neurological pathogen and/or possible causative agents of abortions in horses. BUNV includes 27 strains/isolates with activity around the world. In the US, Panama, Colombia and Guyana it is documented the pathogenic potential of BUNV in both animals and humans because the infection by this virus was found associated with the production of Central Nervous System disease, congenital malformations and abortions. In Argentina, two strains of BUNV have been recovered from mosquitoes, (1) CbaAr-426 (Cordoba, 1964/1965), and (2) AG83-1746 (Santa Fe, 1981). Serological surveys have detected high prevalence of BUNV infection in humans (healthy and undifferentiated febrile syndrome from which 2 new strains of BUNV (SFCrEq231 and SFBzEq232) were isolated; later (July, 2013) in the same area another BUNV strain (SFABCrEq238) was recovered from the brain of an equine abortion. Serologic records in cohabiting horses in Santa Fe province indicate a seroprevalence over 50% (strain CbaAr-426). Genomic amplification and the subsequent phylogenetic analysis of a 250 bp of segment S the 3 new strains showed 100% homology between them and 98,9% with the strain CbaAr426 isolated in Cordoba which although is responsible for high seroprevalence in domestic and wild life animals has not been found associated with disease production. Moreover the phylogenetic analysis of the complete genome of the new strains with the other strains of BUNV available in Genbank showed a homology of 99,4% with strain 86MSP18 (isolated from a human with febrile syndrome in Panama in 1985) for the Segment S. For this final phylogenetic analysis, the strain CbaAr426 It was not included because the whole genome sequence is not available. These findings highlight the circulation of new strains of BUNV in Argentina and constitute the first evidence of a possible association between infection by BUNV and neurological syndrome in domestic animals for our country. Moreover, these results underline the importance of intensifying the surveillance of BUNV to determine its likely real impact and to improve differential diagnosis.

169 Incidence of Equine Protozoal Myeloencephalitis (EPM) in Veterinary Teaching Hospitals

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Equine Protozoal Myeloencephalitis (EPM) is a serious neurologic disease in equids. Although seroprevalence has been reported to be high in horses, the actual prevalence of disease has been estimated between 0.5 to 1%, based on laboratory submission to the University of Kentucky. However, incidence (number of new diagnosed cases) of EPM in horses has not been reported. The purpose of this study was to determine the incidence and breed of EPM cases in equids presented to Veterinary Teaching Hospitals (VTH) in North America and to determine if the incidence of EPM was decreasing over time. A secondary purpose of the study was to survey veterinary practitioners to determine if they perceive that the incidence of EPM is decreasing over time. Equine accesses reported to the Veterinary Medical Data Base (VMBDB) from 1990-2014 were used for this study. Medical records from the VMDB (Systematized Nomenclature of Medicine [SNOMED] and Systematized Nomenclature of Veterinary Diseases and Operations [SNVDO]) were accessed for overall EPM proportional morbidity rates (PMR), PMR by database, PMR compared by breed, and annual PMR from 1990-2014. Also, results of an on-line survey questionnaire (Zoomerang.com) were summarized. The on-line survey, containing 12 questions, was completed by equine practitioners who were members of either the Equine Clinicians Network (ECN) or the American Association of Equine Practitioners (AAEP) list-serves. There were 2,748 cases of EPM per 312,222 accessions resulting in an overall PMR of 0.88%, among hospitals reporting to the VMBDB during the study period. The PMR was not significantly different between the two databases (SNOMED and SNVDO). Proportional diagnoses increased from 0.293% in 1990 to 1.952% in 1997, which was a significantly (P<0.05) higher than the overall PMR (0.88%). Although, there was a significant increase in the PMR of EPM cases from 1995-1998, there was no evidence of a significant temporal increase over the 24-year study period. The PMRs were significantly (P<0.05) higher in Standardbreds (1.592%), Walking Horses (1.367%), Thoroughbreds (1.314%), and Warmbloods (1.096%) than all breeds combined (0.88%). However, the PMRs were significantly (P<0.05) lower in Draft breeds (0.518%), Ponies (0.591%), and Quarter Horses (0.694%), when compared to the PMR for all breeds. For the survey, there were 221 respondents and 153 (69%) were from private practice and 68 (31%) were from academia. The average number of practitioners per practice was 8. Thirty-eight percent of the equine practitioners believed that it had not changed over the previous 2-4 years. Our findings seem to suggest breed predispositions, with higher PMRs being observed in Standardbreds, Walking Horses, and Thoroughbreds and lower PMRs being observed in Draft breeds, ponies and Quarter Horses. The majority of practitioners surveyed thought that the incidence of EPM had either not changed or decreased in the previous 2-4 years. Although there was a significant increase in EPM case diagnosed from 1995-1997, it appears from these data that the overall proportion of EPM cases has essentially not changed over the past 24 years.

081 Practical management of EHV-1 neurological disease outbreaks in the United Kingdom: costs and benefits from three tiers of approach

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Latent infections are the cornerstone of EHV-1’s continued success. Consequently, EHV-1, unlike equine influenza and even strains (S. equi) is not an infection that can be practically eradicated from equine populations. Confirmation and containment of EHV-1 outbreaks is necessary to avoid spread of infection and to minimise the potential for significant impact to the equine industry from more widespread outbreaks, in particular, large and widely disseminated outbreaks of EHV-1 neurological disease. In recent years, experience gained through management of EHV-1 neurological disease outbreaks in the United Kingdom (UK) has led to establishment of protocols to control the spread of disease and minimise equine industry losses. To that end the Animal Health Trust, working with the UK equine industry, has proposed three possible tiers of approach as to how EHV-1 neurological outbreaks can be practically managed. The three tiers of approach are referred to as Gold, Silver and Bronze and whilst they are all based on the same set of principles of Segregation of the population, Collection and Testing of samples and Observation of clinical
disease, there are clear differences between them in terms of the strength of evidence accrued, the time required and the costs incurred. Segregation into smaller discrete groups is common to all three approaches and is believed to be key to a successful strategy through minimising the spread of disease through the population and allowing clearance and release of quarantine measures as soon as possible. The Gold tier option provides insight into both the extent of recent infectious spread (based on agent detection tests, especially PCR) in each segregated group while allowing risk management to then be optimised specifically to each group. This option also facilitates prompt removal to an isolation area of infectious horses from segregated groups, so reducing likelihood of ongoing infectious transmission within the group and overall morbidity. The Silver tier option incurs fewer costs than the Gold option as whole blood samples are not screened for viraemia by virus isolation (qPCR is not optimised for this purpose), but it only provides an insight into the extent of actively shedding and exposed horses without detecting potentially infectious viraemic animals. The Bronze tier option provides a retrospective insight into the extent of infectious spread but relies on observation of neurological signs and/or abortions/neonatal foal deaths to trigger further investigations, thereby potentially missing subclinical infectious spread, which is a recognised phenomenon in propagating EHV-1 outbreaks. This option may be the cheapest of the three with respect to laboratory costs but the lack of initial information about the infectious status in all segregated groups might ultimately lengthen the time for effecting overall control of the outbreak and depending on how many segregated groups are further investigated, may ultimately not significantly reduce laboratory costs.

**092 A Recombinant Fusion Protein Consisting of West Nile Virus Envelope Domain III Fused in-frame with Equine CD40 Ligand Induces Anti-viral Immune Responses in Horses**

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West Nile Virus (WNV), since its introduction in 1999, is endemic in the US and causes severe neurologic disease in horses. There is no effective pharmaceutical treatment for WNV infection, so vaccination is the only approach to prevention and control of disease. The purpose of this study was to evaluate a recombinant vaccine containing Domain III (DIII) of the WNV envelope glycoprotein with and without a natural adjuvant CD-40L in producing virus neutralizing antibodies in horses. Fifteen Thoroughbred horses were randomly divided into five groups and were immunized per the schedule in Table 1. Venous blood samples were collected from each horse prior to vaccination, the 1 and 2 weeks after initial vaccination, and biweekly thereafter to 16 weeks. Serum IgGa concentration in horses vaccinated with the DIII-CD40L+TiterMax and DIII-CD40L groups were increased after the second booster vaccination and were significantly (p<0.05) higher compared to other groups starting from Week 8. The results demonstrated that vaccination with recombinant WNV E DIII-CD40L protein induced specific immune response in healthy horses that might be protective against WNV-associated disease. CD40L could be utilized as a non-toxic, alternative adjuvant to boost the immunogenicity of subunit vaccines in horses.

**Table 1**: Vaccination schedule

<table>
<thead>
<tr>
<th>Group</th>
<th># of horses</th>
<th>1st vaccination</th>
<th>2nd vaccination</th>
<th>3rd vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 0</td>
<td>Week 2</td>
<td>Week 6</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1ml Vetera® Gold + VEE</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.2mg DIII</td>
<td>0.2mg DIII</td>
<td>0.2mg DIII</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.2mg DIII+T</td>
<td>0.2mg DIII+T</td>
<td>0.2mg DIII+T</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.2mg DIII-CD40L</td>
<td>0.2mg DIII-CD40L</td>
<td>0.2mg DIII-CD40L</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.2mg DIII-CD40L+T</td>
<td>0.2mg DIII-CD40L+T</td>
<td>0.2mg DIII-CD40L</td>
</tr>
</tbody>
</table>

T: TiterMax adjuvant was purchased from CytRx Corporation.

**140 Effect of age and vaccination status of the dam on the serological response of foals to vaccination with an activated rabies vaccine**

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Vaccination of domestic animal against rabies is important for preventing this fatal infection, which in turn reduces the risk of human exposure in rabies-endemic areas. These public health concerns provided the main impetus for the American Association of Equine Practitioners (AAEP) to include rabies as a core vaccine for all horses in North America in its vaccination guidelines. The result is that a higher proportion of horses, including broodmares, are now rabies antibody-positive, as are their foals via passive transfer of maternally derived antibodies (MDA). Current label recommendations for primary vaccination against rabies include administration of one dose of inactivated vaccine at three months of age or older, followed by a second dose at one year of age. MDA has been shown to inhibit the response of three-month-old foals to inactivated vaccines directed against several important equine pathogens; however, the impact of MDA on the response of foals to rabies vaccine has not previously been investigated. The serologic response to two doses of an inactivated rabies vaccine administered one month apart was evaluated using the rapid fluorescent focus inhibition test (RFFIT) in three-month-old and six-month-old foals born to rabies-vaccinated mares. Foals from non-vaccinated mares served as antibody-negative controls. Rabies antibodies were not detected in samples collected before vaccination from foals born to non-vaccinated mares. In contrast, high titers of antibody were present in post-nursing samples from foals born to vaccinated mares. Titers declined with a half-life of approximately 30 days; however, rabies antibody was still detectable at 6 months of age in most foals. Both three-month-old and six-month-old foals from non-vaccinated mares mounted a substantial serologic response after administration of one dose of vaccine, and experienced a further increase in titer after administration of the second dose four weeks later. In contrast, MDA’s present at the time of initial vaccination completely blocked the response of three-month-old foals from vaccinated mares to administration of two doses of vaccine. The response of these foals to a third dose administered at one year of age was much poorer than the response seen in