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Increase of Muc5ac and Muc5b glycoprotein in equine airway mucus accumulation and characterisation of Muc5ac and Muc5b from primary equine airway epithelial cells in culture

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Aims: Horses are affected by respiratory problems which are associated with mucus accumulation. Young racehorses suffer from inflammatory airway disease and a major pathological problem is the accumulation of airway mucus leading to breathing difficulties and lower performance. In previous work, we identified Muc5b and Muc5ac as the major mucins in equine airway mucus (Rousseau et al. 2008). However, the mucin composition of accumulated mucus and the events leading to mucus accumulation are unknown. Our aims are to quantify mucins in accumulated mucus and to develop an air-liquid interface cell culture system for equine airway epithelial cells in order to investigate the effect of inflammatory cytokines which have been shown to be implicated in airway inflammation.

Methods: Mucins were quantified by Western blotting using Muc5b and Muc5ac specific equine antibodies after agarose gel electrophoresis. The cell culture system was developed using equine tracheas obtained from our local abattoir and the media electrophoresis. The cell culture system was developed using

References: Available on request from the author.

Equine multinodular pulmonary fibrosis in 6 horses

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Aims: Equine multinodular pulmonary fibrosis (EMPF) is a progressive fibrosing interstitial lung disease, which has been associated with γ-herpesviruses. This case series describes 6 horses with EMPF, which were PCR-positive for equine herpesvirus-5 (EHV-5). They presented between November 2008 and December 2010. Methods: Three horses, two 2-year-old fillies and a 22-year-old mare were subjected to euthanasia due to other diseases and diagnosed with EMPF at post mortem. EHV-5 DNA was identified in all cases by PCR. Two mares, an 8- and a 16-year-old, presented with dyspnoea and weight loss and were diagnosed with EMPF, but died despite treatment with corticosteroids. Furthermore a 22-year-old gelding was presented with recurrent pyrexia and dyspnoea, after intramuscular back infiltration with triamcinolone. The laboratory findings, the results of BAL (intranuclear eosinophilic inclusion bodies in macrophages), thorax radiographs and ultrasound, a positive EHV-5 PCR and lung biopsy were suggestive of EMPF. The horse recovered after one week of treatment with valacyclovir (40 mg/kg bwt t.i.d. per os) and was reported to be clinically healthy one year later.

Conclusions and practical significance: Aetiopathogenesis of EMPF is thought to be similar to human idiopathic pulmonary fibrosis (IPF), which is associated with Epstein Barr Virus (EBV), also a γ-herpesvirus. An inflammatory process seems to induce a dysregulated repair mechanism causing progressive pulmonary fibrosis and γ-herpesviruses might play a role in either initiating or exacerbating this process. The presumed predominance of TH2 cytokines in EMPF and IPF could be induced by EHV-5 and EBV, respectively, as both are reported to possess genes encoding for interleukin 10-like protein. As in humans with IPF horses suffering from EMPF have not responded favourably to corticosteroids. Horses with EMPF have been treated with acyclovir with varying results in the past. To our knowledge this is the first report describing a case responding to treatment with valacyclovir.

Effect of exercise and lower airway inflammation on plasma levels of surfactant protein D


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Introduction: Surfactant protein D (SP-D), mainly synthesised by alveolar type II cells and nonciliated bronchiolar cells, is one important component of innate pulmonary immunity. In humans, circulating concentrations of SP-D are routinely used as
biomarkers for pulmonary injury. Aims: To investigate plasmatic SP-D concentrations at rest and after exercise in horses with inflammatory airway disease (IAD) and controls. Methods: Venous blood samples were collected from 42 trained Standardbred racehorses at rest and 60 min after performing a standardised treadmill exercise test. Tracheal wash and bronchoalveolar lavage fluid (BALF) samples were collected after exercise. According to BALF cytology, 22 horses were defined as IAD-affected (>10% neutrophils and/or >2% mast cells and/or >1% eosinophils) and 20 horses were classified as controls (normal BALF cytology). EDTA plasma was kept frozen until SP-D levels were assessed using a commercially available ELISA kit, and statistically compared between groups of horses and sampling times. Results: Plasma SP-D levels in IAD-affected horses were significantly higher than those of control horses, both at rest (mean ± s.d.; respectively 72.2 ± 31.2 ng/ml vs. 27.0 ± 10.7 ng/ml) and after exercise (73.5 ± 37.1 vs. 26.2 ± 9.7 ng/ml). Within each group of horses (IAD and control), no significant effect of the treadmill test was noticed on SP-D levels; pre- and post exercise values were furthermore highly correlated (r² = 0.975; P<0.001). No significant correlation was found between plasma SP-D concentrations and inflammatory cell percentages in either respiratory fluid. Conclusions and practical significance: This is the first study determining plasma SP-D concentrations in a noninfectious, naturally occurring form of lower airway inflammation in horses. The results highlight that IAD is associated with a detectable, though moderate, increase of circulating SP-D levels. This parameter could then be a potentially useful and readily accessible blood biomarker of equine lower airway inflammation.

09.15–09.30
Experimental challenge with equid herpesvirus-2 is associated with long-lasting inflammation of the intermediate airways


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Introduction: While lower airway inflammation is of paramount importance concerning equine performance, a lack of knowledge relating to the possible implication of viruses as cofactors for syndrome of tracheal inflammation or inflammatory airway disease is currently still reported. Aims: To experimentally investigate the putative role of equid herpesvirus-2 (EHV-2) in respiratory diseases of adult horses and especially its involvement in airway inflammation. Methods: Four horses were respectively submitted to intra-nasal and intra-tracheal EHV-2 inoculation (LK strain). Corticosteroid treatment (dexamethasone) was performed before infection (0.2 mg/kg bw; 3 consecutive days) and as reactivation stimulus 84 days post infection (1 mg/kg bw; 3 days). Two other horses, used as controls, received dexamethasone prior to mock infection and were not submitted to a reactivation stimulus. Because of immunodepression, virus-specific PCR were systematically performed for all EHV-2. Clinical and endoscopic signs being exhibited were investigated, as was the association between EHV-2 detection and modifications of cytological profiles. Results: Mild clinical signs, including tracheal hyperaemia and hyperreactivity were observed throughout both periods of the trial. EHV-2 shedding was observed in all horses (including controls) following corticosteroid treatment. Viral DNA (wild-type or reference strain) was detected in nasal swabs and respiratory fluids up to 21 and 14 days, respectively. Moderate to severe neutrophilia was transiently detected respectively in bronchoalveolar lavage and tracheal wash; while cytological evaluation furthermore revealed a significant association between EHV-2 detection and either concomitant neutrophilia or morphological abnormalities of the tracheal epithelial cells. Conclusions and practical significance: This study is the first trial reporting systematic respiratory fluids analyses over the course of an experimental EHV-2 infection, including both viral detection and cytological evaluation. Clinical and laboratory findings reproduced in this trial allowed experimental confirmation of EHV-2 being a possible co-factor of lower airway inflammation. EHV-2 should then probably be suspected and investigated in poorly performing horses.
Mean ± standard deviation of echocardiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 10)</th>
<th>Moderate AR (n = 7)</th>
<th>Severe AR (n = 7)</th>
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<tr>
<td>FS (%)</td>
<td>36.0 ± 2.7</td>
<td>39.7 ± 3.4</td>
<td>38.7 ± 3.6</td>
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<td>Global SL (%)</td>
<td>-24.61 ± 1.50</td>
<td>-25.93 ± 1.13</td>
<td>-23.80 ± 3.15</td>
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<td>Global SR (%)</td>
<td>62.80 ± 3.66</td>
<td>66.07 ± 3.74</td>
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<td>Septal DRS (mm)</td>
<td>17.39 ± 3.10</td>
<td>21.11 ± 4.18</td>
<td>27.14 ± 3.79</td>
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<td>Septal VS (cm/s)</td>
<td>-4.35 ± 1.24</td>
<td>-8.32 ± 1.16</td>
<td>-10.75 ± 1.90</td>
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Aims: To determine if systemic markers of inflammation correlate with biopsy-diagnosed inflammation in horses with hepatic disease. Methods: The records of 145 horses in which liver biopsy had been performed were retrospectively examined. Information obtained included values for systemic markers of inflammation as well as biopsy inflammation scores. Systemic markers of inflammation evaluated included total white cell count, serum amyloid A (SAA) concentration, fibrinogen concentration and globulin concentration. The degree of inflammation seen on biopsy was scored (either absent/mild; moderate; or severe) using a previously developed scoring system (Durham et al. 2003). Results: All horses had at least one systemic marker of inflammation recorded. Globulin concentration was measured in 133/145 (91%), total white cell count in 110/145 (82%), fibrinogen concentration in 119/145 (75%) and SAA in 50/145 (34%). There were 82 horses with either absent or mild inflammation, 54 with moderate and 9 with severe inflammation on the biopsy. There was no significant association between any of the systemic markers of inflammation and the degree of inflammation seen within the liver on biopsy. Conclusions and practical significance: Liver disease is a commonly diagnosed condition of horses. Diagnosis typically relies on a combination of testing, including biochemical analysis, ultrasonography and hepatic biopsy. A hepatic biopsy is considered the gold standard for diagnosing hepatic disease, and the results can be used to guide therapeutic options as well as prognosis. Based on the findings of this study, a diagnosis of hepatic inflammation is best made via biopsy, as systemic markers of inflammation are not typically increased despite evidence of hepatic inflammation. Further investigations including evaluation of other markers of inflammation may provide more information.

Reference: Available on request from the author.

Adrenocorticotropic hormone in domestic donkeys - reference values, seasonality and association with laminitis
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Aims: To determine reference values and seasonality of adrenocorticotropic (ACTH) in donkeys and any associations with age, sex, laminitis and obesity. Methods: Blood samples were collected from 422 donkeys during routine clinical evaluation of new relinquishments to The Donkey Sanctuary, UK and ACTH was measured by chemiluminescent immunoassays. Age, sex, body condition score (BCS; /5), history of previous laminitis and clinical examination findings were recorded. Donkeys were divided into 4 groups: not obese not laminic (NONL- reference group; 277), obese not laminic (ONL; 86), not obese previously laminic (NOP; 28) and obese previously laminic (OPL; 31). Kruskal-Wallis and Mann-Whitney tests were used to determine associations of ACTH to different groups, seasonality and sex, and regression analyses to look at ACTH against age. Results: The median age was 10 years (range 0.5–38). Of 422 donkeys, 14% had a history of previous laminitis and 27.7% were obese (BCS>3.5). In NONL donkeys (24.7 [21.3–27.1]) there was no significant relationship between ACTH and age (P = 0.43), but ACTH values were significantly higher in geldings than females (P = 0.03). There was a distinct seasonality in ACTH, with significantly lower values in...
November to June (median = 17.8 [IQR = 16.5–19.5]) than July to October (37.9 [28.9–36.9]) in NONL donkeys (P<0.001). This seasonality was significant in all groups (P<0.003), except NOPL. OPL donkey ACTH values (34.8 [24.3–42.9]) were significantly greater than in NONL and ONL (17.7 [16.6–23.3]) (P<0.03) donkeys. NOPL donkey ACTH values (31.4 [24.9–47.9]) were significantly greater than in ONL donkeys (P = 0.013).

Conclusions: The reference range for donkey ACTH values have been established (24.7 [21.3–24.7]), and a distinct seasonality has been demonstrated. A history of previous laminitis, but not clinical evidence of obesity, was associated with higher ACTH values. Practical significance: This study has determined values for ACTH in donkeys with no history of laminitis or clinical signs suggestive of PPID (NONL).

NOTES