**10th IEIDC Abstracts-Emerging and Re-emerging Diseases**

**137 Viral load kinetics and liver disease following equine hepavivirus and equine pegavirus infection**

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In recent years three new members of the *Flaviviridae* family, equine hepavivirus (EHCV), equine pegavirus (EPgV) and Theiler’s disease associated virus (TDAV), have been identified in association with clinical and subclinical liver disease in horses. Several studies have been conducted in the USA, United Kingdom, Germany, Japan, and Brazil to determine the prevalence of EPgV and EHCV infection. Seroprevalence and frequency of viremia has been reported as high as 32-67% for EPgV, and 19-43% for EHCV. We have described the viral replication kinetics, viral-specific antibody responses, and associated liver damage following experimental EHCV infection as well as EHCV/EPgV co-infection. Although we identified high levels of EHCV in liver tissue, we found no significant difference between plasma and liver EPgV viral loads in co-infected horses, consistent with plasma contamination of liver tissue. Thus, EPgV does not appear to be hepatotropic, and its contribution to the development of liver disease in the horse has not been confirmed. EPgV is related to human pegivirus which has questionable pathogenicity, infects mononuclear cells, and has not been convincingly demonstrated to replicate in the liver. To determine if infection with one or more of the above equine viruses was associated with liver disease in racehorses, we obtained plasma samples from ten actively racing Thoroughbreds previously evaluated for decreased performance that had increased serum gamma-glutamyltransferase (GGT) activity. All samples were tested for EHCV, EPgV, and TDAV by RT-PCR. Eight of these ten horses were positive for EPgV, but not EHCV nor TDAV. To determine if EPgV could cause liver disease, we experimentally inoculated three horses with plasma containing only EPgV and measured weekly EPgV viral loads, serum GGT, and serum sorbitol dehydrogenase (SDH). These horses became viremic within one week and developed significant elevations in GGT and SDH within 9-14 weeks post inoculation. Our preliminary results support the conclusion that both EHCV and EPgV can cause liver disease in the horse, and that EPgV may be associated with otherwise unexplained elevations in GGT and poor performance in racing Thoroughbreds.

**058 Landing sites and diel activity in Culicoides midges attacking Fjord horses in the Netherlands**

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In the Old World, African horse sickness (AHS) and equine encephalitis are transmitted to equids by *Culicoides* midges. AHS has a case-fatality-rate of 95% in horses. Though endemic to sub-Saharan Africa, AHS virus is able to incur northwards and to disseminate widely within Mediterranean countries. For example, >1,000 equines died during the AHS-epidemics affecting Spain, Portugal and Morocco (1987-1991). The virus was isolated not only from *Culicoides imicola*, but also from species endemic to Europe: *C. obsoletus* and *C. pulicaris*. In cattle and sheep, the unforeseen emergence of bluetongue virus in NW Europe in 2006, followed by Schmallenberg virus five years later, has led to more midge species being added to the vector list: *C. chiopterus*, *C. dewulfi* and *C. scoticus*. These species feed opportunistically on all breeds of livestock, including horses. In Europe, with the lack of an AHS-vaccine, reducing the contact between the vector and the host is a protective measure: protective stabling of horses and the use of protective blankets. In the Netherlands, approximately 50% of the horses are maintained outdoors (housing is never provided, or not available). Therefore, there is a need for understanding the feeding habits of *Culicoides* midges in relation to horses. In our study (early summer of 2014), we focused on midge landing and biting sites on a pair of Fjord horses, and on their hours of activity across the diel. Furthermore, *Culicoides* were reared from the dung of the Fjord horses that were located in an area without cattle (within a 1km radius). The Fjord horses were maintained permanently at pasture in the central Netherlands. Eleven body regions of the horse were screened for midges, with observations confined to the hour immediately before and after sunset; each body region was sampled at random and for 5 minutes, using a handheld mouth aspirator (pooter). Midge were obtained from all areas of the horse, in particular the belly (64.3%), the legs (8.2%), the flank (5.8%), the shoulders (4.6%) and the hind quarters (4.6%). Subsequently, between June and September, midge activity on the horses was measured across the diel, from sunrise to one hour after sunset. Insect activity was distinctly bimodal, surging both at sunset and an hour after sunrise. The mean attack rate (AR) in *C. chiopterus*, *C. punctatus*, *C. obsoletus* complex, and *C. dewulfi* around sunset was 11.7, 8.6, 3.3 and 3.0 midges/minute, respectively. Mean AR in the first 5 hours after sunrise of midges...
Mosquito species presence on equine premises in the UK

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Background: Globally there has been increasing concern over emerging infectious diseases. In particular arboviral diseases represent a significant threat to animal and human health and the introduction of West Nile virus into North America has demonstrated the potentially significant effects of these viruses on a naïve host population. In Europe recent arboviral emergence events include Usustu, chikungunya, and dengue and in the UK, bluetongue and Schmallenberg viruses. A number of arboviruses affecting horses have been noted as important zoonoses with potential for emergence in Europe, including Japanese encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus and Venezuelan equine encephalitis virus. West Nile virus already causes outbreaks in equines in Europe including in Northern Ireland and in Southern France in 2015. Information regarding the species presence and numbers of mosquitoes on equine premises in the UK are lacking. This information is important in assessing the future risk to horses from mosquito borne disease. The purpose of this study was to add to information on mosquito presence and abundance on equine premises and to investigate the practicalities of mosquito surveillance techniques on equine premises in the UK. Initial fieldwork has concentrated on the species and relative numbers of mosquitoes present on equine premises and to provide baseline information for future mosquito surveillance. This study involved mosquito sampling on 8 premises in each of 4 widely distributed regions of England. Stratified sampling of four mosquito habitats in proximity to equine premises was utilised: Fenland, Woodland, Urban, and Saltmarsh. Each region was visited 3 times during the mosquito season and the timing of these visits was based around the average peaks of different mosquito species abundance. The main trap used for sampling was the Mosquito Magnet™ and this was run for approximately 72 hours at each visit. Larval sampling and a resting box trap were also used on all sites, and resting mosquito sampling, and sweep netting were also used where appropriate. Mosquitoes were identified as far as possible morphologically and by molecular methods for the Culex pipiens complex. Preliminary results indicate that species presence is highly correlated with expected presence based on proximity to mosquito breeding habitat. Cx pipiens complex mosquitoes and Culiseta annulata were two of the most commonly found species. Further conclusions may be drawn after completion of the study and analysis of data.

Long-lasting non-primate hepacivirus infection and transmission of the virus from dams to infants in horses

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Non-primate hepacviruses (NPHVs) that infect horses are the closest relatives of hepatitis C virus (HCV) described to date. HCV and NPHV belong to the genus Hepacivirus, within the family Flaviviridae of single-stranded, positive sense RNA viruses. In this study, we analyzed the NPHV prevalence in stallions, broodmares and yearlings in Japan. We used 35 serum samples from stallions at Farm A and 122 and 125 serum samples from broodmares and yearlings at Farm B and Farm C, respectively, obtained in November 2013. All of the horses were Thoroughbred. Most of the yearlings were reared at Farm B with their dams until 6 to 7 months of age, and then moved to Farm C. NPHV RNA in serum samples was detected by a nested RT-PCR targeting conserved sequences in the nonstructural 3 (NS3) protein coding region. Four (11.4%) of the 35 stallions, 7 (5.8%) of the 122 broodmares and 4 (3.2%) of the 125 yearlings were NPHV RNA-positive. The NS3 region sequences of all 15 NPHV RNA-positive PCR products were determined. Phylogenetic analysis showed that all of the 15 NS3 sequences clustered with sequences previously classified as NPHV. The analysis revealed that vertical transmission from one broodmare to her infant and horizontal transmission among yearlings occurred. A retrospective and follow-up survey of NPHV RNA-positive horses revealed that most of the horses had chronic infection and that only a few horses had acute infection. Two horses showed viremia over a ten-year period. In 4 NPHV RNA-positive stallions, GGT, AST and LDH levels in serum were almost all within reference ranges. Therefore, hepatitis was not observed. This study suggested that NPHV infections are widespread in Japanese horses as well as in horses in other countries, such as UK, Germany, USA and Brazil, and that both vertical transmission and horizontal transmission occur.

Genetic and antigenic analysis of Getah virus isolated in 1978 and 2014 in Japan

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Getah virus is a mosquito-borne virus that causes fever, rash on the body, and edema of the legs in horses and pigs. An inactivated vaccine has been used since the first outbreak occurred in 1978 at the Miho training center of the Japan Racing Association, and no outbreaks had occurred in vaccinated horses until 2014, when the disease resurfaced among racehorses at the same training center in September and October of that year [1]. As we reported previously [2], indirect causes of this outbreak likely include the existence of susceptible horses that have not completed the vaccination program and an increased risk of exposure to the virus because of epizootic Getah virus infection around the training center. However, the direct cause of the 2014 outbreak remains unclear. To determine whether mutation of the virus was directly responsible for the outbreak, we performed genetic and antigenic analysis of the vaccine strain (MI-110) isolated in 1978 and a strain isolated in 2014 (14-I-605). We sequenced the complete genomes of two cloned MI-110 strains and two cloned 14-I-605 strains. In addition, the original MI-110 and 14-I-605 were inoculated into seronegative horses. Antisera collected from these horses were used in a cross-neutralizing test. The complete genome sequences of the cloned 14-I-605 strains had 98.6% nucleotide identity to those of the cloned MI-110 strains. The nucleotide lengths of non-structural polyprotein (nsP1234, 7404 nucleotide lengths of non-structural polyprotein (nsP1234, 7404