

168 Diagnosis and control of Equine Infectious Anemia in a horse farm located in Buenos Aires province, Argentina

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Although Equine Infectious Anemia (EIA) is endemic in the northern area of Argentina, its epidemiological situation varies along the extent of the territory, with a low prevalence in the central region and free-status in Patagonia (southern region). Argentinian legislation imposes the immediate euthanasia of horses detected as positive by agar gel immunodiffusion test (AGID). This report describes the consequences of EIA virus infection in a farm dedicated to the production of crossbreeding horses (Criollo, Arabian and Quarter horse) located in Baradero, 150 km northwest of Buenos Aires. This horse population (n: 124) had not been tested for EIA in the last 3 years, and there was no EIA control on animals admitted into the farm. The observation that motivates the EIA screening was the death, with no attributable causes, of 10 horses during last year. On March 21st 2015, the adult population of horses was tested for EIA by AGID. Twenty-four out of 109 (22%) horses were found positive. The Animal Health Authorities (SENASA) were notified and these animals were immediately euthanized, and the farm quarantined. After this, three additional AGID tests were conducted on adult horses, and other three on the whole population (including foals at foot). The obtained results are shown in the following table.

<table>
<thead>
<tr>
<th>Horses tested</th>
<th>March 21st</th>
<th>May 15th</th>
<th>June 23rd</th>
<th>July 30th</th>
<th>August 28th</th>
<th>September 17th</th>
<th>October 5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>109</td>
<td>83</td>
<td>67</td>
<td>68</td>
<td>83</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>AGID Positives</td>
<td>24</td>
<td>20</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prevalence</td>
<td>22%</td>
<td>24%</td>
<td>7%</td>
<td>4%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

As result of EIA infection, 53 horses in all (43% of total population) had to be euthanized. The 29 horses which tested positive during the six-month period of additional testing either contracted the infection during this period or had been already infected when introduced into the farm. The inappropriate handling, with horses being either ignored in one of the tests or introduced into the farm during quarantine (shown by the incongruity in the number of horses tested in each occasion), could be the reason for the long time taken to control the infection. Even though all horses resulted negative in the last two tests, the farm still remains quarantined; another control 60 days after, with negative results, is required. The present work illustrates the devastating consequences of EIA infection, as it has brought along important economic losses for the owner, due both to the loss of positive EIA horses, which have been euthanized, as to the restrictions for the movement and sales of horses from this breeding farm. Moreover, this report emphasizes the importance of promoting EIA surveillance in the area considered as "low prevalence", considering this area is where the most valuable horses are bred in Argentina. It is also necessary to raise awareness that it is mandatory to strictly comply with legislation when dealing with EIA infection, which implies control of all horses (even foals on foot) in the facility at the same time and prohibition of entry/exit of new individuals if untested.

132 Identification of Equine arteritis virus immunodominant B cell epitopes using a peptide microarray

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Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease of equids. Affected adult horses make a complete recovery, but a percentage of infected stallions may become long-term carriers. The genome of EAV has a length of 12.7kb and contains seven open reading frames (ORFs). ORFs 1a and 1b represent seventy-five percent of the viral genome and code for replicase and polymerase activities. ORFs 2-7 are nested and code for the structural proteins of the virus. EAV is widely distributed in equine populations around the world and although there is only one serotype for EAV, considerable variation exists between field strains. At present, the serum neutralisation test (SNT) is the principal serological assay used to detect EAV, but the SNT is expensive and time consuming to perform. Additionally, several laboratories have reported problems relating to serum cytotoxicity in lower serum dilutions. To overcome these limitations, considerable efforts have been undertaken to develop an ELISA, but with limited success so far. The antibody response of an individual horse differs depending on its host MHC (ELA) genotype, the EAV strain, and the duration/type of infection. A peptide microarray was developed using the commercially available PEPperCHIP® microarray platform to identify immunodominant B cell (antibody) epitopes of EAV. The whole EAV strain Bucyrus amino acid sequence was transformed into a total of 625 peptide overlapping peptides that were synthesized and spotted in duplicate onto a microarray slide. A panel of 28 field samples representing a selection of sera generated against a variety of known EAV genotypes was tested using the microarray. Of the 625 peptides, 97 peptides (15.52%) showed reactivity with the EAV positive samples. No single peptide was detected by all the positive serum samples. Seven peptides repeatedly showed reactivity above the cut-off and were considered to have diagnostic potential. Five of these peptides were within the immunodominant GP5 protein and two within the replicase polyprotein region NSP2 and NSP10 located in ORF1. Based on the results obtained in this study, a peptide ELISA offers a promising approach as an alternative to the SNT for the detection of antibodies to EAV. Further development and optimization is required to determine if the peptide candidates identified through this study offer the sensitivity and specificity required for the diagnosis of EAV.

143 Performance of the iELISA in horses with long term guttural pouch carriage of Strep equi equi

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Strangles, a very contagious disease, is caused by Streptococcus equi subsp. equi (S. equi). The aim was to study if asymptomatic horses carrying S. equi in their guttural pouches could be identified by serology. Thirty-seven horses from 5 farms that showed positive results for S equi by real-time PCR [1-2] in samples from their guttural pouches once (n=18) or several times (n=19) after the clinical signs of strangulations had subsided were included, resulting in 66 sampling occasions. The time to samplings was 1-23 months from the start of an acute outbreak in their stable (50 samples) or entering a stable with known carriers of S equi (16 samples). Serum antibodies against antigens A and C from S. equi was examined by iELISA with a cutoff of OD >0.5 (Dr Andrew Waller, Animal Health Trust, Newmarket [3]). The results showed that at the recommended cut-off of OD 0.50, 10 horses were positive to both antigen A and C included in the test, 13 horses only to antigen A, 17 horses only to antigen C and 26 horses were seronegative. The sensitivity for the iELISA to detect guttural pouch carriers of S equi was 61% with a cut-off of OD 0.50, and 73% with a cut-off of OD 0.30. Interestingly, some horses went to become carriers without passing a stage of overt clinical disease. In conclusion, serology may be of help to identify asymptomatic carriers when using a cut-off value of OD >0.3.

Acknowledgements

The Animal Health Trust is gratefully acknowledged for the iELISA antigens.

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Serological survey of some major equine viral diseases in the Eastern Caribbean

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There is a relative dearth of sound data on the prevalence of major equine infectious diseases in the Caribbean Basin. To begin to address this, we analyzed banked sera from 180 equines (40 donkeys and 140 horses), mean age 6.3 years collected between 2006 and 2015. Samples originated from 3 different islands in the Leeward group (St Kitts, Nevis and Sint Eustatius) and were tested for antibodies against several equine viral diseases notifiable to the World Organization for Animal Health in licensed and commercially available ELISA test kits. Kits for the detection of antibodies against Equine Infectious Anemia (EIA), Equine Arteritis (EA), Equine Influenza (EI), Equine Herpes Virus (EHV) 1 and 4, and West Nile (WNV) were used. All samples were EIA test-negative. Only 1 animal (0.57%) a donkey, female, 4 years old from Sint Eustatius was positive for EAV. A total of 49 equines (27%) of which 1 donkey and 48 horses, mean age 7.5 years were positive for Equine Influenza. A total of 39 equines (24%) including 4 donkeys and 35 horses, mean age 6.7 years were positive for EHV1. For EHV4, serological testing revealed a total of 138 positives equines (83%) of which 18 donkeys and 120 horses, mean age 6.3 years. A total of 32 equines (18%) of which 8 donkeys and 26 horses, mean age 6.5 years were positive for WNV. The immunization history of most horses was not available to us, but we suspect some of the racing horses may have been immunized against EI and EHV. Donkeys as well as some horses previously used for teaching purposes had no history of vaccination for any of the tested diseases. Prevalence for both EHV1 and EHV4 was similar to previous reports from other parts of the world, confirming the presence of these 2 viruses in equines around the world. There is no official report of WNV in humans in the tested islands; however, other flaviviruses such as Dengue have been reported and several mosquito species known as vectors for WNV as well as migratory birds that travel between North America and the Caribbean are known to be present in the areas from where the samples were collected. Taken together, this study documents serologic evidence of several major equine viral diseases in the area of Eastern Caribbean. Further studies are needed to define the presence and rates of transmission, to characterize local virus strains, and to study their impact on these populations.

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Determining optimal sampling site for Streptococcus equi subsp equi carriers using loop-mediated isothermal (LAMP)

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We hypothesized guttural pouch sampling would be more sensitive than the nasopharynx to identify carriers of Streptococcus equi (S. equi) and that the eqbE LAMP assay would be more sensitive than real time Seel polymerase chain reaction (PCR). Three samples were collected from each horse previously infected with S. equi: nasopharyngeal flocked nylon swab (NPS), nasopharyngeal wash (NPW), and endoscopically-guided guttural pouch lavage (GPL). The following tests were performed: NPS LAMP assay for S. equi, NPW LAMP assay for S. equi, GPL (split into 3 aliquots: S. equi culture, Seel PCR, LAMP assay); Logistic regression and area under the receiver-operator curve were performed using STATA 13. P-values < 0.05 were considered significant. 1/41 NPS, 6/38 NPW and 24/44 GPL samples were positive by eqbE LAMP from 44 S. equi convalescent horses. 18/44 GPL were positive with Seel PCR. S. equi was isolated from 4/44 GPL samples. GPL was the best sample to detect carriers compared to NPS (OR 48.0, P<0.001) and NPS (OR 6.4, P=0.001). Sensitivity and specificity of eqbE LAMP GPL samples when compared to the presence of guttural pouch empyema were 92% and 61%; ROC=0.80, 70% were correctly classified. Sensitivity and specificity of Seel PCR GPL samples was 92% and 80%: ROC=0.92, 84% correctly classified. Sensitivity and specificity of GPL eqbE LAMP was 83% and 65% compared to GPL Seel PCR: ROC=70, 73% correctly classified. Our study demonstrates that GPL should be used to detect S. equi carriers and LAMP PCR was comparable to Seel PCR.