“Underlying concepts in chronic lung disease; from injury to dysregulated repair”

Royal College of Surgeons, Edinburgh, UK
8th – 10th October, 2015

Principal sponsor: Boehringer Ingelheim
Additional sponsor: British Association for Lung Research
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FOREWARD

We are extremely honoured and proud to be hosting the 33rd Veterinary and Comparative Respiratory Society Symposium in Edinburgh and we extend a warm welcome to all speakers and delegates.

The theme of the meeting is “Underlying concepts in chronic lung disease, from injury to dysregulated repair”, an area of recent focussed attention in both human and veterinary medicine. Within this overall theme, five sub-themes will be developed over the course of the meeting: these include pulmonary fibrosis, bronchiectasis, animal models, tools for monitoring disease progression, pulmonary vascular remodeling. We are extremely fortunate to have a wealth of local expertise within this area and many of our speakers are leading authorities in their respective fields of research. Additionally, we have been fortunate to secure the commitments of speakers from further afield, who bring their own expertise and experiences to the symposium. We are extremely grateful to all speakers for their willingness to contribute to the success of the meeting.

We also extend our sincere gratitude to all who submitted an abstract for inclusion in the program. We appreciate your willingness to share both preliminary and more extensive datasets with the delegates, an exercise which we are confident will promote stimulating discussions throughout the duration of the meeting. Furthermore, we are delighted to welcome all delegates who hail from near and far (11 countries across 3 continents) and are grateful for your efforts to attend this meeting. We hope you enjoy the scientific content, the comradery of the Society and the hospitality that Edinburgh has to offer.

We are sincerely grateful to our principal sponsor, Boehringer Ingelheim for their continued generous support of the VCRS symposium; their financial backing undoubtedly and significantly enables the host institute to maximise the quality of the program, whilst maintaining affordable delegate fees. We would also like to thank the British Association for Lung Research for their kind support, through the provision of travel and abstract awards and their promotion of the meeting to their membership.

Finally, we would like to reiterate our warm welcome and hope that you have an enjoyable and memorable experience in our city.

2015 VCRS Symposium Scientific Committee

Prof Scott Pirie                 Dr. Dave Collie                    Prof Bruce McGorum                    Dr Gerry McLachlan
(University of Edinburgh)           (Roslin Institute)                                 (University of Edinburgh) (Roslin Institute)
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<tr>
<th>Speakers</th>
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<td>Bedi Pallavi</td>
<td>University of Edinburgh MRC Centre for Inflammation Research</td>
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<td>Church Colin</td>
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ACKNOWLEDGEMENT TO SPONSORS

BOEHRINGER INGELHEIM: The VCRS is indebted once again to Boehringer Ingelheim for their continued and generous financial support of the annual VCRS symposium. The scope of their monetary contribution enables the symposium organising committee and the Society to develop a high quality program with invaluable input from international speakers of renowned reputation, whilst maintaining the delegate fees at an appropriate level to encourage and enable attendance by both senior researchers/clinicians and doctoral and post-doctoral students / clinical residents. This breadth and depth of experience within the delegate body is considered pivotal to the aims and objectives of the Society.

BRITISH ASSOCIATION FOR LUNG RESEARCH: The VCRS would like to acknowledge the BALR for their support of this meeting. In addition to their active role in promoting and disseminating information about the 2015 meeting to their membership, BALR have kindly provided travel awards to 3 delegates to facilitate their attendance at the meeting to present short communications. Furthermore, the BALR have provided an early career investigator prize (oral presentation) and a poster prize.
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<tr>
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<tr>
<td>08:15</td>
<td>Scott Pirie</td>
<td>Welcome address</td>
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<td>08:45</td>
<td>Moira Whyte</td>
<td>Repair after acute lung injury: molecular mechanisms and therapeutic opportunities</td>
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<td>09:30</td>
<td>Nik Hirani</td>
<td>Pulmonary Fibrosis in humans - an aberrant response to injury</td>
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<td>10:45</td>
<td>Kurt Williams</td>
<td>Comparative pathology of lung fibrosis</td>
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<td>11:15</td>
<td>Minna Rajamäki</td>
<td>Natural and experimental animal models: Canine pulmonary fibrosis</td>
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<td>Amy Miele</td>
<td>Natural and experimental animal models: Asinine pulmonary fibrosis</td>
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<td>Alison MacKinnon</td>
<td>Natural and experimental animal models: Murine models of pulmonary fibrosis</td>
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<td>M. Melamies</td>
<td>Treatment of Eosinophilic Bronchopneumopathy in Dogs--Efficacy of Oral Versus Inhaled Corticosteroids</td>
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<td>Mechanisms of Airway Smooth Muscle Remodeling Reversal in Heaves</td>
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<td>J. Paxson</td>
<td>Using In-Vitro Assays to Investigate the Role of Age in Lung Repair</td>
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<td>William Wallace</td>
<td>Pathology of bronchiectasis</td>
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<td>Pallavi Bedi</td>
<td>Bronchiectasis - the vicious cycle</td>
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<td>T. Lee-Fowler</td>
<td>Evaluation of HISTOGELt and GELFOAMtm Embedded Bronchoalveolar Lavage Fluid Specimens in Comparison to BALF Cytospin and Sediment Smear Preparations</td>
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<td>H. Hoskinen</td>
<td>Effects of General Anaesthesia in Dorsal Recumbency to Bronchoalveolar Lavage Cytology of Healthy Horses</td>
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<td>S. Dos Santos</td>
<td>Microbial Culture is of Limited Use in Assessing the Microbial Properties of Healthy Horses and Horses Suffering from Recurrent Airway Obstruction</td>
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Friday 9th October

09:00 Mathilde Leclere In vivo monitoring of remodelling - sequential lung biopsy in equine asthma

09:20 Carol Reinero In vivo monitoring of remodelling – computed tomography in feline asthma models

09:40 Kev Dhaliwal Real time point of care evaluation - novel approaches

10:25 DISCUSSION / OPEN MIKE

10:45 BREAK

11:15 Short communications

  R. Elodie Standardized Characterization of Thoracic High Resolution Computed Tomographic Findings in West Highland White Terriers with Canine Idiopathic Pulmonary Fibrosis: Effect of Sedation Versus Anesthesia in Healthy and Affected Dogs

  K. Lascola The Use of Quantitative CT Techniques to Characterize Pulmonary Disease in Neonatal Foals

  M. Grabman Characterization of Airway Microbiota in Healthy and Bronchitic Dogs

  E. Richard Cytokine Profiles in Bronchoalveolar Lavage Fluid from Horses with Unilateral IAD-Consistent Cytology

12:25 LUNCH

13:55 Alistair Church Pathobiology of pulmonary hypertension and vascular remodelling in humans

14:40 Kurt Williams Veno-occlusive remodelling in horses and dogs

15:20 DISCUSSION / OPEN MIKE

15:40 BREAK

16:10 Short communications

  M. Davis The Effect of Acute Exposure to High Altitude on Pulmonary Mechanical Properties and Exercise Capacity in Dogs

  S. Hansen Evaluation of Airway Inflammation in Icelandic Horses Under Different Management Systems

  L. Couetil Identification of Fungal Aerollergens Associated with Recurrent Airway Obstruction in Horses by Immunobloting

  M. Bullone Meteoropathy and Heaves: Looking for the Missing Link

17:20 FINISH

EVENING SYMPOSIUM AND AWARDS DINNER
Saturday 10th October

09:00  Paul Nicklin  *En route* from molecule to clinic – where do animal models lie?

09:30  Elspeth Hulse  Experiences with an ex-vivo ovine lung model

09:50  David Collie  Experimental ovine model of chronic infection

10:10  David Griffiths  Spontaneous ovine model of chronic lung cancer

10:30  Mathilde Leclere  Spontaneous equine model of asthma

10:50  DISCUSSION / OPEN MIKE

11:10  BREAK

11:40  Amanda Tatler  Lungs on a plate; PCLS - a clearer line of sight to the clinic

12:10  Gerry McLachlan  GM large animal models - moving on from Dolly

12:30  DISCUSSION / OPEN MIKE

12:50  FINISH
REPAIR AFTER ACUTE LUNG INJURY: MOLECULAR MECHANISMS AND THERAPEUTIC OPPORTUNITIES

Moira Whyte
**Opening Lecture:** REPAIR AFTER ACUTE LUNG INJURY: MOLECULAR MECHANISMS AND THERAPEUTIC OPPORTUNITIES

*Professor Moira Whyte OBE PhD FRCP FMedSci*

**Biography:**

Moira Whyte is Professor of Respiratory Medicine at the University of Edinburgh and Director of the MRC/University of Edinburgh Centre for Inflammation Research (www.cir.ed.ac.uk/investigator/Professor-Moira-Whyte). Moira is currently Registrar of the Academy of Medical Sciences and Chair of the MRC Clinical Training and Careers Panel. Moira trained in medicine at St Bartholomew’s Hospital Medical College, University of London and undertook postgraduate training at the Hammersmith Hospital, London, including an MRC Training Fellowship in Respiratory Cell Biology. She subsequently held a Wellcome Advanced Fellowship at the University of Nottingham and the Imperial Cancer Research Fund Laboratories, Lincoln’s Inn Fields, London. She was appointed to Sheffield in 1996 where she headed the Academic Unit of Respiratory Medicine and the Department of Infection and Immunity before moving to Edinburgh in September 2014. Her research group have interests in basic mechanisms of innate immunity and she has clinical interests in chronic obstructive pulmonary diseases (COPD) and interstitial lung diseases. She has served on a number of MRC, NIHR and Wellcome Trust grants panels.

**NOTES:**
Session 1

— LUNG FIBROSIS —

PULMONARY FIBROSIS IN HUMANS - AN ABERRANT RESPONSE TO INJURY
Nik Hirani

COMPARATIVE PATHOLOGY OF LUNG FIBROSIS
Kurt Williams

NATURAL AND EXPERIMENTAL ANIMAL MODELS: CANINE PULMONARY FIBROSIS
Minna Rajamäki

NATURAL AND EXPERIMENTAL ANIMAL MODELS: ASININE PULMONARY FIBROSIS
Amy Miele

NATURAL AND EXPERIMENTAL ANIMAL MODELS: MURINE MODELS OF PULMONARY FIBROSIS
Alison MacKinnon
PULMONARY FIBROSIS IN HUMANS - AN ABERRANT RESPONSE TO INJURY

Nik Hirani

Biography:
Nik Hirani qualified from Nottingham University and continued his respiratory training in Nottingham and the South West. He took up a Wellcome Trust Clinical Training Fellowship in Edinburgh in 1996 and then a GlaxoSmithKline clinician scientist award before his current post as senior clinical lecturer and honorary consultant at the Edinburgh Royal Infirmary and PI in the University of Edinburgh MRC Centre for Inflammation Research. He is clinical director of Respiratory Medicine in Lothian and he leads the Edinburgh Interstitial Lung Disease service and founded the Scottish Interstitial Lung Disease (ScILD) network. He is past chair of the British Thoracic Society and National Institute for Clinical Excellence lung fibrosis guideline groups. He has research interests in the natural history of ILDs, early-phase translational studies in lung fibrosis and in macrophages biology.

Executive summary:
Some 200 disease entities make up the the interstitial lung diseases in man, but the actual repertoire of pathological sequelae following lung injury are limited to just a few ‘patterns’ of disease. These histological patterns of disease have radiological and clinical correlates and are associated with complete resolution, resolution with scarring or progressive ‘irreversible’ fibrosis. The latter is a characteristic feature of ‘usual interstitial pneumonia’ (UIP), which is the most common form of lung fibrosis and is associated with the poorest outcome. Unfortunately there are few, if any pre-clinical models that accurately mimic any of the human interstitial lung diseases, and this is particularly true for UIP. After 2 decades of ‘negative’ clinical trials, two drugs have recently been shown to be effective in the treatment of UIP, and this has ignited interest in ‘fast-tracking’ other potential therapies from the bench into man. The role of animal models of lung fibrosis in this new era is evolving.
COMPARATIVE PATHOLOGY OF LUNG FIBROSIS

Kurt Williams

Biography:
Kurt Williams received his DVM from Michigan State University, advanced training in pathology at Cornell University and a PhD at UC Davis. He is currently an Associate Professor in the Department of Pathobiology at MSU. Dr. Williams is the author of numerous peer-reviewed papers and book chapters pertaining to respiratory diseases of domestic animals. His research and teaching interests are centered on respiratory diseases in domestic animals, and he has a particular interest in comparative lung biology and disease and the integration of spontaneous diseases of domestic animals as ‘models’ of respiratory diseases of humans. He was the first to identify and describe spontaneous idiopathic pulmonary fibrosis in cats and canine pulmonary veno-occlusive disease. He identified and described equine multinodular pulmonary fibrosis (EMPF) and established the relationship between pulmonary infection with EHV 5 and the development of EMPF. Most recently, through his research interests in equine exercise-induced pulmonary hemorrhage (EIPH), he has identified regional pulmonary venous remodeling as critical to the pathogenesis of EIPH.

NOTES:
NATURAL AND EXPERIMENTAL ANIMAL MODELS: CANINE PULMONARY FIBROSIS

Minna Rajamäki

Biography:
Minna Rajamäki is currently a researcher and internist at the University of Helsinki, Faculty of Veterinary Medicine. She graduated from the University of Helsinki, Finland in 1991 and joined soon the department of Equine and Small Animal Medicine, where she is now an Adjunct Professor. She defended her PhD thesis describing inflammation and role of matrix metalloproteinases in canine eosinophilic bronchopneumonopathy 2005. Her main interests include upper and lower respiratory tract diseases in dogs, with special emphasis on idiopathic pulmonary fibrosis, eosinophilic bronchopneumopathy and brachycephalic syndrome. She is the author of several scientific publications. She is the head of a canine lung research group supervising several PhD students on various subjects.

Executive summary:
Minna M. Rajamäki DVM, PhD and Henna P. Laurila DVM
Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease of unknown etiology (Raghu et al., 2011). The first case series of canine IPF (CIPF) in West Highland white terriers (WHWTs) was published in the late 1990s (Corcoran et al. 1999). Reports of CIPF in other dog breeds were described around the same time by Lobetti et al. (2001) and Corcoran et al. (1999). The prevalence and incidence of (CIPF) are currently unknown and can be difficult to estimate.

The etiologies of CIPF and IPF are unknown. The etiological factors are difficult to trace as they may damage the lung for several years before CIPF is eventually diagnosed. It is likely that IPF is the end result of a complicated dialogue between genetic and environmental factors. Involvement of genetic factors is also suspected in dogs because of the strong predisposition of the WHWT breed and studies are currently being conducted. Previously, a genetic study of CIPF by Eriksson et al (2009) analyzed SP B and C and found that SP C was absent in one dog. In this dog, a mutation was detected at SFTPC exon 5. Krafft et al. (2013) investigated the pulmonary gene expression from lung samples of dogs with CIPF and healthy control dogs by
microarray analysis. A quantitative reverse transcriptase PCR analysis confirmed the change of expression for genes coding chemokine (C-C) ligand (CCL) 2, CCL7, chemokine (C-X-C) ligand 8 (CXCL8), CXCL14, fibroblast activation protein and the palate, lung and nasal associated protein (PLUNC).

CIPF usually affects middle-aged to older WHWTs. Human IPF more often affects males, however, no sex predisposition has been reported in dogs. The usual age at the time of diagnosis varies from eight to fifteen years, but younger WHWTs with CIPF have also been reported (Lilja-Maula et al. 2014). Some of the non-WHWTs with CIPF have been substantially younger (Lobetti et al. 2001). In humans, IPF typically manifests in the sixth and seventh decades (Raghu et al. 2011). CIPF is considered an inevitably progressive disease. The mean duration of clinical signs when presented to the veterinarian has been estimated to be 8-13 months with a great individual variation (Lilja-Maula et al. 2014). Dogs with CIPF are usually bright and alert due to adaptation to slowly developing respiratory impairment. The most typical clinical signs are exercise intolerance and chronic cough. Eventually CIPF can cause respiratory difficulty, cyanosis and respiratory failure (Heikkilä et al. 2011). 6-minute walk test, a common test measuring submaximal exercise test in humans, has been studied in dogs showing that WHWTs with CIPF walked a shorter distance, than healthy controls (Lilja-Maula et al. 2014).

CIPF has a negative impact on life expectancy, but individual survival varies considerably from some months to some years; median survival time has been reported as 32 months from the beginning of clinical signs (Lilja-Maula et al 2014). Most human patients with IPF die within 5 years of diagnosis. However, several different progression patterns are recognized both in humans and WHWTs. In some patients, progression is slow whereas in others, stable phases are interrupted by acute exacerbations (Lilja-Maula et al. 2014, Selman et al. 2007).

The diagnosis of CIPF is based on anamnestic information, findings in clinical examinations and diagnostic imaging, and exclusion of other respiratory diseases since histopathological examination of lung is rarely possible. Bilateral, inspiratory Velcro crackles are a characteristic finding on lung auscultation both in dogs and humans (Heikkilä et al.2011). Arterial blood gas analysis is an objective estimate of lung function. Hypoxemia is a common finding in dogs with CIPF. Heikkilä et al (2011) reported that 90% of the WHWTs with CIPF were hypoxemic (PaO₂ less than 80 mmHg, and 45 % had severe hypoxemia (PaO₂ less than 60 mmHg).
Thoracic radiographs of dogs with CIPF commonly show a bronchointerstitial pattern (Johnson et al. 2005). Pulmonary hypertension (PHT) develops in a large number of WHWTs with CIPF. Schober et al. (2006) estimated that PHT was a frequent finding affecting more than 40 % of the WHWTs in their study. Similarly, PHT is very common in humans with IPF and is related to increased mortality (Smith et al. 2013). In human IPF, HRCT plays a crucial role in the diagnostic decision making, and a HRCT diagnosis of IPF has a very high positive predictive value. The scanning can be done under general anesthesia, sedation or sometimes in wake animals. The HRCT findings described are ground glass opacity, parenchymal bands, subpleural lines, subpleural interstitial thickening, peribronchovascular interstitial thickening, the interface sign, traction bronchiectasis and honeycombing (Heikkilä et al. 2011). Consolidation can also occur, and in some dogs the bronchial walls can appear thickened. The distribution of the lesions can be patchy and the predilection site is reported to be the dorsocaudal lung lobes. In human IPF, honeycombing and traction bronchiectasis appear to be more common than in CIPF.

Bronchoscopic findings detected in dogs with CIPF are nonspecific. Many dogs with CIPF seem to have some degree of bronchial involvement. It is not known whether this is an individual phenomenon or related to underlying CIPF. The bronchoscopic changes reported in dogs with CIPF are tracheal collapse, bronchial mucosal irregularity, increased amount of bronchial mucus, bronchomalacia, dynamic airway collapse, and bronchiectasis (Heikkilä et al. 2011). Tracheal collapse and the increase in bronchial mucus are usually graded as mild to moderate. In human IPF, bronchoscopy is not commonly (Collard et al. 2007).

In CIPF, the BAL fluid (BALF) analysis shows usually an increase in the total cell count due to increased numbers of macrophages, neutrophils, and mast cells. In the differential cell counts, only a lower lymphocyte percentage was detected in dogs with CIPF when compared to healthy dogs. The usual BAL cell pattern in IPF is defined by increased macrophages and neutrophils, and also mild to moderate eosinophilia can be present. The lack of prominent lymphocytosis supports the IPF diagnosis (Meyer et al. 2012).

At the moment, there is no effective treatment for CIPF. Treatment is mainly used to reduce clinical signs on an individual basis and secondly to alleviate possible complications. No clinical treatment trials have been performed on dogs with CIPF and only anecdotal evidence exists for an effect of any drug. At the moment, there is no treatment that can reverse the chronic fibrotic changes of human IPF. Pirfenidone and nintedanib are approved drugs for the
treatment of human IPF. The pharmacokinetics of pirfenidone has been studied in dogs (Bruss et al. 2004) but there are no published reports of its clinical use in dogs.

Corticosteroids can be used in the treatment of NSIP. Based on the potential benefit of corticosteroids in human NSIP, and also on the fact that many dogs have concurrent bronchial changes, oral corticosteroids might have a role in the treatment of CIPF. Corcoran et al. (1999) reported previously that some dogs with CIPF appear to respond to corticosteroid treatment. Based also on the authors’ experience, corticosteroids seem to alleviate cough in many dogs although no clear effect on arterial oxygenation is detected.

Treatment with proton pump inhibitors or histamine-2 receptor blockers could be considered. This is important especially if corticosteroid therapy is started, because hypoxemia can make the dog more prone to the gastrointestinal adverse effects. In human patients with IPF, gastroesophageal reflux, either symptomatic or occult, is very common, and microaspiration is speculated to have a role in the pathogenesis of IPF (Gribbin et al 2009).

In CIPF, search for suitable biomarkers is ongoing and some potential markers have already been found. Procollagen type III amino terminal propeptide (PIINP) is a marker of fibroblast activity and enhanced collagen turnover. PIINP levels are elevated in humans with IPF. In dogs with CIPF, PIINP levels are elevated in BALF and can be used to differentiate CIPF from CB with reasonable accuracy, however, serum PIINP concentrations cannot distinguish between different chronic lung diseases (Heikkilä et al. 2013). Endothelin-1 (ET-1), a vasoactive, pro-inflammatory and pro-fibrotic peptide is elevated in humans with IPF, both in serum and BALF. Serum ET-1 is also significantly elevated in dogs with CIPF when compared to dogs with eosinophilic bronchopneumopathy, healthy dogs, or dogs with CB (Krafft et al. 2011).

Proteomics has also been used to examine a large scale of expressed proteins in BALF in order to find those specific for CIPF. The comparison of BALF proteomes between healthy dogs and dogs with CIPF or CB revealed no CIPF specific proteins. We have demonstrated previously that CIPF dogs’ BALF showed enhanced gelatinolytic and caseinolytic activity in zymography. Matrix metalloproteinase -2, -7 and -9 activities were higher than in dogs with CB (unpublished data).

Roels et al. (2014) showed that high CXCL8 blood concentrations and possibly CCL2 concentrations might be related to the breed predisposition of the WHWT for CIPF. Krafft et al. (2014) reported that the serum concentration of TGFβ-1 was elevated in WHWTs with CIPF. The concentration was also higher in healthy WHWTs when compared with healthy dogs of
all other investigated breeds except the Scottish terrier. Activin B, a cytokine of the TGF-β family, was detected in BALF of WHWTs with CIPF suffering from acute exacerbation of the disease (Lilja-Maula et al., 2014a). Because CXCL8 and TGFβ-1 are elevated in both healthy and sick WHWTs but not in dogs of several other breeds, these markers could also be related to the breed predisposition of WHWTs to CIPF (Roels et al., 2015).

REFERENCES
List of other references is provided on request
NATURAL AND EXPERIMENTAL ANIMAL MODELS: ASININE PULMONARY FIBROSIS

Amy Miele

Biography:
Amy graduated from the Royal (Dick) School of Veterinary Studies in 2006 and spent 5 years in mixed and small animal practice. She returned to The University of Edinburgh in 2011 and has recently been awarded her PhD titled ‘Comparative Pulmonary Fibrosis: imaging fibroproliferation in donkey and man’.

Executive summary:

Amy Miele, Kev Dhaliwal, Nicole Du Toit, John T Murchison, Catharine Dhaliwal, Harriet Brooks, Sionagh H Smith, Nik Hirani, Tobias Schwarz, Chris Haslett, William A Wallace and Bruce McGorum

Pulmonary fibrosis is a chronic and debilitating condition that proposes several challenges to both veterinary and medical clinicians. Despite considerable research, many fibrotic lung diseases remain elusive in terms of aetiology, pathogenesis and treatment. Furthermore, progress is hindered by the lack of a translatable animal model with durable and persistent fibrosis. Asinine Pulmonary Fibrosis (APF) is a spontaneous syndrome of aged donkeys with high prevalence (35%). No previous detailed characterisation of APF has been performed and disease diagnosis remains a challenge.

APF was studied with regard to clinical, pathological and molecular features and the suitability of this condition as a model for a rare fibrotic lung disease in humans known as pleuroparenchymal fibroelastosis (PPFE) was assessed. Whole lungs were collected from 32 aged donkeys at routine necropsy. Gross examination revealed pulmonary fibrosis in 19 donkeys (APF cases), while 13 (controls) had grossly normal lungs. HRCT images and histology sections were reviewed independently and blindly for each of the lungs. Ten of 19 APF lungs were categorised as being ‘consistent with’ PPFE according to previously defined histological and imaging criteria. All 10 PPFE-like lungs had marked pleural and subpleural fibrosis, predominantly within the upper lung zone, with accompanying intra-alveolar fibrosis and elastosis. In conclusion, APF is a chronic untreatable condition of aged donkeys that shares
key pathological and imaging features with PPFE, further study of APF may yield valuable information to help elucidate the aetiopathogenesis of this emerging human disease.

(Miele et al, *Chest.* 2014; 145:1325-1332)

**NOTES:**
NATURAL AND EXPERIMENTAL ANIMAL MODELS: MURINE MODELS OF PULMONARY FIBROSIS

Alison MacKinnon

**Biography:** My work focusses on the regulation of chronic inflammation in the lung and other organs by galectin-3 and modulation of disease with genetic deletion models and small molecular inhibitors of galectin-3.

**NOTES:**
Session 2
– BRONCHIECTASIS –

PATHOLOGY OF BRONCHIECTASIS

William Wallace

BRONCHIECTASIS - THE VICIOUS CYCLE

Pallavi Bedi
PATHOLOGY OF BRONCHIECTASIS

*William Wallace*

**Biography:**
Dr Wallace graduated from Edinburgh University BSc(Hons) in 1984 and MBChB (Hons) in 1986. After obtaining the MRCP in 1989 he began training as a pathologist in Edinburgh and developed an interest on respiratory and thoracic pathology. In 1995 he obtained a PhD following a study identifying auto-antibodies to a lung epithelial antigen in patients with ‘cryptogenic fibrosing alveolitis’ now referred to as idiopathic pulmonary fibrosis. He obtained his MRCPath in 1997 and was appointed Consultant Pathologist at the Northern General Hospital, Sheffield in 1998. In 2001 he returned to Edinburgh as lead consultant for respiratory and thoracic pathology at the Royal Infirmary of Edinburgh. He has authored, or co-authored, over 100 scientific and clinical articles on a wide variety of respiratory / thoracic topics including a number of collaborative studies with colleagues in veterinary medicine and veterinary pathology at the Roslin Institute and Royal (Dick) School of Veterinary Medicine. In addition he has contributed chapters on lung pathology to major international textbooks and has been a member of international guideline development groups for SIGN and the BritishThoracic Society. He has been an invited speaker at a range of large international pathology meetings including the International Academy of Pathology, the European Pathology Congress, US Pulmonary Pathology Society and in November 2015 will be a guest speaker at the Japanese Pathology Society in Tokyo. In 2010 Dr Wallace was appointed Honorary Reader in Pathology at the University of Edinburgh.

**Executive summary:**
Bronchiectasis was first described in the early 1800s when it was identified in patients with tuberculosis. Despite the much lower prevalence of TB bronchiectasis remains a relatively common condition. Pathologically this is characterised by inflammation and damage to bronchi and bronchioles which results in fibrosis and dilatation. While TB remains a recognised cause there is a very wide range of other aetiologies which can result in either localised or widespread bronchiectatic changes. Thus bronchiectasis can be localised to a segment or lobe of lung, as may be seen with bronchial obstruction from a foreign body, or may be generalised affecting both lungs in a diffuse manner as in cystic fibrosis. Dilatation of airways and development of bronchiectasis can also be seen as a consequence of fibrosis of the alveolar...
lung parenchyma following, for example, following radiotherapy or in some cases of idiopathic parenchymal lung disease such as UIP. In this context collagen laid down contracts as it matures and this pulls and dilates the airways (traction bronchiectasis). Airway damage results in disturbance of the normal physiological functions involved in the clearances of secretions and host defence from infection which may further exacerbate the condition. As well as direct damage to the airways patients with bronchiectasis often develop secondary changes in the lung parenchyma distal to the bronchiectatic airways with variable inflammation and fibrosis. This may lead to respiratory failure, pulmonary hypertension, cor pulmonale and death of the patient. Rarely serious systemic complications may also arise in some patients with the development of amyloidosis and metastatic abscess formation.
BRONCHIECTASIS - THE VICIOUS CYCLE

Pallavi Bedi

Biography:
Dr Bedi is a respiratory trainee in Edinburgh. She has taken time out of clinical medicine and she initially did an MD where she investigated the role of Atorvastatin as an anti inflammatory agent in bronchiectasis. She is currently in the final year of her PhD, where she is investigating the role of lipid mediators in bronchiectasis. After completing her PhD, she will complete speciality training to obtain a CCT in respiratory medicine. She would like to apply for an intermediate fellowship to pursue translational research in bronchiectasis in the future.

Executive summary:
She will be talking about the pathogenesis of bronchiectasis and in particular about the vicious circle in the airways. The aim of treatment in this ‘orphan’ disease is to break this vicious circle from perpetuating. The current mainstay of treatment in bronchiectasis is long-term antibiotics and chest physiotherapy. However over the last few years there has been a drive towards novel non-antibiotic therapy in bronchiectasis. Several trials have been done with macrolides and they have been shown to increase the time to next exacerbation. Statins have also been recently trialed in bronchiectasis. She will summarize recent trials and advances in the area and conclude by talking about future perspectives.

NOTES:
Session 3

– DEFINING DISEASE PROGRESSION –

IN VIVO MONITORING OF REMODELLING - SEQUENTIAL LUNG BIOPSY IN EQUINE ASTHMA
Mathilde Leclère

IN VIVO MONITORING OF REMODELLING – COMPUTED TOMOGRAPHY IN FELINE ASTHMA MODELS
Carol Reinero

REAL TIME POINT OF CARE EVALUATION - NOVEL APPROACHES
Kev Dhaliwal
IN VIVO MONITORING OF REMODELLING - SEQUENTIAL LUNG BIOPSY IN EQUINE ASTHMA

Mathilde Leclère

Biography:
Mathilde Leclere obtained her DVM degree from University of Montreal in 2001. She completed an internship in equine medicine Montreal and a residency in large animal internal medicine at UC Davis. She is an ACVIM diplomate since 2005. She pursued a PhD in veterinary sciences at the University of Montreal and McGill University under the supervision of Dr Jean-Pierre Lavoie, while working as a part time clinician in equine medicine. She is an assistant professor at the University of Montreal since 2012. While she is now working on the lung microbiome in health and allergic airway disease, her PhD was mostly focused on airway remodeling in equine asthma.

Executive summary:

Equine heaves (also called RAO) is a common chronic disease affecting mature horses in temperate climates. Affected animals present recurrent episodes of severe breathing difficulties leading to early retirement, poor quality of life, and often euthanasia. The term Inflammatory Airway Disease (IAD) is used to describe a similar, but mild to moderate airway disease that will progress to heaves in a small subset of IAD-affected horses. Currently there are no means to identify horses with IAD that will eventually develop heaves, and no cure for this debilitating disease. While treatment strategies (antigen avoidance, corticosteroids and bronchodilators) aim at improving lung function and inducing clinical remission, it is believed that progression of the disease is at least in part due to structural changes in the airway wall (airway remodelling). We previously showed that horses with heaves have evidence of marked peripheral airway remodelling (1-3) that is associated with altered lung function. We therefore initiated a research program to investigate the possible reversibility of airway remodelling.

Peripheral airways
Large peripheral lung biopsies are required in order to prospectively study the remodelling affecting the airways of asthmatic horses. Transthoracic lung biopsies generally do not provide enough cross-sectional airways to allow histomorphometric analysis. Furthermore, additional
tissues are required in order to investigate the molecular changes associated with remodelling for mechanistic studies. Large peripheral lung biopsies can be obtained under thoracoscopic guidance with different techniques:

- **Endoscopic linear cutter-stapler**: Lugo et al. first reported that thoracoscopically guided pulmonary wedge resection in horses is well tolerated by standing sedated healthy horses and horses with heaves (4). The technique was described with a 45-mm endoscopic linear cutter-stapler and provides biopsies of sufficient size to perform histology. Although very effective, it may be cost-prohibitive for prospective studies in research settings.

- **Pre-tied ligating loop**: Our group showed that pre-tied ligating loops were an inexpensive alternative to staples, and could be used in asymptomatic heaves-affected horses as well as in horses in exacerbation (5). Biopsies were of adequate size for histology but tended to be smaller than with the endoscopic linear cutter-stapler technique. Also, in one study, for 31% (9/29) of the biopsies, additional procedures were necessary to secure the biopsy site (additional loop, linear stapler or cauterization).

- **Bipolar tissue sealing system**: In the first study performed with the tissue sealing system, it was considered a rapid and effective alternative to the cutter-stapler technique and the ligating loops (6). Thermal damage was limited to less than 4 mm and few complications occurred. However, in a follow-up study, the complication rate we observed was unacceptably high, and this method cannot be recommended at present time. It appears that between the two studies, the manufacturing of these instruments had changed after the company distributing these instruments was sold.

Of note, while bilateral pneumothorax often develops during thoracoscopy, the procedure is well tolerated even in horses with heaves in severe exacerbation. Minimal complications were observed when adequate seal of the biopsy site was obtained.

From these studies we found that current therapy (corticosteroids, or decreased antigen exposure associated with improved feeding and barn conditions), even after one year, only partially reversed the airway smooth muscle (ASM) remodelling affecting the peripheral airways of these animals (7). A 30% decrease in ASM was observed after 6 months of therapy with inhaled corticosteroids, and did not further improve with an additional 6 months of treatment. With antigen avoidance strategy, a similar decrease was only observed after 1 year. Furthermore, and with either treatment, there was still approximately twice as much
ASM as observed in the airways of control horses. Surprisingly, the deposition of collagen in the lamina propria improved to a greater extent with these treatments. We recently also observed that a 3-month treatment regimen with inhaled corticosteroids, alone or combined with a bronchodilator, led to a similar degree of improvement in remodelling affecting both the ASM mass and the extracellular matrix (Bullone, unpublished data). The changes were not observed at 1 month. Taken together, these results suggest that part of the remodelling process may not be reversible, at least not with inhaled corticosteroids or antigen avoidance strategies.

Central airways
Until recently, study of central airway remodelling was limited to post-mortem specimens (1) and very little was known of the dynamic of the changes affecting the central airways in heaves due to the lack of available tools. We therefore investigated different means to study these airways for prospective studies.

- **Endobronchial biopsies** are easily collected in the standing sedated horses during bronchoscopy (3). While it allows to adequately sample the epithelium and the lamina propria, it harvest incompletely the ASM layer, and therefore is of limited use for the study of the reversibility of ASM remodelling in heaves (8). However, endobronchial biopsies contain enough tissue to allow for myocytes marking by immunohistochemistry or other techniques, or for gene expression studies. Using a proliferation marker (PCNA), we found that the percentage of proliferating myocytes was increased in the large airways of heaves-affected horses in exacerbation compared with healthy horses (3). We also found that the fast contracting myosin isoform (+(+) insert) was significantly increased in central and peripheral airways of heaves-affected horses in clinical exacerbation when compared horses with horses in remission and controls. Both corticosteroids administration and antigen avoidance led to a significant reduction of the (+) insert expression in the airways, to or below expression levels in healthy horses, indicating that this remodelling feature is completely reversible by corticosteroids administration and antigen avoidance (9).

We recently developed a scoring system for endobronchial biopsies based on features of inflammation and remodelling, which allows differentiation of horses with heaves in exacerbation of the disease from controls, or horses in remission (Bullone, unpublished data). This may prove helpful also in a clinical setting to monitor the response to therapy.
- **Endobronchial ultrasonography (EBUS)** is performed by inserting a special ultrasound probe within the biopsy channel of a video-endoscope, which is advanced into large bronchi. We recently reported that EBUS can be performed in standing sedated horses, and therefore allows the study of the temporal remodelling changes occurring the central airways in heaves with a minimally invasive technique (10). Using this technique, we found a decrease of approximately 30% of the layer corresponding to ASM (and to a lesser degree to the extracellular matrix), in treated horses, which was similar to what was observed in the peripheral airways of the same horses (Bullone unpublished data).

- **Narrow Band Imaging endoscopy: NBI** is a non-invasive technique that enhances the visualization of submucosal vessels, commonly employed for the study of angiogenesis. While we found no difference between groups for the volume density of both superficial and deep vessels at the carina or intermediate bronchi, in the trachea, the volume density of superficial vessels was increased in horses with heaves compared to controls (Herteman, in revision, JVIM). NBI imaging of the airways was easily performed in standing sedated horses. The significance of the increased tracheal vascularity in heaves remains to be ascertained.

We now have many tools to investigate the remodelling affecting both the central and the peripheral airways of horses. Due to the invasive nature of the sampling protocol, the study of the peripheral airways will likely be limited to research settings at the moment, but both endobronchial biopsies and EBUS could be performed in clinical settings, with minimal invasiveness and great potential for long term monitoring.

**References**


NOTES:
IN VIVO MONITORING OF REMODELLING – COMPUTED TOMOGRAPHY IN FELINE ASTHMA MODELS

Carol Reinero

Biography:
Carol is an Associate Professor (Comparative Medicine Emphasis) and Director of the Comparative Internal Medicine Laboratory, Department of Veterinary Medicine & Surgery, University of Missouri, Columbia MO. Her basic science research activities have centered on allergic asthma research, with an emphasis on immunomodulation, clinical drug trials, and mechanisms of mucosal tolerance using a feline model of chronic asthma. Her laboratory is set up to evaluate the hallmark features of allergic asthma using a variety of immunologic assays (on blood, bronchoalveolar lavage fluid and exhaled breath condensate), ventilator-acquired pulmonary mechanics (using bronchoprovocation) as a standalone test or in combination with computed tomography. A newer area of research is the respiratory microbiome in health and disease.

Executive summary:
The hallmark features of allergic asthma include airway eosinophilia, airway hyperresponsiveness and airway remodeling. To study spontaneous asthma in pet cats and in humans, a feline model which replicates the major disease features has been used. Serial monitoring of airway eosinophilia and airway hyperresponsiveness is possible without sacrifice using bronchoalveolar lavage and ventilator-acquired pulmonary mechanics; however, monitoring progressive remodeling via repeated histopathologic evaluation is problematic. Computed tomography (CT) has the advantage of being a non-invasive diagnostic modality which captures global architectural changes in the lung. In an experimental feline asthma model using general anesthesia and inspiratory breath holds, progressive increases in attenuation with regions of ground glass opacity, consolidation and parenchymal bands were noted in comparison to control cats up to 1 year of age. Additionally, increases in bronchial wall thickness were noted only in experimentally asthmatic cats. Histopathology in these cats at the 1 year time point showed lesions affecting both the proximal and distal airways including epithelial cell hyperplasia, smooth muscle hypertrophy and hyperplasia, submucosal gland hyperplasia and peribronchiolar inflammation. In a subsequent study, delayed remodeling could be documented with therapeutic intervention
using adipose derived mesenchymal stem cells compared to untreated controls. While general anesthesia is preferred for the highest quality scans, use of a positioning/restraining device in awake cats led to satisfactory quality scans. Comparison of experimental research and spontaneous pet asthmatic cats showed increased attenuation compared with healthy research cats. Collectively these results suggest CT is a powerful, non-invasive tool that can be used to objectively measure changes in airway remodeling in asthmatic cats.

NOTES:
REAL TIME POINT OF CARE EVALUATION - NOVEL APPROACHES

Kev Dhaliwal

Biography:
Dr Kev Dhaliwal graduated from the University of Edinburgh and completed general professional training in London before returning to Edinburgh to undertake a period of consolidated laboratory research in the Lung Inflammation Group. During his PhD he developed close links with the Department of Chemistry (Prof Mark Bradley) and along with Prof Chris Haslett, the group initiated a strategic partnership to develop and translate optical molecular imaging in pulmonary disease. Dr Dhaliwal completed specialist training in Respiratory Medicine and was employed as a Senior Clinical Lecturer in Pulmonary Molecular Imaging in 2013. He leads a translational group working across multiple disciplines with an ethos of 'molecule to man '. In 2014, he cofounded Edinburgh Molecular Imaging (a UoE spinout) that is now progressing the clinical and commercial development of optical molecular imaging. Dr Dhaliwal leads the interdisciplinary hub of the Proteus Project (www.proteus.ac.uk) and is performing translational studies in collaboration with the Intensive Care Unit at the RIE. Additionally he has helped to establish an international network of sites to implement clinical trials. He is passionate about developing interdisciplinary research and training the next generation of optical imagers with a one health approach to develop solutions that are applicable across species.

Executive summary:
The talk will outline the clinical and technical challenges in developing and initiating clinical trials for pulmonary endomicroscopy. The talk will describe the physical sciences and biological hurdles and how these are being addressed through the Proteus Project. The ultimate aim is to develop novel point-of-care tools that will be widely applicable across species to understand pulmonary pathobiology.
Session 4

– VASCULAR REMODELLING –

PATHOBIOLOGY OF PULMONARY HYPERTENSION AND VASCULAR REMODELLING IN HUMANS
Alistair C. Church

VENO-OCLUSION REMODELLING IN HORSES AND DOGS
Kurt Williams
PATHOBIOLGY OF PULMONARY HYPERTENSION AND VASCULAR REMODELLING IN HUMANS

Alistair C. Church

Biography:
The Scottish Pulmonary Vascular Unit (SPVU) is the national centre for the treatment of all patients in Scotland with the condition of pulmonary hypertension. This is a rare condition with a terminal prognosis but is important to recognize as early diagnosis and treatment can make a significant improvement in patients mortality. It has grown from 1 consultant and 1 nurse to now 3 consultants, 3 nurses, 3 clinical fellows and a clinical research nurse.

The unit was set up under the leadership of Professor Andrew Peacock, and with the two other consultants, Dr Martin Johnson and Dr Colin Church, the unit has an international reputation for first class clinical care and both clinical and basic science research. The unit has published research in the areas of clinical imaging, clinical physiology and the understanding of the molecular pathways in pulmonary hypertension. Dr Church is involved in the basic science laboratory and has an interest in the role of inflammation in the development of pulmonary hypertension. He trained in Glasgow, Sydney, Cambridge and Papworth before becoming a consultant in pulmonary vascular medicine in Glasgow in 2013. He has published in the biology of p38MAPK and the role of inflammation in pulmonary hypertension. He is lead investigator on a number of clinical trials in pulmonary hypertension including one looking at the use of an anti-IL-6 antibody.

Executive summary:
Pulmonary hypertension is a devastating illness which leads to progressive narrowing of the pulmonary circulation, right ventricular failure and eventual death. The processes leading to the circulatory narrowing has been termed pulmonary vascular remodeling and in the last decade or so there has been profound discoveries that have led to a better understanding of the biology of the condition.
This talk will describe the pathology of pulmonary vascular remodeling and explore the pathways that have been implicated and indeed have led to the development of successful therapies for patients. In addition novel molecular and genetic pathways that have more recently been identified as being important will be discussed.

The basic abnormal principles underlying the development of pulmonary hypertension are felt to be vasoconstriction, pulmonary vascular remodelling, loss of pulmonary vessels (vascular pruning) and pulmonary vascular thrombosis. These all occur to varying degrees depending on the underlying aetiology of the pulmonary hypertension but the hallmarks of all forms of PH are recognised as sustained vasoconstriction and vascular remodelling.

This pathobiological process is characterised by pulmonary vascular cellular proliferation, cellular hypertrophy, cellular migration, increased extracellular matrix deposition and inflammation. One of the key features is the appearance of smooth muscle containing cells within the small arterioles in the vasculature, so called distal muscularisation. These features can occur throughout the vessel wall and result in progressive narrowing of the lumen with increased resistance to flow. In addition abnormal endothelial proliferation and function are felt to be responsible for one of the pathognomonic features of PAH pathology, the plexiform lesion. This is a complex vascular structure characterised by endothelial and myofibroblast lined vascular channels, which can lead to obstruction of the vascular lumen.

The cellular changes associated with pulmonary vascular remodelling involve more than just the cells intrinsically linked to the pulmonary vessel. Indeed new exciting research has demonstrated the involvement of circulating fibrocytes and endothelial progenitor cells in this process and suggested an increasing role for inflammatory cells in the initiation and propagation.

Key molecular pathways involved include Nitric oxide, endothelin and prostacyclin. These topics will be discussed in this talk.
VENO-OCCCLUSIVE REMODELLING IN HORSES AND DOGS

*Kurt Williams*

**Biography:**
Kurt Williams received his DVM from Michigan State University, advanced training in pathology at Cornell University and a PhD at UC Davis. He is currently an Associate Professor in the Department of Pathobiology at MSU. Dr. Williams is the author of numerous peer-reviewed papers and book chapters pertaining to respiratory diseases of domestic animals. His research and teaching interests are centered on respiratory diseases in domestic animals, and he has a particular interest in comparative lung biology and disease and the integration of spontaneous diseases of domestic animals as ‘models’ of respiratory diseases of humans. He was the first to identify and describe spontaneous idiopathic pulmonary fibrosis in cats and canine pulmonary veno-occlusive disease. He identified and described equine multinodular pulmonary fibrosis (EMPF) and established the relationship between pulmonary infection with EHV 5 and the development of EMPF. Most recently, through his research interests in equine exercise-induced pulmonary hemorrhage (EIPH), he has identified regional pulmonary venous remodeling as critical to the pathogenesis of EIPH.

**NOTES:**
Session 5

– ANIMAL MODELS AND ALTERNATIVES –

EN ROUTE FROM MOLECULE TO CLINIC – WHERE DO ANIMAL MODELS LIE?
Paul Nicklin

EXPERIENCES WITH AN EX-VIVO OVINE LUNG MODEL
Elspeth Hulse

EXPERIMENTAL OVINE MODEL OF CHRONIC INFECTION
David Collie

SPONTANEOUS OVINE MODEL OF CHRONIC LUNG CANCER
David Griffiths

SPONTANEOUS EQUINE MODEL OF ASTHMA
Mathilde Leclère

LUNGS ON A PLATE; PCLS - A CLEARER LINE OF SIGHT TO THE CLINIC
Amanda Tatler

GM LARGE ANIMAL MODELS - MOVING ON FROM DOLLY
Gerry McLachlan
EN ROUTE FROM MOLECULE TO CLINIC – WHERE DO ANIMAL MODELS LIE?

Paul Nicklin

Biography:
Paul Nicklin is a qualified Pharmacist with a PhD in transepithelial transport mechanisms. He has 25 years of drug discovery experience in the Pharmaceutical Industry and is a visiting Professor at the University of Hertfordshire. He has a focus and track record in the identification of new treatments for the unmet medical needs in asthma, COPD, cystic fibrosis, pulmonary fibrosis and pulmonary arterial hypertension. He currently directs the IPF Research Program at Boehringer Ingelheim and also leads the Exploratory Research team for Respiratory Diseases. Developing and implementing open innovation models for translational drug discovery with academic partners is an area of high interest.

Executive summary:
The utility and importance of animal models for enabling drug discovery in respiratory diseases will be reviewed. Specifically, the multi-facetted use of in vivo studies to identify new targets, target engagement readouts, disease biomarkers and to translate new therapies for lung fibrosis will be exemplified. Finally, the limitations of animal models for discovering the next-generation of IPF treatments will be discussed.
EXPERIENCES WITH AN EX-VIVO OVINE LUNG MODEL

Elspeth Hulse

Biography:
Elspeth studied medicine at the University of Aberdeen (1997-2002) before undertaking her Royal Navy general duties on board HMS LIVERPOOL in the Caribbean, HMS YORK in the Baltics and Mediterranean and in Iraq on Operation TELIC as an emergency medicine trainee.
She gained a diploma in medical toxicology from Cardiff University (2010-2011) whilst working as a junior anaesthetist at MDHU Derriford, Plymouth.

She is a currently senior anaesthetic registrar working at the Royal Infirmary of Edinburgh and is undertaking a PhD in toxicology at the Centre for Cardiovascular sciences at the University of Edinburgh.

Her research interests include acute lung injury, blast lung injury, organophosphorus (OP) pesticide poisoning and the understanding and treatment of pulmonary aspiration secondary to OP poisoning. In her work she has developed, an in-vivo minipig model using a high fidelity intensive care facility for 48 hour studies, and an ovine ex-vivo lung perfusion (EVLP) model. She has also conducted human studies in intensive care facilities in Peradeniya, Sri Lanka.

She currently holds tenure as a military lecturer in anaesthesia and toxicology as part of the Academic Department of Military Anaesthetics and Critical Care (ADMACC).

Executive summary:
Suicide through ingestion of organophosphorus (OP) pesticide is a huge problem in rural Asia with an estimated 250,000 deaths every year. OP pesticides cause respiratory failure, increased bronchoalveolar secretions, vomiting and paralysis through excessive cholinergic stimulation. If patients are lucky enough to make it to hospital, around a third are intubated, and of those up to 50% die. Our research team proposed that the reason for this high mortality might be due to a lung injury created through pulmonary aspiration of the patient’s gastric contents, including the OP, and the solvent in which it was emulsified.

To explore this hypothesis, work was mainly done within an in-vivo porcine model (48 hour studies) and human observational work in Sri Lanka. However, to further investigate the
pulmonary pathophysiology of the aspiration of OP and gastric contents, an ex-vivo lung perfusion (EVLP) model was also designed.

As well as discussing the EVLP study results, this talk will focus on how to design an EVLP model from scratch with little or no budget, the expertise required, and tips for success.
EXPERIMENTAL OVINE MODEL OF CHRONIC INFECTION

David Collie

Biography:
Dr David Collie graduated from the R(D)SVS in 1986 and returned there as a clinical demonstrator in 1988 after a spell in mixed general practice in SW Scotland. Completing an MPhil in the pathophysiology of chronic respiratory disease in calves in 1991 he went on to complete his PhD on pathophysiological correlations in Maedi-visna in 1994. After a two year post-doctoral fellowship in the Inhalation Toxicology Research Institute (now LRRI), Albuquerque, NM, USA spent working on mouse and dog models of asthma, he returned to the R(D)SVS in 1996 as a lecturer in comparative respiratory medicine. He maintains a teaching role within the school and his research interests, which are all lung-oriented, centre around his current role as a group leader within the Roslin Institute, R(D)SVS. These interests include developing and optimising strategies for lung-directed gene therapy, understanding the pathophysiological basis of inflammatory lung disease, the normal repair response in the airways and the role of the lung microbiome in dictating susceptibility to lung disease.

Executive summary:
Chronic lung infection with Pseudomonas aeruginosa is a major contributor to morbidity, mortality and premature death in cystic fibrosis. A new paradigm for managing such infections is needed, as are relevant and translatable animal models to identify and test concepts. We recently developed a lung segmental model of chronic Pseudomonas infection in sheep and this presentation will reflect on data collected as part of an investigation into the lung microbiota changes associated with chronic P. aeruginosa lung infection and the impact of systemic therapy with colistimethate sodium (CMS).
SPONTANEOUS OVINE MODEL OF CHRONIC LUNG CANCER

David Griffiths

Biography:
David Griffiths graduated from the University of Manchester in 1992 with a B.Sc. in Biochemistry. He gained a Ph.D. in Virology from the University of London in 1996 for his studies on the potential role of retroviruses in human rheumatic disease. Following a post-doctoral position at the Chester Beatty Laboratories London, in 1998 he was awarded an Arthritis Research Council Postdoctoral Fellowship at University College London. In 2003, Dr Griffiths moved to the Moredun Research Institute as a Principal Research Scientist to study virus infections in livestock. His current research interests include the pathogenesis of ovine pulmonary adenocarcinoma and the development of viral vectors for use as vaccines in ruminants.

Executive summary:
Lung cancer is the leading cause of cancer deaths worldwide. Animal models are valuable tools for studying oncogenesis in lung cancer, particularly during the early stages of disease where tissues are rarely available from human cases. Murine models have been widely used to study cancer pathogenesis, but there are a number of limitations in using mice for studying human disease and alternative species may have some advantages. Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring lung cancer of sheep caused by retrovirus infection and has several features in common with adenocarcinoma of humans, including a similar histological appearance and activation of common cell signaling pathways. In addition, the similar size and organization of sheep and human lungs facilitates experimental approaches in sheep that are not available in mice. Therefore, OPA presents opportunities for studying lung tumor development that can complement murine models. Here I will discuss the potential value of OPA as a model for human lung adenocarcinoma, with a focus on the in vivo and ex vivo experimental systems available for studying the disease.
SPONTANEOUS EQUINE MODEL OF ASTHMA

Mathilde Leclère

Biography:
Mathilde Leclère obtained her DVM degree from University of Montreal in 2001. She completed an internship in equine medicine Montreal and a residency in large animal internal medicine at UC Davis. She is an ACVIM diplomate since 2005. She pursued a PhD in veterinary sciences at the University of Montreal and McGill University under the supervision of Dr Jean-Pierre Lavoie, while working as a part time clinician in equine medicine. She is an assistant professor at the University of Montreal since 2012. While she is now working on the lung microbiome in health and allergic airway disease, her PhD was mostly focused on airway remodeling in equine asthma.

Executive summary:

Mathilde Leclère and Jean-Pierre Lavoie. University of Montreal.

Spontaneous exacerbations of asthma-like symptoms are rare in mammals other than humans. Heaves in horses (Recurrent Airway Obstruction, previously equine COPD) shares many features of human asthma, including lower airway inflammation, reversible airflow obstruction, bronchial hyperresponsiveness and airway remodelling (1). While heaves exacerbations develop when susceptible horses are exposed to barn antigens, primarily those found in hay, the summer form of the condition, Summer Pasture Associated Pulmonary Disease (SPAOPD), develops when horses are pastured. Both heaves and SPAOPD can exist in the same animal.

Heaves is obviously a disease of its own importance in equine medicine, with an impact on performance and on the quality of life of affected horses. But heaves is also a unique model to study phenomena observed in human asthma. One aspect of heaves that makes it an interesting model, is that exacerbations can be induced by an exposure to environmental antigens. Airway obstruction is reversible with sustained antigen avoidance and with treatment with common anti-asthmatic drugs such as corticosteroids and bronchodilators. A somewhat similar approach can be used when studying SPAOPD, although clinical exacerbations are limited to summer months. Importantly, and contrary to other models of asthma that are based on intraperitoneal or aerosolized allergen sensitization and exposure
over short periods (days to weeks), equine heaves/SPAOPD, as human asthma, likely results from a complex immune response occurring over many years.

Heaves develops in adult horses, is associated with neutrophilic inflammation, and by definition, with severe respiratory impairment during crisis (2). Similarly, neutrophilic human asthma is also more common in older individuals, and is associated with disease severity. Human patients with neutrophilic asthma are considered poorly responsive to corticosteroids administered by inhalation, and often require oral corticosteroids to improve their lung function. This distinction is more difficult to make in horses as most are treated with oral corticosteroids for convenience and economical reasons, and not necessarily for lack of response to inhaled corticosteroids. Furthermore, both in human asthma and equine heaves, corticosteroids used alone are poorly effective at controlling airway inflammation.

**Heaves in horses, a natural model of chronic asthmatic remodelling**

Equine heaves is likely the best "model" to study the complex remodelling processes present in chronic asthma, and its possible reversibility. Interestingly, the studies of comparative pulmonary morphology show that the horse's lung more closely resembles the human lung (3, 4) and their lifespan (30-35 year-old) is closer to human than small rodents. The prospective study of equine cohorts with well-controlled exposure to hay in an experimental setting allows the analysis of disease mechanisms in the asymptomatic and symptomatic stages of the disease evolution, an approach not feasible in humans. Heaves is a particularly appealing model to study airway remodelling in the chronic asthmatic response as histological features of heaves resemble those of asthma including epithelial detachment and regeneration, goblet cell hyperplasia, increased airway smooth muscle mass, and deposition of the extracellular matrix in the airway lamina propria. An additional advantage of the equine model is that it allows the concurrent measurement of multiple variables, the harvest of tissues and fluids in search of possible mediators, and the study of animals multiple times over extended periods (5). The size and docile temperament of horses allow physiological measurements and tissue sampling in standing awake animals. This is not possible in rodents, as anaesthesia, which alters many variables, is required. Lung function and bronchoalveolar lavage (BAL) are well-standardized techniques used in research and clinical settings.

**Equine asthma**

Horses also develop a mild and moderate form of asthma, as seen in human, which may be associated with eosinophilic, neutrophilic, or pangranulocytic inflammation call IAD (Inflammatory Airway Disease). While increased mast cells are not commonly reported in
sputum of patients with asthma, it is common in BAL fluid of horses with IAD. However, this may be due to the sampling method, as mast cells are also rare in tracheal secretions of horses with IAD.

Based on these similarities, we recently proposed to use the term "Equine Asthma" to described what is now called heaves, RAO, SPAOPD and IAD. This term would facilitate communication between veterinary internists, general practitioners and client, as currently, only specialists in the fields are not confused by the ever-changing terminology for these equine conditions.

References

LUNGS ON A PLATE; PCLS - A CLEARER LINE OF SIGHT TO THE CLINIC

Amanda Tatler

Biography:
Amanda Tatler is currently an NC3Rs Fellow and Senior Research Fellow working within the Division of Respiratory Medicine at the University of Nottingham, UK. Her research focuses primarily upon mechanisms of TGFβ-mediated tissue remodelling in the lung in the context of a variety of respiratory conditions such as asthma, pulmonary fibrosis and viral infections. More specifically her research examines how activation of TGFβ by integrins including αvβ5 and αvβ6 contributes to disease pathogenesis.

Amanda was awarded a Royal Society Travel Fellowship in 2011 allowing her to undertake a period of additional post-doctoral training at the Lung Biology Centre, University of California San Francisco. While working at UCSF, Amanda gained invaluable experience using in vivo and ex vivo models to investigate respiratory diseases, including the precision-cut ex vivo lung slice model. Following her return to the UK the NC3Rs awarded her a David Sainsbury Fellowship to further investigate the role of bronchoconstriction in promoting asthmatic airway remodelling via activation of TGFβ. As part of this fellowship, Amanda has spent 6 months at Harvard University Medical School in the US, advancing her knowledge of the ex vivo lung slice model utilising a method to mimic tidal breathing in the slices. Recently, Amanda has been awarded an Asthma UK Mid-career fellowship to pursue this work further and investigate the dynamic relationship between breathing, deep inspiration and activation of TGFβ.

Amanda has sat on the committee of the British Association for Lung Research (balr.co.uk) since 2010, and as Meetings Secretary since 2013. Amanda also sits on the Website Committee of the Respiratory Cell and Molecular Biology Assembly of the American Thoracic Society. She is an active STEM Ambassador and regularly participates in activities aimed to inspire children and young adults in to careers in Science, Technology, Engineering and Maths. Furthermore, Amanda is an Associate Faculty Member for Faculty1000, acting to peer review published scientific articles. Amanda is a member of several professional organisations including The Biochemical Society, the European Respiratory Society, the American Thoracic Society and the British Lung Foundation.
Executive summary:
Dr Amanda Tatler will present an overview of the use of ex vivo precision cut lung slices (PCLS) for investigating a variety of lung pathologies including asthma, pulmonary fibrosis and COPD. PCLS can be created from virtually any species and allow in depth studies to be performing on intact living tissue ex vivo. The presentation will cover both the advantages of using the PCLS model but also highlight the limitations and discuss how these may be overcome.

NOTES:
GM LARGE ANIMAL MODELS - MOVING ON FROM DOLLY

Gerry McLachlan

Biography:
Dr McLachlan gained his PhD at the Department of Medicine and Therapeutics, University of Aberdeen in 1992. His early postdoctoral research in the lab of Prof David Porteous at the MRC Human Genetics Unit, Edinburgh involved the characterization of a Cystic Fibrosis (CF) Knock-Out Mouse model and the development of Gene Therapy protocols for CF. He was awarded a Wellcome Trust Research Fellowship in 1998 to study beta-defensins in the ovine lung. He continued in the theme of innate immunity in the lung as an MRC Research Fellow at the Respiratory Medicine Unit, MRC Centre for Inflammation Research. Dr McLachlan then returned to the field of CF Gene Therapy and moved to the School of Veterinary Medicine in the position of Senior CF Trust Research Fellow (2002) within the UK Cystic Fibrosis Gene Therapy Consortium, a collaborative program involving groups at the University of Oxford and Imperial College London. The main focus of this research has been developing the sheep lung as a model for pre-clinical development of CF gene Therapy protocols to evaluate both safety and efficacy of candidate gene transfer agents. This work supported the recent and successful large-scale multi-dose clinical trial in CF patients. Dr McLachlan is now a Group Leader at The Roslin Institute.

Executive summary:
There is a growing perception that the progress of research in respiratory diseases has been hampered by the lack of good animal models. The ability to genetically manipulate mouse embryonic stem cells and generate transgenic animals has proved a powerful tool in understanding disease however there are limitations in the ability to translate potential therapeutic strategies developed in such models into clinical use. Large animals with greater similarity in lung architecture, physiology and immunity may provide a better alternative however the lack of availability of ES cells from large animal species has meant that specific gene knockout has relied on homologous recombination in somatic cells combined with somatic cell nuclear transfer (SCNT). This is an expensive and relatively inefficient process. The recent advent of genome editing technologies such as zinc finger nucleases, TALENS and CrispR/Cas have opened up new possibilities in generation of GM large animals. These targetable DNA cleavage systems result in double strand breaks at precise DNA sequences
which can be repaired either by non-homologous end joining, resulting in deletions, or homologous recombination from an exogenous template to generate precise mutations. The potential applications in modelling human disease, biotechnology and animal breeding will be discussed.

NOTES:
SHORT COMMUNICATION ABSTRACTS (ORAL)

Marika Melamies*. TREATMENT OF EOSINOPHILIC BRONCHOPNEUMOPATHY IN DOGS – EFFICACY OF ORAL VERSUS INHALED CORTICOSTEROIDS.

Michela Bullone*. MECHANISMS OF AIRWAY SMOOTH MUSCLE REMODELING REVERSAL IN HEAVES.

Julia Paxson. USING IN-VITRO ASSAYS TO INVESTIGATE THE ROLE OF AGE IN LUNG REPAIR.

Tekla Lee-Fowler. EVALUATION OF HISTOGEL™ AND GELFOAM™ EMBEDDED BRONCHOALVEOLAR LAVAGE FLUID SPECIMENS IN COMPARISON TO BALF CYTOPIN AND SEDIMENT SMEAR PREPARATIONS.

Heini Koskinen. EFFECTS OF GENERAL ANAESTHESIA IN DORSAL RECUMBENCY TO BRONCHOALVEOLAR LAVAGE CYTOLOGY OF HEALTHY HORSES.

Scott Dos Santos*. MICROBIAL CULTURE IS OF LIMITED USE IN ASSESSING THE MICROBIAL PROFILES OF HEALTHY HORSES AND HORSES SUFFERING FROM RECURRENT AIRWAY OBSTRUCTION.

Roels Elodie*. STANDARDIZED CHARACTERIZATION OF THORACIC HIGH-RESOLUTION COMPUTED TOMOGRAPHIC FINDINGS IN WEST HIGHLAND WHITE TERRIERS WITH CANINE IDIOPATHIC PULMONARY FIBROSIS: EFFECT OF SEDATION VERSUS ANESTHESIA IN HEALTHY AND AFFECTED DOGS.

Kara Lascola. THE USE OF QUANTITATIVE CT TECHNIQUES TO CHARACTERIZE PULMONARY DISEASE IN NEONATAL FOALS.

Megan Grobman*. CHARACTERIZATION OF AIRWAY MICROBIOTA IN HEALTHY AND BRONCHITIC DOGS.

Eric Richard. CYTOKINE PROFILES IN BRONCHOALVEOLAR LAVAGE FLUID FROM HORSES WITH UNILATERAL IAD-CONSISTENT CYTOLOGY.

Michael Davis. THE EFFECT OF ACUTE EXPOSURE TO HIGH ALTITUDE ON PULMONARY MECHANICAL PROPERTIES AND EXERCISE CAPACITY IN DOGS.

Sanni Hansen. EVALUATION OF AIRWAY INFLAMMATION IN ICELANDIC HORSES UNDER DIFFERENT MANAGEMENT SYSTEMS.

Laurent Couetil. IDENTIFICATION OF FUNGAL AEROALLERGENS ASSOCIATED WITH RECURRENT AIRWAY OBSTRUCTION IN HORSES BY IMMUNOBLOTTING.

Michela Bullone*. METEOROPATHY AND HEAVES: LOOKING FOR THE MISSING LINK.

*Candidate for Joan O’Brien Graduate Research Award
TREATMENT OF EOSINOPHILIC BRONCHOPNEUMOPATHY IN DOGS – EFFICACY OF ORAL VERSUS INHALED CORTICOSTEROIDS, preliminary results

Marika Melamies, S. Viitanen, M.M. Rajamäki
Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, Viikintie 49, 00014 University of Helsinki, Finland

Purpose of the study: Treatment of canine eosinophilic bronchopneumopathy (EBP) requires long-term administration of corticosteroids. When compared to treatment with inhaled corticosteroids (ICSs) oral medication is more often accompanied with unpleasant adverse effects such as iatrogenic hyperadrenocorticism. Purpose of the current study was to evaluate the efficacy of oral and ICS therapy in dogs with EBP.

Methods used: This was a prospective, randomized, noninferiority clinical trial conducted in the small animal hospital at the University of Helsinki. Diagnosis of idiopathic EBP was based on typical respiratory symptoms, clinical, radiographic, hematological and bronchoscopic findings, and bronchoalveolar lavage fluid (BALF) eosinophil percentage of ≥ 20%. Exclusion criteria were other respiratory diseases than EBP or known causes of eosinophilia. Altogether 14 dogs fulfilled criteria; 8 dogs were allocated to prednisolone (PRED) treatment group (0.5 mg/kg bid), and 6 dogs to ICS treatment group (5 dogs received budesonide 200 µg bid and 1 dog fluticasone propionate 250 µg bid). Control visit with BAL was arranged after 3 months on treatment.

Summary of results: At the time of diagnosis median BALF eosinophil percentages were 40% (range, 25-69%) in the PRED group and 57% (range, 21-94%) in the INH group. After treatment BALF eosinophil percentages decreased below 14 % in 8/8 dogs in the PRED group (median, 3%; range, 0-13%) and in 5/6 dogs in the ICS group (median, 7%; range, 2-11%). Blood eosinophilia was originally detected in 3/14 patients, and in none of the dogs after treatment. Pulmonary densities detected in thoracic radiographs improved in 13/14 dogs. All dogs responded to therapy according to symptom scores assessed by the owners.

Conclusions: After 3 months treatment both oral and ICSs decreased pulmonary eosinophilic inflammation and other clinical findings associated with EBP.
MECHANISMS OF AIRWAY SMOOTH MUSCLE REMODELING REVERSAL IN HEAVES

Michela Bullone¹, M Chevigny¹, Y Elce¹, and JP Lavoie¹.

¹ Department of Clinical Sciences, Faculty of Veterinary Medicine, Université de Montréal
* Current address: Royal (Dick) School of Veterinary Sciences, The University of Edinburgh

Purpose of the study: Heaves exacerbations are characterized by severe episodes of bronchospasm, as demonstrated by the rapid effectiveness of bronchodilators at reversing symptoms. Increased airway smooth muscle (ASM) mass is the major determinant of airway obstruction. We have previously demonstrated that ASM mass is increased both in peripheral and in central airways of horses with heaves, the latter likely having a critical implication in the severity of the disease. While peripheral ASM mass remodeling is partly reversible, no data are currently available regarding central ASM. This study hypothesizes that central and peripheral ASM remodeling is reversible to the same extent, and investigates the possible mechanisms implicated in these processes.

Methods: Clinical exacerbation was induced in 12 horses with heaves by means of 4-weeks of stabling and hay feeding. Then, 6 horses were treated only with antigen avoidance (kept at pasture with no hay) and 6 were administered fluticasone/salmeterol by inhalation (2500/250 mcg q12h) while kept in the offending environment for 12 weeks. Pulmonary function data, endobronchial ultrasound images, endobronchial biopsies (EBBs), and caudodorsal lung biopsies were obtained before (baseline) as well as 4 and 12 weeks after treatment. Morphological analysis of corrected ASM mass (ASM area/airway perimeter²) was performed on EBUS images and on histological samples of peripheral lung. Immunohistochemistry for smooth muscle actin (SMA) and proliferating cell nuclear antigen (PCNA) was performed on EBB samples and peripheral lung biopsies. The density of proliferating myocytes was studied counting PCNA⁺ cells/area occupied by SMA⁺ cells. Composition of ASM layer (ASM:collagen:elastic fibers) was studied by point counting and expressed as a percentage.

Results: Both treatments significantly reduced airway obstruction in heaves-affected horses. However, fluticasone/salmeterol treatment had a more rapid effect, with lung function normalizing in all horses after only 1 week of treatment. Both central and peripheral ASM mass were significantly reduced in the fluticasone/salmeterol group, but not in the antigen avoidance group, after 12 weeks of treatment (p<0.05). Similarly, the density of proliferating myocytes in central airways was significantly reduced in the fluticasone/salmeterol group, but not in the antigen avoidance group, after 12 weeks of treatment (p<0.01). In the peripheral airways, proliferating myocyte density decreased in both groups but the difference was significant only for the antigen avoidance group (p<0.05). Antigen avoidance did not alter ASM layer composition, but fluticasone/salmeterol treatment significantly reduced collagen (p<0.05) and elastic fiber (p<0.05) content of the ASM layer in central and peripheral airways, respectively.

Conclusions: Heaves-associated ASM remodeling of the central airways is partly reversible by a 3-month treatment with inhaled fluticasone/salmeterol. The magnitude of ASM reversal is similar in both central and peripheral airways. Significant decreases in myocyte proliferation and extracellular matrix deposition among ASM fibers are likely to be the main contributors to the observed reduction in ASM mass along the bronchial tree. Whether a reduced ASM mass can reduce the severity of heaves symptoms remains to be ascertained.
USING IN-VITRO ASSAYS TO INVESTIGATE THE ROLE OF AGE IN LUNG REPAIR

Julia Paxson PhD, DVM, DACVIM, College of the Holy Cross

Purpose of the study: We are interested in understanding how age affects the ability of the lung to repair after injury, and specifically how aging of lung mesenchymal stem cells (LMSCs) might contribute to the progression of chronic lung disease. In this study we investigated the use of three in-vitro assays to examine the capacity of young and old lung mesenchymal stem cells to respond to injury.

Methods used: In this study, we optimized and compared three different in-vitro assays to examine different aspects of cell function after injury. Mouse fibroblasts and LMSCs (isolated from either 3 month or 12 month mice) were damaged using soluble cigarette smoke extract (CSE) for 24 hours. Damage was assessed using measures of cell viability (MTT assay), cell proliferation (EdU incorporation assay), and migration (quantitative scratch assay). Damaged cells where also incubated with young or old LMSC-conditioned media for 24 hours, and the same assays were performed compared to controls that were not incubated with MSC-conditioned media.

Summary of results: Cell damage after exposure to CSE was detected using all three in-vitro assays. However, the assays showed varying sensitivity in detecting changes in the capacity of LMSCs to response to repair after cell damage. Our results indicate that LMSCs are much more resistant to damage compared to immortal mouse fibroblasts (NIH 3T3 cell line). Furthermore, our results suggest that the capacity of LMSCs to contribute to lung repair may decline with age.

Conclusions: Our results show that these in-vitro may be useful in elucidating the role of age in lung repair, and that the age of lung mesenchymal stem cells may reduce the capacity of these cells to contribute to lung repair after injury.
EVALUATION OF HISTOGEL™ AND GELFOAM™ EMBEDDED BRONCHOALVEOLAR LAVAGE AND TRANSTRACHEAL WASH SPECIMENS IN COMPARISON TO CYTOSPIN AND SEDIMENT SMEAR PREPARATIONS

Lee-Fowler Tekla M\(^a\), Haysom L\(^a\), Spangler E\(^b\)

\(^a\)Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Alabama 36849
\(^b\)Department of Pathobiology, College of Veterinary Medicine, Auburn University, Alabama 36849

**Purpose of the study:** Storage and temperature have been demonstrated to significantly impact bronchoalveolar lavage fluid (BALF) analysis. Shipment of samples to a pathologist is often necessary, particularly those collected in private practices. Alternative cell preparation methods could limit storage and temperature effects. This study aimed to determine if Histogel and Gelfoam airway wash preparations were comparable to cytospin and sediment smear preparations.

**Methods used:** Eleven bronchoalveolar lavage and 3 transtracheal wash samples were available for interpretation, including 8 canine, 1 feline and 5 equine samples. Cytospin and sediment smear preparations were created. Two milliliters of fluid was reserved for further analysis. Total nucleated cell count (TNCC) was determined via hemocytometer. The remaining fluid was used for Histogel and/or Gelfoam preparations. Each preparation was analyzed by a single board certified clinical pathologist and assigned a cellularity score (1-3) and a morphology score (1-4). Differential cell counts were also evaluated.

**Summary of results:** Histogel and Gelfoam preparations resulted in poorer cellularity and morphology in comparison to cytospin preparations but did not differ significantly in comparison to sediment smear preparations. Cellularity scores for sediment smear, Histogel and Gelfoam preparations were inversely correlated with TNCC.

**Conclusions:** Cytospin preparations resulted in the best cellularity and morphology, and are therefore recommended whenever possible. Neither Histogel nor Gelfoam demonstrated any advantage over sediment smear preparations, and both performed poorly when compared to cytospins. Therefore, we do not recommend use of these methods. Total nucleated cell count impacts the cellularity of sediment smear, Histogel and Gelfoam preparations.
EFFECTS OF GENERAL ANAESTHESIA IN DORSAL RECUMBENCY TO BRONCHOALVEOLAR LAVAGE CYTOLOGY OF HEALTHY HORSES

Heini Koskinen, Marja Raekallio, Minna Rajamäki, Heidi Tapio, Anna Mykkänen

Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki

Purpose of the study: Pneumonia has been described as a potential complication of general anaesthesia in horses. Anaesthesia is known to increase the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) of horses. Our aim was to determine when the neutrophil percentage reaches maximum and how rapidly BALF cytology returns to normal after anaesthesia.

Methods used: Four healthy horses were anaesthetized with detomidine, midazolam, ketamine and anaesthesia maintained with isoflurane for one hour. Horses were placed in dorsal recumbency and ventilated mechanically. The horses were subject to BAL one, 20, 48, 72 and 156 h after recovery using 240 mL saline to each lung. A similar series of control samples were taken from each horse without anaesthesia.

Summary of the results: After anaesthesia the neutrophil percentage increased in all horses and in both lungs and reached the peak in the right lung at 48 h in 2/4 horses and at 72 h in 2/4 horses. In the left lung the peak was reached at 20 h in 2/4 horses, at 48 h in one horse and at 72 h in one horse. In the control series repetitive sampling did not increase the neutrophil percentage. All horses had normal cytology in the right lung and all but one horse in the left lung 156 h after anaesthesia.

Conclusions: General anaesthesia causes neutrophilic inflammation in both lungs, which is reversible at 156 h. This finding suggests that increased neutrophil percentage in BALF may not indicate pneumonia until it persists over 156 h.
MICROBIAL CULTURE IS OF LIMITED USE IN ASSESSING THE MICROBIAL PROFILES OF HEALTHY HORSES AND HORSES SUFFERING FROM RECURRENT AIRWAY OBSTRUCTION.

Scott J Dos Santos1,2, JB Montgomery1, KL Lohmann3, LA Johnson2, BL Chaban2, JI Steedman3, JE Hill2.
1 University of Surrey, Faculty of Health and Medical Sciences, Guildford, UK, GU2 7TE.
2 Western College of Veterinary Medicine, Veterinary Microbiology, Saskatchewan, Canada, S7N 5B4.
3 Western College of Veterinary Medicine, Large Animal Clinical Sciences, Saskatchewan, Canada, S7N 5B4.

Purpose of the study: To characterise and compare the microbial profiles of horses suffering from recurrent airway obstruction (RAO) and healthy controls, using culture-based methods and culture-independent DNA sequencing techniques.

Methods used: Horses were recruited from the client base of the Western College of Veterinary Medicine, Saskatchewan, Canada. Horses were grouped as ‘healthy’ or ‘RAO’ based on history, clinical examination and bronchoalveolar lavage cytology. Samples were collected 2 weeks apart (±2 days). Tracheal aspirates (TAs) were collected transendoscopically and sent to a commercial diagnostic laboratory for culture (MacConkey and Columbia blood agar). Colony PCR using primers targeting the universal cpn60 gene was performed on up to 12 isolated colonies per plate. PCR products were purified and sequenced using the amplification primers. Additionally, microbiome analysis was performed by pyrosequencing of cpn60 amplicon libraries generated from total DNA extracts of TAs.

Summary of results: Forty-seven samples (31 RAO, 16 controls) from 27 horses that fulfilled inclusion criteria were studied. Bacteriological culture reports from the diagnostic laboratory included largely genus-level identifications. Many plates showed confluent growth or were culture-negative. Overall, there were no evident differences in culture results between horses suffering from RAO and healthy controls. A total of 177 colonies from 25 horses were characterised with PCR, yielding 119 unique cpn60 sequences. Bacteriological observations were generally supported by colony PCR sequencing data. Comparison to a curated reference sequence database (cpnDB) resulted in species-level identification in many cases where previously, only the genus was known. A sequence similarity of >95% to a cpnDB reference sequence was observed for 40/119 sequences, with 15 sequences showing >99% similarity. A marked proportion of the colonies cultured from the equine respiratory tract represented species or strains that had no match in cpnDB. Furthermore, the composition of the ‘cultured microbiota’ varied considerably in many cases between sampling time-points. Phyllogenetic analysis of all Streptococcus-like cpn60 sequences was performed and showed considerable diversity within the taxon that was not described in the clinical bacteriology results. Only 15/44 TAs (from 5 RAO horses and 3 controls) yielded sufficient cpn60 PCR product for direct microbiome profiling by means of pyrosequencing. A total of 516 operational taxonomic units were identified, 298 of which were unique to the RAO group. Phylum-level profiles were dominated by Firmicutes, Actinobacteria and Gammaproteobacteria. The profiles of each individual horse generally showed consistency between sampling time-points. Colony PCR gave rise to 35 unique cpn60 sequences between the 8 horses. Among these, two exact matches to sequences also seen in the microbiome profiles were present, along with 6 other sequences with a >99% similarity to a microbiome sequence.

Conclusions: A comparison of cultured species vs. microbial pyrosequencing profiles shows that microbial culture only selects for a sub-population of the resident respiratory microbiota that are ‘culture-adapted’. However, the relative abundance of such organisms can only be discerned with culture-independent techniques. Given these results, future RAO research involving microbial culture should be complemented by metagenomic techniques to fully assess the microbiota.
STANDARDIZED CHARACTERIZATION OF THORACIC HIGH-RESOLUTION COMPUTED TOMOGRAPHIC FINDINGS IN WEST HIGHLAND WHITE TERRIERS WITH CANINE IDIOPATHIC PULMONARY FIBROSIS: EFFECT OF SEDATION VERSUS ANESTHESIA IN HEALTHY AND AFFECTED DOGS

Roels Elodie(1), Couvreur T.(2), Soete C.(1), Clercx C.(1), Verschakelen J. (3), Bolen G.(4) (1)Internal Medicine Section, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Liege, Belgium; (2)Department of Radiology, CHC Liege, Belgium; (3)Department of Radiology, Faculty of Medicine, KU Leuven, Belgium; (4)Diagnostic Imaging Section, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Liege, Belgium

Purpose of the study: Canine idiopathic pulmonary fibrosis (CIPF) is a progressive interstitial lung disease mainly affecting West Highland white terriers (WHWTs). The aims of the present study were to (1) describe thoracic high-resolution computed tomography (T-HRCT) findings obtained in control and CIPF WHWTs using the glossary of the Fleischner Society and (2) compare T-HRCT images obtained under general anesthesia (T-HRCTGA) with those obtained under sedation (T-HRCTS).

Methods used: T-HRCT images from 11 WHWTs affected with CIPF and 9 age-matched control WHWTs were retrospectively reviewed by three observers (1 veterinarian and 2 physicians) in consensus. Specific T-HRCT features were independently assessed for presence/absence and scored according to their distribution extend (score ranging from 0 to 18) when possible. Overall T-HRCT quality and presence of motion artefacts were also graded. The Fisher’s exact test was used for the statistical analysis. P-value ≤ 0.05 was considered statistically significant.

Summary of the results: Ground glass opacity (GGO) was observed in all CIPF WHWTs and in 5/9 of controls (P=0.026). In control WHWTs, GGO was localized to right and/or left cranial lung lobes with an overall GGO score ≤ 2. In WHWTs affected with CIPF, GGO was present in all lung lobes in 8 dogs and in all but 2 lobes in 1 dog with an overall GGO score ranging from 9 to 18. In the remaining 2 CIPF dogs, GGO was localized to both cranial lung lobes in addition to the accessory lobe in 1 dog, with an overall GGO score was ≤ 4. Consolidations were observed in 5/11 CIPF WHWTs but not in controls (P=0.038), without lobar predilection. Overall consolidation score ranged from 1 to 6. A mosaic pattern, suggestive of air trapping, was noticed in 8/11 CIPF WHWTs but not in controls (P=0.001). The mosaic pattern was observed in all lung lobes in 4 dogs (global score from 10 to 18) and in 2 to 4 lung lobes in the 4 other dogs (global score from 2 to 10). Nodules were present in 3/11 CIPF WHWTs but not in controls. Reticulation, subpleural bands and parenchymal bands were noticed in 1, 1, and 3/11 CIPF WHWTs respectively. Honeycombing, emphysema, pleural effusion and pleural thickening were never observed. Bronchial wall thickening and bronchiectasis were present in 6/11 and 3/11 CIPF WHWTs respectively but not in controls (P=0.014 and P=0.218). The overall T-HRCTS quality was good in 10/17 examinations compared with 16/20 for T-HRCTGA (P=0.279). Motion artefacts were present in 15/17 T-HRCTS examinations compared with 7/20 for T-HRCTGA (P=0.002). However, those T-HRCTS motion artefacts were most frequently graded as mild (11/15) rather than moderate (2/15) or severe (2/15) (P=0.0004). T-HRCTS allowed identification of a mosaic pattern in 2 additional CIPF WHWTs, while consolidation could not be identified in 2 others. There was no other difference between T-HRCTGA and T-HRCTS.

Conclusions: GGO, consolidation, mosaic pattern and bronchial wall thickening are the main T-HRCT features of CIPF in WHWTs. Honeycombing, the major feature of IPF in humans, was never observed, which suggests a different disease pathophysiology or severity between the two species. T-HRCTS can be used for CIPF diagnosis.
THE USE OF QUANTITATIVE CT TECHNIQUES TO CHARACTERIZE PULMONARY DISEASE IN SICK NEONATAL FOALS.

Kara M Lascola, Stephen Joslyn, Benjamin Rivard, Bob O’Brien, Pamela Wilkins, Scott Austin
University of Illinois College of Veterinary Medicine, Urbana, Illinois, USA

Purpose of the Study:
The objectives of this study were to compare quantitative histogram analysis of CT images in surviving and non-surviving neonatal foals with pulmonary disease and to compare manual and semi-automated 3-D lung segmentation of CT images in diseased foals.

Methods Used:
Seven foals ≤ 14 days diagnosed with pulmonary disease were recruited for CT imaging. Semi-automated lung segmentation was performed using DICOM viewing software (OsiriX®) and specialized segmentation software (MiaLite®). Manual segmentation was performed using OsiriX® alone. Attenuation values within segmented lung were averaged to obtain mean whole lung attenuation values. Software-generated histogram analysis for the frequency distribution of attenuation values for the lung was performed using both segmentation techniques. Histograms were compared between surviving and non-surviving diseased foals and healthy foals, and between lung segmentation protocols for all sick foals.

Summary of Results:
7 hospitalized neonatal foals were recruited. Mean lung attenuation for diseased foals (-251 ± 48) was greater than healthy foals (-479 ± 45; P = 0.003) but did not differ between diseased surviving (-264 ± 59) and non-surviving (-241 ± 44) foals (P = 0.577). Histogram distributions differed between healthy, surviving diseased (n = 3), and non-surviving diseased (n = 4) foals. Semi-automated segmentation was faster compared to manual segmentation (< 10 versus 50-90 minutes) Histogram differences were not identified between techniques.

Conclusions:
Differences in CT histograms between surviving and non-surviving foals may reflect differences in disease distribution in the lung. The semi-automated lung segmentation technique represents a promising tool for CT quantitative image analysis with potential applications to other veterinary species.
CHARACTERIZATION OF AIRWAY MICROBIOTA IN HEALTHY AND BRONCHITIC DOGS

Megan Grobman, Alexa R. Personett, Aaron C. Ericsson, Hansjörg Rindt, and Carol R. Reinero

Department of Veterinary Medicine and Surgery (Personett, Grobman, Rindt, Reinero), Department of Veterinary Pathobiology (Ericsson), University of Missouri, Columbia, MO

**Purpose:** Canine chronic bronchitis (CCB) is an inflammatory lower airway disorder often of unknown etiology. Culture-independent techniques in healthy humans have demonstrated a core airway microbiota that differs in and may contribute to chronic airway diseases. Dysbiosis deserves study in CCB.

**Objectives/Hypothesis:** Study objectives included characterizing the microbiota of upper and lower airways in healthy dogs, and comparing the lower airway microbiota to bronchitic dogs. We hypothesized that the canine airway microbiota differs in health and disease.

**Methods:** Nasal, oropharyngeal, and bronchoalveolar lavage fluid (BALF) samples from healthy research dogs (n=16) and BALF samples from client-owned dogs with CCB (n=14) were collected. Samples underwent DNA extraction, purification, and next-generation sequencing of the 16S rRNA amplicons using the Illumina MiSeq platform. Sequence data was annotated using the Greengenes database. Microbial communities were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) software. One way ANOVA on ranks and students T tests were used; P <0.05 was significant.

**Results:** Healthy dogs had a core airway microbiota similar in types and abundance of operational taxonomic units (OTUs) present, with microbial families often from the environment. Lack of clustering on Principle Component Analysis suggested dissimilar communities between healthy and bronchitic dogs. Significantly more microbial families found in the fecal microbiome were present in CCB samples (P= <0.05).

**Conclusions:** Next generation sequencing of the canine microbiome demonstrated the existence of a core airway microbiota that differs in health and disease and is relevant to better understanding of disease pathogenesis and future development of novel targeted therapies.
CYTOKINE PROFILES IN BRONCHOALVEOLAR LAVAGE FLUID FROM HORSES WITH UNILATERAL IAD-CONSISTENT CYTOLOGY

E. Hue a,b, M. Orard a, M. Depecker c, A. Couroucé-Malblanc c, R. Paillot d, S. Pronost a,b, Eric Richard a

a: LABÉO Frank Duncombe; 1 Route de Rosel, 14053 Caen Cedex 4, France
b: Normandie Univ; UNICAEN, SF4206 ICORE, EA 4655 U2RM, 14032 Caen, France
c: LUNAM Université, ONIRIS, UPSP 5304, Atlantpôle - La Chantrerie, BP40706, Nantes, 4307, France.
d: Animal Health Trust, Landwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, United Kingdom

Purpose of the study: Only few data on BALF cytokine profiles are available for racehorses with inflammatory airway disease (IAD); cytological diagnosis being most frequently made from one lung only per horse. The aim of the study is to compare cytokine mRNA expressions and protein concentrations in BALF from both lungs of horses with unilateral IAD-consistent cytology.

Methods used: As part of a larger study, 250ml saline was randomly instilled in one lung and 500ml in the contralateral lung of 30 clinically healthy Standardbred racehorses. This procedure was repeated 72h later, inversing the volume per lung. Cytological cut-off values for IAD diagnosis was neutrophils proportions >10% when instilling 250ml. For these samples, mRNA expression and concentrations of IL-1β, IL-4, IL-8, IL-10, IL-17, TNF-α and IFN-γ were determined by RT-qPCR and ELISA.

Summary of results: Eleven horses exhibited BALF with IAD- and control (CTL)-consistent cytology from respectively each lung, and were enrolled in the study (22 samples). Data were not significantly influenced by the sampling day, and there was no significant difference between left and right lung for BALF total cell counts or cytokine concentrations. Relative mRNA expression of IL-1β (3.887±3.082; p=0.01) and IL-10 (3.225±1.710; p=0.005) were significantly higher in BALF of IAD- compared to CTL consistent lungs (respectively 1.408±1.118 and 1.488±1.393). Expressions of both cytokine were significantly correlated (r=0.62; p=0.002), and also correlated to neutrophil proportions (respectively r=0.54; p=0.01 for IL-1β and r=0.65; p=0.001 for IL-10).

Conclusions: Differences in cytokine mRNA expression were associated with IAD- or CTL-consistent BALF cytology in the same racehorses in training. These findings suggest that specific local immune reactions or regulation within the lower airways should be further considered in IAD.

Ethical Animal Research: The study was approved by the Regional Animal Ethic Committee (CEEA-PdL 2015.70), and informed consent was provided by all owners.

Competing Interests: Authors disclose no conflict of interest.

Sources of Funding: Financial support was provided by LABÉO, CISCO-Oniris and AVEF (French Association of Equine Practitioners).
THE EFFECT OF ACUTE EXPOSURE TO HIGH ALTITUDE ON PULMONARY MECHANICAL PROPERTIES AND EXERCISE CAPACITY IN DOGS

Michael S. Davis¹, C. Royer² and David Irwin³. ¹) Oklahoma State University, Stillwater, OK; ²) Lovelace Respiratory Research Institute, Albuquerque, NM; ³) University of Colorado, Denver, CO.

Purpose of the study: High altitude (>4,000m) is associated with impaired exercise capacity due to reduced blood oxygen content and can be complicated by deterioration of pulmonary function due to High Altitude Pulmonary Edema. These effects can be attenuated with time and gradual exposure to the high altitude environment, but during modern warfare combat units of both humans and dogs may be introduced into a high altitude environment without any time for acclimatization. The purpose of this study was to quantify the effects of high altitude on unacclimatized athletic dogs.

Methods used: Studies were performed with 24 sedentary dogs (Unconditioned) and 26 aerobically-conditioned dogs (Conditioned). In both studies, endurance exercise capacity (time to fatigue) was measured on a high-speed treadmill, followed by general anesthesia and measurement of diffusion capacity and pulmonary blood flow using inert gas inhalation and dynamic respiratory compliance and respiratory resistance using positive pressure ventilation. After 24 hr of recovery, Unconditioned dogs were housed in a normobaric hypoxia altitude simulation chamber for 4, 24, or 48 hr, with assessment of endurance capacity repeated at these times while in the altitude simulation chamber. After completion of the exercise assessment, dogs were removed individually from the chamber and pulmonary function measurements performed immediately. Conditioned dogs were housed in the same chamber for up to 48 hr or for 24 hr with chamber set at sea-level (0 hr of altitude exposure) to account for the possible conditioning effects of the initial sea-level exercise endurance test. All data were expressed as a percentage of the initial sea-level values and analyzed as a single factor ANOVA.

Summary of results: There was no effect of simulated altitude on exercise capacity in unconditioned (p = 0.17) or conditioned dogs (p = 0.40), with the mean exercise endurance of unconditioned dogs ranging from 91 to 104% of their sea-level endurance and the mean exercise endurance of the conditioned dogs ranging from 99% to 127% of their sea-level capacity. There was no effect of altitude exposure in unconditioned dogs on diffusion capacity (p = 0.12), or dynamic respiratory compliance (p = 0.73). Respiratory resistance was significantly increased by altitude exposure (p = 0.005) and there was a trend for an increase in pulmonary blood flow (p = 0.07) after 48 hr of altitude exposure. In fully conditioned dogs, there was no effect of altitude exposure on diffusion capacity (p = 0.12), pulmonary blood flow (p = 0.21), or dynamic respiratory compliance (p = 0.14). Respiratory resistance was significantly increased by altitude exposure (p < 0.001), with the largest increase occurring after 48 hr of altitude exposure.

Conclusions: Healthy athletic dogs are remarkably resistant to the deleterious effects of high altitude hypoxia, showing no signs of HAPE and demonstrating the capacity to maintain endurance exercise capacity in the face of reduced oxygen availability. The mechanisms of this apparent rapid acclimatization to this environmental extreme merit further study as possible therapeutic targets for humans undergoing similar environmental challenges.
EVALUATION OF AIRWAY INFLAMMATION IN ICELANDIC HORSES UNDER DIFFERENT MANAGEMENT SYSTEMS

Sanni Hansen¹, K. Klintø², M. Austevoll³, K. E. Baptiste⁴ and J. Fjeldborg¹
¹Departments of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark
²Hoejgaard Hesteklinik Aarhus, Nodlige Bjergevej 7-9, Hoejbjerg, Denmark
³Boehringer Ingelheim Vetmedica, Stroeamvej 52, DK-2100 Copenhagen, Denmark
⁴Danish Health and Medicines Authority, Department of Veterinary Medicine, DK-2300 Copenhagen South, Denmark

Purpose of the study: To evaluate if difference in management system (conventional stabling, loose-housing and outdoors) affects the amount of airway inflammation evaluated by endoscopy, tracheal aspiration (TA) and bronchoalveolar lavage (BAL).

Methods used: A descriptive cross-sectional study was designed, dividing the included horses into 3 different management groups. Clinical examination, blood sample, and endoscopic examination including mucus score, TA and BAL were performed in 84 Icelandic horses (aged 8.1 + 4.6) which were all used for pleasure riding. The horses were housed in three different management systems, conventional stabled (n=29), loose-house systems (n=29) and pasture (n=26). TA and BAL cytology including total cell counts (TCC) and differential cell counts (DCC).

Summary of results: A significant higher BAL neutrophil cell count was found in the two groups of horses with indoor access compared to the outdoor group. A significant higher TA TCC was found in the two groups of horses with indoor access compared to the group outdoor.

Conclusions: A larger part of the horses with indoor access showed evidence of subclinical airway inflammation characterized by an increase in TA and BAL neutrophils in the absence of poor performance or signs of systemic inflammation.
IDENTIFICATION OF FUNGAL AEROALLERGENS ASSOCIATED WITH RECURRENT AIRWAY OBSTRUCTION IN HORSES BY IMMUNOBLOTTING

Couetil L¹, Kamarudin M¹, Aime M², HogenEsch H³, Rosenthal F⁴.

¹ Veterinary Clinical Sciences, College of Veterinary Medicine, ² Botany and Plant Pathology, College of Agriculture, ³ Comparative Pathobiology, College of Veterinary Medicine, ⁴ School of Health Sciences, College of Health and Human Sciences, Purdue University, West Lafayette, IN

Purpose of the study: Recurrent Airway Disease (RAO) is a common chronic respiratory disease in horses. Previous work suggested that it is a hypersensitivity reaction to inhaled molds however, the identity of specific fungal species responsible for this response is not known precisely. The objectives of this study were 1) to identify fungal species contained in inhalable dust collected from RAO and control horses fed with moldy and good quality hay, 2) to identify fungal species that were immunogenic in RAO horses.

Methods used: Nine RAO horses in remission and 6 age-matched control horses were recruited. Horses were exposed to good and moldy hay up to two weeks with a 4-week wash out period between exposures. Clinical assessment, including physical examination, pulmonary function testing, and bronchoalveolar lavage (BAL) were performed before and after hay exposure. During exposure period, horses were equipped with personal air sampling devices to collect inhalable dust in the breathing zone. Dust filters were eluted and plated on Potato dextrose agar. Pure cultures were sorted into morphospecies and DNA was extracted and amplified using fungal specific primers for identification. Molecular cloning was also conducted to confirm fungal identification. Eluted dust samples and fungal proteins extracted from individual fungal cultures underwent electrophoresis and were subjected to immunoblotting using BAL fluid as a source of primary antibodies. Results were compared between RAO and control horses before and after exposure to good and moldy hay.

Summary of results: Horses fed with moldy hay were exposed to 2.3 fold higher concentrations of fungi (cfu/m³) particulates in their breathing zone than when fed with good quality hay (p = 0.005). In total 20 different mold species were isolated from culture method and 31 different species were identified using molecular cloning method. The majority of the species were found both in good and moldy hay samples with Aspergillus flavus, Alternaria alternata, Penicillium chrysogenum, Wallemia sebi, Eurotium amstelodami the most commonly isolated species. Four fungal species showed strong binding patterns with BAL fluid antibodies from RAO but not control horses.

Conclusion: Horses fed good or moldy hay are exposed to similar fungal species in inhalable dust samples but only a few fungal species result in unique immunogenic reactions with BAL fluid from RAO horses.
Purpose of the study:
Clinical exacerbations of heaves are frequently reported during winter months, when horses are exposed to airborne dusts during stabling. However, we have also observed clinical symptoms worsening on days of heatwave. We sought to investigate the effect of temperature and humidity on the clinical signs and lung function of heaves-affected horses during clinical exacerbation of the disease.

Methods:
Data of a group of 14 horses with heaves exposed to a dusty environment and evaluated clinically on a regular basis were analyzed retrospectively. Indoor temperature and relative humidity (RH) values were obtained and air enthalpy was calculated. Pearson test was used for correlating mean daily clinical scores of horses and environmental variables, including indoor temperature, humidity, and enthalpy as well as outdoor airborne pollen and spore concentrations. Lung function parameters recorded at 4-day interval during hot (25°C, 60% RH, indoor values) and warm (18°C, 61% RH) environmental conditions were also compared with paired t-test.

Results:
Significant positive correlations were observed between the mean daily clinical score and relative humidity ($r=0.7$, $p=0.02$) as well as with air enthalpy ($r=0.73$, $p=0.03$), while only a tendency was observed with temperature ($p=0.09$, $r=0.56$). Mean daily clinical scores of the horses were not correlated with the total concentrations of airborne pollens or spores of the same day, of the previous day, or of the 3 previous days ($p>0.1$). However, when pollens were analyzed separately, a significant correlation was observed between the mean daily clinical score and the airborne concentration of *Morus spp* pollens ($r=0.78$, $p=0.01$). Higher barn temperature and humidity, in absence of changes in the management of horses, was also associated with increased transpulmonary pressure ($p=0.005$), pulmonary resistance ($p=0.008$), and elastance ($p=0.005$). This data, in absence of significant changes in the breathing frequency ($p=0.48$) or lung tidal volume ($p=0.12$), suggest bronchoconstriction to be the major cause of the observed worsening of clinical symptoms of heaves-affected horses during heatwave.

Conclusions:
Providing a cold environment could help attenuate the severity of airway obstruction in uncontrolled exacerbation of heaves. Also, the increased presence of pollen (particularly from *Morus spp* trees) and spores in the air during hot days could worsen symptoms by means of non-specific airway irritation. In conclusion, variations in environmental temperature and humidity should be taken into account when evaluating the response to therapy in clinical or research settings.
SHORT COMMUNICATION ABSTRACTS (POSTERS)

**Laurent Couetil.** Efficacy of inhaled levalbuterol compared to albuterol in horses with recurrent airway obstruction.

**Roels Elodie*.** Canine idiopathic pulmonary fibrosis is not associated with herpes virus infection.

**Michelle Husulak*.** Does antimicrobial therapy improve outcomes in horses with recurrent airway obstruction and a positive tracheal wash culture?

**Chung-Hui Lin*.** Cyto logical results between the left and right lungs using non-bronchoscopic bronchoalveolar lavage in cats with naturally acquired lower airway disease.

**Esther Siegers.** The effect of ionization on air quality in horse stables.

**Irene Tosi.** Characterisation of TLR 7/8 in equine pulmonary alveolar macrophages.

**Yoshiki Yamaya.** Exposure to environmental tobacco smoke enhances activation of CCR4+ T-lymphocytes and sensitization to allergens in dogs with lymphoplasmacytic rhinitis.

*Candidate for Joan O’Brien Graduate Research Award*
EFFICACY OF INHALED LEVALBUTEROL COMPARED TO ALBUTEROL IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION

Couëtil L., Arroyo M. G., Nogradi N., Kamarudin M, Ivester K. M.
Veterinary Clinical Sciences, Purdue University College of Veterinary Medicine,
West Lafayette, Indiana, USA.

Purpose of the study: The (R)-enantiomer of the racemic albuterol is responsible for the bronchodilation while the (S)-enantiomer has adverse effects causing bronchoconstriction and airway hyperresponsiveness in human airways, animal models and equine isolated bronchi. In people, levalbuterol, the (R)-enantiomer of racemic albuterol improves pulmonary function of asthmatics with a longer duration of effect compared to albuterol. Therefore, the objective of this study was to determine the effect and duration of action of inhaled levalbuterol in horses with recurrent airway obstruction (RAO) in comparison to racemic albuterol.

Methods used: Nine RAO horses in disease exacerbation were enrolled in a randomized, crossover trial where each horse was treated with nebulized albuterol and levalbuterol with at least 24 h washout in between treatments. Pulmonary function was measured before and after the cumulative bronchodilator challenge for up to 3 hours. Maximum transpulmonary pressure change (DPmax) was measured during the challenge. Data was summarized as median and interquartile range (IQR) and analyzed by one-way analysis of variance for repeated measures with a significance level set as P ≤ 0.05.

Summary of results: The dose of bronchodilator that resulted in a maximum bronchodilation (EDmax) was not significantly different between the albuterol and levalbuterol (EDmax = 125.0 [125- 125 µg] EDmax = 188 [125 – 188 µg] respectively; P=0.068). The average duration of bronchodilator effect was 60 minutes for albuterol and 120 minutes for levalbuterol (P<0.05). The magnitude of bronchodilation was not significantly different between the two treatments (57.2% and 57.7% reduction in DPmax for albuterol and levalbuterol, respectively).

Conclusion: Levalbuterol is as effective a bronchodilator as albuterol but the relatively short duration of action makes this drug non-practical for therapy of RAO.
CANINE IDIOPATHIC PULMONARY FIBROSIS IS NOT ASSOCIATED WITH HERPES VIRUS INFECTION


(1)Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Liege, Belgium; (2)Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Belgium; (3)Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland

Purpose of the study: An association between gammaherpesvirus infection and pulmonary fibrotic disorders has been suggested in humans, horses and rodents. In dogs, canine idiopathic pulmonary fibrosis (CIPF), a fibrotic lung disease of unknown origin and poorly understood pathophysiology, occurs principally in West Highland white terriers (WHWTs) at an advanced age. Therefore, the aim of the present study was to investigate the potential association between herpesvirus infection and CIPF.

Methods used: Pan-herpesvirus PCR assays, using either degenerate or deoxyinosine-substituted primers targeting highly conserved regions of the DNA polymerase gene (DPOL) of herpesviruses was used on lung samples issued from WHWTs affected with CIPF (n = 28) and controls (n = 18) in a nested format. DNA of gammaherpesvirus-positive murine (Murid herpesvirus-4) and bovine (Bovine herpesvirus-4) spleen samples were included as positive controls. Water samples were tested as negative controls.

Summary of the results: Herpes virus DPOL sequence could not be amplified from the 46 lung samples included.

Conclusions: An association between herpes virus infection and CIPF is unlikely. Investigation of other etiologic agents is warranted.
DOES ANTIMICROBIAL THERAPY IMPROVE OUTCOMES IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION AND A POSITIVE TRACHEAL WASH CULTURE?

Michelle L. Husulak, ST Manning, H Kosolofski, JI Steedman, AC Williams, LP Riddell, SJ Dos Santos, MD Meachem, HJ Burgess, TY Epp, JB Montgomery

1Department of Large Animal Clinical Sciences, 2Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan, Canada, S7N 5B4

Purpose of the study: To determine if horses with recurrent airway obstruction (RAO) and a positive tracheal wash (TW) bacterial culture benefit from treatment with antibiotics combined with a conventional treatment of systemic corticosteroids, bronchodilators, and environmental management.

Methods used: Twelve horses with clinical RAO were examined with written consent by their owners and assigned a clinical score based on their abdominal respiratory effort and severity of nostril flaring. Transendoscopic TW and bronchoalveolar lavage (BAL) was performed on all horses. Cytological analysis of BAL samples and bacterial culture of TW aspirates were evaluated. All horses had a minimum of 20% neutrophils on the BAL differential cell count and a positive TW bacterial culture. The bacterial growth was mild to moderate with at least one species of bacteria present, all of which were sensitive to ceftiofur, a third generation cephalosporin antimicrobial. The horses were randomly assigned to two groups of six horses. Both groups received treatment with dexamethasone (0.08 mg/kg PO q24 hours initially, tapering the dose after 4 days, ending at 0.04 mg/kg PO q48 hours at 16 days post initiation of treatment), clenbuterol hydrochloride (0.8 μg/kg PO q12 hours for 7 days), and a change in management to reduce the airborne dust in their environment. The principal (antibiotic) group received long acting ceftiofur (6.6 mg/kg IM, 2 doses 4 days apart) and the control group received 0.9% sodium chloride solution, IM, at the same volume as a dose of ceftiofur. The investigator who examined all of the horses (MLH), and the horse owners were blinded to the treatment groups. The horses were re-examined with the same protocol within 24 hours of completing the treatment regimen, 16 days following the initiation of treatment. BAL fluid supernatant was frozen at -80°C and subsequently analyzed by neutrophil myeloperoxidase (MPO) activity assay and tumor necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA). The principal and control groups were compared with a Mann-Whitney U test and the pre- and post- treatment values were compared with a Wilcoxon signed rank test with the p value < 0.05 considered significant.

Summary of results: There were no differences between the principal and control groups clinical exam score, BAL neutrophil counts, MPO activity, or TNF-α concentration. Treatment with antimicrobials significantly improved the post-treatment clinical score of the principal group compared to the pre-treatment score, whereas there was no difference in the clinical score pre- versus post- treatment in the controls. The principal group also had significantly less MPO activity post-treatment than pre-treatment. There was no difference in MPO activity pre- versus post-treatment in the control group. No differences were noted in pre- versus post- treatment BAL neutrophil counts or TNF-α concentrations in either group.

Conclusions: Antimicrobial therapy should be considered in horses with RAO which have a positive TW bacterial culture, as it improves post-treatment clinical scores and decreased MPO activity.
CYTOLOGICAL RESULTS BETWEEN THE LEFT AND RIGHT LUNGS USING NON-BRONCHOSCOPIC BRONCHOALVEOLAR LAVAGE IN CATS WITH NATURALLY ACQUIRED LOWER AIRWAY DISEASE

Authors: Chung-Hui Lin (1) (2), Pei-Ying Lo (2), Jih-Jong Lee (1) (2) (3), Chen-Hsuan Liu (1) (4) (5)

(1) Graduate Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
(2) Section of Small Animal Internal Medicine, National Taiwan University Veterinary Hospital, Taipei, Taiwan
(3) Graduate Institute of Veterinary Clinical Sciences, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
(4) Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
(5) Section of Diagnostic Pathology, National Taiwan University Veterinary Hospital, Taipei, Taiwan

Purpose of the study: It was previously reported that total and differential cell counts in bronchoalveolar lavage (BAL) fluid can differ between lung segments in cats undergoing bronchoscopy, despite the presence of diffuse radiographic changes. The aim of this study was to evaluate the difference between the left and right lungs when using non-bronchoscopic BAL (NB-BAL) technique.

Methods used: Cats receiving bilateral NB-BAL were enrolled. Exclusion criteria included: (1) no separate analyses for the left and right samples; or (2) having focal rather than diffuse infiltration on chest radiographs. Cytological diagnoses included eosinophilic (≥17% eosinophils), neutrophilic (≥7% neutrophils), and mixed inflammation (≥17% eosinophils and ≥7% neutrophils). The total nucleated cell count (TNCC), eosinophil %, neutrophils %, lymphocyte %, and macrophage % of the samples from the left and right side were compared. The agreement of cytological assessment was determined by Kappa statistic.

Summary of results: There were no statistically significant differences between BALF from left and right side for TNCC and differential cell counts in eleven cats (P > 0.05). However, the cytological diagnoses were different at left and right side in 5/11 (45.5%) cases. The Kappa value for agreement on cytological diagnoses from the left and right lung BALF was 0.24 (95% CI, -0.22 to 0.69), indicating only a fair level of agreement.

Conclusions: The difference between the left and right lung did not significantly affect quantification of results of BALF analysis. However, the cytological diagnosis in individual case can be different, mostly resulting in single cell inflammation at one side and mixed inflammation at the other side. The classification of inflammatory type might be of question in cases with nonuniform inflation in different lung segments.
THE EFFECT OF IONIZATION ON AIR QUALITY IN HORSE STABLES

Esther W. Siegers, F.J.C.M. van Eerdenburg, I.M. Wouters, M. Anthonisse, C.M. Westermann

Purpose of the study: Horses kept in stables are likely to be exposed to high levels of organic dust. Organic dust increases the risk of inflammatory reactions and is associated with respiratory diseases. Ionizing devices are manufactured to improve air quality and have been studied in housing of man and poultry with conflicting results.

Objectives: To determine the effect of the ionization of air on dust, fungal and endotoxin levels in horse stables, and the effect of stabling horses on shavings and feeding them haylage versus stabling on straw and feeding dry hay.

Materials and methods: Four units of six boxes equipped with an ionization installation were used. In two units horses were kept on shavings and were fed haylage, in the other two horses were kept on straw and were fed dry hay. Ambient inhalable dust samples were collected on 5 fixed, similar positions within each unit. Measurements were performed with and without activated ionization, during daytime and nighttime, and repeatedly over the course of a week. The dust samples were analyzed for endotoxins, fungi and dust. Statistical analysis was performed using a General linear model, Wilcoxon signed rank test. $P$ values <0.05 were considered significant.

Results: 156 samples were examined for dust levels and 154 samples were examined for endotoxin levels. Highest dust and endotoxins levels were found in the units with straw and dry hay during daytime. 86 samples were taken for fungal growth. Samples taken in straw units showed higher growth of fungal colonies than samples taken in units where horses were kept on shavings. Nighttime sampling showed less fungal growth than daytime sampling. No difference in dust, endotoxins or fungal growth were found when ionization was activated, except for fungi on shavings.

Conclusions: The installation of an air purifier in the form of a negative ionizator in the horse stable, under the conditions used in this study, does not contribute to a healthier climate in the stable, since it had no effect on the reduction of dust, endotoxins and viable fungal spores. The enormous effect of dust free bedding and feeding is confirmed.
CHARACTERISATION OF TLR 7/8 IN EQUINE PULMONARY ALVEOLAR MACROPHAGES

Irene Tosi (1, 4), Linda Frellstedt (1, 4), Dimitri Pirottin (2, 4), Sophie El Abbas (1, 4), Ingrid Waldschmidt (3, 4), Marie-Capucine Dupuis (3, 4), Pierre Lekeux (1, 4) and Tatiana Art (1, 4).

(1) Center of Equine Sports Medicine, University of Liège, Liège, Belgium.

(2) Laboratory of Cellular and Molecular Physiology, GIGA-Research, University of Liège, Liège, Belgium.

(3) CIRALE, National Veterinary School of Alfort, Goustranville, France.

(4) Hippolia Foundation, Caen, France.

Corresponding author: irene.tosi@ulg.ac.be

Purpose of the study: In both human and equine athletes, viral infections are common causes of respiratory diseases and of a sudden deterioration of expected performances. In both species, the underlying mechanisms are still unclear, and an involvement of Toll-Like Receptors (TLRs), a fundamental link between innate and adaptive immunity, has been advocated. Our objectives were to verify the presence of TLR7 and TLR8, responsible for the early anti-viral response in mammals, in equine pulmonary alveolar macrophages (PAMs) and to assess their function through specific stimulation.

Methods used: Equine PAMs were collected by broncho-alveolar lavage (BAL), isolated by adherence and stimulated with specific TLR7/8 ligands (an imidazoquinoline compound and single-stranded RNA), mimicking a viral attack. The expression of TLR7/8 was evaluated by rt-PCR and the ligand-induced production of cytokines (type I-IFNs and TNF-α) was assessed via ELISA.

Summary of results: Our study demonstrated the expression of TLR7/8 in equine PAMs. QPCR analyses showed a high relative expression of genes coding for TLR7 and TLR8 on equine PAM. Stimulation with specific TLR7/8 ligands resulted in significantly up-regulated production of IFN-β and TNF-α, thereby confirming that TLR7/8 are functional in equine PAMs and that they play a role in the early pulmonary antiviral response.

Conclusions: This study shows that TLR7 and TLR8 are present and functional in equine PAM and that they could play a role in the early pulmonary antiviral response. In terms of future perspectives, it is interesting to suggest that the extensively demonstrated efficacy of TLR7 and TLR8 synthetic ligands in the treatment of viral diseases in human medicine could motivate the pursuit of clinical trials in the equine patient for the therapeutic management or prevention of viral respiratory infections.
EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE ENHANCES ACTIVATION OF CCR4+ T-LYMPHOCYTES AND SENSITIZATION TO ALLERGENS IN DOGS WITH LYMPHOPLASMACYTIC RHINITIS

Veterinary Anesthesiology & Respiratory Research Laboratory, College of Bioresource Sciences,
Nihon University, Kameino 1866, Fujisawa, Kanagawa 252-0880, Japan

Canine lymphoplasmacytic rhinitis (LPR) is a type of chronic rhinitis in dogs caused due to various reasons, including immune-mediated or allergic responses, such as fungal hypersensitivity. In addition, exposure to environmental tobacco smoke (ETS) is known to cause allergic rhinitis in humans. Therefore, we hypothesized that exposure to ETS may enhance the allergic response in canine LPR. Here, the relationship between atopy and exposure to ETS in dogs with LPR was investigated by measuring the proportion of CC chemokine receptor 4+ (CCR4+) cells among CD4+ peripheral blood cells (CCR4+/CD4+) and serum levels of specific immunoglobulin E (IgE) antibodies (Abs) and cotinine. In brief, blood samples were obtained from the jugular vein of 21 dogs with LPR, centrifuged, and the collected sera samples were stored immediately at 4°C until assayed. Serum cotinine levels were measured using a Cotinine for Passive Smoking ELISA Kit (Cosmic Corporation, Tokyo, Japan) and divided into two groups of low (<0.5 ng/ml; Lo-ETS) and high (≥0.5 ng/ml; Hi-ETS) concentrations. The proportion of CCR4+/CD4+ cells was determined by flow cytometry using an Alexa 647-conjugated anti-canine CD4 Ab and R-phycoerythrin-conjugated, anti-human, CCR4 Ab, as previously reported. All analyses were performed by Animal Allergy Clinical Laboratories, Inc. In addition, serum antigen-specific IgE Ab levels were measured using a fluorometric enzyme-linked immunosorbent assay, following a previously reported protocol. A dog was considered sensitized to the allergen (ETS) if the antigen-specific IgE Ab concentration was >100 ng/ml.

On the basis of serum cotinine levels, 11 (52%) and 10 (48%) dogs were classified to the Lo- and Hi-ETS groups, respectively. The mean percentage of CCR4+/CD4+ cells in the Hi-ETS group was significantly higher than that in the Lo-ETS group (39.6 ± 11.8% vs. 31.8 ± 5.5%, respectively). Moreover, serum levels of 11 allergen-specific IgE Abs in the Hi-ETS group were significantly higher than those in the Lo-ETS group. In addition, the sensitized rate to only Penicillium notatum of 41 allergens in the Hi-ETS group was significantly higher than that in the Lo-ETS group (60%, 6/10 vs. 9%, 1/11; P = 0.0209).

The control of environmental factors, such as EPS, which are known to induce allergic responses, may be important for the treatment of dogs with LPR. For example, restriction from rooms containing ETS may help to lessen the effects of LPR in dogs.