine-specific microarray for gene expression profiling in the lung", S P Smith, P Dicksinson, V Ogilvie, R Talbot, D David S Collie, **pg. 59**

"Complete cDNA sequence encoding the porcine MX1", A Thomas, M Palm, A Broers, M Leroy, D Desmecht, **pg. 61**

"Validation of plethysmography as a discriminating tool to evaluate susceptibilitiy patterns to lung infections in mice", T Flandre, M Leroy, D Desmecht, **pg. 62**

"Activator protein-1 activity in bronchial brushing samples from horses with recurrent airway obstruction", L L Couëtil, T Art, F Bureau, P Lekeux, **pg. 63**

"Effect of inhaled fluticasone on airway reactivity and inflammation in cats", N Kirschvink, J Leemans, F Delvaux, F Snaps, C Clercx, P Gustin, **pg. 65**

"Immunohistochemical expression of tryptase in the lung of control and heaves susceptible horses during challenge", K J Dacre, C Berney, L R Bartner, N E Robinson, J K Brown, A D Pemberton, B C McGorum, **pg. 66**

"Adjustment in functional residual capacity due to external loading does not explain the 'fixed resistor effect'", J Lofgren, D Bedenice, E Rozanski, T Oura, J Abrams, A Hoffman, **pg. 67**

"Stat5 promotes granulocyte survival during lung inflammation", L Fiévez, C Desmet, G Seumois, B Pajak, L Gillet, A Vanderplasschen, P Lekeux, F Bureau, **pg. 69**

"Assessment of viral-induced lung injury in mice using monitoring of carbon monoxide uptake: comparison with ventilatory pattern and histology", P Faisca, M Leroy, D Desmecht, **pg. 70**

"Selective Blockade of NF-κB Activity in Airway Immune Cells Inhibits the Effector Phase of Experimental Asthma", C Desmet, P Gosset, B Pajak, D Cataldo, M Bentires-Ali, P Lekeux, F Bureau, **pg. 71**

"Implication of avian respiratory physiology on the use of capnometry in the monitoring of CO_2 dynamic in anesthetized birds, M Desmarchelier, Y Rondenay, G Fitzgerald, S Lair, **pg. 72**

"Myeloperoxydase concentration in bronchoalveolar lavage from healthy and heavy horses", T Art, C Desmet, P Lekeux, B de Moffarts, L Couëtil, M Becker, T Franck, S Kohnen, G Deby-Dupont, D Serteyn, **pg. 74**

"Lung function in horses over 4 consecutive days following a short elective surgical procedure (castration) in dorsal recumbency", S Leinker, K Riedelberger, U Auer, **pg. 76**

"Endothelin and nitric oxide production by equine bronchial epithelial cells cultured under air-liquid interface conditions", L R R Costa, K O'Reilly, R Truax, T Foster, J R Johnson, R M Moore, **pg. 78**

"The influence of feeding on the concentration of urea and ammonium in exhaled breath condensate and peripheral blood", P Reinhold, A Langenberg, M Rothe, G Becher, **pg. 79**

12:00-14:00 **Lunch and Poster viewing** *(all posters are up)*

ORAL PRESENTATIONS

14:00-14:15 "Differential gene expression in acute equine recurrent airway obstruction", M V Crisman, J R Tschetter, S L Woody, L M Beex, **pg. 14**

14:15-14:30 "DNA damage in respiratory epithelial cells of non-RAO and RAO-affected horses", T. L Cuff, C M Deaton, D Kingston, R Williams, D J Marlin, **pg. 15**

14:30-14:45 "Tryptase mRNA transcript regulation in control and heaves susceptible horses during challenge", K J Dacre, R S Pirie, C Berney, L R Bartner, N E Robinson, A D Pemberton, B C McGorum, **pg. 16**

14:45-15:00 "Comparison of different stains for detection of mast cells in equine BALF", M Leclère, M Desnoyers, JP Lavoie, **pg. 17**

15:00-15:15 "Ovine elafin compartmentalizes the lung's response to bacterial LPS", T Brown, D Collie, JM Sallenave, **pg. 18**

15:15-15:30 "Measurement of exhaled nitric oxide and carbon monoxide in the horse", C M Deaton, C Maxted, A Fehmi, D J Marlin, **pg. 19**

15:30-15:45 "Concentration of NO in BALF of horses with heaves", S Macieira, D Jean, JP Lavoie, **pg. 20**

15:45-16:00 "Efficacy of theophylline associated with a low dose of dexamethasone in the treatment of horses with heaves", C Cesarini, E Hamilton, V Picandet, JP Lavoie, **pg. 21**

16:00-16:15 "Retrospective study: canine chronic nasal disease 1998-2003", M Dunn, E Meler, M Lécuyer, **pg. 22**

16:45 Departure for the Sugar Bush party

Saturday, October 2nd, 2004

8:00-9:00 "**Indications and limitations of current methodologies for the assessment of mechanical changes due to airway remodeling**" Dr. Jason Bates, Research Professor, College of Medicine, University of Vermont, **pg. 23**

ORAL PRESENTATIONS

9:00-9:15 "Analysis of the respiratory impedance at frequencies below 5 Hz using impulse oscillometry and FAMOS", J Jaeger, HJ Smith, P Reinhold, **pg. 28**

9:15-9:30 "Use of whole body plethsymography to assess influences of rat strain and age on nonspecific airway responsiveness", J A Dye, D W Winsett, N Haykal-Coates, D L Costa, **pg. 30**

9:30-9:45 "Molecular mechanics of smooth muscle myosin molecules in the latch-state", R Léguillette, AM Lauzon, **pg. 31**

9:45-10:00 "Effect of obesity on airway function in healthy retrievers", J Bach, E Rozanski, D Bedenice, D Chan, L Freeman, J Lofgren, T Oura, A Hoffman, **pg. 33**

10:00-10:30 Coffee break

10:30-10:45 "Interferon alpha-induced resistance to bovine parainfluenza type 3 virus is not mediated through the three classic pathways", M Leroy, E Baise, D Desmecht, **pg. 35**

10:45-11:00 "Autonomic dysfunction in horses affected by recurrent airway obstruction (RAO)", S Norman, D J Marlin, R Newton, R Eager, N Waran, P Harris, C M Deaton, **pg. 36**

11:00-11:15 "Airway smooth muscle remodeling in horses with heaves", D Ramos-Barbon, B Herszberg, M Tamaoka, J G Martin, JP Lavoie, **pg. 37**

11:15-11:30 "Persistent mucus accumulation – A consequence of delayed apoptosis in RAO-affected horses?", L R Bartner, Y Tesfaigzi, N E Robinson, **pg. 38**

11:30-11:45 "Exposure of horses to total and respirable particle endotoxin concentrations generated by specific feed and bedding materials", P J Spendlove, J L Hodgson, D R Hodgson, N Malikides, **pg. 39**

11:45-12:00 "Indoor air quality in a boarding stable", M May, N E Robinson, **pg. 41**

12:00-12:15 "Is a cough just a cough?", A Hoffman, D Bedenice, M Mazan, **pg. 42**

12:15-12:30 "Climatic and aeroallergen risk factors for chronic obstructive pulmonary disease", L L Couëtil, M P Ward, **pg. 44**

12:30-12:45 "Prevalence of inflammatory airway disease in Michigan pleasure horses – winter 2003-4", N E Robinson, S J Holcombe, F J Derksen, E A Carr, **pg. 45**

13:00-18:00 Lunch and field trip (Biodome de Montréal)

19:00-20:00 VCRS Banquet and Presentation of the Joan O'Brien Award

20:00-21:00 Evening lecture: "**Life, health and the second law of thermodynamics**"
Dr. Peter Macklem, Professor Emeritus, McGill University, **pg. 46**

Sunday, October 3rd, 2004

8:30-9:30 **"Comparative Aspect of breathing in Newborns"**
Dr. Mortola, Professor, Department of Physiology, McGill University, **pg. 47**

ORAL PRESENTATIONS

9:30-9:45 "Metabolic acidosis and hypocalcemia increase the PaO_2 of anesthetized dogs", E Aguilera-Tejero, I Lopez, A J Felsenfeld, J C Estapa, M Rodriguez, **pg. 49**

9:45-10:00 "Acid base balance in arterial blood in horses with heaves", V Picandet, D Jean, JP Lavoie, **pg. 50**

10:00-10:30 Coffee break

10:30-10:45 "Comparison of dexamethasone and the MAPK p38 inhibitor MRL-1EQ in equine heaves", J.-P. Lavoie, D. Thompson, E. Hamilton, M Debrue, F. David, G. Hickey, **pg. 51**

10:45-11:00 "Sonography compared with radiography in revealing rib fracture in newborn foals in an equine intensive care unit", D Jean, V Picandet, S Macieira, G Beauregard, **pg. 52**

11:00-11:15 "Defining the role of organ-based adaptation to localized insult using the sheep as a model system", D D Collie, S P Smith, S Tate, P Dickinson, V Ogilvie, **pg. 53**

11:15-11:30 "Evaluation of lung function in pigs experimentally infected with *Chlamydia suis",* P Reinhold, A Langenberg, K Sachse, **pg. 54**

11:30-11:45 "A preliminary investigation of exhaled NO & CO in healthy cats and cats with airway inflammation", D Marlin, A Fehmi, A Sparkes, E Mardell, C M Deaton, V Adams, **pg. 56**

11:45-12:00 VCRS Business Report

AIRWAY REMODELING IN ASTHMA

Jim Martin, Professor, Dept. of Medicine, Director,
Meakins-Christie Laboratories, McGill University

Introduction

The airways in asthma are altered structurally and functionally so as to contribute to the phenomenon of airway hyperresponsiveness, which is a defining characteristic of asthma. The structural alterations are generally considered under the rubric, airway remodeling. Although some authors have chosen other definitions, including airway inflammation the former definition is more usual. Airway remodeling results from the complex interactions of various inflammatory mediators on airway structural cells such as epithelium and smooth muscle. The changes observed microscopically include epithelial detachment, goblet cell hyperplasia, subepithelial fibrosis, new vessel formation, smooth muscle growth and adventitial thickening. There is a growing conviction that chronic disease is maintained by airway remodeling.

Epithelial changes in asthma

Epithelial changes include metaplasia and goblet cell hyperplasia. Epithelial detachment has been described by some but not all investigators. There is agreement that the epithelium is at least fragile. There are also substantial changes related to the expression of pro-inflammatory molecules by the epithelium but they are not generally considered as remodeling. An increase in the numbers of intraepithelial dendritic cells, professional antigen presenting cells that are crucial for allergic responses, follows allergen exposures. Under the epithelium there is thickening of the sub-epithelial basement membrane region that is caused by collagen deposition. Although this finding was thought to be typical of asthma is can be seen in other pathologies such as COPD. The importance of epithelial changes for asthma symptoms and signs is uncertain.

Airway smooth muscle remodeling in asthma

The increase in airway smooth muscle (ASM) by hyperplasia is arguably the most important of the changes in that it could account for excessive airway narrowing in asthma which is by far the most significant cause of symptoms. An increase in ASM can overcome the mechanical impedances imposed by the lung parenchyma under normal circumstances that are critical for maintaining the airways in a dilated state and preventing much airway narrowing when inflammatory mediators are present. Many studies of the asthmatic airway have shown that ASM mass is increased. Few studies have addressed the mechanisms of the increase. Hyperplasia has been deduced to have occurred, based on a count of nuclei and its comparison with the area of muscle. In some subjects hypertrophy also contributes to the change in mass. Of particular significance for severe asthma is that an increase in ASM in the larger airways is associated with fatal asthma whereas increase in ASM mass in the peripheral

airways is a feature of both non-fatal and fatal asthma. Since the severe narrowing of the airways that is required to trigger a life-threatening degree of asthma requires that much of the airway tree is affected, then coincidental narrowing of a few central airways is an event that has a greater probability of occurring than the coincidental narrowing of a large number of peripheral airways.

Modeling of airway smooth muscle growth

A variety of small animal models of asthma have been used to study ASM growth. Most studies have been performed in the Brown Norway rat, which is an animal whose immune system is Th2 biased and shows typical eosinophilic airway inflammation following allergen challenge. Repeated exposure to sensitizing antigen such as ovalbumin results in an increase in airway smooth muscle mass, assessed by morphometric techniques. The increase in ASM mass in the large airway correlates with the change in airway responsiveness to methacholine that is observed after the repeated allergen exposures. Several investigators have shown that hyperplasia occurs, through the demonstration of the incorporation of bromodeoxyuridine, a marker of the S phase of the cell cycle. Cysteinyl leukotrienes and endothelin are both involved in the hyperplastic response of the ASM cells *in vivo* but the cell sources of these mediators are as yet uncertain. These mediators are also present in elevated amounts in the airways in human asthma. Activated macrophages, mast cells and the airway epithelium are potentially of importance as sites of synthesis. Indeed mast cells are found in high numbers interspersed among the smooth muscle fibers in subjects suffering from asthma.

Theoretically an increase in ASM mass could results either from hyperplasia or from a reduction in the normal turnover of the tissues, more specifically through the inhibition of apoptosis. Even the normal rat shows a considerable turnover of the tissues of the airway, not only the epithelium which would not be surprising as epithelial surfaces are in general in a more rapid state of renewal than other tissues but also mesenchymal cells such as smooth muscle. Cells proliferating and undergoing apoptosis are not rare, suggesting a rapid and dynamic turnover of tissues. Interestingly the balance between these two phenomena appears to be finely controlled because the mass of muscle is a constant throughout the airway tree and is similar from animal to animal. How these processes are coupled has not been established but one is tempted to speculate that the pro-mitogenic signals for the growth of new ASM may originate also from the cells that remove apoptotic cells such as the tissue macrophages. These cells are certainly a source of cys-leukotrienes and growth factors.

T cells and airway remodeling *in vivo*

$CD4^+$ T cells of the Th2 phenotype play a pivotal role in the inflammatory response associated with asthma. It is therefore of importance to the understanding of airway remodeling to determine whether these cells participate in airway remodeling. T cells can exert mitogenic effects on ASM directly *in vitro*

but may also mediate effects on ASM and other airway structures through their cytokines or by driving other cells of the immune system. Direct contact between activated T cells and activated smooth muscle can evoke a proliferative response of the muscle, involving CD44, hyaluronic acid and integrins. One technique for the implication of T cells in allergic airway responses *in vivo* is adoptive transfer. Cells harvested from sensitized donors can transfer allergen sensitivity to naïve recipient rats such that these animals develop late allergic airway responses, eosinophilic inflammation and a typical Th2 profile of cytokines after challenge. We have employed the technique of adoptive transfer to investigate whether primed $CD4^+$ T cells could mediate allergen-induced airway remodeling using an *in vivo* rat model. We focused on changes in the ASM and the epithelium. $CD4^+$ T cells were purified from the cervical lymph nodes of ovalbumin (OVA)-sensitized donors and transferred to naive recipients.

Actively sensitized rats that undergo repeated allergen challenge have airway remodeling that includes a hyperplastic response of both the epithelium and the ASM. We explored a similar protocol of challenge in rats that were "sensitized" to OVA through T cell transfers. CD4+ T cells were purified from OVA-sensitized rats and transferred *i.p.* to unsensitized recipients that underwent three OVA challenges at three day intervals. We found that both ASM and epithelium showed proliferative responses by $PCNA^+$ immunostaining. The intensity of the proliferative response varied from airway to airway but was similar for both the epithelium and ASM in individual airways, suggesting that common mediators might be involved in the responses of both tissues. In addition to proliferation there was also evidence that mass of ASM is regulated through changes in the rates of programmed cell death. Animals that received CD4+ T cells from OVA sensitized donors and that underwent OVA challenges showed a reduction of the density of apoptotic cells in both the epithelium and ASM, as assessed by TUNEL staining. We confirmed also that the changes in these signals were associated with an increase in ASM mass by a detailed morphometric analysis smooth muscle specific alpha actin positive tissue. It appears that CD4+ T cells are sufficient for the triggering of ASM and epithelial remodeling after allergen challenge. The relative importance of the inhibition of apoptosis and stimulation of hyperplasia as mechanisms for altering tissue mass requires to be established.

We have successfully identified enhanced green fluorescent protein (EGFP) expressing T cells that were ovalbumin specific in the airways of rats challenged with ovalbumin. These observations support the plausibility of the hypothesis that direct interactions of CD4+ T cells with ASM or direct effects of T cell derived cytokines such as IL-13 and IL-4 on the ASM may occur. The former enhances mitogenic responses to cys-LT1 receptor agonists by increasing cys-LT1 receptor expression on the ASM but IL-4 appears to be anti-proliferative. Interestingly, IL-4 has been recently reported to induce apoptosis of lipopolysaccharide stimulated monocytes, by activating the caspase cascade. Whether IL-4 has effects of this sort on ASM has not been reported. The plausibility of direct interactions of T cells with ASM to an extent that could have a significant impact on airway remodeling

requires that these cells be present in the ASM layer in sufficient numbers. However, to date there appear to be no quantitative studies of the density of T cells in the airway smooth muscle layer.

Airway smooth muscle remodeling in an equine model of asthma

The validity of the mechanisms of tissue remodeling in small animals for human disease needs to be determined because it is conceivable that the rat and other small animals may have a higher intrinsic rate of tissue turnover. The results of studies of tissue remodeling in larger mammals are of interest in this regard. The horse suffers from a condition called heaves, which is a spontaneous form of reversible airways obstruction and airways hyperresponsiveness caused by sensitization to constituents in moldy hay. The disease behaves clinically like asthma and is responsive to bronchodilators and corticosteroids similarly to asthma. It can result in persistent airflow obstruction also, similar to chronic asthma. Bronchoalveolar lavage demonstrates a neutrophilic inflammation but there is a Th2 pattern of cytokines expressed in the airways. Therefore by several criteria heaves is a pertinent model for human asthma.

We have examined lung tissues from horses with heaves for evidence of remodeling by standard morphometric techniques. Both the mass of ASM, as defined by smooth muscle specific alpha-actin immunoreactivity and the density of PCNA+ cells within the smooth muscle layer of the airways were determined. The mass of muscle was markedly increased (approximately three-fold) compared to control horses and the PCNA+ cells were almost nine times as frequent. This confirmed that even in large mammals hyperplasia of ASM could occur and potentially account for the increase in mass. There was also increase in the numbers of cells undergoing apoptosis, suggesting a homeostatic regulation of the mass of ASM in the airway wall. We have not attempted to address the issue of hypertrophy in this model and indeed in human asthma there is very limited information about its occurrence and its mechanisms.

Conclusions

Airway remodeling is an important part of asthma. There are several changes in the airways that are noteworthy, including epithelial changes and changes in ASM mass. These changes can be modeled in animals and can be driven by allergy. Airway smooth muscle growth can be clearly demonstrated to occur over a short time scale in small animals models of allergic asthma. Allergen specific CD4+ T cells transfers allergen sensitivity to recipient animals. Repeated allergen challenge elicits a hyperplastic response of ASM and a reduction in the rate of apoptosis. Cysteinyl leukotrienes and classical growth factors are likely important in causing some of the remodeling changes. Whether intimate contact between the T cell and ASM is necessary is unknown but does raise the possibility that cell-to-cell contact might occur and lead to ASM proliferation as has been shown *in vitro* or that cytokines such as IL-13 might facilitate growth by enhancing the responsiveness of the ASM to the actions of mediators such as cysteinyl-leukotrienes. Somewhat problematic is the fact that

the literature to date has not shown that ASM hyperplasia occurs *in situ* in asthma. However there are several possible reasons for failure to make such observations. Subjects may have been adequately treated with anti-asthmatic medications prior to biopsy or ASM may be recruited by migration of myofibroblasts in the sub-epithelial region. Perhaps the rate of tissue turnover in asthmatic subjects is relatively slow and therefore any increases in rate of proliferation may be small and hard to detect. Reduction in the rate of apoptosis is an alternative mechanism for increase in ASM mass. All of these possibilities will need to be explored in order to resolve the issue of whether ASM grows from existing bundles of muscle or is added from other sites. The therapeutic strategies that might be ultimately employed to prevent airway remodeling will depend on the elucidation of the biology of the process.

DIFFERENTIAL GENE EXPRESSION IN ACUTE EQUINE RECURRENT AIRWAY OBSTRUCTION

JR Tschetter, SL Woody, LM Beex and MV Crisman
VA-MD Regional College of Veterinary Medicine, Molecular Diagnostics Lab, Blacksburg, VA

Purpose: The objective of this study was to identify genes that are differentially expressed in the acute Recurrent Airway Obstruction (RAO) lung compared to lung from a clinically normal horse.

Methods: Lung biopsies were harvested from a horse with previously diagnosed RAO following challenge with straw bedding and moldy hay. A lung biopsy was also harvested from a healthy control horse. Suppressive subtractive hybridization-polymerase chain reaction (SSH-PCR) and reverse Northern blots were used to identify differentially expressed genes in lung tissue from the two horses (acute RAO vs Normal).

Summary: RAO remains one of the most challenging respiratory conditions of adult horses. Central to the development of novel diagnostic and therapeutic strategies is the elucidation of the molecular events that drive this complex disease. SSH PCR was used to identify differentially expressed genes in acute RAO versus normal lung tissue. Two subtractions were completed (acute RAO minus normal and normal minus acute RAO) to identify genes that are either up or down regulated during acute disease. All clones were checked for differential expression by the printing of PCR products or purified plasmid on nylon membranes and probing with radiolabeled forward and reverse probes. Searches using the NCBI BLAST program revealed clones with homology to known genes, ESTs and clones with homology to no published sequences.

One disadvantage of using SSH-PCR is that this procedure, in general, requires a five-fold difference in the level of gene expression for selection. To increase the pool of differentially expressed genes, a library of lung clones was screened by reverse Northern blot analysis. mRNA from normal and acute RAO lung tissue was used as probe. These reverse Northern blots resulted in the detection of additional differentially regulated genes. Reverse Northern blot analysis was also used to confirm the differential expression of the clones identified by SSH-PCR. Preliminary evaluation suggests differential regulation of genes encoding for stress-induced proteins, regulators of apoptosis, T cell activators, immune response proteins and metabolic-growth related genes.

Conclusions: Results confirm the effectiveness of SSH-PCR to detect differential gene expression in horses with RAO. Selected genes will be used to produce a custom gene macroarray to further evaluate differential gene expression in horses with RAO.

DNA DAMAGE IN RESPIRATORY EPITHELIAL CELLS OF NON-RAO AND RAO-AFFECTED HORSES

Thea L Cuff, Christopher M Deaton[1], Demelza Kingston, Rachel Williams and David J Marlin[1]
Hartpury College, Hartpury House, Gloucester, UK, GL16 3BE
[1]Centre for Equine Studies, Animal Health Trust, Lanwades Park, Kentford, Suffolk, UK, CB8 7UU

Previously we have shown that peripheral blood mononuclear cells (PBMC) from horses affected by recurrent airway obstruction (RAO) in clinical remission have a significantly higher endogenous level of DNA damage (quantified by the Comet assay) than non-RAO horses[1]. Since RAO is a pulmonary disease, we hypothesised that airway cells from RAO horses in clinical remission would also show greater DNA damage compared with non-RAO controls. Airway epithelial cells were obtained from the distal trachea of 4 non-RAO and 4 RAO-affected horses with a cytology brush *via* an endoscope. The cells were immediately placed in freezing media (10%DMSO/90%FCS) and slow frozen to –80°C over 24h and then transferred to –180°C. Cytology revealed that the samples comprised >95% epithelial cells. Cells were thawed and 'viability' determined by Trypan Blue exclusion (mean 5±4(SD)%). However, as we have reported previously for PBMC, the basal DNA damage in these cells was low. In addition, these cells could be cultured for at least 21 days suggesting that the viability was underestimated by Trypan Blue. Thus, we conclude that membrane integrity rather than viability *per se* was compromised by brushing. In addition, cells were only allowed ~25 min to recover from thawing prior to determining viability to limit further DNA damage. For the Comet assay, cells were re-suspended in PBS, mixed with low-melting point agarose and applied to coated slides (Trevigen). The cells were lysed in 2.5M NaCl, 100mM Na_2EDTA, 10mM Tris and 1% Triton-X100, incubated in alkaline electrophoresis buffer for 40 min, following which electrophoresis was undertaken at 4°C for 30 min at 0.8v/cm. Cells were stained using SYBR Green and scored manually with the scorer blinded to the identity of the samples. The CV for a QC sample run 4 times was 17%. Neither endogenous nor exogenous (after incubation with 50 umol/l hydrogen peroxide) DNA damage was different between non-RAO or RAO affected horses ($P>0.05$). However, following stabling on shavings for 72 h, RAO horses had a significant increase in endogenous DNA damage (Pre 85±64(SD)[arbitrary units]; Post 179±47: $P=0.04$). There was no change in exogenous DNA damage following stabling. In conclusion, whilst PBMC from RAO horses in clinical remission have greater DNA damage, this does not appear to be mirrored by greater damage in tracheal epithelial cells. Stabling on wood shavings, which resulted in mild neutrophilic tracheal inflammation did result in a significant increase in epithelial cell endogenous DNA damage.

[1]Marlin, D.J., Johnson, L., Kingston, D.A., Smith, N.C., Deaton, C.M., Mann, S., Heaton, P., Van Vugt, F., Saunders, K., Kydd, J. and Harris, P.A. (2004) Application of the comet assay for investigation of oxidative DNA damage in equine peripheral blood mononuclear cells. *J Nutr* **134**, 2133S-2140S.

TRYPTASE mRNA TRANSCRIPT REGULATION IN CONTROL AND HEAVES SUSCEPTIBLE HORSES DURING CHALLENGE

Dacre, K.J.[1], Pirie, R.S. [1], Berney, C. [2], Bartner, L.R. [2], Robinson, N.E. [2], Pemberton, A.D. [1] and McGorum, B.C. [1]. [1]Royal (Dick) School of Veterinary Studies, Midlothian, UK, [2]Michigan State University, East Lansing, USA

The mast cell specific protease tryptase has previously been shown to be significantly increased in bronchoalveolar lavage fluid (BALF) from clinically affected heaves horses compared to controls or heaves horses in remission (Dacre et al, 2003). The aim of this study was to investigate tryptase mRNA transcript regulation in control and heaves susceptible horses during natural hay / straw challenge.

BALF cell pellets were harvested from control (n=3) and heaves susceptible (n=6) horses before and immediately after a 48h natural hay / straw challenge. Additionally, bronchial and bronchiolar tissue samples were collected *post mortem* from control (n=4), challenged control (n=5) and heaves horses in early resolution phase post challenge (5d in a hay / straw challenge environment and then 7d in a low dust environment) (n=7). mRNA was extracted from BALF cell pellets and tissue samples, reverse transcribed and subjected to quantitative PCR for transcripts of tryptase and the housekeeping gene β-actin.

Post 48h hay / straw challenge tryptase transcripts were upregulated five-fold in BALF cell pellets from heaves horses compared to challenged controls ($p=0.07$). In bronchiolar tissues tryptase transcripts were down-regulated seven-fold in heaves horses in early resolution phase post challenge compared to challenged controls ($p=0.02$) and eleven-fold compared to unchallenged controls ($p=0.01$).

In conclusion, tryptase transcripts appear to be upregulated in BALF mast cells following challenge of heaves susceptible horses and down-regulated in bronchiolar mast cells following removal of challenge.

Dacre, K.J., Deaton, C., Marlin, D., Pirie, R.S., Brown, J., Pemberton, A.D. and McGorum, B.C. (2003) Mast Cell Protease Concentrations In Equine Bronchoalveolar Lavage Fluid From Control And Heaves Affected Horses. *Proceedings of 21st Veterinary and Comparative Respiratory Symposium*, San Antonio, Texas, USA.

COMPARISON OF DIFFERENT STAINS FOR DETECTION OF MAST CELLS IN EQUINE BRONCHOALVEOLAR LAVAGE FLUID

M. Leclere, M. Desnoyers and J.-P. Lavoie. Département des Sciences Cliniques (Leclere, Lavoie), Département de Pathologie et Microbiologie. (Desnoyers), Faculté de médecine vétérinaire, Université de Montréal, Québec, Canada

Mast cells are part of normal equine bronchoalveolar lavage fluid (BALF), but they usually represent less than 2 % of the cells. Increased numbers of mast cells have been found in horses with inflammatory airway disease, but there is a large variation in the numbers reported by different authors. Since the presence of an abnormal mast cell count can have a clinical importance, it seems essential to have an accurate and sensitive method of detecting them in BALF.

In a prospective study, a clinical pathologist blindly evaluated four staining techniques on the cells from BALF of 24 horses presented for various respiratory diseases. A differential was made on 400 cells and the percentage of mast cells obtained with each staining technique was compared using a repeated measure ANOVA, along with a Fischer's PLSD.

A fast Romanosky stain (Diff-Quik®) was compared to the following metachromatic stains: an automated Romanosky stain (Hematek®), May-Grundwal Giemsa and Toluidine Blue. With the Diff Quik technique, the metachromatic granules of mast cells were not stained and their identification was based on morphological criteria. The percentage of mast cells in BALF was 1.01 ± 0.22, 1.71 ± 0.44, 1.91 ± 0.55 and 2.15 ± 0.46 for Diff-Quik, Hematek, May-Grundwal Giemsa and Toluidine Blue, respectively. The percentage obtained with the Diff-Quik technique was significantly lower than with the three other methods. The number of horses identified as having an increased number of mast cells (above 2 %) was also lower with Diff-Quik. Toluidine blue allowed for the detection of the highest number of mast cells but was inadequate for performing a complete differential. In conclusion, the fast Romanosky stain (Diff-Quik) is inadequate for the detection of mast cells in equine BALF and the automated Romanosky stain (Hematek), May-Grundwal Giemsa and Toluidine Blue provide metachormatic staining of mast cell granules.

OVINE ELAFIN COMPARTMENTALISES THE LUNG'S RESPONSE TO BACTERIAL LPS

T. Brown[1], D. Collie[2] and J-M. Sallenave[1]; [1]Rayne Laboratory, Medical School, University of Edinburgh; [2]Wellcome Trust Centre for Research in Comparative Respiratory Medicine, Easter Bush Veterinary Centre, Roslin, Edinburgh.

Introduction and Aims

Our laboratory is 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 constructed a replication deficient adenovirus (Ad) coding for the low molecular weight serine antiprotease ovine elafin (Ad-o-elafin). We have previously shown that the use of calcium phosphate/adenovirus co-precipitates leads to enhanced infection of both alveolar epithelial cells and also alveolar macrophages. In order to investigate the potential for elafin to modulate the response to intra-pulmonary LPS, two groups of sheep were bronchoscopically instilled with calcium phosphate/Ad into distinct lung segments with a dose of 108 pfu per segment. One group received Ad-o-elafin and the other Ad-GFP as a control. 10 days later *Mannheimia haemolytica* LPS was instilled into both adenovirus treated segments (referred to as Ad+/LPS+ segments) and also a naïve segment in the contra-lateral lung (Ad-/LPS+). These segments, and an additional 'new' naïve segment (Ad-/LPS-), were sampled by bronchoalveloar lavage 4 days later to assess inflammation by total and differential cell counts. Haematology was also performed at each time point.

Results

Ad-o-elafin treated animals showed a significant increase in BALF total cell counts and BALF neutrophil counts in both Ad+/LPS+ and Ad-/LPS+ segments when compared to the naïve segment (Ad-/LPS-). Ad-GFP instillation led to no significant changes in these parameters in the BALF.

In Ad-o-elafin treated animals there was a relative decrease in the circulating white cell count that occurs following LPS instillation in the lung seen in Ad-GFP treated animals (significant increase in total cell count at day 14 compared with pre-experimental values and a significant increase in circulating lymphocyte counts at day 14 compared to day 10 in Ad-GFP treated animals).

Conclusion

The up-regulation of ovine elafin in the lung leads to an exaggerated local response to LPS but a diminution of the systemic LPS response. Hence anti-proteases may have important functions in the compartmentalisation of inflammation within the lung.

MEASUREMENT OF EXHALED NITRIC OXIDE AND CARBON MONOXIDE IN THE HORSE

C.M. Deaton, C. Maxted[1], A. Fehmi[1] and D.J. Marlin
Centre for Equine Studies, Animal Health Trust, Newmarket, UK.
[1]School of Animal, Rural and Environmental Sciences, The Nottingham Trent University, Nottinghamshire, UK.

Analyses of gases such as nitric oxide (NO) and carbon monoxide (CO) in exhaled breath have been extensively studied in human beings for use as markers of airway inflammation. In healthy human subjects the concentrations of NO and CO in exhaled breath range from 2 – 20 ppb and 0.5 – 5ppm, respectively. The concentrations of both NO and CO have been demonstrated to be elevated in the exhaled breath of human asthmatics. Analysis of exhaled gases has the potential to provide a non-invasive diagnostic indicator of airway inflammation in horses. The aim of the present study was therefore to evaluate the collection and measurement of NO and CO in the exhaled breath of the horse. Collection of exhaled breath was performed using a sealed facemask and non-rebreathing valve. A 25-litre Tedlar bag was connected to the expiratory port of the valve. Concentrations of NO and CO were measured by chemiluminescence. The concentration of exhaled NO in 6 healthy control horses at pasture was 0.7 ± 0.2 ppb (mean ± SD). The concentration of exhaled CO was below the limit of detection of 0.1 ppm. Measurements of background air NO and CO were always made in parallel. The coefficients of variation in the concentration of exhaled NO for three consecutive measurements on the same sample and three samples collected consecutively in 6 healthy horses were 22 ± 13% and 41 ± 11%, respectively. The concentration of exhaled NO in the Tedlar bag was stable for at least 30 minutes when kept at room temperature. There was no significant diurnal or day-to-day variation in the concentration of exhaled NO in 3 control horses and 3 horses with a history of recurrent airway obstruction (RAO), which were asymptomatic at the time of sampling. Feeding of a pelleted concentrate had no effect on the concentration of exhaled NO or CO. In contrast, one-hour after feeding haylage (20g/kg bodyweight), the concentration of exhaled CO increased from below the limit of detection prior to feeding to 9.0 ± 2.9 ppm, returning to below the limit of detection by 2 hours after feeding. In order to determine if exhaled NO and CO are sensitive indicators of mild airway inflammation, four RAO-affected horses in remission were stabled for 3 days with a bedding of wood shavings. Stabling resulted in a small increase in airway inflammation from 7 ± 5 to 17 ± 6 neutrophils/μl. The concentration of NO prior to stabling (0.92 ± 0.28 ppb) was not increased at the end of the challenge (0.90 ± 0.14 ppb). CO was not detected in the exhaled breath either before or after challenge. In conclusion, the concentrations of NO and CO in the exhaled breath of horses without airway inflammation are low compared to that in human beings, which is consistent with previous published data for exhaled NO in horses. However, measurement of the concentrations of these exhaled gases is simple, reproducible and well tolerated by horses. The sensitivity of exhaled NO and CO as markers of airway inflammation therefore warrants further investigation.

CONCENTRATIONS OF NITRIC OXIDE IN BRONCHOALVEOLAR FLUID IN HORSES WITH HEAVES

Susana Macieira, Daniel Jean, Jean-Pierre Lavoie
Faculté de médecine vétérinaire, Université de Montréal, Qc, Canada

Nitric oxide (NO) has been shown to modulate the immune response in various inflammatory diseases. NO is synthesized by numerous cells normally present in the respiratory tract and could therefore be implicated in amplification and perpetuation of airway inflammation in heaves. The purpose of this study was to determine the concentrations of nitric oxide in bronchoalveolar lavage fluid of heaves-affected horses and normal horses.

Ten adult horses weighing approximately 450 to 650 kg were studied. Horses had normal CBC results and endoscopy of the upper respiratory tract did not reveal any abnormalities. Horses were diagnosed with heaves (n=5), or were considered to be free of respiratory disease (control horses; n=5) on the basis of history, clinical examination, and pulmonary function measurements. All horses were stabled in the same barn and were exposed to a dusty environment and moldy hay for several weeks before the beginning of the study. BAL was performed under endoscopic guidance in the right lung using two 250 ml bolus of warm isotonic saline and aspirated with a suction pump. The same procedure was then repeated in the left lung. BAL fluid was centrifuged and the sediment was frozen at –80ºC until analyzed. Concentration of NO in each BAL sample was indirectly measured by determining the concentrations of reactive nitrogen intermediate (nitrate and bound NO) using a chemiluminescent method.

There was a significant difference ($p<0.05$) between the lung function of horses with heaves (PL, 12-67 cm of H_2O) compared to that of controls (PL, 6-7 cm of H_2O). The percentage of neutrophils in BAL was also significantly greater in horses with heaves compared to controls, although pulmonary neutrophilia was present in both groups of horses. BAL fluid concentration of nitric oxide in heaves-affected horses was slightly but not significantly greater in the right lung than in the left lung. However, nitric oxide concentration in BAL fluid did not differ between the 2 groups of horses and was not correlated with BAL neutrophilia.

In conclusion, the results of this study suggest that NO is not likely to be a major mediator of chronic airway inflammation in horses with heaves.

This study was funded by the Fonds du Centenaire and Merck Research Laboratories.

EFFICACY OF THEOPHYLLINE ASSOCIATED TO A LOW DOSE OF DEXAMETHASONE IN THE TREATMENT OF HORSES WITH HEAVES

C Cesarini, E Hamilton, V Picandet, JP Lavoie
Faculté de médecine vétérinaire, Université de Montréal, Qc, Canada

Corticosteroids are currently the most effective drugs for the treatment of heaves, although their long-term administration is often hampered by the fear of the development of severe side effects. Theophylline is a bronchodilator with anti-inflammatory effects but its narrow therapeutic index has limited its use for the management of equine heaves. Several recent clinical studies demonstrated that the administration of low dosages of theophylline had a steroid-sparing effect in human asthmatics. The aim of the present study was to evaluate if theophylline potentates the effects of low dose dexamethasone in heaves in horses.

Ten heaves affected horses in clinical exacerbation were randomly allocated to 5 treatment groups during three experimental periods, using an incomplete block design. Horses were administered daily, for 7 days, either 25 mg of dexamethasone IV (Group A) or PO (Group B), 10 mg of dexamethasone PO (Group C) alone or combined with 5 mg/kg q12h of theophylline (Group D), or the same dose of theophylline alone (Group E). All horses were stabled together, bedded on straw and exposed to mouldy hay. A 4-week washout period separated each experimental treatment. Respiratory mechanics were performed before drug administration and on days 3 and 8. Clinical scores were evaluated daily during the study.

There was a improvement in the airway function of all horses from Group A ($p<0.05$). While non significant, there was an improvement in the respiratory condition of 3 of the 6 horses in Groups B and D, and 2 of the 6 horses in Group C. The respiratory condition of horses treated with theophylline alone (Group E) remained unchanged. Peak and trough serum levels of theophylline measured on days 2 and 5 were within the expected therapeutic range and the airway function of all horses improved following atropine administration.

Results of this study indicate that the oral administration of theophylline for 7 days at 5 mg/kg does not improve the airway function of horses with heaves. Furthermore, a potentiation of the effects of a low dose of dexamethasone by theophylline was not observed.

This study was funded by Vetoquinol Canada, Merck Research Laboratories and GREMEQ.

RETROSPECTIVE STUDY: CANINE CHRONIC NASAL DISEASE 1998-2003

Erika Meler DMV, Manon Lécuyer DMV, ACVIM, Marilyn Dunn DMV, MVSc, ACVIM. Faculté de médecine vétérinaire,
Université de Montréal, C.P. 5000, Qc, Canada

Purpose : To describe clinical, radiographic, endoscopic and cytologic findings in canine nasal disease and critically evaluate our ability to obtain an etiologic diagnosis.

Methods : Retrospective study of cases presenting to the Small Animal Teaching Hospital of the University of Montreal for evaluation of chronic nasal disease. Case selection : Cases must have clinical signs relating to the nasal cavity of at least one month's duration and have undergone diagnostic testing. Medical records of these cases were selected from 1998-2003.

Results : 80 cases were retained for the study. Average age at presentation was 7 years with a range of 4 months to 16 years. 57.5% (46/80) were males and 42.5% (34/80) were females. Brachycephalic breeds represented 16% (13/80) of the cases. Nasal discharge was present in 72.5% (58/80), sneezing was present in 66% (53/80) and epistaxis was found in 41% (33/80). Radiographs were performed in 65 cases. 46% (30/65) showed increased density of the nasal cavity, 22% (14/65) had signs of bone lysis and 23% (15/65) had frontal sinus involvement. Rhinoscopy was performed in 56 cases. Erythema was seen in 82% (46/56), a mass was seen in 16% (9/56), white, yellow and green plaques were seen in 13% (7/56), parasites and a foreign body were each seen in one case and 9% (5/56) cases presented no visible lesions. Nasal flush cytology was performed in 45 cases. Mixed inflammatory cells were seen in 51% (23/45) of cases, neoplastic cells were seen in 16% (7/45) and fungal hyphae were seen in 11% (5/45). Histologic analysis of nasal biopsies was performed in 56 cases. Inflammatory infiltrates were seen in 61% (34/56); 44% (15/34) neutrophilic, 35% (12/34) lymphocytic-plasmacytic and 21% (7/34) eosinophilic. Neoplasia was identified in 13% (7/56) and fungal disease was found in 9% (5/56). Bacterial culture was performed in 53 cases and mycology was positive in 14% (5/36) cases. MRI was performed in only 2 cases. In both cases a mass lesion in the nasal cavity was identified. Overall, an etiologic diagnosis was achieved in 64% (51/80) of cases; 24% (19/80) inflammatory rhinitis, 15% (12/80) neoplasia, 9% (7/80) mycotic rhinitis, 9% (7/80) oronasal fistula, 4% (3/80) cleft palate, parasitic rhinitis, a foreign body and bacterial rhinosinusitis were each diagnosed in one case. A diagnosis could not be established in 36% (29/80) of cases.

Conclusions: The results of this study indicate that inflammatory rhinitis, neoplasia and fungal disease are the most common etiologies of chronic nasal disease in the dog. Surprisingly, no etiology is identified in one third of cases despite an extensive medical evaluation. Only two cases underwent advanced imaging. As advanced imaging techniques become more widely available, it is expected that the number of undiagnosed cases will decrease. Future retrospective studies will be needed in order to assess the overall usefullness of advanced imaging in the diagnosis of canine nasal disease.

INDICATIONS AND LIMITATIONS OF CURRENT METHODOLOGIES FOR THE ASSESSMENT OF MECHANICAL CHANGES DUE TO AIRWAY REMODELING

Jason H.T. Bates, PhD, DSc
Research Professor
Vermont Lung Center
Departments of Medicine and Molecular Physiology and Biophysics
University of Vermont

INTRODUCTION

Assessment of lung mechanics is an exercise in what an engineer would call system identification, or inverse modeling. This begins with the application of some suitable input to the system under study, together with a measurement of the resultant output. The input and output are then related to each other in terms of a mathematical model encapsulating the important functional components of the system. The parameters of the model, which are evaluated by matching the model's behavior to that of the system, are taken as measures of corresponding quantities within the system itself.

Conventionally, flow (considered the input, while the pressure (P) required to generate that output. P is determined by

METHODS BASED ON THE SINGLE-COMPARTMENT LINEAR MODEL

The simplest model of lung mechanics that is physiological reasonable can be visualized as an elastic compartment (servo) served by a flow-resistive conduit. The equation relating V and P in this model is

where R, E and P_0 are parameters. If P is transpulmonary pressure (the difference between airway opening and esophageal pressures) then R is pulmonary resistance and E is pulmonary elastance. P_0 accounts for any positive end-expiratory pressure that might be present. This model describes P data accurately when most of the power in

simple model, so there are no values for R and E that will make the right-hand side of Eq. 1 match the left-hand side exactly. However, it is possible to find values for R and E that match the two sides of the equation as closely as possible. This used to be achieved, prior to the widespread use of digital computers, by the electrical subtraction method of Mead and Whittenberger [9]. Now, however, R and E are conveniently evaluated by multiple linear regression (Fig. 1).

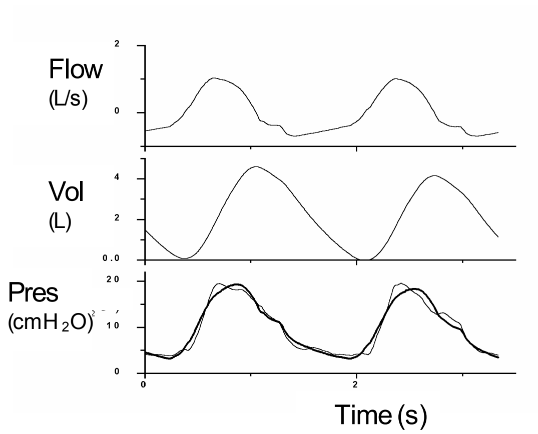


Figure 1 : An input flow signal (top) is applied to the lungs to produce an output pressure (bottom, thin line). The associated volume signal (middle) is obtained by integrating flow with respect to time. Employing Eq. 1, we find the best prediction of pressure (bottom thick line) by adding scaled version of flow and pressure together with an addition constant (P_0). The scaling factors applied to flow and volume are *R* and *E*, respectively.

The single -compartment linear model is readily extended to include nonlinearities of both *R* and *E*. For example, if airflow in the lungs is turbulent, the resistance of the airways will depend on flow [13]. Alternatively, ela stance may increase with volum e, particularly in injured lung or at high lung volumes [3].

FREQUENCY DEPENDENCE OF RESISTANCE AND ELASTANCE

The physiological interpretations of *R* and *E* appear to be obvious – *R* is the flow resistance of the airways and *E* is the stiffness of the lung tissues. However, invasive studies with alveolar capsules in animals have shown [1, 5] that this is incorrect in the case of *R*, which is composed of both airway resistance (*Raw*) plus tissue resistance (*Rt*). *R* depends markedly on the frequency of ventilation because, while *Raw* is independent of frequency, *Rt* decreases asymptotically toward zero as frequency increases.

Frequency dependence of *R* and *E* is not something that the single -compartment linear model is able to account for. The question, therefore, is h ow the single -compartment linear model should be extended to more accurately represent the mechanical properties of a real lung. There are several physiologically plausible possibilities (Fig. 2); two elastic compartments connected in parallel [12], two compartments connected in series [10], and a single alveolar compartment surrounded by viscoelastic tissue [11]. The viscoelastic model shown in Fig. 3 features an airway resistance (R_1) together with a double spring and dashpot assembly known as a Kelvin bo dy (R_2, E_1 and E_2) that describes the tissues.

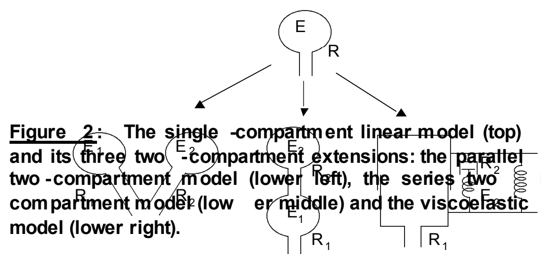


Figure 2: The single -compartm ent linear model (top) and its three two -compartment extensions: the parallel two -compartment model (lower left), the series two compartment model (low er middle) and the viscoelastic model (lower right).

It has been shown that the viscoelastic model is the most appropriate for describing a normal lung [1]. However, it has also been shown that the lung becomes markedly heterogeneous under pathological con ditions [4, 7], so some combination of all three models is likely necessary in disease.

IMPEDANCE

A more general approach to the multi - compartment behavior of the lung is provided by a quantity known as input impedance, Z_{in}, which is really nothing more t han a description of how *R* and *E* vary with frequency over some range. Z_{in} can be obtained, in principle, by oscillating the lungs with flow at a particular frequency, calculating *R* and *E* via Eq. 1, moving on to another frequency, and so on until the desire d frequency range has been covered. However, it is

more usual and much more convenient, to use the so-called forced oscillation technique [14]. Here, a complex

loudspeaker, piston pump), while P is measured. Computational techniques employing the fast Fourier transform are then used to separate V and P into their individual frequency components, and R and E determined for each component.

The calculation of Z_{in} is based on the assumption that the lung is a linear dynamic system. This means that it behaves like a single-compartment linear model (Eq. 1) at each frequency, even though the values of R and E may not be same at all frequencies. Consequently, interpreting Z_{in} globally requires a model of the lung that allows for R and E to be frequency dependent. The models show in Fig. 2 are possible candidates. However, a better description of the normal lung is provided by the so-called constant-phase model [5, 15]. This is a uniformly ventilated viscoelastic model much like the one shown in Fig. 2, except that its tissue properties are described slightly differently.

Z_{in} for the constant-phase model is

R_N is a Newtonian resistance that usefully approximates that of the pulmonary airways, I_{aw} is the inertance of the gas in the airways, G_t characterises viscous dissipation of energy within the lung tissues during inflation and deflation, and H_t characterises energy storage in the tissues. G_t is thus related to tissue resistance while H_t is related to tissue elastance. The two terms R_{aw} and $i2\pi f I_{aw}$ thus constitute the impedance of the single model airway, while the quantity $(G_t - iH_t)/(s\pi f)^{\alpha}$ is the impedance of the tissues. Of particular note is that the ratio of the real to the imaginary parts of tissue resistance is constant with f, and hence the name "constant-phase".

Figure 3: Fit of Eq. 2 (solid lines) to Z_{in} (mean ± SE) from normal mice (n = 11) under baseline conditions (from [15]).

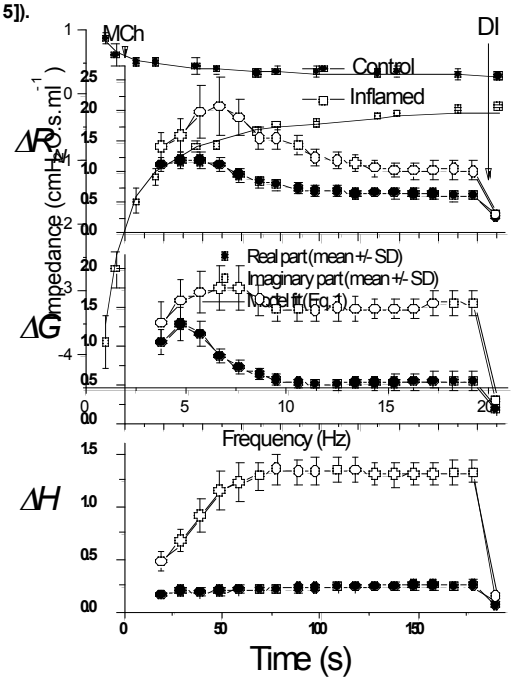
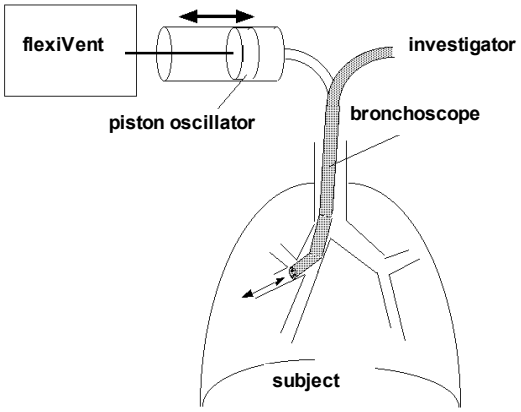


Figure 4: The parameters of the constant-phase model (Eq. 2) expressed as fractional changes above baseline in control and allergically inflamed mice following a 40 s challenge with an aerosol of methacholine. MCh indicates time of completion of delivery of methacholine. DI indicates time of delivery of two deep lung inflations to 25 cm H_2O (from [15]).



is able to reproduce the differences between normal and an allergically mice simply by increasing the epithelial thickness lining the airways and the tendenc y of the airways to close [15].

Figure 5: The subject lies supine while the bronchoscope is wedged by the investigator into a subsegment of the right middle lobe. A plastic catheter connects the piston of the oscillator to the instrument channel of the bronchoscope, through which is delivered an oscillatory volume signal (from [6]).

The forced oscillation technique is becoming increasingly used in human subjects. In a variant of this technique [6], flow oscillations were applied to a segment of the lung through a wedged bronchoscope (Fig. 5). The resulting real and imaginary parts of impedance were found to vary significantly when methacholine was instilled into the wedged segment through the bronchoscope (Fig. 6). This technique was used to directly demonstrate that the lung periphery in asthmatics is hyperresponsive to bronchial challenge [6].

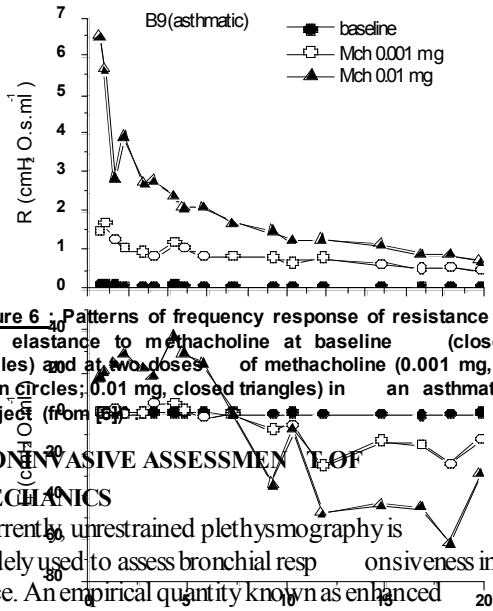


Figure 6 : Patterns of frequency response of resistance and elastance to methacholine at baseline (closed circles) and at two doses of methacholine (0.001 mg, open circles; 0.01 mg, closed triangles) in an asthmatic subject (from [8]).

NONINVASIVE ASSESSMEN T OF MECHANICS

Currently, unrestrained plethysmography is widely used to assess bronchial resp onsiveness in mice. An empirical quantity known as enhanced pause (Penh) is derived from the plethysmographic box pressure $P_b(t)$ and assumed to be an index of bronchoconstriction. However, $P_b(t)$ is determined largely by gas conditioning when normal mice br eathe spontaneously inside a closed chamber in which the air is at ambient conditions [8]. When the air in the chamber is heated and humidified to body conditions, the changes in $P_b(t)$ are significantly reduced (Fig. 7).

The remaining changes are thus due to gas compression and expansion within the lung.

$$\int_0^{Ti} P_b(t)\,dt = \frac{V_T\, R_{aw}\, FRC}{V_b}$$

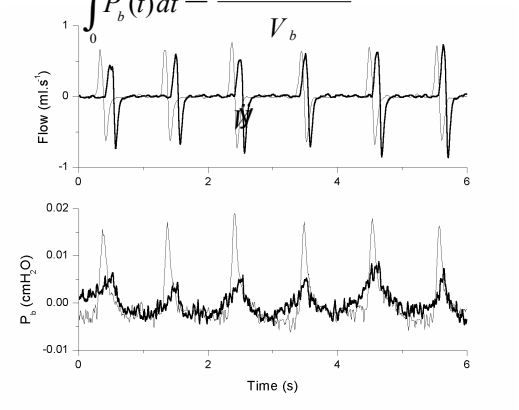


Figure 7: Records of P_b made with a mouse breathing spontaneously while enclosed in the chamber with the gas inside the chamber at 23 °C and relative humidity 29% (thin lines), and after the chamber gas was heated to 37 °C and humidified to 85% (thick lines). Inspiratory flow is positive (from [8]).

When the chamber gas is appropriately conditioned, the time-integral of $P_b(t)$ over inspiration is determined by R_{aw}, FRC and V_T thus

where V_b is the volume of the plethysmograph. Thus, unless these various quantities can be either controlled or measured, unrestrained plethysmography is not likely to be of practical use as a means of obtaining accurate measures of mechanical lung function. This applies in particular to Penh, which should not be used as a means of assessing bronchial responsiveness.

It may be possible to estimate changes in R_{aw} using Eq. 4 if some independent means for measuring changes in lung volume can be used while P_b is being measured. This would be extremely useful, even though it could never provide measurements of the precision of the forced oscillation technique in anesthetized tracheostomized animals [2].

REFERENCES

1. Bates JHT, Ludwig MS, Sly PD, Brown K, Martin JG, Fredberg JJ (1988) Interrupter resistance elucidated by alveolar pressure measurement in open-chest normal dogs. J Appl Physiol 65: 408-414
2. Bates JHT, Irvin CG (2003) Measuring lung function in mice: the phenotyping uncertainty principle." J Appl Physiol 94: 1297-1306, 2003
3. Bersten AD (1998) Measurement of overinflation by multiple linear regression analysis in patients with acute lung injury. Eur Respir J 12: 526-532
4. Fredberg JJ, Ingram RH, Castile RG, Glass GM, Drazen JM (1985) Nonhomogeneity of lung response to inhaled histamine assessed with alveolar capsules. J Appl Physiol 58: 1914-1922
5. Hantos Z, Daroczy B, Suki B, Nagy S, Fredberg JJ (1992) Input impedance and peripheral inhomogeneity of dog lungs. J Appl Physiol 72: 168-178
6. Kaminsky DK, Irvin CG, Lundblad LKA, Moriya HT, Lang S, Allen J, Viola T, Lynn M, Bates JHT (2004) Oscillation mechanics of the human lung periphery in asthma. J Appl Physiol (in press)
7. Ludwig MS, Romero PV, Sly PD, Fredberg JJ, Bates JHT (1990) Interpretation of interrupter resistance after histamine-induced constriction in the dog. J Appl Physiol 68: 1651-1656
8. Lundblad LKA, Irvin CG, Adler A, Bates JHT (2002) A reevaluation of the validity of unrestrained plethysmography in mice. J Appl Physiol 93: 1198-1207
9. Mead J, Whittenberger JL (1953) Physical properties of human lungs measured during spontaneous respiration. J Appl Physiol 5: 779-796
10. Mead J (1969) Contribution of compliance of airways to frequency-dependent behavior of lungs. J Appl Physiol 26: 670-673
11. Mount LE (1955) The ventilation flow-resistance and compliance of rat lungs. J Physiol (Lond) 127: 157-167
12. Otis AB, McKerrow CB, Bartlett RA, et al (1956) Mechanical factors in distribution of pulmonary ventilation. J Appl Physiol 8: 427-443
13. Otis AB (1986) History of respiratory mechanics. In: Handbook of Physiology, The respiratory system, vol III. American Physiological Society, Bethesda, pp 1-12
14. Peslin R, Fredberg JJ (1986) Oscillation mechanics of the respiratory system. In: Handbook of Physiology, The respiratory system, vol III. American Physiological Society, Bethesda, pp 145-177
15. Wagers S, Lundblad LKA, Ekman M, Irvin CG, Bates JHT (2004) The allergic mouse model of asthma: normal smooth muscle in an abnormal lung? J Appl Physiol 96: 2019-2027

ANALYSIS OF THE RESPIRATORY IMPEDANCE AT FREQUENCIES BELOW 5 HZ USING IMPULSE OSCILLOMETRY AND FAMOS

Julia Jaeger[1], H.-J. Smith[2], Petra Reinhold[1]
[1]Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Institute of Molecular Pathogenesis, Jena, Germany & [2]VIASYS Healthcare, Hoechberg, Germany

Objective: When using impulse oscillometry in its standard configuration, the respiratory impedance is usually calculated within the frequency range of 5 – 35 Hz. However, frequencies below 5 Hz are thought to be most informative with respect to peripheral airways and/or mechanical properties of the lung tissue. The aims of this study were (i) to explore information in the frequency range 1 – 5 Hz by recalculation of original IOS-data using the software FAMOS (Fast Analysis and Monitoring of Signals) and (ii) to compare recalculated data with IOS-data in calves and pigs.

Material and methods: Twelve calves (age: 31 – 77 days, body weight: 52 – 94 kg) and 20 pigs (age: 5 – 27 weeks, body weight: 7 – 100 kg), all free of respiratory symptoms, were included in the study. Each calf underwent eight lung function tests within two months, whilst 23 lung function tests were performed in each pig over a period of six months.

The "Masterscreen-IOS" (VIASYS Healthcare) impulse oscillometry system was used for lung function testing. Each time point for each animal comprised three consecutive IOS-measurements (measurement period: 60 seconds, 3 impulses per second, 32 sampling points per impulse). All three IOS-measurements per time point and animal were used for evaluation, providing a total of 1164 IOS-data files recalculated using FAMOS (n = 285 in calves, n = 879 in pigs). For recalculation, 32 sampling points per impulse were used, too.

For statistical comparison between original IOS-data and recalculated FAMOS-data, respiratory resistance [R], respiratory reactance [X] and coherence were used at identical frequencies (3, 5, 10, 15 and 20 Hz). Statistical analysis was undertaken by *Statgraphics Plus* Version 4.0 for windows (Manugistics, inc., USA) using both Mann-Whitney-Wilcoxon-test and linear correlation analysis.

Results: Spectral results of respiratory impedance – expressed as respiratory resistance and respiratory reactance – are given in Figure 1 for both calves and pigs. FAMOS-data did reflect IOS-data at 3, 5, 10, 15, and 20 Hz in an acceptable way, but provided more information within the frequency range between 1 and 10 Hz. Coefficients of linear correlation between original IOS-data and recalculated FAMOS-data were excellent as shown in Table 1. Although most of coherence values were significantly higher in FAMOS-data compared to

original IOS-data, both IOS-derived coherence and FAMOS-derived coherence did reflect the high quality of respiratory impedance measurements.

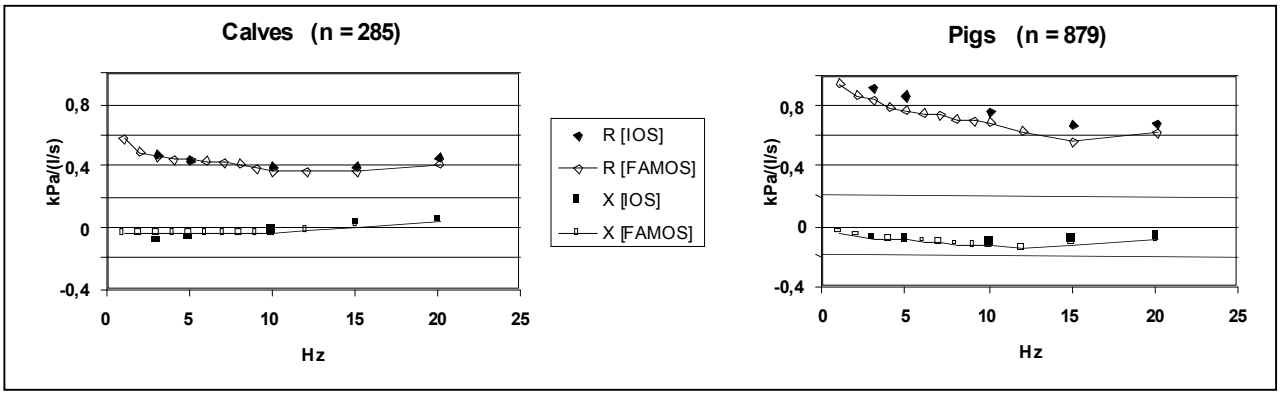


Figure 1: Medians of spectral respiratory impedance measured originally by IOS and recalculated using FAMOS in calves and pigs

Table 1: Coefficients of linear correlation (r) between original IOS data and recalculated respiratory impedance data using FAMOS in calves and pigs ($p \leq 0.001$)

	n	$R_{3\ Hz}$	$R_{5\ Hz}$	$R_{10\ Hz}$	$R_{15\ Hz}$	$R_{20\ Hz}$	$X_{3\ Hz}$	$X_{5\ Hz}$	$X_{10\ Hz}$	$X_{15\ Hz}$	$X_{20\ Hz}$
calves	285	0.92	0.92	0.91	0.91	0.91	0.78	0.88	0.94	0.91	0.89
pigs	879	0.96	0.97	0.96	0.96	0.96	0.95	0.96	0.95	0.89	0.93

Conclusion: The software program FAMOS allows evaluation of the respiratory impedance at frequencies lower than 5 Hz and facilitates examination of the peripheral respiratory system in calves and pigs for further diagnostic purposes.

USE OF WHOLE BODY PLETHSYMOGRAPHY TO ASSESS INFLUENCES OF RAT STRAIN AND AGE ON NONSPECIFIC AIRWAY RESPONSIVENESS

JA Dye, DW Winsett, N Haykal-Coates, DL Costa.
US EPA, ORD, ETD, Pulmonary Toxicology Branch, RTP, NC, USA.

Increased airway responsiveness (AR) is a well-established characteristic of asthma that epidemiological evidence suggests may be linked to air pollutant exposure. Establishing the biologic basis between pollutant exposure and subsequent adverse public health outcome requires an integrated toxicological approach — a key component of which is the ability to quantify pollutant-induced changes in pulmonary physiology and AR in laboratory species. Increasingly, whole body plethysmography (WBP) is being used to "document" air pollutant effects. This is due in part to its ability to allow non-invasive, repeated assessment of the breathing pattern/ventilatory effort of unrestrained, untrained animals. Therefore, in these studies we examined: (1) whether rat strain-related differences in AR could be determined using the WBP-based enhanced pause ("Penh", a unitless parameter) during stepped aerosolized methacholine (MCh) bronchoprovocation testing, and (2) whether age further influenced this assessment. The strains evaluated included 4 commonly used in air pollutant research, namely outbred Sprague-Dawley (CD), Wistar-Kyoto (WKY), spontaneously hypertensive (SH), and "atopy-prone" Brown-Norway (BN) rats. Results demonstrated that strain differences in AR to MCh were apparent in young adult (4-mo-old) male rats with: CD≥WKY>SH≥BN. These results were consistent with previous AR assessments in anesthetized, intubated, and ventilated 4-mo-old WKY and SH rats based on changes in airway opening pressure during IV acetylcholine challenge. Lastly, assessment of the effects of aging on AR in CD rats using WBP/Penh revealed relatively minor influences with the following overall pattern: 8-mo-old > 4-mo-old ≥ 17-mo-old ≥ 24-mo-old rats. Similarly, in SH rats, AR to MCh in 8-mo-old > 17-mo-old > 4-mo-old rats. We conclude that while WBP is seemingly sufficiently sensitive to evaluate AR non-invasively in rats, prudent use of appropriate strain- and age-matched controls will be critical to interpreting and extrapolating air pollutant-induced effects on AR in laboratory rats.

(This abstract does not reflect US EPA policy).

MOLECULAR MECHANICS OF SMOOTH MUSCLE MYOSIN MOLECULES IN THE LATCH-STATE

R. Léguillette. Supervisor: **A.-M. Lauzon**
Meakins-Christie Laboratories, McGill University, Montreal, QC, Canada, H2X 2P2

Rationale: Asthmatic airway smooth muscle (SM) is hypercontractile and has an impaired capacity to relax. The altered contractility of asthmatic SM could be due to enhanced intrinsic SM mechanical properties. Smooth muscle (SM) cells are unique in their ability to maintain force for long periods of time with very low MgATP consumption. This property of force maintenance at low levels of myosin phosphorylation and cross-bridge cycling rate is called the latch-state and differs among smooth muscle types. The latch-state is more prominent in tonic (tone-maintaining, e.g. blood vessels) than phasic (fast contracting, e.g. intestine) SM. Two isoforms of the SM myosin heavy chain (SMMHC) differ by the absence [(-)insert] or presence [(+)insert] of a 7 amino acid insert in the motor domain.The (-)insert is expressed predominantly in tonic while the (+)insert is found mostly in phasic muscle. Because MgADP release from myosin is necessary for its detachment from actin, an attractive mechanism to explain the latch-state is that myosin dephosphorylation simply reduces the rate of MgADP release from cross-bridges and thus slows their detachment. Another possibility is that dephosphorylated myosin, while cycling slowly, reattaches to actin and maintains force. However, evidence has accumulated to suggest that dephosphorylated myosin has a conformation that is incompatible with binding to actin.

Goal: The purpose of the study was to determine, at the molecular level, 1) the role of MgADP affinity in the latch state and 2) if unphosphorylated myosin can attach to actin and maintain or generate force.

Methods1: We used an *in vitro* motility assay to measure fluorescently labeled actin filament velocity (v_{max}) when propelled by purified phasic SM (chicken gizzard) or tonic SM (bovine aorta) myosin, at increasing [MgADP] from 0.1 to 1mM. Myosin was 25% thiophosphorylated/ 75% unphosphorylated, approximating latch-state conditions.

Methods2: We used a laser trap to measure the unbinding force of unphosphorylated (+) and (-)insert SM myosin molecules.

Results1: v_{max} decreases with increasing [MgADP] for both phasic and tonic muscle myosin but decreases more for tonic muscle myosin. Linear regression shows a significant difference ($p<0.001$) in the slope of v_{max} vs [MgADP] and the inhibition constants (K_I) values between phasic and tonic muscle myosin.

Results2: Both tonic and phasic unphosphorylated SM myosin molecules can attach to actin. The unbinding force measured with the laser trap is

approximately 0.5pN per myosin molecule of SM, and there was no significant difference between phasic and tonic SM myosin.

Conclusions: The affinity for MgADP of tonic muscle myosin is greater than that of phasic muscle myosin. We also showed, for the first time, that unphosphorylated myosin can attach to actin and exerts a load. Thus the latch-state may be explained by the combination of a high affinity for MgADP, allowing dephosphorylation while attached, thereby generating a strong cross-bridge, and a reattachment of dephosphorylated myosin contributing to tension maintenance.

EFFECT OF OBESITY ON AIRWAY FUNCTION IN HEALTHY RETRIEVERS

Bach J, Rozanski E, Bedenice D, Chan D, Freeman L, Lofgren J, Oura T, Hoffman A.
Lung Function Testing Laboratory, Tufts University, North Grafton, MA 01536

Obesity is a common problem in pet dogs. Various deleterious effects of obesity including increased incidence of orthopedic disease, shortened lifespan, and risk of death from pancreatitis have been documented. The aim of this study was to evaluate the effect of body condition on respiratory function in healthy retrievers. Retriever dogs without a history of respiratory disease were eligible for study inclusion. Dogs were scored using the Purina® body condition score (BCS), with a range of 1-9, with 9 being obese. All dogs had routine thoracic radiographs performed to evaluate for subclinical lung disease. Subjects less than one year of age and with a history of upper airway or thoracic disease were excluded. Dogs underwent pulmonary function testing as follows:

1) Measurement of specific airway resistance (sRaw) using head out whole body plethysmography (Bedenice et al, ACVIM proceedings 2003). sRaw was calculated during inspiration and expiration during normal breathing and hyperpnea was induced by rebreathing CO_2 in a closed system. Hyperpnea was evident by a significant increase in tidal volume (TV) for all dogs and within groups.

2) Measurement of functional residual capacity (FRC) and diffusing capacity (DLCO) using a multiple breath helium and carbon monoxide dilution technique respectively (Amis, AJVR 1984) (45 and 20 seconds respectively).

3) The formula (sRaw / FRC = Raw) was used to calculate airway resistance (Raw).

4) Respiratory variables (TV, MV, Ti, Te, PIF, PEF, Te/Ti, PEF:PIF) were measured via pneumotachography.

5) Collection of an arterial blood gas sample for determination of the PaO_2 and $PaCO_2$ and subsequent calculation of the A-a gradient [(150 - $PaCO_2$ / 0.8) - PaO_2].

Dogs were divided on the basis of BCS into normal weight (BCS 5.5 or less) and moderately obese (BCS 6 and 6.5) and markedly obese (BCS ≥7). Groups were compared at rest and during hyperpnea using univariate analysis (ANOVA) and Student-Newman-Keuls.

Thirty-six dogs were included. There were 28 Labradors, 7 Golden Retrievers and 1 Chesapeake Bay retriever. Eleven dogs were normal (BCS ≤ 5.5), 14 were moderately obese (BCS = 6 or 6.5) and 11 were markedly obese (BCS ≥ 7). FRC / kg varied inversely related to BCS, and was significantly different between groups ($p \leq 0.001$). The mean FRC / kg ± SD for the normal dogs was 47.5 ml ± 8.2 ml, for the moderately obese dogs 40.0 ml ± 3.3 ml and for the markedly obese dogs 31.4 ml ± 6.7 ml. During hyperpnea, the expiratory specific airway resistance (sRaw) was significantly greater in the markedly obese dogs (15.3 ± 7.4 cmH_2O/s) as compared with the normal dogs (8.3 ±0.95 cmH_2O/s) ($p = 0.009$). Additionally during hyperpnea, the expiratory airway resistance (Raw) was significantly greater in the markedly obese dogs (13.2 ± 6.6 cm $H_2O/L/s$) as compared with the moderately obese (8.7 ± 3.6 cm $H_2O/L/s$) and the normal dogs (6.1 ± 2.2 cm $H_2O/L/s$) ($p = 0.002$). No other values were significantly different between groups of dogs, or between rest and hyperpnea.

Morbidly obese dogs (BCS=9), a population which may have the greatest respiratory compromise, were not available. However, this study confirmed the presence of increased expiratory resistance in obese retrievers during hyperpnea.

Funding provided by Nestlé Purina

INTERFERON ALPHA-INDUCED RESISTANCE TO BOVINE PARAINFLUENZA TYPE 3 VIRUS IS NOT MEDIATED THROUGH THE THREE CLASSIC PATHWAYS

Leroy M., Baise E., Desmecht D.
Dept. of Pathology, Faculty of Veterinary Medicine, B-4000 Liège, Belgium.

In the bovine species, parainfluenza type 3 virus (BoPi-3), which is involved in the respiratory disease complex, is inhibited by type I interferons through an unknown mechanism. Until now, three IFN-induced pathways have been well characterized: the double stranded RNA-dependent protein kinase R (PKR), the 2'-5' oligoadenylate synthetase/RNase L pathway, and the Mx protein. Among these, we have previously shown that the 2'-5' oligoadenylate synthetase/RNase L pathway was not involved in this antiviral process. In contrast, the human MxA protein (HuMxA) was able to limit the replication of the virus in Vero cells. In this study, we first wanted to evaluate whether the bovine Mx1 protein could also block the viral replication and protect cells against the cytopathic effects of BoPi-3. We then wanted to evaluate the involvement of the protein kinase R pathway in the inhibition of viral replication.

To asses the antiviral effect of BoMx1, we used our double transgenic Vero cell clone conditionally expressing the protein under tetracycline stimulation. Cells were infected at a multiplicity of infection of 0.1 and viral yield reduction assay was performed at day 5 post-infection. No differences were seen whatever the cells were stimulated or not, suggesting that, in contrary to HuMxA, there is no antiviral activity of BoMx1 against BoPi-3. To confirm our results, and to test whether the bovine Mx1 protein could protect the cells against the cytopathic effect of the viral replication, we used an MTS-based assay. Again, whatever the viral load, no difference could be recorded. We next tested the double stranded RNA-dependent protein kinase R pathway through its ability to phosphorylate the eukaryotic initiation factor alpha (eif2-α), leading to the inhibition of protein translation of cellular and of viral origin. In our study, cells were stimulated with 1000 U/ml of recombinant type I IFN for 18h before being infected for 6h by BoPi-3 virus. Cells were then lysed and analyzed for eif2-α phosphorylation through an SDS-PAGE immunoblotting analysis. No differences were recorded in the phosphorylated status of eif2-α, thus suggesting that the IFN-dependent antiviral activity against BoPi-3 is not due to the implementation of the PKR pathway.
In conclusion, this study suggest that even if type-1 IFNs can drastically reduce the replication of the virus, this antiviral effect is independent of the three most characterized pathways. Recently, a single-stranded RNA-specific 3'-5' exonuclease that is produced by *ISG20* gene upon type-1 IFNs induction was described in another preparation, which may play a role here. Alternatively, the hypothesis of a pathway not yet discovered should also be taken in consideration.

AUTONOMIC DYSFUNCTION IN HORSES AFFECTED BY RECURRENT AIRWAY OBSTRUCTION (RAO)

Susannah E Norman[1], David J Marlin[1], Richard Newton[2], Rachel Eager[3], Natalie Waran[3], Pat Harris[4], Christopher M Deaton[1]
Centres for Equine Studies[1] and Preventive Medicine[2], Animal Health Trust, Lanwades Park, Kentford, Suffolk, UK, CB8 7UU
[3]Division of Animal Health and Welfare, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK
[4]WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK

Analysis of heart rate variability (HRV) is a useful, non-invasive tool for assessment of autonomic function. Both low (LF) and high frequency (HF) power are reduced in human COPD and asthmatic patients. As far as we are aware, there are no published studies which have attempted to determine whether RAO has a component of systemic autonomic dysfunction. We set out to test the hypothesis that RAO affected horses would show decreased HRV compared with non-RAO controls, after controlling for effects of other potentially confounding variables, including temperament. Resting ECG recordings were obtained from 12 healthy horses (non-RAO; 3 mares, 9 geldings), with a mean age of 9±4 (SD) years. ECG recordings were also obtained from 11 RAO-affected horses in remission (4 mares, 7 geldings) with a mean age of 16±6 years. Horses were habituated to the study environment and recording equipment for at least 1h prior to the first recording session. ECG's were recorded using a telemetry system (Lifescope 8, Nihon Kohden, UK). The ECG signal was then digitised at 1000Hz using a PC-based data acquisition system (Po-Ne-Mah, Gould Instrument Systems Inc., OH, USA). Sets of 2048 beats from each horse were subjected to both time and frequency domain heart rate variability analysis. As temperament has been shown to modulate HRV in horses, temperament was assessed using a startle response test. Multiple linear and polynomial regression analysis using a forward stepwise modelling procedure was then used to relate the observed HRV values simultaneously with different forms of the predictive variables. There was no difference in RMSSD or pNN50 between non-RAO and RAO-affected horses ($P>0.05$). Total power was significantly lower in RAO compared with non-RAO affected horses ($P=0.039$). This was predominantly due to lower LF power in RAO horses compared with non-RAOs ($P=0.033$). There was a positive correlation between total power and temperament in non-RAO horses but not in the RAO horses. The final multiple regression analysis model of significant predictive factors for HRV demonstrated that age (modelled polynomially; $P=0.0061$), RAO status ($P=0.001$), temperament score (modelled linearly; $P=0.0002$) and an interaction term between RAO status and temperament score ($P=0.0003$) were all significantly predictive of HRV in horses in this dataset of 21 animals. The significant negative interaction term indicates that the positive effect of higher temperament scores on HRV is eliminated in RAO animals but remains in non-RAO horses. This study found a significant relationship between HRV and temperament, which was modified by RAO status. This implies that horses affected with RAO are in some way limited in producing sympathetic responses and might be considered to have autonomic dysfunction.

AIRWAY SMOOTH MUSCLE REMODELING IN HORSES WITH HEAVES

D. Ramos-Barbon[1], B. Herszberg[2], M. Tamaoka[1], J.G. Martin[1], J.P. Lavoie[3]
[1]Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada. [2]Ecole Nationale Veterinaire d´Alfort, Maisons-Alfort, France. [3]Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, Quebec, Canada.

Rationale: The increase in airway smooth muscle mass (ASM) observed in human asthma is likely to be an important mechanism causing airway hyperresponsiveness. Airway smooth muscle hyperplasia occurs in rodent models of allergen-driven experimental asthma, but the relevance of such finding in spontaneously occurring disease in large mammals is unknown. We examined horses affected with heaves, a naturally occurring form of chronic asthma-like disease, related to sensitization and exposure to moldy hay. We hypothesized that airway remodeling occurs in heaves and shares disease mechanisms with human asthma. The development of airway remodeling in association with chronic airway inflammation in heaves may contribute to irreversible deterioration of lung function, even if the horses receive effective symptomatic treatment, as it is the case in human asthma. The study of the mechanisms of airway remodeling may contribute a better understanding of the physiopathogenesis of both heaves and human asthma.

Methods: We quantified the amount of airway smooth muscle corrected for airway size, and the numbers of proliferating and apoptotic airway smooth muscle cells in 5 horses with heaves, and 5 control horses using morphometric techniques. Cell proliferation was detected in tissue sections by immunostaining for proliferating cell nuclear antigen (PCNA) and apoptotic cells were detected by terminal UTP-nucleotide end labeling of fragmented DNA (TUNEL). Both signals were co-localized with smooth muscle specific alpha-actin.

Results: The airway smooth muscle mass (area corrected for the basement membrane perimeter squared) in the heaves affected horses was nearly triple that of the controls ($9.15 \pm 1.38 \times 10^{-3}$ *versus* $3.21 \pm 0.23 \times 10^{-3}$, dimensionless index; P=0.003). The frequency of $PCNA^{+}$ airway myocytes was 7-fold in heaves compared with controls (6.41 ± 1.26 *versus* 0.89 ± 0.27 cells/ mm^2; P=0.003), and the frequency of apoptotic myocytes was 6-fold (2.37 ± 0.49 *versus* 0.39 ± 0.09 cells/mm^2; P=0.0004). The increases in airway smooth muscle mass, proliferating and apoptotic myocytes was greater the smaller the airways.

Conclusions: Horses with heaves had an increase in the amount of smooth muscle in the airways, associated with increased myocyte proliferation and apoptosis. These data suggest that airway smooth muscle remodeling occurs in heaves, and that myocyte hyperplasia accounts at least in part for the smooth muscle growth. The accompanying increase in myocyte apoptosis may reflect a compensatory homeostatic mechanism, associated with an accelerated smooth muscle cell turnover. Airway smooth muscle remodeling may play a comparable role in the mechanisms of airway hyperresponsiveness and chronic lung function impairment in heaves and human asthma.

PERSISTENT MUCUS ACCUMULATION—A CONSEQUENCE OF DELAYED APOPTOSIS IN RAO-AFFECTED HORSES?

L. R. Bartner[1], Y. Tesfaigzi[2], N.E. Robinson[1]. [1]Dept. of Large Animal Clinical Sciences, Michigan Sate University, East Lansing, MI 48824, USA. [2]Lovelace Respiratory Research Institute, Albuquerque, NM 87108, USA

Accumulation of mucus within airways is a hallmark of recurrent airway obstruction (RAO) in horses. This accumulation is due to increased secretion (eqMUC5AC upregulation) and decreased clearance (via increased viscoelasticity) of mucus. The purpose of this study was to examine the role of delayed mucous cell (MC) apoptosis as a contributing factor to mucus accumulation. To do this, we determined the number of Bcl-2-positive MCs in the bronchi of RAO-affected and control horses. Bcl-2 is an anti-apoptotic member of the Bcl-2 protein family.

Six RAO-affected and six control horses were stabled and fed hay for 5 days in order to induce inflammation and then fed pellets for 1 week to induce partial resolution. Lung function, mucus score, and BALF cytology were measured after the 5 days of hay and the 7 days of pellets after which horses were euthanized. Four-mm diameter bronchi were harvested from 8 regions of lung and immunohistochemically stained for Bcl-2 and then with alcian blue to identify MC. The number of Bcl-2-positive and negative MCs was counted per mm basal lamina around the airway. Serial sections also were stained with alcian blue/periodic acid Schiff's reagent in order to stain acidic and neutral mucins and measure the amount of stored intraepithelial mucosubstance (Vs).

RAO-affected horses had more airway obstruction and higher mucus score after feeding both hay and pellets. After 5 days on hay, neutrophil numbers did not differ significantly between the two groups of horses, but after pellets the neutrophil numbers decreased in the control but not the RAO-affected group. Bcl-2-positive MCs occurred almost exclusively in RAO-affected animals so that both their number and percentage were significantly greater in RAO-affected horses than in controls. Horses having more than 10 neutrophils per microliter of BALF tended to have Bcl-2 staining in more than half of their MCs, whereas the horses with less inflammation had less expression. There were, however, no differences in MC number or Vs between RAO and control horses but in RAO-affected animals, Vs decreased as BALF neutrophil numbers increased.

As in the airways of rats challenged with LPS, allergen, or cigarette smoke, MCs of RAO-affected horses show immunoreactivity to Bcl-2 antibody. However, because of the lack of difference in MC numbers in RAO-affected and control horses, a conclusive role for Bcl-2 in prolonging MC life cannot be determined.

Supported by USDA/CSREES 2002-35204-1259.

EXPOSURE OF HORSES TO TOTAL AND RESPIRABLE PARTICLE ENDOTOXIN CONCENTRATIONS GENERATED BY SPECIFIC FEED AND BEDDING MATERIALS

P.J. Spendlove, J.L. Hodgson, D.R Hodgson, N. Malikides
Faculty of Veterinary Science, University of Sydney, Australia

Dust is ubiquitous in equine stables and endotoxin is associated with this particulate matter. Exposure to airborne dust and endotoxin in stable environments is known to contribute to the aetiopathogenesis of neutrophilic Inflammatory Airway Disease (IAD) in horses. Two major contributors to aerosolised dust are feedstuffs and bedding materials as a result of prehension of feed and horse movement within the stable respectively. Preliminary studies have demonstrated that endotoxin concentrations within mixtures of feeds and certain bedding types are highly variable. However, the contribution of individual feeds and bedding types to the exposure of horses to dust and particle endotoxin in their breathing zone has not been determined.

In this study we investigated total and respirable (<5µm) particles and particle endotoxin concentrations generated by four feed types (hay, chaff, oats and pellets) and four bedding materials (shavings, straw, sawdust, rice hulls). Total and respirable particle samples were collected from the breathing zone of a horse via Personal Air Sampling (PAS) devices. Exposures to breathing zone particles and particle endotoxin generated by the four feeds and four bedding materials were each assessed separately on six occasions using a crossover Latin Square design.

Mean total and respirable particles and particle endotoxin concentrations to which horses were potentially exposed when standing on different bedding materials and eating different feedstuffs are presented in Tables 1 and 2.

Table 1. Mean (±SE) total and respirable particle exposures (mg/m^3) and particle endotoxin exposures (ng/m^3) generated from four different stable bedding materials (n=6 for dust; n=2-5 for endotoxin).

Bedding	Total Dust (mg/m^3)	Total Endotoxin (ng/m^3)	Respirable Dust (mg/m^3)	Respirable Endotoxin (ng/m^3)
Straw	19.4±3.2^	76,753±32,166	8.8±1.4	2,329±594*
Shavings	8.2±2.7	732±319	1.5±0.4	565±270
Sawdust	3.5±0.7	836±661	2.2±0.4	170±103
Rice hulls	132.5±18.8*	620,524±169,616#	39.3±5.8*	122,737±61,099#

* Significantly higher than other variables (One-way ANOVA; P<0.05)
^ Significantly higher than sawdust (P<0.05)
# Not included in analysis.

Table 2. Mean (±SE) total and respirable particle exposures (mg/m^3) and particle endotoxin exposures (ng/m^3) generated from four different feeds (n=6 for dust; n=2-5 for endotoxin).

Feed	Total Dust (mg/m^3)	Total Endotoxin (ng/m^3)	Respirable Dust (mg/m^3)	Respirable Endotoxin (ng/m^3)
Oats	6.6±3.1	2,100±1,276	4.4±1.1	118±69
Hay	12.1±3.1	2,394±849	4.3±1.1	286±143
Pellets	9.6±4.1	237±92	7.5±2.9	302±132
Chaff	4.2±0.7	2,031±575	2.8±1.2	433±278

The average exposure to breathing zone total and respirable particles generated from rice hulls was significantly higher than other bedding types. In addition, the average exposure to breathing zone respirable endotoxin concentration generated by straw was significantly higher than produced by shavings and sawdust. Although the mean concentration of particle endotoxin measured in the breathing zone of horses bedded on rice hulls was substantially higher than the other bedding types, this variable was not included in analyses due to insufficient dilution of samples during endotoxin assay. Finally, there was no difference between total and respirable particles and particle endotoxin exposures generated by the four feed types. Based on these results we suggest horses are potentially exposed to high concentrations of breathing zone particles and particle endotoxin generated by specific bedding types and feedstuffs. This may have deleterious effects on the health of their respiratory tract.

Acknowledgement: This project was supported by the Waltham Foundation.

INDOOR AIR QUALITY IN A BOARDING STABLE

Melissa May and N.E. Robinson
Dept. Large Animal Clinical Sciences, Michigan State University, E. Lansing, MI

Attacks of airway inflammation in stabled horses are thought to be induced by dusts in the environment. The aim of *this* study was to begin to characterize the physical and chemical characteristics of dusts commonly found in the stable environment.

Airborne particulate concentrations were continuously quantified by use of two Dust-Trak™ aerosol monitors fitted with 'conditioners', which established particle size cut points of 10 and 2.5 micron in diameter and smaller. Particles of this size are considered 'respirable', and have the ability to reach the lower airway. Multiple samples were taken in the horses breathing zone under various circumstances to determine the potential daily particle load exposure. Airborne dust concentrations were measured during riding activities, feeding/eating various types of food, and regular barn cleaning activities under various conditions and with varying moisture content.

In the riding arena under dry circumstances particulate concentrations were as high as 55.34 mg/m^3 PM 10 and 60.58 mg/m^3 PM 2.5, but when arena footing moisture content was greater than 6%, particulate levels were not higher than background. Breathing zone levels of particulates for horses consuming clean dry hay were as high as 93.2 and 19.3 mg/m^3 of PM 10 and 2.5 respectively with levels in dry 'dusty hay' being greater than 140 and 81 mg/m^3 respectively. These values decreased significantly under wet conditions.

Particle size distribution in arena footing samples as well as dust samples taken from wall surfaces ranged from < 4 um in diameter to > 2000 um in diameter. These samples were also analyzed to determine their chemical composition. Components of interest including iron, copper, manganese, and crystalline silica were all shown to be present.

Dragar™ pumps and sampling tubes sampled ammonia and carbon dioxide as indicators of ventilation status in the stable. Ammonia levels ranged from non-detectable to 59 ppm, and were a function of distance from doorways, season, and whether or not the doors were open or closed. Similar results were obtained for carbon dioxide, with results ranging from non-detectable to 400 ppm.

Particulate loads under dry conditions exceeded those causing bronchitis in people, and particle size distributions were consistent with those shown to be of interest in the current literature. Certain components of the dust samples (e.g. iron and crystalline silica) that are known to cause an inflammatory response in the lung were present in sufficient quantities to warrant further study. Ammonia was also present at levels sufficient to cause mucus membrane irritation, and levels were highly dependent upon ventilation status. Significant variation in particle size and concentration were found during routine stable activities and between various forms of feedstuffs. Adding moisture reduced particulate load to background or near background levels. Supported by Michigan Animal Health Foundation.

IS A COUGH JUST A COUGH?

Daniela Bedenice, Melissa Mazan, Andrew Hoffman.
Pulmonary Function Laboratory, Tufts University School of Veterinary Medicine (TUSVM), 200 Westboro Rd, North Grafton, MA 01536

While cough is commonly accepted as a sign of inflammatory airway disease (IAD, Havemeyer Foundation Monograph No.9; DHS 2002), there are few studies which have statistically associated cytological or physiological variables with cough. The purpose of this study was to evaluate the association between cough, broncho-alveolar cytology (BAL) and pulmonary function in horses at a referral center (n=137) or in field practices (n=142). This retrospective study was divided into two parts: **Part 1 = Field**: Evaluation of clinical history, physical examination and BAL-cytology from our private practice database. **Part 2 = Referral**: same as "**Field**", plus forced oscillatory mechanics (FOM) and histamine broncho-provocation of horses presented to TUSVM for non-infectious respiratory disease. Horses with a history or current signs of heaves were excluded. The following variables were extracted from consult letters (**Field**) or TUSVM medical records (**Referral**): age, sex, discipline, exercise intolerance, cough, nasal discharge, abnormal lung sounds, increased respiratory rate (>20/min), EIPH (Part 1 only), and history of infectious disease. A diagnosis of IAD was based on BAL analysis, and included patients with BAL mast cells >2%, neutrophils (PMN) >5%, or eosinophils >0.1 % (DHS 2002). The following FOM variables were recorded (**Referral**): Respiratory system mechanics (R_{RS}, X_{RS} 1-3 Hz; and f_{res}), provocative histamine concentration that doubles R_{RS} at 1 Hz ($PC100R_{RS}$) and frequency dependence ($R_{RS}1Hz/R_{RS}3Hz$) were recorded for referral cases. Univariate statistical analyses were employed to compare independent groups, and logistic regression to derive odds ratios (OR) for variables related to cough.

Results: **Field** – Cough showed a positive association with high BAL neutrophil counts (mean PMN: 28.8 vs. 5.1% in non-coughers, P<.001; OR for PMN>5% = 3.4; 95% CI: 1.7-9.6) and was negatively related to a history of exercise intolerance (P< 0.002; OR=0.21) and increased RR (P<.001; OR=0.14).
Referral – Cough was predicted by a high BAL neutrophil count (mean PMN: 16.4 vs. 4.6%, P=0.001; OR for PMN>5% = 4.4; 2.1-9.2) and the presence of nasal discharge (P=0.001; OR 4.5; 2.1-9.6). Additionally, referred sport horses were significantly more likely to cough than race horses (P<0.001, OR 6.7, 3-14.9). Additionally, older horse (>7yrs) were statistically more likely to cough than young horses in both study groups (P<0.001; **Field**-OR 3.99, **Referral**-OR 4.17). Respiratory system mechanics, EIPH, sex, BAL %mast cell counts or %eosinophils were not associated with cough. However, cough predicted IAD in both study groups (P<0.05). Further characterization of IAD via forward logistic regression revealed that IAD was predicted by cough (p<.0001; OR: 4.5) in **Field**

patients, and by frequency dependence (P=.034; OR 13.3) and PC100 (P=.025; OR: 0.87) in **Referral** patients.

In conclusion, cough was more prevalent in horses >7yrs and was best characterized by a high BAL neutrophil count (indicative of IAD) and nasal discharge. The BAL-cytology and its relationship to cough were highly comparable between study groups (**Field** vs. **Referral**). Exercise intolerance and increased resting RR were less common in coughers vs. non-coughers. Furthermore, frequency dependence of R_{rs} and airway hyperreactivity were characteristic of IAD. Thus, IAD but not cough is strongly associated with pulmonary dysfunction.

CLIMATIC AND AEROALLERGEN RISK FACTORS FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Michael P. Ward, Laurent L. Couëtil, Departments of Veterinary Pathobiology (Ward) and Veterinary Clinical Sciences (Couëtil), School of Veterinary Medicine, Purdue University, West Lafayette IN 47907-2027, USA.

Purpose—To estimate the association between the prevalence of chronic obstructive pulmonary disease (COPD) in horses and climate and airborne pollen and fungi.

Methods—Study is retrospective, involving 1,444 COPD cases diagnosed among 157,791 hospitalized horses. The Veterinary Medical Database (VMDB) was used to identify records of horses admitted to Veterinary Teaching Hospitals (VTH) in the United States and Canada between 1990 and 1999. For each VTH's geographic location, monthly rainfall, mean minimum and maximum temperature, and maximum pollen and fungal spore counts were recorded for each month of data reported to the VMBD. Associations between climatic and aeroallergen data, and monthly prevalence of COPD diagnosis were estimated using cross-correlation and logistic regression models.

Results—Significant positive correlations were found between the prevalence of COPD and rainfall three months prior to diagnosis, minimum temperature one and two months prior, total pollen counts measured 3 months prior and total mold counts measured during the same month and one month prior. The risk of COPD in months with pollen counts in the second (OR 1.97, 95% CI 1.22 – 3.17), third (OR 2.37, 95% CI 1.46 – 3.82) and fourth (OR 2.86, 95% CI 1.76 – 4.63) quartiles was greater than in months with pollen counts in the first quartile (<30 particles per cubic meter of air).

Conclusion—Outdoor aeroallergens and climatic factors may contribute to the occurrence of COPD.

PREVALENCE OF INFLAMMATORY AIRWAY DISEASE IN MICHIGAN PLEASURE HORSES – WINTER 2003-4

N.E. Robinson, S.J. Holcombe, F.J. Derksen, E.A. Carr
Dept. of Large Animal Clinical Sciences, Michigan Sate University, East Lansing, MI 48824, USA.

A recent Havemeyer workshop on inflammatory airway disease (IAD) in horses (http://www.havemeyerfoundation.org/PDFfiles/monograph9.pdf) recommended epidemiological studies to determine the prevalence of the syndrome in different geographic regions and horse populations. In this study, we determined the prevalence of IAD in Michigan pleasure horses and examined the association between tracheal neutrophils, mucus score and a history of coughing.

We examined endoscopically the airways of 260 unselected horses from 17 stables in 6 geographic regions of the state during winter 2003-4. A score was assigned for tracheal mucus accumulation (0-4) and a cytological evaluation was made of tracheal lavage fluid.

The prevalence of mucus scores of 0, 1, 2, 3 and 4 was 42, 37, 13, 7, and <1 % respectively, while the prevalence of tracheal wash neutrophil percentages of 0-20, 21-40, 41-60, 61-80, and >80 was 23, 27, 17, 14, and 19 %, respectively. Tracheal lavage neutrophils increased significantly with mucus score. Mucus scores of 0 and 1 did not have significantly different numbers of neutrophils but each score above 1 differed significantly from one another. There was also a significant relationship between mucus score and the presence of cough. The presence of cough was associated with a higher mucus score and more neutrophils. While almost all horses with mucus score > 2 had > 20 % neutrophils, the majority of horses with > 20 % neutrophils in the trachea did not have high mucus scores.

The recent workshop on IAD suggested that racehorses should have < 20 % neutrophils in the trachea. Applying that criterion to our horse population, 77 % of the horses would have been diagnosed with IAD. An alternative diagnostic criterion is a mucus score of 2 or greater. Applying this criterion, the prevalence of IAD was 21 %. The presence of many of neutrophils in the tracheal wash of clinically normal horses indicates a need for investigation of the role of these cells in the airway.

Supported by the MI Animal Coalition and by Boehringer-Ingelheim.

LIFE, HEALTH AND THE SECOND LAW OF THERMODYNAMICS

Peter T. Macklem, Emeritus Professor of Medicine, McGill University, Adjunct Professor of Medicine, Queen's University

Schrodinger wrote in his famous monograph "What Is Life?" that a major feature of life was the stunning order that living things display that seems to violate the second law of thermodynamics. The second law states that systems will become progressively more disordered with time, whereas in Darwinian evolution, and fetal development, life becomes progressively more ordered. Schrodinger did not solve the increasing order of living things but Progogine discovered that if systems were driven away from thermodynamic equilibrium, e.g., by heating them, order could spontaneously appear, as it does in the bathtub when the potential energy of the water is converted to kinetic energy by pulling the plug. When this happens a highly ordered little whirlpool appears with an inverted cone of air extending well below the surface of the water, defying the laws of gravity. Prigogine quantified the relationships between the amount of energy dissipated, the distance from thermodynamic equilibrium and the rate of entropy production (a measure of disorder).

Living things are highly ordered in a far from equilibrium state, because we continually consume and dissipate energy by a process we call metabolism. This suggests that health requires the metabolic rate of physiologic systems to be just right, and that disease results when systems are too close or too far from thermodynamic equilibrium. Congestive heart failure results when the failing heart is unable to adapt to the increased energy demands of exercise; the inadequate energy consumption causes the system to be too close to thermodynamic equilibrium. This is characterized by a decreased heart rate variability signifying the inability to adapt to changing conditions. Conversely, asthma, a condition in which airway smooth muscle is excessively activated so that it consumes and dissipates too much energy, is too far from thermodynamic equilibrium. It is characterized by excessive variability of impedance to air-flow in and out of the lungs. Because a value of impedance is determined by the configuration of the tracheobronchial tree, excessive variability signifies a continually changing tracheobronchial configuration that is abnormally hyperdynamic. Normal configurations are rarely seen. The measurement of variability of homeostatically controlled systems may prove useful in determining if systems are healthy, too far or too close to thermodynamic equilibrium. Because normal and abnormal variability follows a power law like earthquakes do, this may permit accurate predictions of probabilities of adverse events and thus quantitative prognosis.

COMPARATIVE ASPECTS OF BREATHING IN NEWBORN MAMMALS / SUMMARY

Jacopo P. Mortola
Dept. Physiology, McGill University
Montreal, Quebec, H3G 1Y6

Pulmonary ventilation (VE) and the pattern of breathing result from a combination of many factors, which assume different priorities at different times. Out of all, the metabolic needs of the organism and the mechanical characteristics of the respiratory apparatus are probably the most fundamental constraints. The former dictates the level of ventilation (VE), which determines alveolar and blood gases. The latter dictates which combination of tidal volume and breathing frequency are most appropriate for a particular level of VE. The relationships between metabolism, respiratory mechanics, VE and the breathing pattern have been studied under various conditions and with numerous experimental approaches. The comparison of species of different body size (allometry) has been an approach often adopted to understand the basic rules governing these relationships. Taking advantage of the inter-species allometric approach, the aim of this lecture is to look at the breathing pattern of the neonatal mammal, emphasising the most common characteristics and the differences from the adults.

1- Metabolism and breathing pattern

In adults, specific metabolic rate (i.e., metabolism normalised by weight, W) increases the smaller the animal size. Many eutherian newborns are 10-20 times smaller than adults, and the difference can exceed 1000 times in marsupials. In addition, newborns have the additional metabolic requirements related to the energetic cost of tissue growth. Hence, one may anticipate that the specific oxygen uptake (VO_2/W) in the newborns of the smallest species (<80-100 g) should be extraordinarily high. On the contrary, this is not the case. Small newborns have a rather low specific VO_2, and consume less O_2 than adults of similar W. The exponent of the allometric curve of VO_2 in newborns is 0.9, a value significantly higher than 0.75, which is the standard exponent not only in adult mammals but also in many other classes of animals. Hence, differences in specific metabolism among newborn species are less pronounced than among adults. The reason for the unusually high allometric exponent of the newborn=s VO_2 is still unclear. Because, as in adults, also in newborns VE is directly proportional to VO_2, it follows that $VE \propto W^{0.90}$, meaning that among neonates inter-species differences in VE/W are less pronounced that they are in adults. Tidal volume is directly proportional to W. Hence, the inter-species differences in VE/W are entirely accounted for by differences in breathing rate ($\propto W^{-0.10}$).

The proportionality between VO_2 and VE breaks down in the smallest marsupials. In fact, in such small animals, at birth a great deal of gas exchange

occurs through the skin. In these cases, pulmonary ventilation becomes redundant, and breathing can be a casual event, with low VE.

2- Respiratory mechanics

As in adult species, also in newborns the compliance of the respiratory system is directly proportional to W. The fact that both tidal volume (VT) and compliance (C) are directly proportional to W implies that their ratio (P = VT/C, which, at rest, is the largest component of the total pressure needed to inhale) is an inter-species constant. Because P is mostly due to surface forces and alveolar dimensions, it follows that alveolar dimensions have similar values among different size species. Indeed, this has been confirmed by morphometric measurements, and implies that the number of alveoli, rather than their dimensions, contributes to the inter-species differences in lung size.

In newborns, the compliance of the chest wall (Cw), relative to that of the lung (CL), is high. This important mechanical difference from the adult favours chest wall distortion during inspiration, lowering the efficiency of inspiration. Also, the high Cw-CL ratio lowers the resting volume of the respiratory system. Newborns compensate this mechanical situation by maintaining the end-expiratory level dynamically elevated through a combination of various mechanisms. One of the most obvious characteristics is the post-inspiratory narrowing of the vocal folds, an expiratory pattern that delays lung emptying.

3 - Response to hypoxia

The hyperventilation is the primary response to a drop in oxygenation. Hyperventilation is defined as an increase in pulmonary convection relative to the metabolic needs, e.g., an increase in VE relative to VO_2. Hence, what is important for an effective hypoxic hyperventilation is the increase in VE-VO_2 ratio, not the absolute values of either VE or VO_2. Adult mammals, especially those of large size, hyperventilate by increasing VE. Most newborn species, on the other hand, hyperventilate by lowering VO_2 (hypoxic hypometabolism). Hypometabolism as a means to achieve hypoxic hyperventilation is a very successful strategy, especially in conditions of severe hypoxia, when an increase in VE would be a costly function with little benefits. Because hypometabolism is largely contributed by the decrease in thermoregulation, body temperature drops. The decrease in body temperature during hypoxia favours survival, and artificial attempts to maintain body temperature at the normoxic value can be counterproductive. From the viewpoint of ventilatory control, though, hypoxic hypometabolism may favour the increase in the strength of inputs inhibitory on breathing. In fact, because carbon dioxide is an important stimulus for VE, the drop of its metabolic production during hypoxia may tilt the balance of ventilatory control in favour of respiratory inhibition. Some experiments on ventilatory reflexes of pulmonary (vagal) or laryngeal origin suggest that during neonatal hypoxia the inhibitory effects of these reflexes can be increased. This may be one of the mechanisms implicated in the patho-physiology of the *Sudden Infant Death Syndrome*.

METABOLIC ACIDOSIS AND HYPOCALCEMIA INCREASE THE PaO_2 OF ANESTHETIZED DOGS

I. Lopez, A. J. Felsenfeld[2], J. C. Estepa, M. Rodriguez[1], E. Aguilera-Tejero
Dept. Medicina y Cirugia Animal, Universidad de Cordoba, Ctra. Madrid-Cadiz km 396, 14014 Cordoba (Spain); [1]Unidad de Investigación, Hospital Reina Sofia de Cordoba (Spain); and [2]Dept. Medicine, West Los Angeles VA Medical Center and UCLA, Los Angeles, California (USA)

In the course of previous studies aimed to investigate the effect of metabolic acidosis on parathyroid hormone (PTH) secretion and its modulation by changes in ionized calcium (Ca^{2+}), we became aware that both metabolic acidosis and changes in Ca^{2+} concentration affected the PaO_2 . Because the combination of acidosis, hypocalcemia and elevated PTH levels are common features in critically ill patients and in patients subjected to general anesthesia, we thought that our findings would be interesting from a clinical perspective. The effect of metabolic acidosis and changes in Ca^{2+} on PaO_2 was studied in anesthetized mechanically ventilated dogs (n=33). Metabolic acidosis, with and without Ca^{2+} clamp, was induced by intravenous HCl administration for 2 hours. Changes in Ca^{2+} were induced by acidosis (hypercalcemia) or an EDTA infusion (hypocalcemia). In the metabolic acidosis group with Ca^{2+} clamp, PaO_2 rapidly increased during induction of acidosis, from 96±2 to 108±2 mmHg (P=0.001) and remained increased during the rest of the study. For the same decrease in pH, the increase in PaO_2 was less in the metabolic acidosis group without Ca^{2+} clamp in which the PaO_2 value of 102±4 mmHg was not different from baseline or the control group. The induction of hypocalcemia resulted in a slow, progressive increase in PaO_2 values from 95±2 to 104±3 mmHg (P=0.016) which correlated with the observed decrease in blood pressure, 156±9/81±5 to 118±10/65±7 mmHg (P<0.05). In conclusion, both hypocalcemia and metabolic acidosis separately increase the PaO_2 of anesthetized dogs while acidosis-induced hypercalcemia attenuates the rise in PaO_2.

ACID BASE BALANCE IN ARTERIAL BLOOD OF HORSES WITH HEAVES

Valérie Picandet, Daniel Jean, Jean-Pierre Lavoie,
Faculté de médecine vétérinaire, Université de Montréal, Qc, Canada

Changes in partial pressure of arterial blood gases associated with heaves are well described, but to our knowledge, little is known concerning the metabolic component of the acid-base balance in this respiratory disease. This retrospective study was conducted in order to obtain preliminary information on the acid-base status of horses with heaves.

Arterial blood gas analyses from 22 horses with heaves were studied. Thirteen clinical cases were presented at the Veterinary Teaching Hospital and 9 cases were from our herd of research horses. The diagnosis of heaves was based on history, clinical signs, bronchoalveolar lavage cytology (n=17) and the exclusion of other respiratory diseases. Parameters evaluated in arterial samples included PO_2, PCO_2, pH, bicarbonate concentration, blood lactate and electrolyte concentrations. Strong ion difference (SID) was also calculated. Horses were divided into 2 groups based on their $PaCO_2$. Horses in Group A (n=14) were normocapnic ($PaCO_2$ 35-45 mm Hg), whereas horses in Group B (n=11) were hypercapnic ($PaCO_2$ > 45 mm Hg).

Horses in Group B had significantly lower PaO_2 and higher bicarbonate concentration than horses in Group A. However, there was no significant difference in arterial pH, lactate concentration and SID between the 2 groups. Furthermore, acidemia (pH<7.35) was observed in only 2 horses of group B (7.407+/-0.015). Using linear regression analysis, a 10 mm Hg increase in $PaCO_2$ in these horses was accompanied with an increase of 5 mmol/L in bicarbonate concentration, which exceeded the 3 mmol/L expected from the traditional rules for the prediction of compensation in acid-base balance.

Results of this study contradict the common wisdom that complete compensation of respiratory acidosis should not occur, as all but 2 horses had either normal pH or were alkalotic. Since there was no significant change in chloride concentration and SID in this study, the reasons for this "more effective" compensation was not determined and deserve further investigation.

COMPARISON OF DEXAMETHASONE AND THE MAPK p38 INHIBITOR MRL-1EQ IN EQUINE HEAVES

J.-P. Lavoie[1], D. Thompson[2], E. Hamilton[1], M. Debrue[1], F. David[1], G. Hickey[2].
Faculté de médecine vétérinaire Université de Montréal[1], Qc, Canada,
Merck&Co., Inc, Rahway, NJ, USA[2]

We investigated the efficacy of mitogen activated protein kinase (MAPK) p38 inhibitor Compound MRL-1EQ to either prevent (Phase 1) or treat (Phase 2) heaves in horses. MRL-1EQ was administered intravenously at a dosage of 0.75-1.5 mg/kg q12hrs. The plasma concentrations achieved in the study resulted in ex vivo suppression of LPS induced TNF production in equine blood.

In phase 1, heaves susceptible horses in clinical remission were divided into 2 groups (n=5/group) based on historical values of respiratory mechanics. All horses were entered in the study in pairs (1 control, 1 treated horse) and exposed to the same environmental challenge (stabling, moldy hay and dusty conditions). The treatment group received MRL-EQ1 for 14 days while the control horses were untreated during the same period.

In phase 2, horses with clinical heaves were ranked by severity of respiratory dysfunction and randomly split into either dexamethasone or MRL-EQ1 treatment groups (n=5/group). Bronchoalveolar lavage fluid (BALF), respiratory mechanic measurements, MRL-EQ1 plasma concentration and TNF whole blood activity were sequentially evaluated.

In phase 1, administration of MRL-EQ1 did not prevent the occurrence of clinical exacerbation and pulmonary inflammation, however treatment was associated with a reduction in severity and a delay in the onset of symptoms and a reduction in pulmonary neutrophilia. In phase 2, MRL-EQ1 did not significantly improve airway inflammation or lung function while dexamethasone (positive control) resulted in a marked and early improvement in lung function in all horses.

MRL-EQ1 administration was associated in a dose dependant manner with behavioral (depression, excitability) and blood changes (neutrophilia, increased serum muscle enzyme concentration). We have not ascertained if the cause of these effects is mechanism or non-mechanism based.

In summary, our data suggests that inhibition of p38 in the horse was partially effective in reducing clinical signs when administered prior to, but not during clinical exacerbation in heaves.

SONOGRAPHY COMPARED WITH RADIOGRAPHY IN REVEALING RIB FRACTURE IN NEWBORN FOALS IN AN EQUINE INTENSIVE CARE UNIT

Daniel Jean, Valérie Picandet, Susana Macieira, Guy Beauregard
Faculté de médecine vétérinaire, Université de Montréal, Qc, Canada

In a prospective field study, we reported that 20% of foals on a large thoroughbred studfarm had evidence of thoracic trauma (rib fracture or costochondral dislocation) based on clinical and radiographic examinations. In humans, previous studies have shown that sonography is more sensitive than radiography for detecting rib fractures. The purpose of our study was to determine the incidence of rib fractures in newborn foals in an equine intensive care unit and to compare the sensitivities of sonography and radiography for revealing rib fractures.

This study was performed at the Centre Hospitalier Universitaire Vétérinaire (CHUV), University of Montreal from March 2003 to May 2004. Twenty-nine newborn foals (15 fillies, 14 colts, mean age = 2.96 days (range 1-10 days)) were examined 2 to 12 days after admission. The foals were presented in emergency for reasons other than thoracic trauma. The thoracic cage was palpated externally for abnormalities and all foals were placed in dorsal recumbency to evaluate thoracic cage symmetry. Chest radiography and rib sonography were performed on 24 and 29 foals, respectively. The incidence, location and degree of fracture displacement revealed by radiography and sonography were compared.

Thoracic radiographs revealed 8 rib fractures in 3 of 24 (12,5%) patients and sonography revealed 49 rib fractures in 19 of 29 (65 %) patients. Of the 19 patients with rib fractures found with sonography, fillies (68%) were significantly over represented than colts (32%) and 1 to 5 ribs were found to be fractured. In addition, one patient had a costochondral dislocation. Seventeen of 19 patients had rib fractures located 1 cm or less from the costochondral junction with the ventral part of the rib displaced laterally in all cases. In two patients, where both thoracic radiographs and sonography detected rib fractures, the site of fractures were located on the mid portion of the rib. Rib fracture was detected only on thoracic radiographs in one patient. Sixty-five percent (32/49) of fractured ribs had a moderate displacement (between 1 to 4 mm).

In conclusion, this study indicates that rib fractures and thoracic trauma occur frequently in newborn foals in an equine intensive care unit. Also, sonographic examination reveals more fractures than does radiographic examination and will reveal fractures in most patients presented in emergency. The high incidence of fracture near the costochondral junction suggests that most thoracic traumas likely occur during parturition. The thoracic trauma seen in this study rarely caused clinical sequelae.

DEFINING THE ROLE OF ORGAN-BASED ADAPTATION TO LOCALISED INSULT USING THE SHEEP AS A MODEL SYSTEM

Stephen P Smith, Steven Tate, P Dickinson, V Ogilvie, D. David S. Collie
College of Medicine and Veterinary Medicine, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK

Aims of proposed investigation: To address the hypotheses (1) that the lung will respond on a whole organ basis to a localised insult and (2) that this response will effect changes in lung epithelial gene expression that will be amenable to assessment at a molecular level.

Method: Animals: 12 commercially sourced crossbred sheep (5 F and 7 M) (bodyweight: 33 - 79 kg)
Study design: One lung segment from both the right and left lung of each animal was selected on bronchoscopic examination and its position carefully mapped. One segment, designated 'direct', was selected for LPS instillation and the segment from the contra lateral lung, designated 'remote', used to assess the whole organ response. Bronchial epithelial cells and BAL fluid were collected from each segment by bronchial brushing and then bronchoalveolar lavage not less than two weeks prior to instillation of LPS and at 6 hrs post instillation.
Analysis: Total and differential cell counts in peripheral blood and BALF. Quantitative RT-PCR for IL-1β, IL-6, IL-8, IL-10 and TNFα in the brushed epithelial cells.

Results: Systemic evidence of inflammation comprised a mild pyrexia (+0.8^{o}C), a circulating neutrophilia and a lymphopenia at 6h.
The direct response to LPS comprised a significant neutrophilic infiltrate in the bronchoalveolar space at 6h. (0h median count: 0.03 x10^{6} cells/ml [range: 0.002-0.76] vs. 6h median count: 5.89 x10^{6} cells/ml [range: 0.23-18.08]). In BALF taken from the remote segment at 6h there was no significant change ($p=0.784$, $n=12$) in the number of neutrophils.
Histopathological analysis confirmed the presence of acute neutrophil-mediated inflammation in the directly challenged segment and the absence of any change in the remote segment.
In epithelial brushings collected from the directly challenged segment IL-1β, IL-6 and TNFα expression levels were significantly up-regulated ($p<0.05$) 6h post-LPS. In the remote segment IL-10 mRNA levels were significantly up-regulated ($p<0.05$) 6h post-LPS.

Discussion: The lung mounts an organ-wide anti-inflammatory response to local gram-negative endotoxic inflammatory challenge. This may be a protective mechanism to prevent organ-wide damage and failure such as that seen in ARDS patients. Further characterisation of this response may elucidate novel preventative or therapeutic pathways for prevention of organ-wide inflammation and lung failure due to bacterial infection. Manipulation of organ-wide responses to inflammation may provide further avenues of intervention in inflammatory lung disease.

Acknowledgements: The authors wish to acknowledge the animal care and welfare team at the Wellcome Trust Centre for Research in Comparative Respiratory Medicine, EBVC, Roslin, Midlothian. This work was funded by the Norman Salvesen Emphysema Research Trust.

EVALUATION OF LUNG FUNCTION IN PIGS EXPERIMENTALLY INFECTED WITH *CHLAMYDIA SUIS*

Petra Reinhold[1], Annelie Langenberg[1], Konrad Sachse[2]
FLI - Federal Research Institute for Animal Health, [1]Institute of Molecular Pathogenesis, [2]Institute of Bacterial Infections and Zoonoses, Naumburger Str. 96a, 07743 Jena, Germany

Objective: To evaluate the influence of *Chlamydia* infection on lung function in pigs.

Animals and study design: Eight pigs aged 39–44 days were included in an aerosol challenge model (Sachse *et al*., 2004). Four animals were exposed to *Chlamydia suis* and four non-infected animals served as controls. For lung function testing, the impulse oscillometry system ("MasterScreen-IOS", VIASYS Healthcare, Hoechberg, Germany) was used as validated for pigs before (Klein & Reinhold, 2001; Klein *et al*., 2003). Each lung function test consisted of three consecutive IOS-measurements (duration of each single measurement was 60 seconds while 3 test impulses were generated per second). The sampling rate was set at 200 Hz (period between two sampling points of 5 ms) selecting 32 sampling points after each impulse. Variables of ventilation (respiratory rate, tidal volume, minute volume), respiratory impedance (expressed as respiratory resistance [R] and respiratory reactance [X] within 3 to 15 Hz and separated between inspiration and expiration [Rin, Rex, Xin, Xex]), and model derived resistance of proximal and distal airways were measured. The total observation period for lung function testing ranged from 2 days before challenge until 7 days after challenge.

Results: While non-infected control pigs did not exhibit any clinical symptoms, animals exposed to *C. suis* showed clinical signs of an acute respiratory infection (fever, severe dyspnoea, dry cough and serous nasal discharge). In pigs exposed to *C. suis*, the respiratory rate increased significantly to up to approximately 100 breathing cycles per minute while the tidal volume decreased by 50 %. Respiratory resistance during expiration increased significantly at 3 and 5 Hz leading to a larger difference between inspiratory and expiratory resistance values. Respiratory reactance of infected pigs was characterized by a strong decrease of Xex at all frequencies (significant at 5, 10, and 15 Hz) and by a decrease of Xin at frequencies higher 5 Hz (significant at 10 and 15 Hz). Resistance of distal airways increased significantly 3 days after *C.-suis*-challenge and remained unchanged in control animals. Resistance of proximal airways was significantly increased in infected pigs 7 days after exposure to *C. suis*.

Conclusions: Experimental infection with *C. suis* led to peripheral and central airway obstruction. The predominant respiratory symptoms caused by *Chlamydia* were comparable to an acute airway infection or asthma exacerbation as typically encountered in humans.

References:

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

Klein, C.; Smith, H.-J.; Reinhold, P.: Respiratory mechanics in conscious swine: effects of face mask, head position and bronchoconstriction evaluated by impulse oscillometry. Res. Vet. Sci. 75 (2003) 71-81

Sachse, K.; Grossmann, E.; Berndt, A.; Schütt, Ch.; Henning, K.; Theegarten, D.; Anhenn, O.; Reinhold, P.: Respiratory chlamydial infection based on experimental aerosol challenge of pigs with *Chlamydia suis*. Comp. Immunol. Microbiol. Infect. Dis. 27 (2004) 7-23

A PRELIMINARY INVESTIGATION OF EXHALED NO & CO IN HEALTHY CATS AND CATS WITH AIRWAY INFLAMMATION

David J Marlin[1], Adem Fehmi[1], Andy Sparkes[2], Ellie Mardell[2], Christopher M Deaton[1] and Vicki Adams[3]
Centres for Equine Studies[1], Small Animal Studies[2] and Preventive Medicine[3], Animal Health Trust, Lanwades Park, Kentford, Suffolk, UK, CB8 7UU.

Exhaled NO and CO have been shown to be markers of airway inflammation in humans. Pulmonary function testing in compliant subjects or endoscopy in larger animals such as the horse are routinely used to diagnose airway disease. In feline medicine, endoscopy of the respiratory tract requires general anaesthesia and is rarely undertaken routinely. The present study was undertaken to investigate a number of factors that might influence exhaled NO and CO in healthy cats and to determine whether these gases are elevated in cats with airway inflammation. Six healthy cats with no previous history or current clinical signs of respiratory disease were studied. They were housed in pairs and fed a diet of commercial dry and tinned food with *ad libitum* access to water. They were regularly dewormed and vaccinated. In addition, samples were collected from 7 cats admitted to the clinic with a history of or concurrent respiratory disease. Samples of exhaled breath were collected over a period of approximately 60 seconds into 360ml Tedlar bags using a cat anaesthesia mask, three-way tap and non-rebreathing valve. Samples were always obtained in duplicate along with a sample of background air. NO, CO and CO_2 in the exhaled breath sample were analysed using an LR2000 analyser (Logan Research Ltd, Rochester, UK). Results are presented uncorrected for background NO and CO. All cats tolerated the collection procedure well. In naïve, healthy cats first sampled at 08:00, 09:00 and 10:00h, NO was highest at 08:00h ($P<0.05$), but lower and not different at 09:00h and 10:00h. CO was not detected in any samples. Mixed exhaled CO_2 was not different between any time point. Neither was there any difference in NO or CO_2 in samples collected at 08:00h on three consecutive days (CO was not detected in any samples). There was evidence of diurnal variation in NO with the highest values recorded at 08:00h and 14:00h, compared with 11:00h and 17:00h. There was no diurnal variation in CO_2 and CO was not detected. The reproducibility (r) of consecutive exhaled breath samples (i.e. paired collections) in 13 cats (6 healthy and 7 with respiratory disease) was 0.97, 0.47 and 0.99 for NO, CO and CO_2, respectively. Mixed exhaled CO_2 was not different between healthy cats and cats with disease. Exhaled CO was significantly increased in cats with respiratory disease ($P=0.0043$). NO was increased in 3/7 cats with respiratory disease ($P>0.05$). All cats with respiratory disease either had increased NO (n=2), increased CO (n=3) or increased NO and CO (n=2). During all collections, background NO and CO were always lower than values measured in exhaled breath. However, subsequent studies at different times of the year have shown that environmental NO can be markedly increased. Therefore as in man, collections should be made using inhaled NO free air. This preliminary study indicates that exhaled NO and CO warrant further investigation as potential markers of airway inflammation in cats.

POSTER PRESENTATIONS

EFFECT OF REPEATED CADMIUM INHALATION ON MMP-2 AND MMP-9 ACTIVITY IN BRONCHOALVEOLAR LAVAGE FLUID IN RATS

Vincke G[1], Kirschvink N[1], Belleflamme M[1], Desmet C[1], Peck M[2] and Gustin P[1]
[1]*Department for Functional Sciences, Faculty of Veterinary Medicine, University of Liège,*[2] *Union Chimique Belge, Pharma Sector, Belgium.*

Rationale: The aim was to investigate the matrix metalloproteinases 2 (MMP-2) and 9 (MMP-9) activity during the pulmonary inflammatory reaction induced by repeated nebulizations of cadmium (Cd), a toxic known to be present in cigarette smoke and which could be associated with COPD.

Methods: 30 rats were divided into 5 groups: one saline exposed group (controls) and 4 Cd-exposed groups. Cd was nebulized for 1 hour 3 times a week. Group Cd1 was killed 24 hours after a single exposure. Groups Cd3w and Cd5w were killed after 3 and 5 weeks of exposition, and group Cd5+2w after 5 weeks of exposition and a follow-up of 2 more weeks. Bronchoalveolar lavage fluid (BALF) was analyzed for cytology, and for MMPs using semi-quantitative gelatin zymography. Mean interwall distance (MID) was determined as index of airspace enlargement.

Table 1: Differential cell count, MMP activity in BAL fluid and MID.

Variable (unit)	Controls (n=15)	Cd1 (n=6)	Cd3w (n=6)	Cd5w (n=6)	Cd5+2w (n=6)
Macroph.(10^3/ml)	129±53	716±90*	233±43*	409±115*	239±127
PMN (10^3/ml)	1.0±1.1	345±68*	466±60*	429±91*	102±73*
MMP-9 (AU)	ND	238±124*	138±62*	156±67*	62±42*
MMP-2 (AU)	42±14	912±182*	292±113*	63±25	73±59
MID (µm)	52.9±4.2	45.9±4.6*	59.2±4.0*	57.7±3.3*	62.3±2.7*

* Significantly different from controls, $p<0.05$

Results: Compared to controls, Cd-treated groups showed higher counts of macrophages and neutrophils. MMP-2 was increased in groups Cd1 and Cd3w whereas MMP-9 was increased in all Cd-exposed groups. MID was significantly increased in Cd3w, Cd5w and Cd5+2w rats, suggesting development of emphysema

Conclusion: A single Cd nebulization induced an acute lung inflammation associated with both MMP-2 and MMP-9 activity. Long term exposure led to persistent inflammation, airspace enlargement and MMP-9 but not MMP-2 activity, suggesting that MMP-2 was not implicated in the late phase of the Cd-induced inflammation.

This abstract is funded by: Région wallonne DGTRE and UCB, Braine l'Alleud, BELGIUM.

DEVELOPMENT AND APPLICATION OF AN OVINE-SPECIFIC MICROARRAY FOR GENE EXPRESSION PROFILING IN THE LUNG

Stephen P Smith, P Dickinson, V Ogilvie, R Talbot[1], D. David S. Collie
College of Medicine and Veterinary Medicine, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK and [1]ARK-Genomics, Roslin Institute (Edinburgh), Roslin Institute, Midlothian EH25 9PS

Aims of proposed investigation: To generate and assess a cDNA based microarray capable of probing the molecular response to inflammatory stimuli in ovine lungs

Method: Known and characterised ovine sequences from the NCBI database were used to query the collected sequences from an available bovine genomic library (USDA MARC 1-4BOV and BARC 5BOV; ARK-Genomics), with clones showing suitably high homology with ovine sequences then being used to represent those ovine products on a microarray. In order to discover elements representing the lower level transcripts and the lung specific or differentially regulated elements a subtractive suppressive hybridisation (SSH) library technique was employed. The SSH technique involved subtracting pooled mRNA from heart, brain, liver, kidney and skeletal muscle tissues from lung-derived mRNA and then normalising the abundance of the sequences in the subtracted population to enrich for rare transcripts.
The two populations of clones (SSH and bovine) were then printed in duplicate onto prepared glass slides (ARK-Genomics) and these microarrays were used to profile the gene expression response of the lung at a site remote from an area subject to local insult.

Study design: One lung segment from both the right and left lung of each animal was selected on bronchoscopic examination and its position carefully mapped. One segment, designated ‘direct’, was selected for LPS instillation and the segment from the contra lateral lung, designated ‘remote’, used to assess the whole organ response. Bronchial epithelial cells were collected from each segment by bronchial brushing not less than two weeks prior to instillation of LPS and at 6 hrs post instillation.

Results: 170 genes were significantly up- or down-regulated in epithelial cells derived from lung segments remote from the site of local insult. Such genes were characterised on the basis of known or perceived function based on prior literature in comparative species where available.

Discussion: The nature and extent of the change in gene expression in remote segments is indicative of a highly complex whole organ response. Further analysis will pave the way towards identifying crucial networks of molecular

interactions involved in innate defence, the manipulation of which may offer potential therapeutic gain.

Acknowledgements: The authors wish to acknowledge the animal care and welfare team at the Wellcome Trust Centre for Research in Comparative Respiratory Medicine, EBVC, Roslin, Midlothian. This work was funded by the Norman Salvesen Emphysema Research Trust.

COMPLETE cDNA SEQUENCE ENCODING THE PORCINE MX1

Thomas A., Palm M., Broers A., Leroy M., Desmecht D.
Department of Pathology, Faculty of Veterinary Medicine, University of Liège, Sart Tilman B43, 4000 Liège – Belgium

The MX protein is one of the antiviral proteins induced by type-1 IFNs and is found in most vertebrates such as mammals, birds and fish. In laboratory mouse strains, allelic polymorphisms at the *Mx1* locus affect the probability of survival after experimental influenzal disease, which raises the possibility that identification of an antiviral MX isoform in pigs might allow selection programmes aimed at improving their innate resistance. A partial cDNA sequence encoding the porcine *Mx1* gene was previously identified from a cDNA library (Müller *et al*., 1992).

In the present study, we were interested in obtaining the entire cDNA sequence encoding the porcine MX1 for further gene structure analysis. This preliminary step is indeed essential to localize the *Mx1* promoter and the different exons. The SMART® RACE technology (Clontech Laboratories) was used to clone the 5'-end, i.e. the segment that was lacking in the published cDNA sequence. Briefly, total RNA was extracted from swine kidney (SK6) cells which had been previously stimulated by IFN□. Using a *Mx1*-specific primer, we amplified a ±1400 bp fragment by RT-PCR. After cloning within the pCRII vector (Invitrogen), sequence analysis of different clones revealed the complete 5'-end sequence. Furthermore, different sequences were repeatedly observed suggesting the presence of an alternative splicing process.

MULLER M., WINNACKER EL., BREM G. (1992) Molecular cloning of Porcine Mx cDNAs: New Members of a Family of Interferon-Inductible Proteins with Homology to GTP-Binding Proteins. J. Interferon Res. 12(2): 119-129.

VALIDATION OF PLETHYSMOGRAPHY AS A DISCRIMINATING TOOL TO EVALUATE SUSCEPTIBILITY PATTERNS TO LUNG INFECTIONS IN MICE

Flandre T., Leroy M., Desmecht D.
Dept. of Pathology, Faculty of Veterinary Medicine, B-4000 Liège, Belgium.

Here, we intended to evaluate whether double chamber plethysmography (Buxco PLY-3351) is sensitive enough to discriminate resistant from susceptible mice strains in a mild infection model with an influenza A virus. Changes in pulmonary function values (PFVs) were obtained by double-chamber plethysmography as previously described (Flandre et al., 2003) implemented in 100 mice from 5 different strains : 4 reputed susceptible to influenza viruses because homozygotes for the Mx^s allele (BALB/cJ, C3H/HEN, DBA/2N and 129/Sv) and one reputed resistant (congenic BALB/c in which the Mx^r allele of A2G had been introgressed). The mice were examined for 6 days after inoculation by porcine influenza virus A/Sw/Belgium/1/98 (H1N1). No death was observed, whereas PFVs significantly changed in susceptible strains and remained stable in the resistant congenic BALB/c line. The respiratory frequency and specific airway resistance were higher from 25% to 75% depending on the strain and minute volume increased only in Mx^s/Mx^s 129/Sv and BALB/c. Overall, pletysmography allowed the 5 strains to be allotted into three groups : (i) resistant, (ii) susceptible (BALB/cJ, C3H/HEN) and (iii) highly susceptible (129Sv and DBA/2N). This grouping could be confirmed by histopathology as well as by titration of lung viral yields. It is concluded that double chamber plethysmography offers an accurate, reliable and sensitive way to identify susceptibility/resistant patterns among mouse strains.

Flandre et al. *Journal of Applied Physiology* 94: 1129-1136,2003.

ACTIVATOR PROTEIN-1 ACTIVITY IN BRONCHIAL BRUSHING SAMPLES FROM HORSES WITH RECURRENT AIRWAY OBSTRUCTION

Laurent L. Couëtil, Tania Art, Fabrice Bureau, Pierre Lekeux, Department of Veterinary Clinical Sciences (Couëtil), School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA and Department of Physiology, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

Purpose—To compare activator protein-1 (AP-1) activity in bronchial brushing samples collected from horses with recurrent airway obstruction (RAO) and controls.

Methods—Seven horses with RAO and six healthy controls were exposed to moldy hay until RAO horses reached a maximum change in pleural pressure (ΔP_{plmax}) > 15 cmH_2O. At that point, pulmonary function tests were performed and AP-1 activity in bronchial brushing samples was measured using electrophoretic mobility shift assays. Tests were repeated after RAO and control horses spent 2 months on pasture.

Results—Exposure to moldy hay resulted in increased ΔP_{plmax} (28.4 ± 14.6 cmH_2O), pulmonary resistance (R_L; 2.4 ± 1.1 $cmH_2O.l^{-1}.s$) and decreased dynamic compliance (C_{dyn}; 0.5 ± 0.4 l. cmH_2O^{-1}) in RAO horses compared to controls (6.4 ± 1.0 cmH_2O; 0.5 ± 0.1 $cmH_2O.l^{-1}.s$; 2.2 ± 0.3 l. cmH_2O^{-1}, respectively; $P<0.01$). During moldy hay challenge, AP-1 activity was significantly higher in bronchial brushing samples of RAO horses compared to controls (Fig. 1; $P<0.01$). After 2 months on pasture, AP-1 activity decreased significantly in RAO horses and was not significantly different from controls.

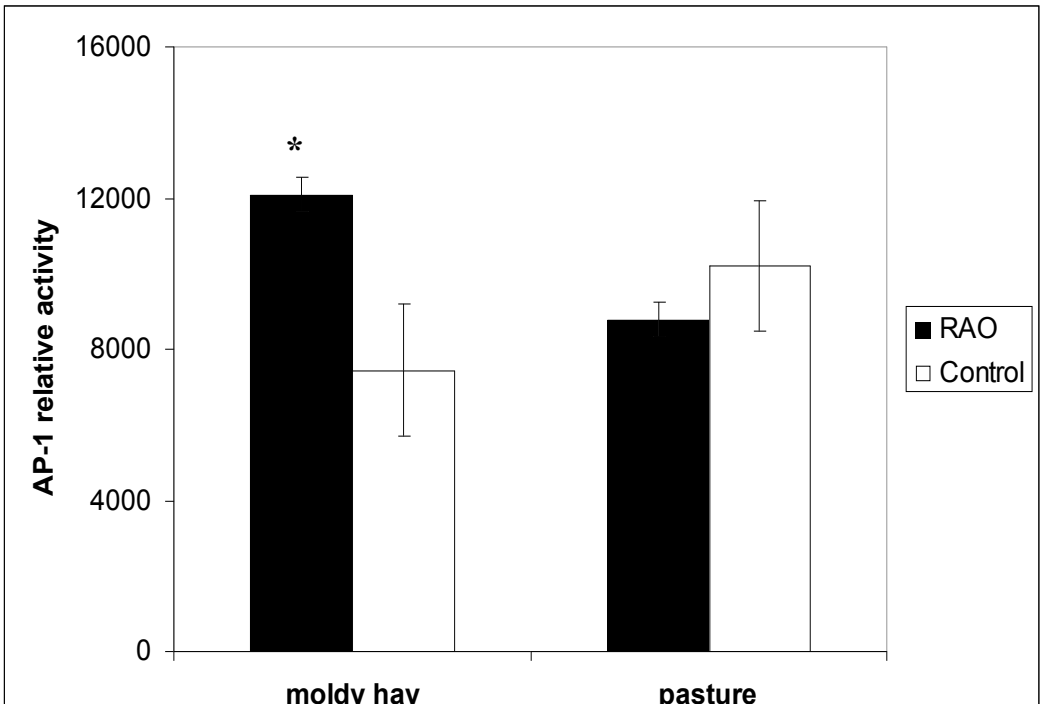


Fig.1: AP-1 activity in RAO and control horses during moldy hay challenge and after 2 months on pasture. *: significantly different from control values (P<0.01).
Conclusion— Results from this study suggest that AP-1 plays a role in RAO pathogenesis.

EFFECT OF INHALED FLUTICASONE ON AIRWAY REACTIVITY AND INFLAMMATION IN CATS

Kirschvink N[1], Leemans J[1], Delvaux F[1], Snaps F[2] ,Clercx C[2], Gustin P[1]
[1]*Department for Functional Sciences,* [2]*Department for Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium.*

Inhaled corticosteroids are commonly used in human patients suffering from asthma or chronic bronchitis to reduce airway inflammation, bronchial reactivity and airway remodelling. Although aerosol therapy is commonly used in feline patients with chronic bronchial disease, quantitative criteria of treatment efficiency are lacking. The aim of the present study was to evaluate the effect of inhaled fluticasone in cats presenting a mild, but persistent neutrophilic lower airway inflammation.

Five adult cats (2 years old, neutered males) were investigated by barometric whole body plethysmography (BWBP), chest radiography and bronchoscopy before and after inhaled fluticasone (250 µg/day via a pressurized metered dose inhaler with spacing chamber and facemask during seven days). Airway reactivity (% CarbPenh300 or concentration of carbachol inducing a 300% increase of Penh, used as an index of bronchoconstriction) was determined by BWBP; a radiography score (min 0, max 9) and a bronchoscopy score (min 0, max 6) were established. Bronchoalveolar lavage fluid (BALF) was analysed cytologically and by zymography for gelatinolytic MMP-9 activity, a marker of airway remodelling. 8-Iso-PGF2alpha, a marker of lipid peroxidation, was determined by enzyme immuno assay.

Table1: Variables before (PRE) and after (POST) fluticasone treatment

	% Carb Penh300	Radio-graphy score	Broncho-scopy score	BAL fluid markers		
				%PMN	MMP-9 (AU)	Iso-PGF2α (pg/ml)
PRE	0.043 ± 0.019	3 (2-3)	4 (2-4)	23 ± 9	199 ± 98	5.09 ± 0.51
POST	0.053 ± 0.020*	3 (2-3)	3 (1-3)*	12 ± 3*	217 ± 105	2.81 ± 0.59*

Data are means ± SD, except scores (median with range); *significantly different from PRE, $p<0.05$; PMN: polymorphonuclear cells.

These results show that 250 µg of fluticasone administrated once daily during seven consecutive days significantly reduced bronchial reactivity, neutrophil percentage and 8-Iso-PGF2α in BAL fluid. The bronchoscopy score was significantly decreased, whereas radiography score and MMP-9 activity in BAL fluid remained unchanged, suggesting that this treatment reduced the inflammatory process but not the proteolytic activity of MMP-9.

Supported by: Région wallonne DGTRE, Belgium.

IMMUNOHISTOCHEMICAL EXPRESSION OF TRYPTASE IN THE LUNG OF CONTROL AND HEAVES SUSCEPTIBLE HORSES DURING CHALLENGE

Dacre, K.J.[1], Berney, C.[2], Bartner, L.R.[2], Robinson, N.E.[2], Brown, J.K.[1], Pemberton, A.D.[1] and McGorum, B.C.[1].
[1]Royal (Dick) School of Veterinary Studies, Midlothian, UK, [2]Michigan State University, East Lansing, USA

Although the number of bronchoalveolar lavage fluid (BALF) mast cells is not increased following hay / straw exposure, clinically affected heaves horses have significantly increased BALF tryptase compared to controls or heaves horses in remission (Dacre et al, 2003). The aim of this study was to evaluate tryptase positive mast cells in the lungs of control and heaves susceptible horses following hay / straw challenge using immunohistochemistry.

Bronchial and bronchiolar tissue samples were collected *post mortem* into Carnoys fixative from challenged control (n=6) and heaves horses in early resolution phase (5d in a hay / straw challenge environment and then 7d in a low dust environment) (n=7). Duplicate sections were probed with rabbit anti-equine tryptase. Mast cell numbers (cells / mm^2) in the epithelium, connective tissue, smooth muscle and alveolar tissue were determined using a combination of manual counting and stereology (10 x 0.044mm^2 fields / slide).

When bronchial and bronchiolar data were combined, there was a strong trend for an increased number of tryptase positive mast cells in the airway epithelium ($p=0.09$). There was no significant difference in the number of tryptase positive mast cells in the other tissues. The areas of alveolar, epithelial, connective tissue and smooth muscle tissue were not significantly different between control and heaves horses.

In conclusion, these results suggest that mast cells are recruited to the airway epithelium in heaves susceptible horses during hay / straw challenge from where they would be in a prime location to secrete tryptase into the airspace.

Dacre, K.J., Deaton, C., Marlin, D., Pirie, R.S., Brown, J., Pemberton, A.D. and McGorum, B.C. (2003) Mast Cell Protease Concentrations In Equine Bronchoalveolar Lavage Fluid From Control And Heaves Affected Horses. *Proceedings of 21st Veterinary and Comparative Respiratory Symposium*, San Antonio, Texas, USA.

ADJUSTMENT IN FUNCTIONAL RESIDUAL CAPACITY DUE TO EXTERNAL LOADING DOES NOT EXPLAIN THE 'FIXED RESISTOR EFFECT'

J, Lofgren, D. Bedenice, E. Rozanski, T.Oura, J.Abrams and A. Hoffman
Pulmonary Function Laboratory, Tufts University School of Veterinary Medicine (TUSVM), 200 Westboro Rd, North Grafton, MA, 01536

Introduction: Upper and lower airway obstructions are common in dogs. The recent development of a rapid non-invasive head-out body plethysmographic (HOP) measurement of airways resistance (Raw) in awake dogs (Bedenice D, *ACVIM*, 2003) was an important step in objectively documenting the degree of airway obstruction accompanying these clinical syndromes. HOP is particularly valuable in quantifying contributions of inspiratory versus expiratory airway obstruction. An unexpected finding in previous studies using HOP in dogs was the exaggerated increase in specific airway resistance (sRaw = Raw*FRC) measured during fixed (versus expiratory or inspiratory) loading. The basis for this "fixed resistor effect" is currently unknown. One potential explanation might be an adjustment in FRC. Adjustments in FRC are known to occur in humans when challenged with an external resistive load (Kelsen, S. *J. Clin. Invest*, 1981), although challenge with a fixed resistor has not been investigated in animals. The purpose of the current study was to determine whether healthy unsedated retriever dogs (n=13) make adjustments in end-expiratory lung volume (FRC) during external loading with inspiratory, expiratory, and fixed resistors. Additionally, the reproducibility of two methods of measuring FRC in the awake dog in a sitting position, helium dilution and respiratory inductive plethysmography (RIP), were evaluated.

Methods: Changes in FRC were measured using two methods: (a) helium dilution by a rebreathing method = FRC_{He}, and (b) respiratory inductive plethysmography. Dogs, in sitting position, were acclimated to each resistor, applied in random order, for 90 sec, and at the end of this period, FRC_{He} or the change in RIP baseline (i.e. end-expiratory lung volume) were measured.

Results: Mean (±SD) FRC_{He} was 1.18L (±0.32) at baseline (no load), vs. 1.16L (± 0.25) after inspiratory, vs.1.28L (±0.33) after expiratory resistive loading, and 1.16L (± 0.28) following a fixed resistive load of 5.6 $cmH_20/L/S$. The coefficient of variation for these measurements ranged from 21-27% for FRC_{He} and 11-13% for the changes in FRC_{He} from baseline. Expiratory loading resulted in a higher FRC than baseline (P=0.033). No significant change was found between baseline and inspiratory or fixed loads. Changes between expiratory, and inspiratory, or fixed loads were significant (P=0.01). There was no significant change in FRC as measured via RIP, although none of the coefficients of variation for RIP were below 60%.

Conclusion: The “fixed resistor effect” can not be explained by *in vivo* adjustments in FRC, but rather must be explained by changes in intrinsic Raw. However, the previously noted 11% discrepancy between observed and expected measurement of Raw after expiratory resistive loading with 5.6 cm/H20/L/s can now be explained by an upward adjustment in FRC (+8.8%) after external loading. The mechanism of increased Raw in dogs with a fixed resistor warrants further investigation. Finally, FRC_{He} is a reproducible method where RIP is poorly reproducible in awake sitting dogs.

Supported by NIH HM62266-03

STAT5 PROMOTES GRANULOCYTE SURVIVAL DURING LUNG INFLAMMATION

L. Fiévez,* C. Desmet,* G. Seumois,* B. Pajak, ¶ L. Gillet, † A. Vanderplasschen,† P. Lekeux,* and F. Bureau*

*Laboratoire de Physiologie Vétérinaire and †Département d'Immunologie-Vaccinologie, Université de Liège, B-4000 Liège, Belgium; and ¶Laboratoire de Physiologie Animale, Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, B-6041 Gosselies,

Delayed granulocyte apoptosis is associated with acute and chronic inflammatory diseases. Numerous inflammatory mediators have been shown to regulate the life span of granulocytes. For example, granulocyte-macrophage colony-stimulating factor (GM-CSF) increases mature neutrophil survival and interleukin (IL)-3, IL-5 and GM-CSF prevent eosinophil apoptosis. Recent research has demonstrated that the effects of GM-CSF are partly mediated through the latent transcription factor, signal transducer and activator of transcription 5 (STAT5). Therefore, the aim of this study was to determine the implication of STAT5 in the apoptosis delay of granulocytes during neutrophilic and eosinophilic inflammation.

To induce neutrophilia, wild-type and Stat5 deficient mice were given aerosolized lipopolysaccharide (LPS) by an ultrasonic nebulizer for 2 hours. Bronchoalveolar lavages (BALs) were performed just before and 1, 2 and 3 days after the exposure and the rate of apoptosis in BAL neutrophils was assessed at each time point of the protocol. The neutrophil viability level was significantly lower in Stat5 deficient mice than in wild-type mice. In order to induce eosinophilia, mice were sensitized twice by intraperitoneal injection of ovalbumin at a 2-week interval. Fourteen days after the second immunization, the sensitized mice were challenged for 1 week with aerosolized ovalbumin. BALs were performed just before and 1, 3, 5 and 8 days after the last aerosol exposure. The rate of apoptosis of BAL eosinophils was assessed at each time point of the protocol. The eosinophil survival rate in Stat5 deficient mice was significantly lower than in wild-type mice.

In conclusion, our results demonstrate that STAT5 is crucial for the delay of neutrophil and eosinophil apoptosis and that STAT5 could be a potential target to reduce inflammation.

ASSESSMENT OF VIRAL-INDUCED LUNG INJURY IN MICE USING MONITORING OF CARBON MONOXIDE UPTAKE : COMPARISON WITH VENTILATORY PATTERN AND HISTOLOGY

Faisca P., Leroy M., & Desmecht D.
Dept of Pathology, Faculty of Veterinary Medicine, B-4000 Liège, Belgium.

The mouse type-1 parainfluenza virus (Sendai) is currently used as a model to study respiratory viral infections. Pathological studies have defined the structural abnormalities, and measurements on ventilation and mechanics of breathing have been recently made but, to our knowledge, there were no attempt to assess impairment of gas exchange directly.

With the objective to determine the reliability and sensitivity of the monitoring of carbon monoxide (CO) uptake to measure lung injury, three different strains of mice with different susceptibilities to Sendai virus (BALB/c as the resistant strain, and DBA/2 and 129Sv as the susceptible ones) were inoculated intranasally with 50μl of a viral suspension containing 1000 PFUs. Pulmonary function values were monitored daily by double-chamber plethysmography while gas exchange was measured by CO uptake. Seven days post-infection, all mice were euthanised, and the lungs processed in a standardized way for histopathology.

The results suggest that CO uptake was not sensible enough to detect lung lesions induced by Sendai virus, at least in the acute phase of infection. While the plethysmography detected an increase in respiratory rate and minute volume, and a decrease in tidal volume starting at day 3 post infection, and the histological examination revealed progressive (epithelial hyperplasia), inflammatory and regressive (necrosis) lesions, especially in the susceptible strains, the CO uptake remain stable during the course of infection.

Even though the CO uptake did not change significantly, in the susceptible strains, it tended to drop from day 0 to day 7 (in 129Sv, from 30.8 ± 1.4 to 26.9 ± 1.4 μl/min and in DBA/2, from 32.1 ± 1.4 to 31.4 ± 1.6 μl/min) while in BALB/c, the resistant strain, it slightly increased (from 29.9 ± 1.4 to 31.3 ± 1.5 μl/min). It remains possible that in the acute phase of infection, the increase of respiratory rate and minute volume compensates the impairment of gas exchanges, and that in more severe pneumonias or in a more chronic phase the CO uptake drops significantly in susceptible animals.

SELECTIVE BLOCKADE OF NF-κB ACTIVITY IN AIRWAY IMMUNE CELLS INHIBITS THE EFFECTOR PHASE OF EXPERIMENTAL ASTHMA

Christophe Desmet,[*] Philippe Gosset,[§] Bernard Pajak,[¶] Didier Cataldo,[†] Mohamed Bentires-Alj,[‡] Pierre Lekeux,[*] and Fabrice Bureau[*]
[*]Laboratoire de Physiologie Vétérinaire, [†]Service de Pneumologie, and [‡]Laboratoire de Chimie Médicale, Centre de Thérapie Cellulaire et Moléculaire, Université de Liège, B-4000 Liège, Belgium; [§]Institut National de la Santé et de la Recherche Médicale, Unité 416, Institut Pasteur de Lille, 59019 Lille Cédex, France; and [¶]Laboratoire de Physiologie Animale, Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, B-6041 Gosselies, Belgium

Knockout mice studies have revealed that Nuclear Factor-κB (NF-κB) plays a critical role in Th2 cell differentiation and is therefore required for induction of allergic airway inflammation. However, the questions of whether NF-κB also plays a role in the effector phase of airway allergy and whether inhibiting NF-κB could have therapeutic value in the treatment of established asthma remain unanswered.

To address these issues, we have assessed in OVA-sensitized wild-type mice the effects of selectively antagonizing NF-κB activity in the lungs during OVA challenge. Intratracheal administration of NF-κB decoy oligodeoxynucleotides to OVA-sensitized mice led to efficient nuclear transfection of airway immune cells, but not constitutive lung cells and draining lymph node cells, associated with abrogation of NF-κB activity in the airways upon OVA provocation. NF-κB inhibition was associated with strong attenuation of allergic lung inflammation, airway hyperresponsiveness, and local production of mucus, interleukin (IL)-5, IL-13 and eotaxin. IL-4 and OVA-specific IgE and IgG1 production was not reduced.

This study demonstrates for the first time that activation of NF-κB in local immune cells is critically involved in the effector phase of allergic airway disease and that specific NF-κB inhibition in the lungs has therapeutic potential in the control of pulmonary allergy.

IMPLICATION OF AVIAN RESPIRATORY PHYSIOLOGY ON THE USE OF CAPNOMETRY IN THE MONITORING OF CO_2 DYNAMIC IN ANESTHETIZED BIRDS

Desmarchelier M, Rondenay Y., Fitzgerald G. & Lair, S.
Service de médecine zoologique, Faculté de médecine vétérinaire,
Université de Montréal, Québec, Canada

The American Society of Anesthesiologists mandates continual monitoring of end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) for all patients receiving general anesthesia. Measurement of $P_{ET}CO_2$ by capnometry has been shown to provide valuable estimations of arterial partial pressure of carbon dioxide (P_aCO_2) in different mammalian species. The clinical use of capnometry has not been extensively studied in avian species, and significant differences between avian and mammalian respiratory physiologies prevent extrapolations from mammals to birds. For instance, the highly efficient parabronchial cross-current gas exchange system present in the avian lung results in CO_2 levels in the air exiting the pulmonary parenchyma higher than the concomitant P_aCO_2.

Correlations between P_aCO_2 and $P_{ET}CO_2$ will be clearly affected by this physiological feature. Furthermore, since most species of birds seen by veterinarians have relatively small tidal volumes and high respiratory rates, measurement of $P_{ET}CO_2$ in these species with high flow side-stream capnometers is likely to result in erroneous readings. In contrast, newly available low flow Microstream® capnometers might be accurate in animals with small tidal volumes.

In light of this, we evaluated a handheld Microstream® capnograph (NPB-75®, Nellcor Puritan Bennett, Plesanton, CA, USA) on different species of birds of prey during general anesthesia. Briefly, each of the nine birds (ranging from 416 g to 2062 g) was anesthetized with isoflurane via face mask, intubated and manually ventilated using a Bain Mapleson D modified non-rebreathing system. Respiratory rates were controlled to achieve different levels of $P_{ET}CO_2$. Concomitant values of P_aCO_2 were measured for each level of $P_{ET}CO_2$ obtained.

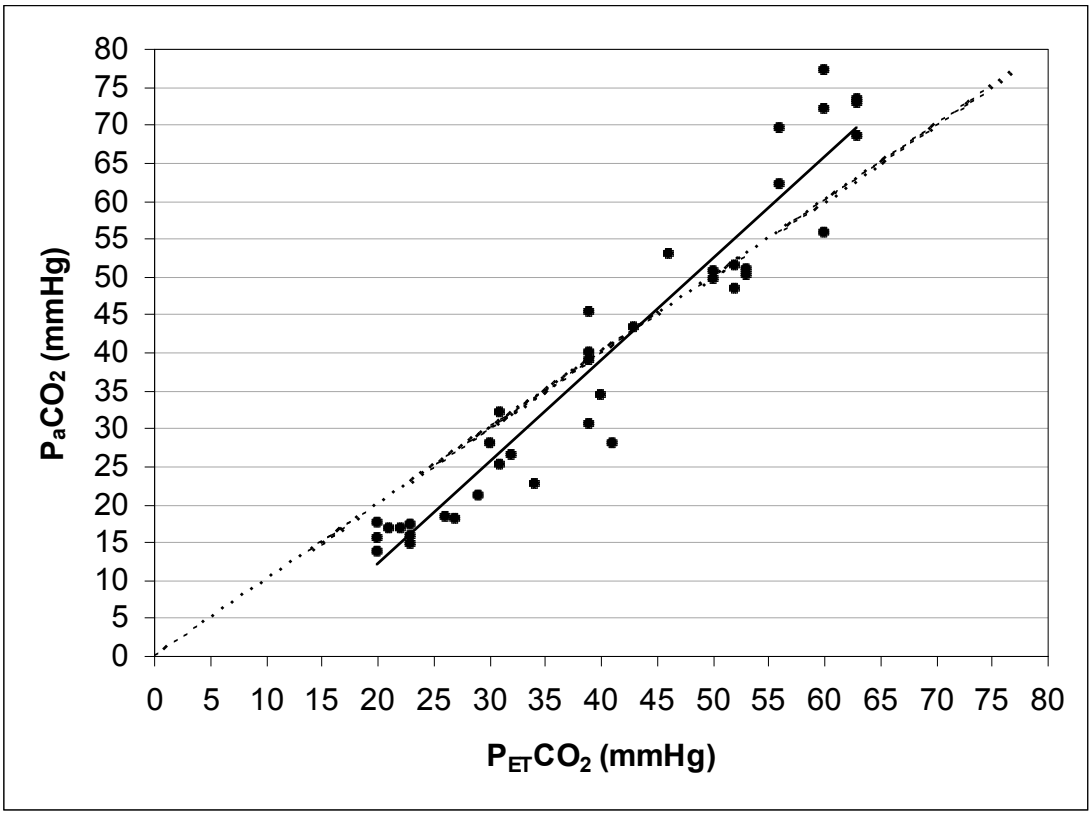
As shown in Figure 1, a strong correlation was observed between $P_{ET}CO_2$ and P_aCO_2 ($r^2 = 0.93$; $p < 0.0001$), as well as between $P_{ET}CO_2$ and arterial pH ($r^2 = 0.90$; $p < 0.0001$) ($n = 38$).

All but one of the $P_{ET}CO_2$ levels measured below 39 mmHg exceed the concomitantly measured values of P_aCO_2 (mean ± SD = 5.7 ± 3.0 mmHg). This overestimation of P_aCO_2 by $P_{ET}CO_2$ is in agreement with the expected effect of the cross-current exchange system in the avian pulmonary parenchyma. Measured levels of $P_{ET}CO_2$ ranging from 39 and 53 mmHg differed from the P_aCO_2 by -7 to 13 mmHg (mean ± SD = 1.5 ± 5.2 mmHg). All but one of the

$P_{ET}CO_2$ levels measured above 53 mmHg were underestimating its concomitantly measured P_aCO_2 level (mean ± SD = -8.8 ± 6.5 mm of Hg). Potential explanations for this frequent underestimation of hypercapnic states include a decrease in the accuracy of the capnograph used within high values of CO_2 and dilution of the CO_2-rich post-parabronchial air by passive exchanges with air present in the air sacs as a result of the low respiratory rate.

Despite strong correlation between $P_{ET}CO_2$ and P_aCO_2, predictability was variable, especially for higher values of $P_{ET}CO_2$. Nevertheless, the Nellcor NPB-75® capnograph could still be a useful non-invasive monitoring tool for the evaluation of P_aCO_2 in birds. Based on the results obtained in the current study, we propose that levels of $P_{ET}CO_2$ should be maintained between 35 and 45 mmHg in anesthetized birds over 400 g.

Figure 1: Scatterplot of P_aCO_2 against $P_{ET}CO_2$ in mmHg (n=38). Continuous line represents the best-fit linear trend line. Dashed line is the line of equality for comparison. $P_{ET}CO_2$ values below and above the dashed line respectively overestimate and underestimate the concomitantly measured values of P_aCO_2.



MYELOPEROXYDASE CONCENTRATION IN BRONCHOALVEOLAR LAVAGE FROM HEALTHY AND HEAVY HORSES

T.Art[1], Ch.Desmet[1], P. Lekeux[1], B.de Moffarts[1], L.Couëtil[1], M.Becker[1], T.Franck[2], S.Kohnen[2], G.Deby-Dupont[2], D.Serteyn[2]
[1] Equine Sports Medicine Center, Faculty of Veterinary Medicine & [2] Center for Oxygen Research and Development, University of Liege, Liege, Belgium.

Introduction—In horses, recurrent airway obstruction or heaves is known to induce a neutrophilic airway inflammation as assessed by the neutrophils counts in bronchoalveolar lavage fluids (BAL). Myeloperoxidase (MPO) is a specific enzyme of neutrophil granules with a strong oxidative activity which most probably plays a role in the pulmonary inflammation observed in horses suffering from heaves. It has never been measured in horse's BAL. The aim of this work was to measure MPO concentration in BAL collected from heavy horses in crisis and in remission and from control horses in order to assess whether MPO could be a marker of neutrophils afflux and activation.

Methods—Seven horses suffering from heaves were exposed to moldy hay until they reached a maximum change in pleural pressure (ΔP_{plmax}) > 15 cmH_2O. At that point, BAL were performed. The BAL cytology, ie total cell count and neutrophils percentages, was immediately performed, while MPO concentration in BAL supernatant (centrifugation 10 minutes at 1000g) was immediately determined using a specific enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies raised against equine MPO (Patent nr 04447027.6) . Tests were repeated on the same horses after they spent 2 months on pasture. Six healthy horses served as controls.

Means were compared by an ANOVA and a probability of > 0.05 was considered as significant. The relationship between absolute and relative neutrophils were assessed by linear regression on the gathered data.

Results— Exposure to moldy induced significant increases in ΔP_{plmax} (28. 4 ± 14.6 cmH_2O), in both absolute and relative neutrophils as well as in the MPO level (figure 1). After 2 months on pasture, the horses recovered a physiologic ΔP_{plmax} (8.1 ± 0.7 cmH_2O), while the absolute and relative number of neutrophils and the MPO concentration decreased significantly (figure 1).
There were no significant differences between neutrophils counts of control horses and heavy horses in remission, but their respective BAL MPO concentration were different, the MPO level from ~~of~~ heavy horses being significantly higher.

Correlation between MPO levels and neutrophils counts were significant, with R^2 values of 0.671 and 0.825 for relative and absolute neutrophils respectively.

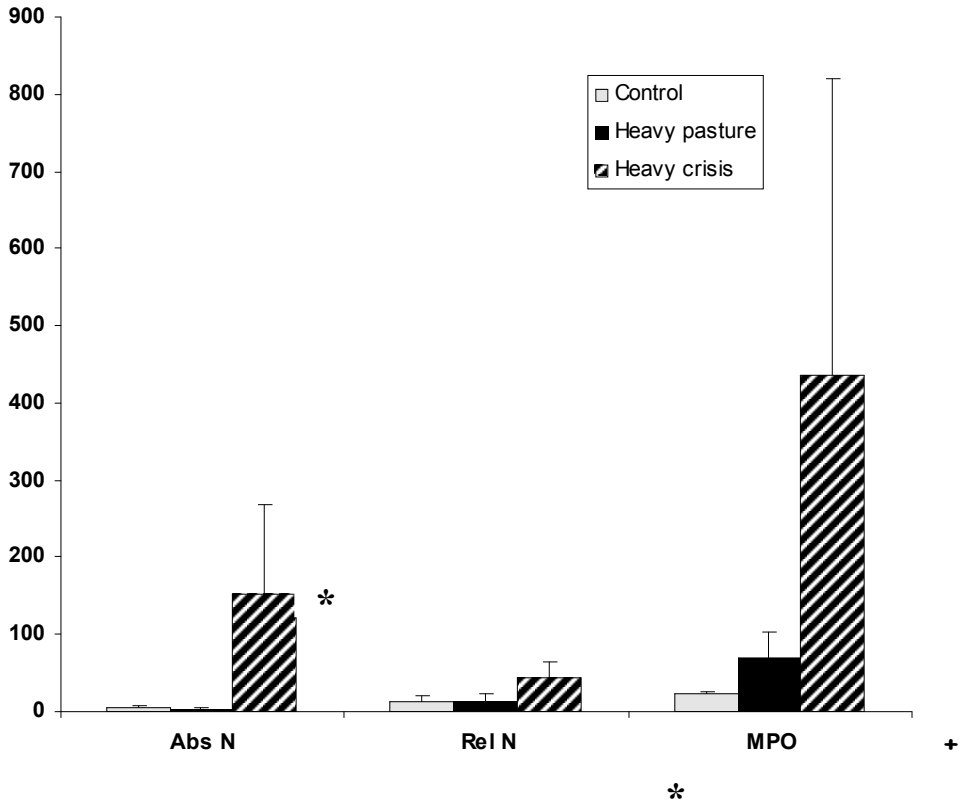


Figure 1: Absolute neutrophils counts (in number of cell 10^4/ml)(Abs N); relative number of neutrophils (in %) (Rel N) and BAL MPO (ng/ml) (MPO) from 7 heavy horses either in crisis or after 2 months on pasture and in control health horses. *: significantly different from healthy horses and heavy horses in remission; + significantly different from healthy horses.

Conclusion— Results (1) lead to the conclusion that the determination of MPO in horse's BAL is technically possible, (2) show that despite a clinical and neutrophils recovery, the horses in remission have a level of neutrophils activation higher than healthy horse and (3) suggest therefore that MPO is a reliable marker of neutrophils presence and activation in the lower airways.

LUNG FUNCTION IN HORSES OVER 4 CONSECUTIVE DAYS FOLLOWING A SHORT ELECTIVE SURGICAL PROCEDURE (CASTRATION) IN DORSAL RECUMBENCY

Leinker S.[1], Riedelberger K.[1], Auer U.[2]
[1]Department of small animals and horses, clinic of internal medicine and infectious disease; [2]Department of small animals and horses, anaesthesia and perioperative intensive care; Veterinary University of Vienna, Austria

In humans atelectasis and impairment of lung function in the postoperative period after abdominal surgery, is a well recognized problem. In one third of patients, atelectasis peristed through the fourth postoperative day.[1] It has been documented, that problems related to respiratory function in large animal anaesthesia are due, at least in part, to atelectasis.[2]

We hypothesised, that lung function, after castration in dorsal recumbency, is impaired over longer period of time post surgery.

The lung function of 17 healthy stallions (aged: 2-5y; weight: 410-620kg) without a history of lung disease was measured with the flowmetric system. We measured in the morning before induction of anaesthesia, 6h after surgery and then every morning until the 4th day post surgery in conscious, unsedated horses. The anaesthesia was premedicated with xylacine and butorphanol, induced with ketamin and valium and maintained with isoflurane in 100% oxygen in combination with tiva, a mixed of midazolame, ketamin and xylacine in 0,9% saline (duration of anaesthesia: 30-75min).

When measuring the lung function, we calculated the PIsf (Peak inspiratory sum-flow), the PEsf (Peak expiratory sum-flow) and their percentage deviation of the base value. Data were analysed with descriptive statistics and ANOVA for repeated measurements. All results were expressed as median +/- SD and differences were considered significant if $p < 0,05$.

No significant changes in PIsf or PEsf were detected. However, 21% and 19% increases relative to baseline values in PIsf and PEsf respectively indicated a tendency toward impaired respiratory function on the first post operative day.

In our study, no statistically significant difference in lung function after a short elective surgical procedure in horses could be detected. A possible way to lower the standard deviation could be the application of a standardized sedation protocol.

17 horses	**prä OP**	**6h post OP**	**1d post OP**	**2d post OP**	**3d post OP**	**4d post OP**
median Pisf +/- SD	3,16 +/- 1,41	3,60 +/- 2,97	3,99 +/- 1,52	3,74 +/- 1,09	3,71 +/- 0,83	3,68 +/- 1,51
median Pesf +/- SD	3,32 +/- 1,61	3,47 +/- 3,1	4,26 +/- 1,43	3,82 +/- 1,25	3,86 +/- 1,02	3,70 +/- 2,0
PIsf mean percent	100% +/- 0%	138,9% +/- 117,8%	120,9% +/- 34,7%	114,1% +/- 29,0%	114,5% +/- 34,2%	118,2% +/- 33,7%
PEsf mean percent	100% +/- 0%	110,0% +/- 27,2%	118,7% +/- 30,6%	111,2% +/- 30,0%	112,7% +/- 33,0%	117,7% +/- 41,5%

References:

1. Lindberg P. et al. Atelectasis and lung function in the postoperative period. Acta Anaesthesiol. Scand. 36, 546-553 (1992)

2. Nyman G. et al. Atelectasis causes gas exchange impairment in the anaesthetised horse. Equine vet. J. 22, 317-324 (1990)

ENDOTHELIN AND NITRIC OXIDE PRODUCTION BY EQUINE BRONCHIAL EPITHELIAL CELLS CULTURED UNDER AIR-LIQUID INTERFACE CONDITIONS

L. R. R. Costa, K. O'Reilly, R. Truax, T. Foster, J. R. Johnson, R. M. Moore;
Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA 70803, USA

Several mediators have been implicated in the pathogenesis of equine airway diseases. Amongst them, nitric oxide (NO) derived from inducible nitric oxide synthase and endothelin (ET) were shown to be increased in airway epithelium of asthmatic and horses affected with summer pasture-associated obstructive pulmonary disease (SPAOPD). Moreover, a number of stimuli, especially cytokines, have been incriminated in the induction of ET and NO synthesis. The overall goal of this study was to stimulate cultures of differentiated equine bronchial epithelial cells with LPS, TNF-alpha and IL-4 and measure the synthesis of ET and NO.

Fresh post-mortem specimens of lung tissue were obtained from two adult horses affected with SPAOPD while horses were in clinical remission (i.e., without signs of respiratory disease, intrapleural pressure difference less than 10 cm of water, and neutrophil in bronchoalveolar lavage less than 15%). The bronchial epithelium was dissected, subjected to cold trypsinization and cultured on Transwells with Dulbecco's modified Eagle's medium:Ham's F12 (1:1 v/v) containing fetal bovine serum and epithelial growth factor (EGF) as previously described. Once cultures were established, they were placed in air-liquid interface (ALI) and maintained in a serum-free media containing low concentration of EGF. After 9 days, the cells were stimulated basolaterally with either LPS (10 ng/ml), human recombinant TNF-alpha (5 and 20 ng/ml) or equine recombinant IL-4 (1%, 10% and 50% v/v). Cell-free supernatants from the bottom of the wells were harvested at 24, 48 and 72 hours and stored at –70C until assayed for ET and NO. ET concentrations were determined using a commercially available sandwich enzyme-linked immunosorbent assay (Biomedica). NO determination was performed using an electrochemical detection system, ISO-NO Mark II. Morphologic differentiation of the cell cultures after 14 to 28 days in ALI was evaluated using light microscopy (thin-sections were stained with Toluene Blue), confocal microscopy (stained for cytokeratin and actin) and transmission electron microscopy.

Stimulation with hrTNF-alpha, LPS or eqrIL-4 for 24 and 48 hours, and eqrIL-4 for 72 hours resulted in increased production of ET (ranging from 1.5 to 4 fold). Stimulation with hrTNF-alpha, LPS and eqrIL-4 for 48 hours and eqrIL-4 for 72 hours induced 1.5 to 2.5 fold increases in NO production by primary bronchial epithelial cell culture. Our results suggest that bronchial epithelial cells represent a potentially important source of ET and NO in response to cytokine (TNF-alpha and IL-4) stimulation. The interactions of these mediators may play a role in the pathogenesis of SPAOPD.

THE INFLUENCE OF FEEDING ON THE CONCENTRATION OF UREA AND AMMONIUM IN EXHALED BREATH CONDENSATE AND PERIPHERAL BLOOD

Petra Reinhold[1], Annelie Langenberg[1], Michael Rothe[2], Gunther Becher[2]
[1]Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Institute of Molecular Pathogenesis, Jena, Germany & [2]Research Company of Lung and Chest Diseases Ltd, Berlin, Germany

Objective: In a previous study, both ammonia and urea in exhaled breath condensate (EBC) were found to be significantly elevated in acute pneumonic inflammation (Reinhold *et al.*, 2002). However, the influence of food intake (which may be reduced in acutely diseased animals) on these variables remained unclear. Consequently, this study aimed to evaluate the influence of food intake on the concentration of urea and ammonium in EBC and blood serum. Clinically healthy pigs were used as models.

Methods: In 12 pigs, 48 EBC and 48 serum samples were collected in parallel (each collection occurred 2-4 hours after feeding). At the end of the study, EBC and blood were collected in non-fed animals (n=12). For EBC collection, the "ECoScreen" (VIASYS Healthcare, Germany) was used.

Urea was analyzed using a commercial test kit based on the Berthelot method (Merckotest, Merck-Diagnostika). Ammonia testing was also done photometrically (Berthelot method), however, no ammonia-releasing urease was included so that only the ammonia already present could be measured. After measuring ammonia, the remaining urea concentration was calculated from the difference (urea = total "urea"$_{measured}$ - ammonia).

Results: As shown in Table 1, urea in blood serum was significantly increased by feeding. Although this effect could not be statistically secured for EBC, higher urea concentrations were found in EBC samples of fed pigs compared to those collected in non-fed pigs. Even in non-fed pigs, no significant correlation between serum and EBC was found for urea.

The concentration of ammonium was not significantly influenced by feeding (neither in blood nor in EBC). A significant rank correlation was found between the concentration of ammonium in blood and those in EBC for non-fed animals ($r_{SPEARMAN} = +0.60$, $p < 0.05$, $n = 12$).

Table 1: Medians (ranges) of urea- and ammonium-concentration in blood, EBC, and BALF (unit: nmol/ml)

	Urea		**Ammonium**	
	after feeding (n=48)	**empty stomach (n=12)**	**after feeding (n=48)**	**empty stomach (n=12)**
Blood serum	4328 (2183 - 6491)	2773 (2100 – 3715)**	812 (586 – 948)	782 (616 – 833)
EBC	30 (6 – 437)	12 (6 –28)	148 (31 – 281)	202 (119 – 333)

** significant influence of feeding [W-test according to Mann-Whitney-(Wilcoxon), $p < 0.001$]

Reference

Reinhold, P; Langenberg, A.; Seifert, J.; Rothe, M.; Becher, G.: *Ammonia and urea in the exhaled breath condensate (EBC) are potential non-invasive markers of pneumonia.* Proceedings of the 20th Symposium of the Veterinary Comparative Respiratory Society, Boston (USA), 4.-6. October 2002, 90-92