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Reasons for performing study: Equine grass sickness (EGS) is of unknown aetiology. Despite some evidence suggesting that it represents a toxico-infection with *Clostridium botulinum* types C and/or D, the effect of EGS on the functional targets of botulinum neurotoxins (BoNTs), namely the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins, is unknown. Further, while it is commonly stated that, unlike EGS, equine botulism is not associated with autonomic and enteric neurodegeneration, this has not been definitively assessed. Objectives: To determine (a) the effect of EGS on the expression of SNARE proteins within cranial cervical ganglion [CCG] and enteric neuronal perikarya, and (b) whether botulism causes autonomic and enteric neurodegeneration. Methods: Immunohistochemistry was used to compare the expression of synaptosomal-associated protein-25 (SNAP-25), synaptobrevin (Syb) and syntaxin (Syn) within CCG neurons, and of Syb in enteric neurons, from horses with EGS, horses with botulism and control horses. The concentrations of these SNARE proteins in extracts of CCG from EGS and control horses were compared using quantitative fluorescent western blotting (QFWB). Light microscopy was used to compare the morphology of neurons in haematoxylin-eosin stained sections of CCG and ileum from 6 EGS horses, 5 botulism horses and 6 control horses. Results: EGS, but not botulism, was associated with autonomic and enteric neurodegeneration and with increased immunoreactivity for SNARE proteins within neuronal perikarya. QFWB confirmed increased concentrations of SNAP-25, Syb and Syn within CCG extracts from EGS versus control horses, with the increases in the latter two proteins being statistically significant. Conclusions and potential relevance: The occurrence of autonomic and enteric neurodegeneration, and increased expression of SNARE proteins within neuronal perikarya, in EGS but not botulism, suggests that EGS may not be caused by BoNTs. Further investigation of the aetiology of EGS is therefore warranted.

## 077

### Clinical prevalence of aural plaque in horses admitted at Centro Universitário de Itajubá's Veterinary Medicine teaching hospital, Itajubá, Minas Gerais, Brazil

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Aural plaque is a dermatopathy caused by *Equus caballus papillomavirus*. Clinically is characterized by epidermis elevation, grayish to whitish colored and with a scaly pattern, may be present uni or bilaterally. Some animals may have sensibility in the ear and mild to severe itch. This illness causes losses to the owner as well as to the horse, such as financial due to treatments and esthetical. It is known that there is no effective treatment to aural plaque, so far. Our teaching hospital is localized in Alto do Sapucaí region, in the south of Minas Gerais State. In order to clarify the real situation about aural plaque in horses of Alto do Sapucaí



**Figure 1.** A horse's ear with whitish, scaly and elevated lesion characteristic of aural plaque.

region, the study was carried out aiming a clinical survey of horses admitted in Centro Universitário de Itajubá's Teaching Hospital. All the animals were admitted for other reasons than aural plaque. The study was performed during 2014 July and 2015 September. Sixty horses were admitted during the study period. Seven (11.66%) had skin lesions clinically characteristic of aural plaque in both ears. Four of them (4/7 – 57.14%) were diagnosed with some level of sensibility and/or itch. Two horses had previous home treatment with no results. One horse was treated with imiquimod 5% cream after the aural plaque diagnostic with complete disappear of lesions after 8 weeks treatment. This study allowed concluding that aural plaque and the virus are in Alto do Sapucaí region, been necessary more studies to establish a therapeutic protocol and avoid dissemination of the disease all over the region.

## 129

### Evidence of Equine Infectious Anemia Virus (EIAV) in horses from Serbia

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Equine infectious anaemia (EIA) is a viral disease caused by Equine Infectious Anaemia virus (EIAV) belonging to the *Retroviridae* family, genus *Lentivirus*, which also includes Human Immunodeficiency Virus (HIV), Bovine and Feline Immunodeficiency Viruses (BIV and FIV) and the visna-maedi virus. The clinical signs associated with the infection appear after an incubation period of one to three weeks. They are mainly characterized by fever, anaemia, oedema and different signs of listlessness. Once the horse is infected, several clinical forms may succeed each other. In the acute form, the horse shows serious clinical symptoms that can lead to death; the chronic form is characterised by a recurrence of clinical phases (such as fever, anaemia and listlessness); and there is also an asymptomatic form. Infected Equidae never eliminate the virus and remain contagious for other Equidae even when there are no clinical signs. The bloodborne virus is transmitted from one animal to another mainly by biting insects or iatrogenically through contaminated needles or dentistry equipment (1). Bloodsucking insects – primarily horse flies and stable flies – are mechanical vectors. Although the virus does not replicate

within the insect, the infectious virus can remain in its mouthparts for several hours after a bite. Several studies have shown that EIAV infection has occurred among horses in North and South America, France, Germany, Italy and Romania. So far no evidence of the presence of EIAV in horses was reported in Serbia. In order to determine EIA prevalence among the 5000 horses of the Vojvodina region of Serbia, 316 horse sera have been collected during 2013 and 2014. Serological analysis was performed using Agar Gel Immuno Diffusion test (AGID) as described by the World Organization of Animal Health (OIE) chapter 2.5.6 and by Enzyme Linked Immuno Sorbent Assay (ELISA). Those sera have been tested with 3 different commercial AGID test (Idexx, ID-Vet and VMRD) and two different commercial ELISA tests (Synbiotics and VMRD). With the three AGID kit 311 (98,4%) among the 316 tested sera were found negative and only five (1,6%) sera were found positive for EIA. With ELISA kits results were slightly different and were dependent of the kit producer. Indeed, Synbiotics ELISA gave the same results as AGID tests but VMRD ELISA found 295 negative samples, 5 positive samples and 16 samples were doubtful. Our study shows for the first time that EIA is present in Serbia and more specifically in the Vojvodina region. Euthanasia and viral characterization of field isolate collected in Serbian horses will be investigated.

### 136

#### Analysis of the large intestinal and faecal microbiota of horses with grass sickness using denaturing gradient gel electrophoresis

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Equine grass sickness (EGS) is a multi-system neuropathy affecting horses, characterized by degeneration of autonomic neurons and stasis of the gastrointestinal tract. Despite almost 100 years of research and the identification of numerous factors associated with disease risk, the precise etiology remains unknown. However, both historical and recent evidence supports a contributory role for *Clostridium botulinum* in disease etiopathogenesis. In this study we compared the microbiota of faeces and caecal and colonic contents from horses with EGS (n=10), control horses (n=4) and healthy co-grazing horses (n=21, only faeces) using denaturing gradient gel electrophoresis (DGGE) in an attempt to identify a profile of the microbial community characteristic of EGS. It was possible to distinguish the caecal and colonic microbiota of horses with EGS from that of control animals in cluster analysis. There was no difference in fecal microbiota between healthy and EGS affected horses; however there was a difference between horses from different premises. Our findings suggest that the change in intestinal microbiota detected in EGS is likely to be a consequence, and not the cause, of the gastrointestinal stasis.

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### 146

#### Equine Infectious Anemia diagnosis in a 3 months old foal

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Equine Infectious Anemia (EIA) is caused by a lentivirus that affects horses, mules and donkeys. It is mechanically transmitted by hematophagous insect vectors or human iatrogenic activities. EIA is endemic in the subtropical region of northern Argentina. In Córdoba province (33°S), Argentina Animal Health Authorities (SENASA) reported an incidence of 0.55% in 2008. The current law requires mandatory reporting and slaughter of animals in which the disease is diagnosed. EIA can be presented in three clinical forms: acute, chronic and unapparent. The latter being the most dangerous since infected horses become silent infected carriers. According to Argentina law the diagnosis should be done by Coggins test. However, some authors propose ELISA technique because of its extreme sensitivity and Coggins test is recommended for its high specificity. A 3 months old colt and his mother were in a riding and equine therapy school of Riobamba town, southern Córdoba, Argentina. The colt showed depression for five days and severe dyspnea two days before veterinary assistance. At clinical examination the foal presented sternal recumbence and depression (Figure 1) but was able to get up, and nurse properly. Oral and ocular mucosae were pale and capillary refill by 3 seconds, tachycardia with respiratory distress worsened when walking. Rectal temperature was 40°C. Mandibular lymph nodes were enlarged. Abdominal ultrasound shows severe splenomegaly. Hematological tests revealed lipemic serum, hyperbilirubinemia (Figure 3), severe regenerative anemia with intravascular hemolysis, thrombocytopenia and lymphocytosis. For initial treatment two liters of whole blood transfusion was performed. Dipyron (22 mg/kbw), flunixin meglumine (1.1 mg/kbw) and water baths were given for fever without clinical response. At 6 hours post transfusion the breathing pattern and



Figure 1. Recumbent colt.