



Cardio-Respiratory Medicine

Chaired by Mark Bowen

Sponsored by University of Liverpool



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

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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

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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

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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

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

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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

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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 \pm standard deviation of echocardiographic measurements

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

09.45–10.00

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

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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.**

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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.**

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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



Epidemiology

Chaired by James Wood

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13.20–13.35

A cross sectional study assessing the current prevalence of trypanosomiasis in the equid population of the central river region of The Gambia

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Aim: In 2006 a cross sectional study of the equid population of the Gambia indicated a high prevalence of trypanosomiasis (Pinchbeck *et al.* 2008). The relative prevalence of different species of trypanosomes and their association with clinical parameters was assessed. A recent outbreak of cerebral trypanosomiasis in the equid population of the Gambia caused by *Trypanosoma brucei* (Peachey *et al.* 2010) suggests that the epidemiology or virulence of this disease is changing. A repeat of this cross sectional study was performed in 2009 to assess this.

Materials and methods: A total of 241 horses and donkeys were sampled at clinics and local markets in the central river region of The Gambia. Blood was preserved on FTA cards (Whatman, UK) and clinical parameters were recorded for each individual. Polymerase chain reaction (PCR) for *Trypanosoma congolense* was performed on all 241 samples and for *Trypanosoma brucei* on a subset of 70.

Results: The prevalence was calculated at 15% for *T. congolense* and 45% for *T. brucei*. Infection with *T. congolense* was significantly associated with pale mucus membranes ($P = 0.04$) and a depressed demeanour ($P = 0.03$). The current prevalence was compared with 2006 values which were 31% for *T. congolense* and 18% for *T. brucei*. This work is ongoing; a complete dataset for all species of trypanosome will be presented. **Conclusion and practical significance:** The results thus far indicate that the prevalence of *T. congolense* and *T. brucei* has decreased and increased, respectively since 2006. In addition *T. congolense* is no longer significantly associated with a low packed cell volume, as was previously found. These data suggest that the epidemiology and pathogenicity of equid trypanosomiasis in The Gambia is changing, this may be associated with the sudden increase in neurological disease in affected animals.

Acknowledgements: RCVS Trust, Gambia Horse and Donkey Trust.

References

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Pinchbeck, G., Morrison, L., Tait, A., Langford, J., Meehan, L., Jallow, S., Jallow, J., Jallow, A. and Christley, R. (2008) Trypanosomiasis in The Gambia: prevalence in working horses and donkeys detected by whole genome amplification and PCR, and evidence for interactions between trypanosome species. *BMC Vet. Res.* **4**, 7.

13.35–13.50

Frequency and speculated causes of equine laminitis in Great Britain

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Aims: To determine the frequency of equine laminitis in Great Britain (GB) reported by veterinary surgeons and to determine owner-suspected causes of the disease. **Methods:** A prospective cohort of horses/ponies under the care of a geographically representative sample of 28 veterinary practices was studied. Veterinarians reported each case of active laminitis by completing a clinical signs checklist ('laminitis reporting form', LRF). Monthly denominator data on the number of horses registered to and attended by the veterinary practices were obtained from a subset of 15 practices. Owners who participated in a nested case-control study of risk factors for laminitis were sent a list of speculative causes of laminitis and were asked to select one option. **Results:** Five-hundred-and-sixteen LRFs were received between March 2009 and January 2011, with case numbers peaking in May and June 2009, January 2010 and May and June 2010. The frequency of veterinary-diagnosed laminitis was 0.75% (95% CI = 0.66–0.84%) in the registered population (275 LRFs; 36,792 horses) and 0.36% (95% CI = 0.32–0.40%) in the attended population (299 LRFs; 82,543 horse visits). Forty-seven responses were received from case owners (48.5% response rate). The most common speculated cause of laminitis was grazing on lush pasture (29.8%), followed by equine Cushing's disease (21.3%), obesity (12.8%) and equine metabolic syndrome (8.5%). Other speculated causes were farrier, contralateral limb lameness, grain overload, cold weather, less exercise, chronic obstructive pulmonary disease and plant poison. Speculated causes not selected by respondents were colic, diarrhoea, drug administration or foaling complications. **Conclusions and practical significance:** Laminitis occurred in <1% of the horse population studied, considerably lower than previously published frequency estimates. Laminitis was most frequent in the spring and winter, consistent with previous epidemiological research. The most common speculated causes indicate that endocrinopathic laminitis may be of major importance in GB. **Acknowledgements:** This project is funded by World Horse Welfare.

13.50–14.05

Assessment of the impact of a charity training programme on health of working horses in Lesotho

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Aims: To assess the impact of an equine charity's farriery, saddlery and nutrition training programme in Lesotho on horse health and owners' knowledge and husbandry practices. **Methods:** One survey (S1) was undertaken before the first training programme began (April–June 2007) and one (S2) 20 months after its completion (August–October 2009). Randomly selected horses (312 and 245 in S1 and S2, respectively) underwent a structured

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clinical examination; blood and faecal samples were obtained for haematology, biochemistry and worm egg counts, respectively. Tack was assessed for condition, cleanliness and fit, following a standardised protocol. Owners were interviewed using a pretested questionnaire administered face-to-face in local language. Differences between survey findings were assessed using Chi-squared and *t* tests. **Results:** Most horses provided ridden transport (S1: 79% vs. S2: 91%). Frequency of forefoot shoeing increased (S1: 14% vs. S2: 29%, *P* = 0.02) but frequency of overgrown forefeet horn was unchanged (S1: 45% vs. S2: 42%). Owners appreciated enhanced skills of trained farriers but reported poor affordability of shoeing. Tack availability, condition and cleanliness remained suboptimal but bridle fit improved (*P* = 0.03). Widespread tack-associated injuries (S1: 58% vs. S2: 78%) and pain on spinal palpation (S1: 53% vs. S2: 72%) persisted; injuries were seen most frequently on spine and withers. Mean body condition score remained suboptimal (S1: 2.5 vs. S2: 2.1) and many owners (S1: 62% vs. S2: 41%) recognised their animal's diet was unbalanced. Approximately one-fifth of horses had low red blood cell counts (S1: 21% vs. S2: 17%). Strongyle infestation was endemic (S1: 88% vs. S2: 89%) and most horses had ticks (S1: 59% vs. S2: 76%). **Conclusions and practical significance:** Although some positive impact has been achieved, key equine health issues remain. Since Lesotho is ranked low (141st/169) on the United Nations' Human Development Index, a sustained intervention period may be required before improvements are seen. Results could aid in selecting topics for future community-based interventions. **Acknowledgements:** World Horse Welfare; Lesotho Department of Livestock.

14.05–14.20

Rates of horse passport compliance as assessed by Local Authority inspections conducted in Great Britain between 2005 and 2010

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Aims: To investigate horse passport noncompliance rates in Britain from 2005–2010 using data from Local Authority horse passport checks; with noncompliance defined as missing passports or passports containing inaccurate information. **Methods:** Data on numbers of passports checked and proportions of these that were compliant, missing or contained inaccurate information were requested for the period 2005–2010 via the Freedom of Information Act from 202 Local Authorities across Britain. Spatial and/or temporal variations in passport checking and noncompliance rates were described. **Results:** Of 202 Local Authorities, 100 (49.5%) checked horse passports and recorded the data, 62 (30.7%) did not check horse passports and the remainder, although they checked passports, data were not stored in an accessible format. Between 2005 and 2010 there were annual increases in numbers of Local Authorities conducting horse passport checks and numbers of checks conducted. Geographical variations in levels of checking and rates of passport noncompliance were seen, with East Anglia having the highest noncompliance rate (25.1%), and the North West the lowest (1.6%). In the period 2005–2010 17,048 individual passports were checked by 64 Local Authorities. Of these, 1558 (9.1%) were

noncompliant, with 963 (5.6%) containing inaccurate information and the remaining 595 (3.5%) being missing passports. A further 12 authorities checked 2772 passports, but recorded individual numbers of noncompliant passports, therefore compliant and noncompliant figures did not equal the denominator data. **Conclusions and practical significance:** A significant proportion of equine passports in Britain were found to be missing or did not comply with statutory regulations. Some caution is required in interpreting these particular data; however, they do suggest that collated passport data in the National Equine Database may contain significant inaccuracies. **Acknowledgements:** This project is funded by the Horserace Betting Levy Board.

14.20–14.35

Equine lameness: Incidence, causes, outcomes and risk factors in a working horse population

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Aims: Lameness is a common equine health problem, yet surprisingly, information regarding UK lameness incidence is restricted to studies of racing or performance horses, and referral hospital populations. This study investigated lameness in a working horse population over a prospective one year period. Study objectives were to determine overall lameness incidence, identify common causes and possible risk factors of lameness, and investigate the incidence, duration and outcome of conditions observed. **Methods:** Lameness questionnaires were distributed to an equine army establishment, and lameness cases recorded. Basic population information was requested for every horse, and further information relating to the nature and outcome of lameness episodes recorded for horses experiencing lameness. Descriptive and univariable statistical (Chi-square/2-sample *t* test, *P*<0.05) analyses were performed. **Results:** Questionnaire response rate was 93%; data for 273 horses were analysed. Overall lameness incidence was 23.3 cases per 100 horses per annum. Mean monthly incidence was 2.12% and a mean of 1.23 lameness episodes per horse in the lame population observed. The most common diagnoses were cellulitis/lymphangitis (18.6%), skin wounds (16.3%) and foot/shoeing problems (11.6%). Other diagnoses included tendon/ligament injuries, arthritis, foot abscesses, muscle bruising and exertional rhabdomyolysis. Age, years of army service and height were similar between lame and nonlame horses. Similar incidence in each work type category was observed. Recent change of rider was reported in 70.6% of cases, and recent exercise/management change in 17.6%. **Conclusions and practical significance:** This study showed a different distribution of lameness causes compared to other studies; however, there are no studies in similar populations, or using similar methodology, for comparison. Type and workload of horses in this study are similar to some of the general equine population, therefore findings from this study may have relevance to the general horse population. **Acknowledgements:** Defence Animal Centre, Household Cavalry Mounted Regiment, Royal Army Veterinary Corps.

14.35–14.50

An investigation of risk factors associated with superficial digital flexor tendon strain in hurdle racing in the UK

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Reasons for performing study: Tendon injuries are common in Thoroughbred racehorses. The risk of tendon injury has been shown to be greater in jump than in flat racing in the UK. The factors associated with this altered risk have not previously been investigated. **Aims:** To identify risk factors for superficial digital flexor tendon (SDFT) strain in Thoroughbred horses racing over hurdles. **Methods:** A retrospective analysis of records from horses running in all hurdle races in the UK between 2001 and 2009 identified cases diagnosed with a strain of the SDFT whilst still at the racecourse. A total of 1031 case starts and 168,637 control starts were then used for univariable and multivariable logistic regression to identify risk factors for SDFT strain at any one start. The potential effect of clustering within horse, race, racecourse, trainer and jockey was also examined by including each of these variables as random effects in mixed-effects models. **Results:** In the final multivariable model several statistically significant risk factors were identified, including: firmer going, increased horse age, having had a previous SDFT injury, having previously run in a flat race and racing in the summer compared to other seasons; all resulting in increased odds of sustaining an SDFT strain. Conversely, being trained by a more successful trainer and having run at least once in the previous 90 days resulted in decreased odds of SDFT strain. **Conclusions:** The risk factors identified provide important information about the risk of SDFT injury. Multiple avenues for further investigation are highlighted, including unmeasured variables at the level of the racecourse and trainer. **Practical significance:** The results of this study will direct future research and shape the development of interventions to minimise the risk in hurdle starts in the future. **Acknowledgements:** The project is funded by the Horserace Betting Levy Board.

14.50–15.05

Associations between exercise and joint injury in Thoroughbred racehorses in training

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Aim: To identify risk factors for carpal and metacarpal/tarsophalangeal (MCP/MTP) joint injury occurrence and/or progression in Thoroughbreds in flat race training, particularly in relation to exercise regimens. **Methods:** In a prospective cohort study, young Thoroughbreds entering training in 13 training yards throughout England were monitored for up to 2 years. Daily exercise records and information on veterinary-diagnosed carpal and MCP/MTP injuries and treatments were collected. Injuries were allocated to one of 4 categories: 1) localised to a carpal or MCP/MTP joint based on clinical examination and/or use of diagnostic analgesia, no diagnostic imaging performed (n = 21); 2) localised to a carpal or MCP/MTP joint, diagnostic images taken but no abnormalities detected (n = 21); 3) evidence of an abnormality of subchondral bone and/or articular margin(s) identified using diagnostic imaging (n = 72) and 4) evidence of

discontinuity of the articular surface identified by diagnostic imaging (n = 70). Cox regression analyses were performed to identify risk factors for injury type and category and injury progression. **Results:** Six-hundred-and-forty-seven horses spent 7785 months at risk of joint injury and 184 injuries were recorded (82 carpal, 102 MCP/MTP injuries). Increasing distances cantered in short periods (daily or weekly) were associated with decreasing risk of any joint injury, injuries in Categories 1, 2 and 3 and MCP/MTP injuries. Category 4 injury risk increased with greater distances cantered in 30 days. Risk of Category 1 injury increased with increasing distances of weekly high-speed exercise. Risk of MCP/MTP injury increased with accumulation of canter or high-speed exercise since entering training. Risk of joint injury progression increased with increasing exercise distances in short time periods. Injuries treated with medication were more likely to progress to a more severe injury. **Conclusions and practical significance:** Modifications to exercise regimens could reduce joint injury occurrence. **Acknowledgements:** Participating trainers and veterinary surgeons, the Horserace Betting Levy Board.

15.05–15.20

Equine grass sickness surveillance in Great Britain from 2000 to 2011: Incidence and aspects of disease epidemiology on affected premises

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Aims: To describe incidence rates and epidemiological aspects of Equine Grass Sickness (EGS) on British premises affected since 2000. **Methods:** A nationwide surveillance scheme using postal and online questionnaires collected retrospective premises-level and prospective case-level information for EGS cases occurring in Great Britain between January 2000 and January 2011. Nonparametric methods were used to test the statistical significance of differences in continuous measures between categories of binary/categorical variables. **Results:** A total of 1517 cases were reported from 1246 locations. There were 249 'recurrent' premises that either reported a history of previous cases or reported multiple cases to the surveillance scheme (median = 2 cases; range = 1–9 cases). Median incidence was not significantly different on 'recurrent' compared to 'nonrecurrent' premises (2.3 cases/100 horse-years-at-risk, and 1.8 cases/100 horse-years-at-risk, respectively, P>0.05). Clinical presentation was recorded for 1324 EGS cases, of which 46.6% (n = 617) were classified as acute, 20.4% (n = 270) as subacute and 33% (n = 437) as chronic. Compared to acute/subacute cases, horses with chronic EGS had been resident for a significantly longer period on both affected premises (median 365 days and 730 days respectively; P = 0.05) and affected paddocks (median 60 days and 90 days respectively; P = 0.009). Total size of premises, paddock size, total number of horses on premises and number of horses per paddock were greater in 'recurrent' premises, compared to premises reporting single EGS cases (all P<0.001). **Conclusions:** Chronic EGS was associated with longer time spent on affected premises and paddocks compared to acute or subacute EGS, which may be consistent with acquisition of partial immunity. Larger premises and those with greater numbers of horses were more likely to report recurrence of EGS. **Practical significance:** Estimates of and factors associated with EGS incidence on premises in Great Britain will be important in developing detailed protocols for future intervention studies such as *Clostridium botulinum* vaccine trials.



General Medicine

Chaired by Mark Bowen

Sponsored by University of Liverpool



15.50–16.05

Role of thymic stromal lymphopoietin (TSLP) in equine allergic diseases

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Aims: Allergic horses react to innocuous environmental substances by exaggerated immune responses. Hallmarks of allergic inflammation are activation of Th2 cells and production of allergen-specific IgE antibodies. Mechanisms involved in Th2 differentiation are not well understood. In other species, thymic stromal lymphopoietin (TSLP) plays a central role in this process. Our aim was to study the role of TSLP in equine allergic diseases. **Methods:** By quantitative RT-PCR we assessed the expression of TSLP in the skin of horses with insect bite hypersensitivity (IBH) and chronic urticaria, and in bronchoalveolar lavage cells of horses with recurrent airway obstruction (RAO). In RAO-affected horses TSLP expression was assessed before and after 30 day exposure to hay. In all these experiments, samples from healthy horses were included as controls. **Results:** TSLP expression was higher (* $P < 0.05$) in lesional skin of 21 IBH-affected horses compared to skin of 10 healthy horses. Urticaria-affected horses ($n = 8$) had also increased expression of TSLP in lesional skin compared to healthy horses ($n = 8$) (* $P < 0.05$). In addition, lesional samples had higher TSLP expression than nonlesional samples taken from the same urticaria-affected horse (* $P < 0.05$, average 46-fold increase). Expression of TSLP in BAL cells isolated from 7 RAO-affected horses after 30 day exposure to hay was significantly higher compared to 8 healthy control horses treated the same way (** $P < 0.01$). In search for possible cellular sources of equine TSLP we found that peripheral blood leucocytes (most likely basophils) upregulate TSLP expression after IgE-crosslinking by allergen or anti-IgE antibody. Primary keratinocytes and bronchial epithelial cells also increased TSLP expression after *in vitro* stimulation with allergens. **Conclusions and potential relevance:** Our findings indicate that TSLP may play an important role in the pathogenesis of equine allergic diseases and could be a potential target for future therapies.

16.05–16.20

Examining the between horse variability in reduction of worm burden using intensively sampled faecal worm egg counts

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Aims: To quantify the effect of performing multiple faecal samples per animal on the accuracy of the faecal egg count reduction test. **Methods:** Intensive faecal sampling using a modified McMasters procedure was performed before and after treatment with pyrantel embonate for 10 horses from the same premises. A total of 10,000 bootstrapped FEC reductions were calculated for several different combinations of numbers of repeats used (between one and 10) and number of McMaster chambers used (between one and 10). Standard errors were produced for each. **Results:** Increasing the number of repeats used had a greater impact on reducing the standard error than increasing the number of chambers. The standard error and probability of falsely declaring resistance was very high for this dataset when using one repeat and 2 chambers, as is commonly used in practice. **Conclusions and practical significance:** This study suggests that due to the large variation in faecal egg counts the standard practice for assessing the efficacy of an equine anthelmintic may be very inaccurate. Performing multiple faecal samples from each individual provides an estimate with much higher confidence. As a result of this study we recommend that in order to accurately calculate a FECR for small groups of horses, 5 faecal samples should be tested from each animal and 6 McMaster chambers read for each sample. This would reduce the standard error substantially, for a moderate increase in the laboratory effort for each individual. This recommendation has particular relevance to current equine clinical practice due to the increasing prevalence of anthelmintic resistance. **Acknowledgements:** James McGoldrick, Callum Wright, E and O Group Laboratories, Moredun Research Institute (joint funding).

16.20–16.35

Accidental lasalocid intoxication on a farm with eighty-one horses

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Aims: Ionophores are known to be very toxic to horses. Our aim is to describe short- and long-term effects of accidental lasalocid intoxication in horses. **Methods:** On a farm with 81 horses, 14 horses showed clinical signs and 2 died shortly after a new batch of concentrates had been fed. Seven horses were presented at the department. Clinical examination, serum biochemistry and cardiac examination were performed. Six horses died. The remaining 72 horses on the farm underwent echocardiography, electrocardiography at rest and during exercise and plasma cardiac troponin I determination. Toxicological analyses were performed. **Results:** Initial clinical signs in 14 horses at the farm were anorexia, lethargy and profuse sweating. Seven horses presented at the clinic showed signs of weakness, cardiac arrhythmias ($n = 7$) and myocardial contractile dysfunction ($n = 5$; fractional shortening 5–24%; decreased radial, circumferential and longitudinal strain). Cardiac troponin I ranged between 1.39 and 816 ng/ml (median 88 ng/ml). Despite antiarrhythmic treatment, one horse died of ventricular



fibrillation 24 h after presentation. Six weeks after the initial signs, mild to severe ataxia and paresis were found in 4 horses due to delayed neuropathy, which gradually normalised after 3 and 9 months in 2 horses. Also in asymptomatic horses, increased cardiac troponin I and arrhythmias were found (0.11–2.01 ng/ml). Toxicology of 2 livers showed 0.5 ppb lasalocid in both. The initial feed on the farm was no longer available for analysis. **Conclusions:** Lasalocid intoxication led to acute signs of cardiomyopathy with arrhythmias, severe contractile dysfunction and death. At a later stage, neuropathy led to ataxia or decubitus, requiring euthanasia in some cases. Long-term cardiac and neurological signs were found. **Practical significance:** Although very uncommon, lasalocid intoxication should be included as a differential diagnosis for unexplained anorexia, depression, cardiomyopathy and ataxia.

16.35–16.50

Evaluation of the efficacy provided by a recombinant canarypox-vectored equine West Nile virus vaccine against virulent challenge with a lineage 2 virus in horses

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Aims: The purpose of this study was to determine the level of cross protection provided by the recombinant ALVAC®-WNV vaccine in a severe lineage 2 WNV challenge model. **Methods:** Horses with no prior exposure to WNV were randomly assigned to one of 2 groups of 10 horses each. *Group 1* horses were vaccinated twice i.m. with ALVAC-WNV on Days 0 and 29. *Group 2* horses received 2 doses of carbomer at the same time and served as negative controls. On Day 43, all horses were challenged intrathecally with a neurovirulent lineage 2 strain of WNV. Serological and clinical responses and WNV isolation were monitored for 21 days after challenge. **Results:** Following challenge, 10 out of 10 controls became viraemic and 9 out of 10 developed WNV disease (3 were subjected to euthanasia for humane reasons). In contrast, none of the vaccinated horses developed WNV disease and only one vaccinated horse became viraemic. The incidence of WNV disease and viraemia were significantly lower in the vaccinated than in the control horses ($P < 0.0001$ for both). At necropsy, the proportion of control horses showing histopathological changes in the brain and/or the spinal cord (10 out of 10) was significantly higher ($P = 0.0031$) as compared to the vaccinated horses (3 out of 10). Neutralising antibodies against both lineages of WNV were seen in all vaccinated horses after vaccination and in all controls after challenge. **Conclusions:** Results from this study demonstrated that the ALVAC-WNV vaccine was effective in protecting naïve horses against viraemia and clinical disease after inoculation with a lineage 2 WNV. **Practical significance:** To our knowledge, this is the first study to demonstrate cross protection provided by a WNV vaccine against a contemporary lineage 2 WNV isolate and may provide veterinarians with a tool to control infections caused by lineage 1 and 2 strains of WNV. ©All marks are the property of their respective owners.

16.50–17.05

Factors associated with mortality of geriatric horses in the United Kingdom

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Aims: To describe mortality rates, causes of death/euthanasia and factors associated with mortality in geriatric horses. **Methods:** Veterinary registered horses (aged ≥ 15 years) randomly selected for a cross-sectional survey on geriatric health were enrolled in this cohort study ($n = 908$). Follow-up information was obtained over an 18 month period via telephone questionnaires. Mortality questionnaires, providing data regarding reasons for death/euthanasia, were completed for 118 cases of mortality. Overall and stratified mortality rates were calculated and Cox proportional hazards models were used to investigate risk factors associated with mortality. **Results:** The majority of horses (94%) were subjected to euthanasia, most frequently due to lameness (24%) and colic (21%). Veterinary advice was important in owner decision making regarding euthanasia of cases of colic or other acute illnesses, while poor quality of life was an important factor where euthanasia was due to chronic diseases or lameness. Overall mortality rate was 11.1 (95% CI 9.2–13.2) per 100 horse-years at risk, with the mortality rate of animals >30 years of age over 5 times the rate in horses aged 15–19 years. Factors associated with an increased risk of mortality included increasing age; cob/cob crossbreeds and Thoroughbred/Thoroughbred crossbreeds; horses considered to be underweight; increasing number of owner-reported clinical signs and the degree to which pain was reported to limit normal daily activities. **Conclusions:** Mortality rates increased with increasing age in geriatric horses. The most common reasons for euthanasia were lameness, colic and chronic illness, and the study has identified a potential requirement for increased veterinary involvement in decision making for euthanasia due to chronic disease. **Practical significance:** This study provides useful information about rates and factors associated with mortality in geriatric horses in the UK, which should be relevant to veterinary surgeons involved in the treatment of aged horses. **Acknowledgements:** This study was generously funded by the Horse Trust.

17.05–17.20

Exploratory survey on acid-base derangements on admission in horses suffering from atypical myopathy

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Aims: 1) To describe acid-base status in horses with atypical myopathy (AM) on admission; 2) determine prognostic indicators; and 3) attempt to explain derangements by linear correlations with indicators of circulatory collapse, respiratory rate and creatine kinase activity (CK). **Methods:** This retrospective study includes 34 AM cases and 15 healthy control horses. Case records were



searched for venous blood gas analysis, electrolytes, total protein, [lactate], packed cell volume (PCV), urea (BUN), CK and heart and respiratory rate on admission. Base excess (BE) due to free water (BE_{fw}), chloride (BE_{cl}), total protein (BE_{tp}), and unidentified anions (BE_{ua}), anion gap (AG) and measured strong ion difference (SID_m) were calculated and shock grades (Grulke *et al.* 2001) were assigned. Statistical analysis compared these variables in AM cases vs. controls and AM survivors vs. AM nonsurvivors. Linear regression was used to explore relationships between acid-base variables and shock grades, respiratory rate and CK activity.

Results: Affected horses often had metabolic acidosis, combined with either respiratory alkalosis or respiratory acidosis. Electrolyte abnormalities and increased AG and [lactate] were common. The pH, pCO₂, HCO₃⁻, BE_{fw}, BE_{ua}, [sodium] and [chloride] were significantly lower in cases compared with controls, and [lactate], AG, SID_m and BE_{cl} significantly higher. Survivors had lower PCV than nonsurvivors. BE_{cl}, Cl⁻ and PCV had strong discriminatory power between survivors and nonsurvivors. BE_{cl}, BUN and heart rate were used to develop an optimal discriminatory tree for survival. There was a positive linear correlation between [potassium] and shock grades and respiratory rate. HCO₃⁻ was negatively correlated to CK, whereas AG and BUN had a positive linear correlation with CK. **Conclusions and practical significance:** The majority of AM cases commonly had acid-base alterations: specifically, hypochloaemic alkalosis, hyponatraemic acidosis, lactic acidosis and respiratory alkalosis or acidosis. These data are clinically important to assess prognosis and will help to refine fluid therapy in individual cases.

Reference

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17.20–17.35

CLCN1 nonsense mutation in a herd of New Forest Ponies associated with congenital myotonia

NOTES

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Aims: To determine the genetic cause of myotonia in a 3-month-old New Forest colt by analysing the *CLCN1* gene as candidate. *CLCN1* mutations cause similar myotonia phenotypes in different mammalian species. **Methods:** EDTA blood of a single patient showing myotonia, its mother, its father, a maternal and 5 paternal half-sibs, 16 related NFP and 21 nonrelated NFP, and 56 horses from different breeds were examined. Total RNA extracted from 4 different skeletal muscles of the affected horse was subjected to reverse transcription-PCR (RT-PCR) to analyse the entire coding sequence of *CLCN1* transcript. All exons and partially introns of the equine *CLCN1* gene were amplified and sequenced using DNA samples from the affected case, as well as a single unrelated control horse. **Results:** The affected horse was homozygous for a nonsense mutation in *CLCN1* exon 15 (c.1775A>C). RT-PCR showed no aberrant splicing of *CLCN1*. The mother, the father, 5 paternal half-sibs, and 2 more distantly related horses were heterozygous 1775A/C. All other relatives and all control horses from NFP and other breeds were homozygous for the wildtype 1775A allele. **Conclusions:** The consequence of the c.1775A>C mutation is the substitution of aspartic acid for alanine in codon 592 of the equine *CLCN1* protein. This amino acid position is highly conserved across mammalian orthologues. Therefore, we identified a horse showing clinical symptoms of congenital myotonia with an associated nonsense mutation of the equine *CLCN1* gene. **Practical significance:** Our finding enables direct genetic testing and the eradication of this genetic disease from the New Forest Pony breeding population. **Acknowledgements:** The cooperating NFP breeders.