

CLINICAL RESEARCH

Neurology and
Spinal Diseases
and Ophthalmology

Chaired by Matthew Smith

Sponsored by University of Liverpool



UNIVERSITY OF
LIVERPOOL

8.30-8.45

Transcranial electrical stimulation (TES)
as a possible novel alternative to
transcranial magnetic stimulation (TMS)
to assess the motor function of the spinal
cord for clinical diagnosis in horses

[†]Journée, S.L., [‡]Delesalle, C.J.G., [§]de Bruijn, C.M.,
[#]Bergmann, W. and [¶]Journée, H.L.

[†]Equine Diagnostics, Burdaard, The Netherlands; [‡]Ghent University, Faculty of Veterinary Medicine, Department of Large Animal Internal Medicine, Belgium; [§]Wolvega Equine Clinic, Wolvega, The Netherlands; [¶]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands; [#]University Medical Center, Department of Neurosurgery, Groningen, The Netherlands. Email: sjournee@hotmail.com

Reasons for performing study: To introduce and assess the feasibility of multipulse transcranial electrical stimulation (TES) in horses. **Objectives:** To assess latency times of muscular motor evoked potentials (MEP) in the *m. extensor carpi radialis* (ECR) and the *m. tibialis cranialis* (TC), elicited by transcranially applied multipulse stimulation in 12 healthy horses. **Study design:** Prospective observational study applied in 12 healthy horses. **Methods:** Horses with a mean age of 11.0 (range 3.6–20.5) years and a height of 160 (s.d. ± 10.0) cm were studied. Horses were sedated with detomidin (1.5 µg/kg bwt) and butorphanol (1.5 µg/kg bwt) i.v. A subcutaneous ring block with lidocaine 2% + adrenaline was placed on the forehead. TES was performed using biphasic high frequency multipulse voltage trains (pulse width: 0.1 ms, interpulse interval: 1.3 ms, 3 pulses/train) applied to 2 subcutaneous needle electrodes bilateral from the centre of the forehead. Latency times (30 V above motor threshold (MT) and amplitude of MEP were recorded bilaterally at the ECR and the TC muscles. Motor latency times are expressed as mean ± 2 s.d. **Results:** Mean latency times (at MT + 30V) for the ECR muscles were respectively 20.18 ± 1.85 ms (left side) and 19.7 ± 1.69 ms (right side) and for the TC muscles respectively: 34.6 ± 2.01 ms (left) and 34.9 ± 2.43 ms (right). **Conclusions:** TES is well tolerated. Interestingly, recorded motor latency times at the level of front and hind legs appear to be shorter for TES when compared to TMS from literature data [1]. **Ethical animal research:** Ethical approval was granted by the animal ethics committee of the Groningen University. **Sources of funding:** JS Center and Wolvega Equine Clinic. **Competing interests:** None.

Reference

1. Nollet, H., Deprez, P., Van Ham, L., Dewulf, J., Declair, A. and Vanderstraeten, G. (2004) Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. *Equine Vet. J.* **36**, 51-57.

08.45-09.00

Prevalence of osseous pathology in
the articular process articulations in
the equine cervical and cranial thoracic
vertebrae

Rombach, N., Stubbs, N.C. and Clayton, H.M.

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48910, USA. Email: office@equinenergy.com

Reasons for performing study: Osteoarthritis (OA) of the articular processes (APs) is recognised as a clinical condition in the equine cervical spine, but there is little information on the prevalence and distribution of OA in the APs of the cervical and cranial thoracic vertebrae. **Objectives:** To determine the prevalence and distribution of OA in the APs of the equine cervical and cranial thoracic vertebrae in relation to vertebral level, age and size of the horse and side of the neck. **Study design:** A *post mortem* longitudinal randomised study of 53 horses. **Hypotheses:** OA is more prevalent and more severe in the APs of the cervicothoracic junction, the prevalence and severity of OA increases with horse age and size, and OA is equally distributed on left and right sides. **Methods:** The cervical (C1–C7) and cranial thoracic (T1–T7) vertebrae of 53 horses were removed at necropsy and boiled out. Based on the size (percentage) of the joint margin that was affected, OA of the 4 APs of each vertebra was graded on a scale of 0 (no osseous lesions) to 3 (severe osseous lesions). Based on these grades, a 3-factor ANOVA was used to test the random effects of horse, age (young, old) and size (small, large), and the fixed factors of side (left, right) and vertebral level (C1 to T7). **Results:** OA lesions were most severe in the mid-cervical vertebrae (C3–C4) followed by the cervicothoracic curvature (C5–T1). Severity of OA increased with age and size of the horse but there was no difference between left and right sides. **Conclusions:** OA is symmetrically present with higher severity in the mid-cervical and cervicothoracic regions and with higher prevalence in older and larger horses. These factors support bilateral injections in specific APs for clinical treatment of OA in the equine cervical spine. **Ethical animal research:** Approval for this study was obtained under Institutional Animal Care and Use Committee number 02-11/020-00. Explicit owner informed consent for participation in this study was not stated but general permission for *post mortem* examination was given. **Sources of funding:** Supported by the CVM Endowed Research Funds and the McPhail Endowment at Michigan State University. **Competing interests:** None.

09.00-09.15

Latero-oblique radiography as a
diagnostic tool for equine cervical
osteoarthritis

Tambaschi, M., Dunkel, B., Mullard, J., Wood, R., Piercy, R.J. and Weller, R.

Royal Veterinary College, North Mymms, Hertfordshire, UK. Email: mtambaschi@rvc.ac.uk

Reasons for performing study: Equine cervical osteoarthritis is a common disease known to contribute to both neck pain and cervical vertebral compressive myelopathy. There are no published studies demonstrating the usefulness of latero-oblique radiography as a diagnostic tool for cervical osteoarthritis. **Objectives:** To determine the sensitivity, specificity and positive

CLINICAL RESEARCH

and negative predictive values of latero-oblique radiography as a diagnostic tool for cervical articular process joint osteoarthritis.

Study design: Prospective cadaver study. **Methods:** Latero-oblique radiographs and CT images were collected, and *post mortem* examinations performed, on 27 cadaver necks from Thoroughbred-type horses of various ages and sexes. Two equine clinicians independently reviewed each set of images for the presence of osteoarthritis. The authors, under the guidance of a veterinary pathologist, reviewed all articular process joints for bony changes indicative of osteoarthritis. **Results:** The prevalence of osteoarthritis identified on CT images was 57.9% (Assessor A) and 23% (Assessor B). The prevalence of lesions identified on *post mortem* examination was 25.6%. Latero-oblique radiography showed a low sensitivity for identifying osteoarthritis when compared to both CT imaging (6.3–16.1%) and *post mortem* examination (2.2–4.4%). However, it showed a high specificity when compared to both CT imaging (92.1–97.8%) and *post mortem* examination (87.2–95.0%). The positive predictive value for identifying osteoarthritis was moderate (40.9–80.0%) when compared to CT imaging and poor (4.2–18.2%) when compared to *post mortem* examination. The negative predictive value was moderate when compared to both CT imaging (42.9–76.3%) and *post mortem* examination (78.0–79.8%). **Conclusions:** Latero-oblique radiography has low sensitivity, but high specificity for the detection of cervical osteoarthritis when compared to both CT imaging and *post mortem* examination. Further investigation comparing the sensitivity, specificity and positive and negative predictive values of latero-oblique radiography vs. laterolateral radiography would determine whether taking latero-oblique radiographs, which can be more difficult to obtain and interpret, is necessary for the diagnosis of cervical osteoarthritis in practice. **Ethical animal research:** Ethical committee oversight not currently required by this congress: The study was performed on material obtained from an abattoir. **Sources of funding:** None. **Competing interests:** None.

09.15–09.30

Cervical computed tomography (CT) and CT myelography in live horses: 16 cases

[†]Kristoffersen, M., ^{*}Puchalski, S., [†]Skog, S. and [†]Lindegaard, C.

[†]Evidensia Equine Hospital Helsingborg, SE-25023 Helsingborg, Sweden; ^{*}Wellington, Florida, USA. Email: mads.kristoffersen@me.com

Reasons for performing study: Cervical spine lesions are often suspected in horses with neurological signs, abnormal head/neck position and obscure forelimb lameness. Computed tomography (CT) has the potential to image the cervical spine in 3 dimensions in superior anatomical detail; e.g. lesions that cause compression of the spinal cord and nerve roots, small fragments, and osteoarthritis of the articular process joints may be more easily detected. **Objectives:** To investigate if CT and CT myelography of the entire cervical spine is possible in horses, describe the technique used, the type of horses and distribution of cervical lesions detected. **Study design:** Retrospective case series. **Methods:** Horses undergoing cervical CT and CT myelography from June 2013 to February 2014 were reviewed. The horses were anaesthetised in left lateral recumbency using continuous intravenous anaesthesia. A Philips Brilliance Big Bore 16 slice scanner and a custom made equine CT table (Solving, Finland) were used. **Results:** Cervical CT was performed on 16 horses, 11 of which also had CT myelography: 12 Warmblood,

2 ponies, 1 Standardbred and 1 Paint Horse. Horses ranged in age from 1 to 21 years, and in weight from 406 to 670 kg. Presenting complaints were: neurological symptoms (n = 8), abnormal head/neck position (n = 5), forelimb lameness (n = 4), Horner's syndrome (n = 1). In all cases (n = 16) the cervical spine from the skull to C7 could be imaged, in 3 cases T1 was imaged and in one case T3. Significant lesions were detected in 14 horses. The sites of the most significant lesions were: C4–C5 (n = 3), C5–C6 (n = 2), C6–C7 (n = 5) and C7–T1 (n = 2), cervical stenosis C5–T2 (n = 1) and muscle injury (n = 1). Length of anaesthesia ranged from 35 to 70 min (median 60 min), with one complicated recovery. **Conclusions:** Cervical CT and CT myelography can be performed in large adult horses. CT may be the future gold standard to evaluate equine cervical lesions. Further studies are needed. **Ethical animal research:** Ethical committee oversight not currently required by this congress: retrospective study of clinical records. Explicit owner informed consent for participation in this study was not stated. **Sources of funding:** None. **Competing interests:** None.

09.30–09.45

Comparison and clinical application of CT and MRI for evaluation of the equine cranial nerves

Dixon, J., Lam, R., Weller, R., Smith, M. and Piercy, R.J.

Equine Referral Hospital, Department of Clinical Sciences and Services, Royal Veterinary College, London, UK. Email: jjdixon@rvc.ac.uk

Reasons for performing study: Advanced imaging modalities enable assessment of the equine skull and brain. Horses are susceptible to neurological dysfunction of the cranial nerves; however, our understanding of these structures' imaging anatomy is limited. **Hypothesis:** Magnetic resonance imaging (MRI) will be superior to computed tomography (CT) for the identification of cranial nerves, but optimal assessment may depend on both modalities. **Objectives:** Identify cranial nerves and compare and contrast the utility of MRI and CT images of cadaver and clinical material. Interpret images from both modalities and determine the cranial nerve imaging anatomy. **Study design:** Prospective cadaver anatomical study combined with retrospective clinical case study. **Methods:** The head of a neurologically normal 9-year-old Thoroughbred gelding was scanned immediately following euthanasia (performed for reasons unrelated to this study). High resolution MRI (1.5 Tesla) and CT examinations were conducted over a 12 h interval following euthanasia. Images obtained were compared with selected clinical cases which were scanned during anaesthesia (MRI; approximately 30–60 min) or standing sedation (CT; approximately 30 s). **Results:** On a high resolution MRI scan of a cadaver equine skull, each of the 12 cranial nerves and their topographic location was readily appreciated. Cranial nerves 1, 2, 3, 5, 7 and 8 were more easily identified in clinically relevant MRI scans. CT allowed visualisation of the stylomastoid foramen, the inner and middle ear and cranial nerves 2, 3, 5 and 7. **Conclusions:** High field MRI allows for excellent visualisation of equine cranial nerves. CT allows for detailed visualisation of the osseous canals and foramina. This study advances anatomical knowledge of the normal equine cranial nerves to aid interpretation in horses that display neurological dysfunction localising to the brain and brainstem. **Ethical animal research:** Horse owners gave their consent for their animals to be included in the study. **Sources of funding:** Institutional. **Competing interests:** None.

CLINICAL RESEARCH

09.45–10.00

The prevalence of ocular diseases in Arabian horses in Poland

[†]*Paschalis-Trela, K., [‡]Cywińska, A., [†]Witkowski, L., [†]Czopowicz, M., [§]Trela, J. and [†]Kita, J.*

[†]Laboratory of Veterinary Epidemiology and Economics, [‡]Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland; [§]Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, USA. Email: anna.cywinska@sggw.pl

Reasons for performing study: Ocular diseases in horses often require long-lasting and costly therapies. Without proper treatment they may lead to partial or full blindness, excluding horses from intended use, and thus pose a serious veterinary and economic problem. **Objectives:** No epidemiological data on equine ocular pathologies in Poland was available. The aim of this study was to evaluate their type and prevalence in pure-bred Arabian horses. **Study design:** The study involved 615 horses (15% of Arabian population) bred and owned by 3 Arabian state stud farms in Poland. All horses underwent standard clinical and ophthalmic examination and medical history from the previous 5 years was analysed. **Methods:** The medical history was based on the data from farms' veterinary archives in the stables and epidemiological interview given by the resident veterinarian. Every horse underwent general clinical and ophthalmic examination. **Results:** The prevalence of ocular diseases was 9.8% (95% CI: 7.7–12.4%). The following pathologies were diagnosed: equine recurrent uveitis (ERU) – prevalence of 5.5% (95% CI: 4.0–7.6%), non-ERU related cataract – 3.3% (95% CI: 1.9–4.7%), post traumatic lesions – 0.8% (95% CI: 0.4–1.9%), glaucoma – one case. Seven horses had one nonvisual eye, one was bilaterally blind. **Conclusions:** Equine recurrent uveitis was the most common ocular disease in this Polish population of Arabian horses. Its prevalence is lower than usually reported in Europe (8–10%) and in the United States (8–25%). However, severe ocular pathologies were observed confirming that they remain an important clinical problem. **Ethical animal research:** The study was approved by the Local Ethics Committee. Explicit owner informed consent for participation in this study was not stated. **Sources of funding:** The research was supported by a grant from National Science Centre on the basis of the decision No DEC-2011/03/B/NZ6/04682. **Competing interests:** None.

Infectious Disease

Chaired by Lutz Goehring

Sponsored by University of Liverpool



UNIVERSITY OF
LIVERPOOL

10.30–10.45

A retrospective study on equine herpesvirus-1 associated myeloencephalopathy in France (2008–2011)

^{†*}*van Galen, G., ^{‡§}Leblond, A., ^{§#}Tritz, P., [†]Martinelle, L., ^{§¶¶}Pronost, S. and [†]Saegerman, C.*

[†]Research Unit of Epidemiology and Risk Analysis applied to Veterinary Science (UREAR-ULg), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Belgium; [‡]UR 346 Animal Epidemiology INRA Theix, Vetagrosup, Equine Department, University of Lyon, France; [§]Réseau d'Epidémiologie-Surveillance en Pathologie Equine (RESPE), Mondeville, France; [#]Veterinary Clinic of Faulquemont, France; [¶]Frank Duncombe Laboratory, Caen, France; ^{¶¶}Normandie Université, Unité Risques Microbiens (U2RM), 14000 Caen, France. *Current address: Large Animal Clinic, Internal Medicine and Surgery, Faculty of Health and Medical Sciences, University of Copenhagen, Højbakkegaard Allé 5, 2630 Taastrup, Denmark. Email: gaby@equinespecialists.eu or gaby@sund.ku.dk

Reasons for performing study: Diagnosis of equine herpesvirus-1 associated myeloencephalopathy (EHM) can be troublesome, but early recognition and knowledge of risk factors are primordial for prevention and control. **Objectives:** 1) Improvement of early clinical recognition, and 2) identification of factors potentially of importance for spread. **Study design:** Retrospective descriptive study of EHM cases and statistical comparison to acutely neurologically affected horses negative for EHM. **Methods:** Files from a French epidemiosurveillance programme for equine infectious neurological diseases (2008–2011) were reviewed. Cases were considered EHM (n = 26) based on presence of acute neurological signs and laboratory confirmation. Cases were considered control cases (n = 29) when reported to be suffering from acute neurological diseases, but negative for EHM. A subgroup of controls was created that excluded cases with peripheral neuromuscular diseases (n = 21). Univariate and multivariate analysis and classification and regression tree analysis between groups were performed to identify diagnostic markers and risk factors. **Results:** EHM had a fatality rate of 46% and occurred often in isolated cases. They frequently showed ataxia, paresis and cauda equina affection, but the clinical picture was variable. Univariate analysis identified the following variables as more associated to EHM than to control groups: new horse introduced in herd, vaccination, cauda equina affection, larger herd size, and saddle horses. In the multivariate analysis, new horse introduced in herd and cauda equina affection could be retained. CART analyses identified herd size, month of occurrence, new horse introduced in herd and cauda equina affection as main predictors for EHM. **Conclusions:** Isolated EHM cases occur frequently, accentuating the diagnostic difficulty. History and clinical examination of acutely neurologically affected horses

CLINICAL RESEARCH

can potentially improve early recognition of EHM. Risk factors were in accordance with other studies, although in a different geographic location and study setup, and therefore strengthen their importance for spread of EHM. **Acknowledgements:** Christel Marcillaud-Pitel and Charlene Daix are gratefully acknowledged for their help with data collection. **Ethical animal research:** Ethical committee oversight not currently required by this congress: retrospective study of clinical records. Explicit owner informed consent for participation in this study was not stated. **Sources of funding:** RESPE. **Competing interests:** None.

10.45-11.00

Sales consignment and nasal shedding of equine herpesvirus-1 (EHV-1) and 4 (EHV-4) in young Thoroughbred horses in South Africa

[†]Badenhorst, M., ^{*}Page, P.C., [§]Ganswindt, A., [†]Guthrie, A.J. and ^{*}Schulman, M.L.

[†]Equine Research Centre; ^{*}Department of Companion Animal Clinical Studies, [§]Endocrine Research Laboratory, Department of Anatomy and Physiology; and [†]Section of Reproduction, Faculty of Veterinary Science, University of Pretoria, Private Bag XO4, Onderstepoort, 0110, South Africa. Email: marcha.badenhorst@up.ac.za

Reasons for performing study: Commingling of horses from various populations, together with stress associated with transport and confinement at a sales complex, may predispose horses to EHV-1 and -4 shedding and transmission. Current information on the prevalence and associated risk factors of EHV-1 and -4 in South Africa is limited. Relevant research could enhance scientific-based risk management strategies for horses attending sales events. **Objectives:** Detect nasal shedding of EHV-1 and -4 at a sales event. Identify the temporal pattern of viral shedding. Investigate the association between clinical signs and EHV-1 and -4 shedding. Identify risk factors for EHV-1 and -4 shedding and transmission. **Study design:** Prospective cohort study. **Methods:** Data was collected over a 9 day period during August 2013 at the National Two Year Old Sales in Germiston, South Africa. The study population included 90 Thoroughbreds (51 colts, 39 fillies) in their second year of life that originated from 8 studs situated in 3 provinces. Nasal swabs were collected from each horse on arrival and on departure from the event. During their stay horses were monitored twice daily for pyrexia and once daily for nasal discharge. Nasal swabs were collected daily from any horse with nasal discharge and/or pyrexia. Nasal swabs were submitted for qPCR to detect EHV-1 and -4. **Results:** No EHV-1 shedding was detected; however, 14.4% of the population shed EHV-4. A biphasic shedding pattern with peaks one day post arrival and on the first day of auction was observed. Pyrexia, with or without nasal discharge, was observed prior to first shedding in 61.5% of EHV-4-positive horses. Province, associated longer travel duration and smaller resident horse populations on farms of origin were associated with increased risk of EHV-4 shedding. **Conclusions:** Young Thoroughbreds consigned to a South African sale shed EHV-4. Pyrexia proved useful to identify impending EHV-4 shedding. **Ethical animal research:** The study was approved by the Animal Ethics Committee (AEC) of the University of Pretoria (Study V040-13). Informed, written consent for participation was obtained from the owners of each of the studs included in the study. **Sources of funding:** Funding for this study was provided by Racing South Africa and the Departments of Companion Animal Clinical Studies and Production Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria. **Competing interests:** None.

11.00-11.15

EHV-5 associated with respiratory disease in a survey of alpha- and gammaherpesviruses in 409 Australian horses

El-Hage, C.M., Mekuria, Z.H., Hartley, C.A. and Gilkerson, J.R.

Equine Infectious Diseases Laboratory, Faculty of Veterinary Science, The University of Melbourne, Victoria 3010, Australia. Email: cmeh@unimelb.edu.au

Reasons for performing study: To determine the prevalence of EHV-1, -2, -4 and -5 in respiratory samples from a large number of horses using quantitative PCR methods. Samples from horses with, and without mild signs of respiratory disease provided an opportunity to examine associations with single or multiple herpesviral infections. **Objectives:** To determine any correlation between quantitative detection of these equine herpesviruses and mild clinical respiratory disease in horses. **Methods:** Nasal swabs were taken from horses with, and without, clinical respiratory disease. Nucleic acid was extracted from all swabs prior to PCR testing for EHV-1, -2, -4 and -5. Clinical signs of respiratory disease included coughing, fever (temperature >38.5°C) or nasal discharge. **Results:** Of the 409 horses, 250 (61%) were clinically normal, 121 (30%) presented with clinical signs consistent with mild respiratory disease and 38 (9%) horses had no traceable clinical history. One-fifth (83/409) of horses sampled were infected with EHV-2 and almost two-thirds 249/409 (60.9%) with EHV-5. Infection with EHV-5 was significantly associated with mild respiratory disease (85/121; 70.2%) compared to nondiseased horses (137/249; 55%) $P = 0.005$. The proportion of EHV-2 infected horses, however, did not differ significantly between those in the diseased (18/121) and nondiseased (61/250) groups. Too few horses were detected with alphaherpesviruses to determine any association with clinical signs of disease. Mean nasal shedding loads of herpesviruses were not significantly different between diseased and nondiseased horses. **Conclusions:** There was a significant association between horses displaying clinical signs of mild respiratory disease and infection with EHV-5, however, no such association was evident for neither horses with EHV-2 nor the alphaherpesviruses EHV-1 and -4. The clinical significance of respiratory gammaherpesvirus infections in horses remains yet to be determined; however, these findings add to the mounting body of evidence incriminating EHV-5 in association with equine respiratory disease. **Acknowledgements:** The authors are grateful to veterinarians and animal health staff who took samples. Garry Anderson provided valued and erudite statistical assistance. **Ethical animal research:** Ethical committee oversight not currently required by this congress: the study was performed on archived material collected for the Victorian Department of Environment and Primary Industries' Equine Influenza surveillance programme. Explicit owner informed consent for participation in this study was not stated. **Sources of funding:** Special Virology Fund at the University of Melbourne and the Chief Veterinary Officer's unit DEPI Victoria. **Competing interests:** None.

CLINICAL RESEARCH

11.15-11.30

Development and efficacy of the recombinant canarypox-based equine influenza (EI) vaccine updated according to the last OIE expert surveillance panel recommendations

***Paillot, R., *Lemaitre, L., *Dancer, A., *Thibault, J.-C. and *Minke, J.**

**Animal Health Trust, Newmarket, UK; *Merial SAS. Email: romain.paillot@aht.org.uk*

Reasons for performing study: Since 2010, the OIE expert surveillance panel (ESP) recommends that EI vaccines contain representative equine influenza virus (EIV) strains from the Florida clade 1 and 2 sub-lineages for optimal protection against EIV currently circulating worldwide. To date, no EI vaccine commercially available in the EU meets this recommendation.

Objectives: This report summarises the development process of a fully updated recombinant canarypox-based EI vaccine, including clinical efficacy results against EIV strain A/eq/Richmond/1/07.

Study design: The EI vaccine ProteqFlu containing 2 recombinant canarypox viruses expressing the haemagglutinin of EIV strains was updated by replacing the A/eq/Newmarket/2/93 strain with the A/eq/Richmond/1/07 isolate (Florida clade 2 sub-lineage) and keeping A/eq/Ohio/03 (Florida clade 1 sub-lineage), to meet the last OIE recommendations. The updated EI vaccine was tested for efficacy in the Welsh mountain pony model. **Methods:** The mode of action, production steps and efficacy of the updated EI vaccine will be presented. Efficacy was tested in a group of 7 ponies vaccinated twice, 5 weeks apart. Protective antibody response were measured and challenged by experimental infection with the A/eq/Richmond/1/07 EIV strain. Clinical signs of disease and virus shedding were compared with control unvaccinated ponies (n = 7). **Results:** Significant protection was measured in vaccinated ponies, which supports the vaccine registration. **Conclusions:** The recombinant canarypox-based EI vaccine was already the first EI vaccine to meet the 2004 OIE ESP recommendations and was successfully used in Australia during the 2007 EI outbreak. This new version will be the first fully updated EI vaccine available in the EU, which will help to minimise the increasing risk of vaccine breakdown due to constant EIV evolution through antigenic drift. EI vaccination remains one of the most effective tools to prevent or limit the impact of EI, as clearly illustrated by the limited frequency and scale of EI outbreaks. **Ethical animal research:** All animal work received ethical approval. **Sources of funding:** The study was funded by Merial SAS. **Competing interests:** R.P. reports no conflict of interest. L.L., A.D., J.-L.T. and J.M. are employed by the study sponsor.

11.30-11.45

Investigation of equine encephalitis cases during the West Nile virus (WNV) epidemics in Greece

***Diakakis, N., *Chaintoutis, S.C., *Bouzalas, I., *Brellou, G.D., *Vlemmas, I., *Papanastassopoulou, M. and *Dovas, C.I.**

**Equine Unit, Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 11 StavrouVoutyra str., 54627, Thessaloniki, Greece; *Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health Sciences,*

*Aristotle University of Thessaloniki, University Campus, 54124, Thessaloniki, Greece; *Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 11 StavrouVoutyra str., 54627, Thessaloniki, Greece. Email: Diakakis@vet.auth.gr*

Reasons for performing study: Clinicopathological and laboratory investigation of horses showing severe neurological signs during the 2010 WNV-lineage 2 human epidemic in Central Macedonia (Northern Greece). Implementation of preventive measures was also addressed. **Objectives:** To describe the clinical signs, supportive treatment and outcome in WNV cases and report the use of an inactivated WNV vaccine used for the protection of horses during the following epidemic periods.

Study design: Clinical study. **Methods:** Laboratory testing included serology, real-time RT-PCR and histopathology. All affected animals received supportive treatment (furosemide, dexamethasone, B-complex vitamins and NSAIDs). The Equip WNV vaccine was used for immunisation of horses. **Results:** WNV-specific IgM antibodies were detected in 17 horses with neurological signs. The specificity of the detected antibodies was confirmed by seroneutralisation tests. The maximum duration of IgM was determined to be 60 days. Clinical signs included weakness of hindlimbs, ataxia and tremors (17/17), altered mental state (10/17), hypersensitivity (7/17), inability to swallow (3/17), recumbency (3/17) and convulsions (3/17). Supportive treatment was successful in 14/17 horses. The 3 recumbent horses died as a result of the infection, or were subjected to euthanasia. Histopathological findings observed in the CNS were mild perivascular cuffing of mononuclear cells, axonal swelling, glial nodules, microhaemorrhages and neuronal necrosis. None of the vaccinated horses showed clinical signs. Three unvaccinated horses showed clinical signs and developed WNV-specific IgM antibodies. **Conclusions:** The Nea Santa-Greece-lineage 2 strain responsible for the massive human epidemic of 2010 was also highly pathogenic for horses. Timely administration of supportive treatment is important to the prognosis of the cases. WNV infection should be included in the differential diagnosis of horses with encephalitis in Greece. Vaccinations can effectively protect horses, especially in areas of Greece where the virus seems to have become endemic. **Ethical animal research:** All owners gave their consent prior to the commencement of the study. **Sources of funding:** Funding for this project came from Zoetis. **Competing interests:** None.

11.45-12.00

Duration of tetanus IgG titres following basic immunisation of horses

***Kendall, A., *Domeij, K., *Gånheim, A. and *Bergström, K.**

**Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; *The Equine Clinic Bollerup, Tomelilla, Sweden; *National Veterinary Institute, Uppsala, Sweden. Email: a.t.kendall@massey.ac.nz*

Reasons for performing study: Recommendations for prophylactic vaccination against tetanus in horses vary greatly between countries and have scarce scientific support in the peer-reviewed literature. In human medicine, recommended booster vaccination intervals are also very variable, but are considerably longer than for horses. **Objectives:** To investigate if the duration of antibody titres previously determined to be protective against tetanus are likely to be longer than indicated by several recommended vaccination intervals for horses. **Study**

CLINICAL RESEARCH

design: Prospective clinical trial. **Methods:** Thirty-four horses were enrolled for basic immunisation with an ISCOM Matrix-combination vaccine (Equilis® Prequenza TE). Horses received the first vaccination at 5–11 months of age, and the second dose 4 weeks later. A third vaccine dose was given 15–17 months after the second dose. Serum tetanus antibody titres were analysed by ToBi ELISA 2 weeks as well as 14–16 months after the second dose. After the third vaccine dose, titres were checked once yearly for 3 years. **Results:** Two weeks after the second dose all horses (34/34) had antibody levels that exceeded 0.03 iu/ml. After 16 months the levels were below 0.03 iu/ml in 5/33 horses. After the third vaccine dose antibody levels remained above 0.03 iu/ml in 25/26 horses for 1 year, 17/17 horses for 2 years, and 9/9 horses for 3 years. **Conclusions:** Horses that undergo basic immunisation with 3 doses of vaccine after the age of 6 months are likely to have serum antibody titres consistent with protection against tetanus for more than 3 years. **Ethical animal research:** This study was approved by the Uppsala Ethical Committee on Animal Experiments and informed owner's consent was obtained prior to enrolment of horses. **Sources of funding:** This study was funded by the Intervet Research Foundation. **Competing interests:** None.

12.00–12.15

Differential expression of microRNAs in equine sarcoids: preliminary results from an *in vitro* model

[†]Terron-Canedo, N., [‡]Britton, C., [§]Nicolson, L. and [†]Nasir, L.

[†]MRC-University of Glasgow Centre for Virus Research; [‡]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow; [§]School of Veterinary Medicine, College of Medicine, Veterinary and Life Sciences, University of Glasgow. Email: n.terrion-canedo.1@research.gla.ac.uk

Reasons for performing the study: Equine sarcoids are locally aggressive fibroblastic cutaneous tumours, representing the third most common health problem in working horses. In developed countries they also have welfare and economic impact (e.g. prepurchase examination). No current treatment offers 100% success rate. It is now well established that Bovine Papillomavirus (BPV) types 1 and 2 play an important role in the pathogenesis of sarcoid tumours. In a number of human cancers, aberrant expression of microRNAs has been linked to the cancerous phenotype. microRNAs are small noncoding RNAs that regulate essential biological and cellular processes. Currently there is much interest in their role as biomarkers and potential therapeutic agents for many diseases. **Objectives:** In an attempt to improve our understanding of the pathobiology of sarcoids, we aim to identify specific microRNAs associated with equine sarcoids. **Study design:** Differential expression of microRNAs was assessed in control equine fibroblasts (EqPalFs) and EqPalFs transformed with the BPV-1 genome (S6-2s). **Methods:** Using a commercially available human microarray, 453 microRNAs were interrogated in RNA samples from both cell lines. Each cell line was analysed in triplicate. **Results:** 219 individual microRNAs were differentially expressed in control (EqPalFs) versus BPV-transformed cells (S6-2s) ($P < 0.05$). Of these, 148 microRNAs (68%) were underexpressed in S6-2 cells relative to EqPalFs. **Conclusions:** We have shown that BPV-1 transformed equine cell lines exhibit a different microRNA profile to control equine

fibroblasts and the aberrant expression of microRNAs in S6-2 cells can be attributed to the presence of BPV-1 genomes. Our future work will focus on studying the expression profiles of a subset of microRNAs in a larger panel of cell lines and sarcoid tumours to help elucidate the significance of the deregulated expression. **Ethical animal research:** Local ethical approval was granted to conduct this study. **Sources of funding:** This work is funded by the Horserace Betting Levy Board with contribution from the Vet Trust Fund (James Herriot Scholarship). **Competing interests:** None.

12.15–12.30

Vaccination with virus-like particles induces long lasting protection from experimentally induced sarcoid-like tumours in horses

[†]Hainisch, E.K., [‡]Harnacker, J., [‡]Shafti-Keramat, S., [‡]Kirnbauer, R. and Brandt, S.

[†]Research Group Oncology (RGO), Equine Clinic, Veterinary University Vienna, Austria; [‡]Laboratory of Viral Oncology (LVO), Division of Immunology, Allergy and Infective Diseases (DIAID), Department of Dermatology, Medical University Vienna, Austria. Email: edmund.hainisch@vetmeduni.ac.at

Reasons for performing study: We have already demonstrated that vaccination with empty BPV1 capsids termed virus-like particles (VLP) protects horses from experimental infection with BPV1 virion. Long-term monitoring of antibody titres in experimental horses and data from other species suggest that this protection is long lasting. **Objectives:** To test protection against experimental infection with BPV-1 virion in horses that were vaccinated with BPV-1 L1 VLP approximately 5 years earlier. **Study design:** Controlled experiment. **Methods:** Seven horses, vaccinated 3 times (boosters after 4 weeks and 6 months) in 2007/2008 with doses of BPV1 L1 VLP ranging from 50 µg to 150 µg/dose and 3 unvaccinated control horses were challenged by intra-dermal inoculation with cow wart derived BPV1 virion (5×10^7 BPV-1 virions per wheal, 10 wheals per horse). Inoculation sites were monitored for 10 weeks. Blood for serum antibody titre determination by pseudovirion neutralisation assay was taken on the day of challenge and after 6 months. **Results:** Six of 7 vaccinated horses had measurable serum antibody titres (1:50 to 1:400). These titres were boosted by inoculation (about one step of dilution). Two of 3 unvaccinated controls remained sero-negative. One control horse showed sero-conversion. All control horses developed tumours at all 10 inoculation sites. Tumours appeared approximately 2 weeks after inoculation and reached maximum sizes of up to 8 mm. Regression was complete by 8 weeks after their first appearance in all horses. All vaccinated horses remained completely free from tumours. No influence of dose rate or antibody titre on the level of protection could be determined. **Conclusions:** BPV-1 L1 VLP vaccination proves to be fully effective in protecting horses from experimental infection and tumour formation 5 years post immunisation. The protection was complete even in horses with low or unmeasurable antibody titres. This is another step towards establishing a vaccination against equine sarcoids. **Ethical animal research:** This experiment was approved by the institutional ethics committee and the national authority under license No: GZ: 68.205/0144-III/3b/2012. **Sources of funding:** Veterinary University Vienna, Austrian Research Fund (FWF). **Competing interests:** None.