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Cardio-Respiratory Medicine

Chaired by Mark Bowen

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08.30–08.45

Increase of Muc5ac and Muc5b glycoprotein in equine airway mucus accumulation and characterisation of Muc5ac and Muc5b from primary equine airway epithelial cells in culture

[†]Rousseau, K., [‡]Cardwell, J., [‡]Newton, R., [§]Clegg, P. and [†]Thornton, D.J.

[†]Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, M13 9PT, UK; [‡]Animal Health Trust, Lanwades Park, Kentford, Newmarket CB8 7UU, UK; and [§]Department of Musculoskeletal Biology, Leahurst Campus, University of Liverpool, Neston CH64 7TE, UK. Email: P.D.Clegg@liverpool.ac.uk

Aims: Horses are affected by respiratory problems which are associated with mucus accumulation. Young racehorses suffer from inflammatory airway disease and a major pathological problem is the accumulation of airway mucus leading to breathing difficulties and lower performance. In previous work, we identified Muc5b and Muc5ac as the major mucins in equine airway mucus (Rousseau *et al.* 2008). However, the mucin composition of accumulated mucus and the events leading to mucus accumulation are unknown. Our aims are to quantify mucins in accumulated mucus and to develop an air-liquid interface cell culture system for equine airway epithelial cells in order to investigate the effect of inflammatory cytokines which have been shown to be implicated in airway inflammation.

Methods: Mucins were quantified by Western blotting using Muc5b and Muc5ac specific equine antibodies after agarose gel electrophoresis. The cell culture system was developed using equine tracheas obtained from our local abattoir and the media reported by Oslund *et al.* 2010. **Results:** We have found that mucus accumulated in the airways of racehorses contains significantly higher amounts of both mucins compared to mucus from healthy horses and that Muc5b remains the most abundant mucin in these airway secretions. We have demonstrated that the trachea epithelial cells can be differentiated into ciliated and mucin producing cells. We have shown that these cells produce Muc5b and Muc5ac and that this system can be manipulated with disease relevant mediators and investigated the effect of such mediators on mucin amounts and properties. **Conclusion and practical significance:** These results indicate that both mucins are up-regulated, and that the cell culture system will be valuable to study mucin regulation. **Acknowledgements:** The project was funded by the Horse Betting Levy Board and The MRC.

References: Available on request from the author.

08.45–09.00

Equine multinodular pulmonary fibrosis in 6 horses

[†]Schwarz, B., [‡]Gruber, A., [‡]Benetka, V., [§]Schwendenwein, I., [#]Bezděková, B., [†]Sárdi, S. and [†]van den Hoven, R.

Equine Clinic, Section Internal Medicine; [†]Institute of Pathology and Forensic Veterinary Medicine; [‡]Clinical Virology and [§]Central Laboratory, University of Veterinary Medicine Vienna, Austria; [#]Equine Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; and [†]Clinic for Large Animals, Szent István University, Hungary.

Aims: Equine multinodular pulmonary fibrosis (EMPF) is a progressive fibrosing interstitial lung disease, which has been associated with γ -herpesviruses. This case series describes 6 horses with EMPF, which were PCR-positive for equine herpesvirus-5 (EHV-5). They presented between November 2008 and December 2010. **Methods:** Three horses, two 2-year-old fillies and a 22-year-old mare were subjected to euthanasia due to other diseases and diagnosed with EMPF at *post mortem*. EHV-5 DNA was identified in all cases by PCR. Two mares, an 8- and a 16-year-old, presented with dyspnoea and weight loss and were diagnosed with EMPF, but died despite treatment with corticosteroids. Furthermore a 22-year-old gelding was presented with recurrent pyrexia and dyspnoea, after intramuscular back infiltration with triamcinolone. The laboratory findings, the results of BAL (intranuclear eosinophilic inclusion bodies in macrophages), thorax radiographs and ultrasound, a positive EHV-5 PCR and lung biopsy were suggestive of EMPF. The horse recovered after one week of treatment with valacyclovir (40 mg/kg bwt t.i.d. *per os*) and was reported to be clinically healthy one year later.

Conclusions and practical significance: Aetiopathogenesis of EMPF is thought to be similar to human idiopathic pulmonary fibrosis (IPF), which is associated with Epstein Barr Virus (EBV), also a γ -herpesvirus. An inflammatory process seems to induce a dysregulated repair mechanism causing progressive pulmonary fibrosis and γ -herpesviruses might play a role in either initiating or exacerbating this process. The presumed predominance of TH2 cytokines in EMPF and IPF could be induced by EHV-5 and EBV, respectively, as both are reported to possess genes encoding for Interleukin 10-like protein. As in humans with IPF horses suffering from EMPF have not responded favourably to corticosteroids. Horses with EMPF have been treated with acyclovir with varying results in the past. To our knowledge this is the first report describing a case responding to treatment with valacyclovir.

09.00–09.15

Effect of exercise and lower airway inflammation on plasma levels of surfactant protein D

[†]Richard, E.A., [‡]Pitel, P.-H., [†]Christmann, U., [‡]Lekeux, P., [‡]Pronost, S. and [‡]Fortier, G.

Frank Duncombe Laboratory; 1 Route de Rosel, 14053 Caen Cedex 4, and IFR 146, ICORE - University of Caen Basse-Normandie, France; [†]CIRALE-ENVA; 14430 Goustranville, France; and [‡]Department of Physiology, Faculty of Veterinary Medicine - University of Liège, 20 Boulevard de Colonster Bat. B42, 4000 Liège, Belgium. Email: e.richard@cg14.fr

Introduction: Surfactant protein D (SP-D), mainly synthesised by alveolar type II cells and nonciliated bronchiolar cells, is one important component of innate pulmonary immunity. In humans, circulating concentrations of SP-D are routinely used as



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biomarkers for pulmonary injury. **Aims:** To investigate plasmatic SP-D concentrations at rest and after exercise in horses with inflammatory airway disease (IAD) and controls. **Methods:** Venous blood samples were collected from 42 trained Standardbred racehorses at rest and 60 min after performing a standardised treadmill exercise test. Tracheal wash and bronchoalveolar lavage fluid (BALF) samples were collected after exercise. According to BALF cytology, 22 horses were defined as IAD-affected (>10% neutrophils and/or >2% mast cells and/or >1% eosinophils) and 20 horses were classified as controls (normal BALF cytology). EDTA plasma was kept frozen until SP-D levels were assessed using a commercially available ELISA kit, and statistically compared between groups of horses and sampling times. **Results:** Plasma SP-D levels in IAD-affected horses were significantly higher than those of control horses, both at rest (mean \pm s.d.; respectively 72.2 ± 31.2 ng/ml vs. 27.0 ± 10.7 ng/ml) and after exercise (73.5 ± 37.1 vs. 26.2 ± 9.7 ng/ml). Within each group of horses (IAD and control), no significant effect of the treadmill test was noticed on SP-D levels; pre- and post exercise values were furthermore highly correlated ($r^2 = 0.975$; $P < 0.001$). No significant correlation was found between plasma SP-D concentrations and inflammatory cell percentages in either respiratory fluid. **Conclusions and practical significance:** This is the first study determining plasma SP-D concentrations in a noninfectious, naturally occurring form of lower airway inflammation in horses. The results highlight that IAD is associated with a detectable, though moderate, increase of circulating SP-D levels. This parameter could then be a potentially useful and readily accessible blood biomarker of equine lower airway inflammation.

09.15–09.30

Experimental challenge with equid herpesvirus-2 is associated with long-lasting inflammation of the intermediate airways

Fortier, G., Richard, E.A., Fortier, C., [†]Lemaitre, L., [†]Jas, D., Legrand, L. and [†]Thiry, E.

Frank Duncombe Laboratory, 1 Route de Rosel, 14053 Caen Cedex 4 and IFR 146, ICORE - University of Caen Basse-Normandie, France; [†]Meriel S.A.S., 29 Avenue Tony Garnier, 69007 Lyon, France; and [†]Department of Physiology, Faculty of Veterinary Medicine - University of Liège, 20 Boulevard de Colonster Bat. B42, 4000 Liège, Belgium.
Email: e.richard@cg14.fr

Introduction: While lower airway inflammation is of paramount importance concerning equine performance, a lack of knowledge relating to the possible implication of viruses as co-factors for syndrome of tracheal inflammation or inflammatory airway disease is currently still reported. **Aims:** To experimentally investigate the putative role of equid herpesvirus-2 (EHV-2) in respiratory diseases of adult horses and especially its involvement in airway inflammation. **Methods:** Four horses were respectively submitted to intra-nasal and intra-tracheal EHV-2 inoculation (LK strain). Corticosteroid treatment (dexamethasone) was performed before infection (0.2 mg/kg bwt; 3 consecutive days) and as reactivation stimulus 84 days post infection (1 mg/kg bwt; 3 days). Two other horses, used as controls, received dexamethasone prior to mock infection and were not submitted to a reactivation stimulus. Because of immunodepression, virus-specific PCR were systematically performed for all EHV-2. Clinical and endoscopic signs being exhibited were investigated, as was the association between

EHV-2 detection and modifications of cytological profiles. **Results:** Mild clinical signs, including tracheal hyperaemia and hyperreactivity were observed throughout both periods of the trial. EHV-2 shedding was observed in all horses (including controls) following corticosteroid treatment. Viral DNA (wild-type or reference strain) was detected in nasal swabs and respiratory fluids up to 21 and 14 days, respectively. Moderate to severe neutrophilia was transiently detected respectively in bronchoalveolar lavage and tracheal wash; while cytological evaluation furthermore revealed a significant association between EHV-2 detection and either concomitant neutrophilia or morphological abnormalities of the tracheal epithelial cells. **Conclusions and practical significance:** This study is the first trial reporting systematic respiratory fluids analyses over the course of an experimental EHV-2 infection, including both viral detection and cytological evaluation. Clinical and laboratory findings reproduced in this trial allowed experimental confirmation of EHV-2 being a possible co-factor of lower airway inflammation. EHV-2 should then probably be suspected and investigated in poorly performing horses.

09.30–09.45

Quantification of left ventricular function in horses with aortic valve insufficiency by tissue Doppler imaging and 2D speckle tracking

Decloedt, A., Verheyen, T., De Clercq, D. and van Loon, G.

Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium. Email: anelies.decloedt@ugent.be

Aims: Significant aortic regurgitation (AR) may cause left ventricular (LV) dilatation and eventually heart failure. The aim of this study was to quantify LV function in horses with AR by tissue Doppler imaging (TDI) and 2-dimensional speckle tracking (2DST). **Methods:** Echocardiographic examinations (GE Vivid 7 Pro) were performed on 10 healthy horses (10 ± 4 years; 509 ± 58 kg) and 14 horses with significant AR (17 ± 4 years; 497 ± 93 kg). Images were recorded from a right parasternal short-axis (TDI and 2DST) and long-axis view (2DST). By 2DST, global radial (SR) and longitudinal (SL) strain were measured. Regional systolic radial displacement (DRS) by 2DST and velocity (VS) by TDI were measured in the interventricular septum and LV free wall. LV end-diastolic internal diameter (LVIDd) and fractional shortening (FS) were measured from a short-axis M-mode image at chordal level. **Results:** Seven horses showed moderate AR (LVIDd range 11.0–12.7 cm), 7 showed severe AR (LVIDd range 13.3–16.9 cm). FS, SR and SL showed no significant differences. However, SL was significantly correlated with LVIDd in normal horses and horses with moderate AR ($r^2 = -0.72$, $P < 0.01$) but inversely correlated in horses with severe AR ($r^2 = 0.82$, $P < 0.05$), suggesting LV contractile dysfunction. Regional changes were present in the interventricular septum. Septal DRS and VS were correlated with LVIDd ($r^2 = 0.667$ and -0.778 , $P < 0.001$) and were increased in horses with moderate and severe AR compared to normal horses ($P < 0.05$). This indicates increased septal motion in horses with AR both with and without LV dilatation. **Conclusions and practical significance:** TDI and 2DST allow quantification of altered LV function due to AR. The prognostic value of these measurements remains to be determined.


Mean ± standard deviation of echocardiographic measurements

	Normal (n = 10)	Moderate AR (n = 7)	Severe AR (n = 7)
FS (%)	36.0 ± 2.7	39.7 ± 3.4	38.7 ± 3.6
Global SL (%)	-24.61 ± 1.50	-25.93 ± 1.13	-23.80 ± 3.15
Global SR (%)	62.80 ± 3.66	66.07 ± 3.74	66.04 ± 5.66
Septal DRS (mm)	17.39 ± 3.10	21.11 ± 4.18	27.14 ± 3.79
Septal VS (cm/s)	-4.35 ± 1.24	-8.32 ± 1.16	-10.75 ± 1.90

09.45–10.00

The importance of *Nicoletella semolina* for horses with respiratory disease
[†]Hansson, L., ^{}Johansson, K.-E. and [§]Riihimäki, M.**

[†]Department of Bacteriology, National Veterinary Institute, SE 751 89 Uppsala, Sweden; ^{**}Department of Biomedical Sciences and Veterinary Public Health; and [§]Department of Clinical Sciences, Section of Large Animal Surgery and Medicine, Equine Internal Medicine, Swedish University of Agricultural Sciences, Box 7054, SE- 750 07 Uppsala, Sweden. Email: Ingrid.Hansson@sva.se

Aims: A new member of the family Pasteurellaceae, *Nicoletella semolina* has been isolated from the respiratory tract of about 200 horses with clinical signs of respiratory disease. Sequence analysis of the 16S rRNA gene showed that *Nicoletella semolina* is closely related to species of the genera *Pasteurella*, *Haemophilus* and *Actinobacillus*. **Reasons for performing study:** To examine whether *Nicoletella semolina* is an emerging pathogen, opportunist or just a part of the normal bacteriological flora. **Methods:** Samples from a healthy control group were compared with samples from horses with a clinical history of respiratory disorders, obtained from the routine laboratory at SVA in Sweden. A total number of 1774 nose swabs and 1194 tracheal aspirates were collected and subjected to bacteriological examination. **Results:** *Nicoletella semolina* were isolated from 11 (5%) of the 207 samples from the nose swabs of the healthy control group, whereas from horses with respiratory disorder *Nicoletella semolina* were found in 42 (3%) of the 1567 samples. In the tracheal aspirates *Nicoletella semolina* was isolated from 7 (3%) of the 211 samples in the healthy control group, which should be compared with 52 (5%) of the 983 samples from horses with respiratory disease. The difference was, however, not significant. In the laboratory examinations, other bacteria were also isolated. The most commonly isolated bacterium was *Streptococcus zooepidemicus* which was isolated in 21% of the tracheal aspirates in the healthy control group and in 33% of the horses with respiratory disease. **Conclusions and practical significance:** *Nicoletella semolina* is an opportunist, because it is found both as pathogen and as commensal in horses. Presences of opportunistic or pathogenic bacteria in the respiratory tract do not always have to be treated with antimicrobial substances. **Acknowledgements:** The authors acknowledge Swedish-Norwegian Foundation for Equine Research for funding the study.

10.00–10.15

Can systemic markers of inflammation be used to diagnose hepatic inflammation in horses?
[†]Johns, I., [†]Stubbs C., [†]Durham, A. and [†]Smith, K.

[†]Equine Referral Hospital, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA; and [†]The Liphook Equine Hospital, Forest Mere, Liphook GU30 7JG, UK. Email: ijohns@rvc.ac.uk

Aims: To determine if systemic markers of inflammation correlate with biopsy-diagnosed inflammation in horses with hepatic disease. **Methods:** The records of 145 horses in which liver biopsy had been performed were retrospectively examined. Information obtained included values for systemic markers of inflammation as well as biopsy inflammation scores. Systemic markers of inflammation evaluated included total white cell count, serum amyloid A (SAA) concentration, fibrinogen concentration and globulin concentration. The degree of inflammation seen on biopsy was scored (either absent/mild; moderate; or severe) using a previously developed scoring system (Durham *et al.* 2003). **Results:** All horses had at least one systemic marker of inflammation recorded. Globulin concentration was measured in 133/145 (91%), total white cell count in 110/145 (82%), fibrinogen concentration in 119/145 (75%) and SAA in 50/145 (34%). There were 82 horses with either absent or mild inflammation, 54 with moderate and 9 with severe inflammation on the biopsy. There was no significant association between any of the systemic markers of inflammation and the degree of inflammation seen within the liver on biopsy. **Conclusions and practical significance:** Liver disease is a commonly diagnosed condition of horses. Diagnosis typically relies on a combination of testing, including biochemical analysis, ultrasonography and hepatic biopsy. A hepatic biopsy is considered the gold standard for diagnosing hepatic disease, and the results can be used to guide therapeutic options as well as prognosis. Based on the findings of this study, a diagnosis of hepatic inflammation is best made via biopsy, as systemic markers of inflammation are not typically increased despite evidence of hepatic inflammation. Further investigations including evaluation of other markers of inflammation may provide more information.

Reference: Available on request from the author.

10.15–10.30

Adrenocorticotrophic hormone in domestic donkeys - reference values, seasonality and association with laminitis
[†]du Toit, N., [†]Shaw, D.J. and [†]Keen, J.A.

[†]Veterinary Department, The Donkey Sanctuary, Sidmouth, Devon EX10 0NU; and [†]Royal (Dick) School of Veterinary Science, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK. Email: Nicole.dutoit@thedonkeysanctuary.com

Aims: To determine reference values and seasonality of adrenocorticotrophic (ACTH) in donkeys and any associations with age, sex, laminitis and obesity. **Methods:** Blood samples were collected from 422 donkeys during routine clinical evaluation of new relinquishments to The Donkey Sanctuary, UK and ACTH was measured by chemiluminescent immunoassays. Age, sex, body condition score (BCS; /5), history of previous laminitis and clinical examination findings were recorded. Donkeys were divided into 4 groups: not obese not laminitic (NONL- reference group; 277), obese not laminitic (ONL; 86), not obese previously laminitic (NOPL; 28) and obese previously laminitic (OPL; 31). Kruskal-Wallis and Mann-Whitney tests were used to determine associations of ACTH to different groups, seasonality and sex, and regression analyses to look at ACTH against age. **Results:** The median age was 10 years (range 0.5–38). Of 422 donkeys, 14% had a history of previous laminitis and 27.7% were obese (BCS>3.5). In NONL donkeys (24.7 [21.3–24.7]) there was no significant relationship between ACTH and age (P = 0.43), but ACTH values were significantly higher in geldings than females (P = 0.03). There was a distinct seasonality in ACTH, with significantly lower values in

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November to June (median = 17.8 [IQR = 16.5–19.5]) than July to October (37.9 [28.9–36.9]) in NONL donkeys ($P < 0.001$). This seasonality was significant in all groups ($P < 0.003$), except NOPL. OPL donkey ACTH values (34.8 [24.3–42.9]) were significantly greater than in NONL and ONL (17.7 [16.6–23.3]) ($P \leq 0.03$) donkeys. NOPL donkey ACTH values (31.4 [24.9–47.9]) were significantly greater than in ONL donkeys ($P = 0.013$).

Conclusions: The reference range for donkey ACTH values have been established (24.7 [21.3–24.7]), and a distinct seasonality has been demonstrated. A history of previous laminitis, but not clinical evidence of obesity, was associated with higher ACTH values.

Practical significance: This study has determined values for ACTH in donkeys with no history of laminitis or clinical signs suggestive of PPID (NONL).

NOTES
