



## 10th IEIDC Abstracts-Respiratory: Other

207

### Equine rhinitis A virus infection and cytokine expression in primary tracheobronchial epithelial cell culture

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Primary bronchial cells and *ex-vivo* explants are commonly used as a model for respiratory viral infections and lung diseases in humans. Similarly, these systems can be used to mimic and study respiratory conditions in the horse. Therefore, equine tracheobronchial epithelial cells (TBEC) could be used to investigate viral respiratory infections and their immune/inflammatory responses in this species. The aims of the present study were to investigate the cytopathic characteristics of equine rhinitis A virus (ERAV) in primary TBECs and the gene expression of IFN-gamma, IFN-Beta, IL-4, and IL-8. TBECs were obtained from the lower trachea, carina and primary bronchi of five euthanized horses. After dissection, the tracheal mucosa was removed, washed and kept overnight on pronase. Twelve to eighteen hours later, the airway mucosa was scraped and cells were mechanically separated. TBECs were then washed, plated on collagen coated flasks/plates using modified DMEM F12 cell culture medium and allowed to propagate to confluency. In order to investigate the cytopathic characteristics, confluent TBEC monolayers were exposed to ERAV and observed daily for 5 consecutive days. Additionally, to investigate cytokine expression by qPCR, TBEC monolayers were exposed to either ERAV or equine influenza virus (EIV) and supernatant samples were collected at 0, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours post-infection. EIV was used as a comparison for cytokine expression. Interestingly, in this study ERAV infected TBECs developed syncytial cell formations and/or cell clumping commonly observed during other *in-vitro* viral infections, such as syncytial or herpesviruses. Contrary to our expectations, significant changes in the expression of interferons and IL-4 were not detectable by qPCR after exposure of TBECs to ERAV or EIV within 24 hours. However, up-regulation of IL-8 after exposure to these two viruses was identified from 2 up to 24 hours post-infection in a similar magnitude. IL-8 is consistently detected in respiratory secretions during viral infections in humans and has been shown to induce chemotaxis and phagocytosis of inflammatory cells in the airways. Therefore, changes in the expression of IL-8 indicate that this chemokine might play an important role during early infections, perhaps as a chemotactic factor and/or phagocytic inductor.

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210

### Voluntary surveillance program for important equine infectious respiratory pathogens in the United States

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Infectious upper respiratory tract disease (IURD) is one of the most common medical conditions encountered by equine practitioners nationwide. A major challenge is to determine the contagious nature of the disease soon enough to properly manage the patient, while reducing risk of exposure to other horses. The rapid turn-around time and accuracy of quantitative PCR (qPCR) makes this molecular technology an ideal tool for prompt diagnosis of infectious respiratory pathogens. The objective of this study was to gain a better understanding of the prevalence and epidemiology of important viral [equine herpesvirus-1/4 (EHV-1, EHV-4), equine influenza virus (EIV), equine rhinitis virus A/B (ERAV, ERBV)] and bacterial (*Streptococcus equi* subsp. *equi*) respiratory pathogens shed by horses with signs of acute IURD. Veterinarians throughout the USA were offered voluntary enrollment in the surveillance program and asked to collect blood and nasal secretions from febrile equine patients with acute onset of IURD and/or neurologic disease. Sampling criteria included unexplained fever and one or more of the following: cough, nasal discharge, depression, acute onset of neurologic disease. A questionnaire was used to collect patient demographic data, clinical signs and vaccination history. Samples were tested by qPCR for the presence of EHV-1, EHV-4, EIV and *S. equi* subsp. *equi*. After the first 54 months of the trial, testing for ERAV and ERBV was initiated. A total of 5,222 horses, mules and donkeys were enrolled in the surveillance program over a 90-month study period. Thirteen hundred and thirty four (25.5%) index cases tested qPCR-positive for one or more of the six pathogens. The highest detection rate was for EHV-4 (431 cases), followed by EIV

(365 cases), *S. equi* subsp. *equi* (286 cases), EHV-1 (183 cases) and ERAV/ERAB (69 cases). There were 47 horses with double infection and 5 horses with triple infection. Detection rate by qPCR for the different pathogens varied with season, age, breed and use. One of the limitations of the study was the lack of a control population. Thus, interpretation of the study results was only possible among specific etiological causes of IURD. While infectious respiratory pathogens were detected in 25.5% of study cases, the etiology for the remaining animals with clinical signs of IURD was not further investigated. Possible explanations for the high percentage of qPCR-negative results include false negative tests associated with wrong timing of sample collection, erroneous sample type, and improper sample storage and processing. Nasal secretions may be less reliable for the detection of *S. equi* subsp. *equi* when compared to nasopharyngeal swabs and washes or guttural pouch lavages. Other poorly characterized viral pathogens (adenovirus, EHV-2, EHV-5) also may be involved with IURD.

## 174

### Rhinosporidiosis Associated Laryngeal Chondropathy. A Case Report

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Rhinosporidiosis can affect both, humans and animals, including the horse. It is caused by a Mesomycetozoa, *Rhinosporidium seeberi* (Family Rhinosporidaeae), which is found in aquatic environments. It is a non-contagious, chronic granulomatous disease, which leads to the formation of polyps, growths or warty, highly vascular, friable and sessile or pedunculated lesions. Frequently affects the mucous membranes of the nasal cavity and nasopharynx, being the rare laryngeal presentation. This paper aims to report an unusual case of laryngeal Rhinosporidiosis in a horse. A horse, male, 10 years old, dapple coat, used for field work; was examined in our service at Teaching Hospital with a history of abnormal breath sounds of two years of evolution. The endoscopic examination of the upper respiratory tract was performed with an Olympus videogastroscope with a CV145 image processor, CLE145 light source and OEV203 monitor. Radiological images were recorded with a x-ray portable equipment AJEX9020H with latero-lateral head incidence. Samples of the doughs found in the larynx were taken by endoscopic biopsy forceps (Olympus FB-25 K1) for subsequent histopathology. Clinical parameters were in the normal range and abnormal inspiratory noise, which was exacerbated by exercise, it was evidenced. Endoscopy showed the presence of multiple nodular-growth, polypoid, mobile, with an irregular and nonulcerated surface, pink in color with bright-red areas on arytenoid cartilage, epiglottis and vocal cords surface. A blockage of about 90% of the rima glottidis was appreciated, with intermittent dorsal displacement of the soft palate. The head latero-lateral radiographs showed nodular images with irregular edges and variable sizes; with increasing density of the soft tissues in the projection of the rima glottidis and cervical trachea lumen, adjacent to the larynx. An increased radiopacity of the arytenoid cartilages and epiglottis, with scalloped edges, was also observed. Histopathology revealed that the masses were composed of fibrovascular tissue, lined with a squamous to columnar, hyperplastic epithelium; with multiple spherical structures corresponded to sporangia, ranging from 80 to 400

microns in diameter, with endospores inside. In the fibrovascular stroma, free endospores, areas of hemorrhage and moderate cell infiltrate composed mainly of neutrophils, macrophages and occasional inflammatory cells; were observed. Based on histological findings, the diagnosis was issued a rhinosporidiosis associated laryngeal chondropathy. Rhinosporidiosis is not a common disease in horses and, when present, its main location is at the mucocutaneous junction of the nostrils and the nasal cavity. Laryngeal Rhinosporidiosis, of which only three cases are reported in the world, it's unusual. To date it has not succeeded in isolation and in vitro culture of this microorganism and serology has no diagnostic value; hence the importance of histopathology to confirm the condition. The latter is essential to differentiate malignant tumors, as opposed to these, Rhinosporidiosis is a good prognosis disease (although it may recur). Based on the information provided in this work, rhinosporidiosis should be included as a differential diagnosis of any laryngeal mass, bearing in mind that there are endemic areas for this disease in Argentina.

## 031

### Detection of a *Pneumocystis carinii*-specific gene in tracheal aspirates from healthy young foals in Japan

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Cases of pneumonia in foals caused by *Pneumocystis carinii* infection have been reported. However, in humans a considerable percentage of healthy infants seroconvert to *P. carinii*, suggesting that the presence of *P. carinii* does not necessarily lead to a definitive diagnosis of disease. The status of *P. carinii* infection in healthy foals remains unknown, although it is suspected that *P. carinii* is an opportunistic pathogen in the same way as it is in human infants. We used real-time PCR to perform surveillance of *P. carinii* in tracheal aspirate (TA) samples collected from Thoroughbred foals born in Japan. TA samples were collected from eight healthy foals. The foals had been bred on the same ranch and pastured with other horses since the age of 3 weeks. TA samples were collected at 3, 4, 6, 8, 10, and 12 weeks of age. Sterile disposable silicone tubes were used for sample collection, and samples were stored at  $-80^{\circ}\text{C}$  until DNA extraction. Samples were homogenized by using a mucus catabolic enzyme before DNA extraction. Specific primers targeting a 75-bp fragment from the large-subunit mitochondrial ribosomal RNA gene region of *P. carinii* were used in real-time PCR assays conjugated with fluorescent SYBR® Green I dye. More than one copy per PCR reaction was considered positive. All eight healthy foals gave positive results. The *P. carinii* gene was detected in two foals at 4 weeks of age, three foals at 6 weeks, and three foals at 8 weeks. Once the foals had become positive they stayed positive through to the end of the study at 12 weeks. The highest number of *P. carinii* gene copies during the surveillance period varied among the foals: the highest copy numbers ranged from 37 to 597 per PCR reaction. These results illustrate that, at least in Japan, foals are commonly exposed to *P. carinii* early in life, suggesting that *P. carinii* is an opportunistic pathogen in horses in the same way as in humans. Therefore, not only molecular diagnosis using clinical samples (e.g. by real-time PCR) but also pathological approaches will be needed to make a definitive diagnosis of *P. carinii* pneumonia in horses.