



## Posters MISCELLANEOUS INFECTIOUS DISEASE

### COMPLICATIONS ENCOUNTERED IN LETHAL EUROPEAN BABESIOSIS

D. Huber<sup>\*</sup>, B. Artuković<sup>\*</sup>, M. Hohšteter<sup>\*</sup>, L. Medven Zagradišnik<sup>\*</sup>, D. Vlahović<sup>\*</sup>, I. Mihoković Buhin<sup>\*</sup>, A. Gudan Kurilj<sup>†</sup>, J. Habuš<sup>†</sup>, I.C. Šoštarić-Zuckermann<sup>\*</sup>. <sup>\*</sup>Department of Veterinary Pathology, Faculty of Veterinary Medicine University of Zagreb, Zagreb, HR; <sup>†</sup>Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine University of Zagreb, Zagreb, HR

**Introduction:** Canine babesiosis is an important emerging vector-borne disease of worldwide distribution. Reported pathological findings of this disease include haemolytic anaemia with jaundice, bilirubin and haemoglobin deposition in tissues, splenomegaly, haemorrhages and pulmonary oedema. Here we report pathological findings so far not described in European canine babesiosis.

**Materials and methods:** The study included 31 dogs that had died due to babesiosis, proven by the detection of merozoites within erythrocytes, but not lymphocytes, in cytological and histological specimens. In all dogs, leptospirosis was excluded by a microscopic agglutination test, and the most common bacterial infections were excluded with a Gram stain.

**Results:** All dogs showed signs of haemolytic disease, and eight dogs exhibited changes consistent with disseminated intravascular coagulation. Three dogs suffered from fibrinous pleuritis accompanied by multifocal to confluent rhabdomyolysis of intercostal muscles, with intralesional vascular thrombi in one dog. Ten dogs showed severe alveolar oedema with alveolar haemorrhage, and seven dogs exhibited a multifocal to coalescing, necrotic mixed cellular myocarditis. Fibrinous peritonitis was seen in five dogs; in three of these this was accompanied by acute necrotic pancreatitis.

**Conclusions:** Acute pancreatitis and rhabdomyolysis have so far only been reported in association with African canine babesiosis, which is presumed to be the most pathogenic form of babesiosis. Other changes (ie, pleuritis, alveolar oedema with haemorrhage and myocarditis) have so far not been associated with canine babesiosis at all. The results emphasise that even though *Babesia* parasites primarily affect erythrocytes, babesiosis represents a multisystemic disease affecting all organs and tissues.

### UNUSUAL PATHOLOGICAL FINDINGS IN A HORSE INFECTED WITH TAPEWORM ANOPOLOCEPHALA PERFOLIATA

D. Stankevica<sup>\*</sup>, D. Gorbacevska<sup>\*</sup>, J. Vanaga<sup>†</sup>, A. Kalnina<sup>‡</sup>. <sup>\*</sup>Preclinical Institute, Latvia University of Life Sciences and Technologies, Jelgava, LV; <sup>†</sup>LBTU Veterinary Clinic, Latvia University of Life Sciences and Technologies, Jelgava, LV; <sup>‡</sup>Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, LV

**Introduction:** *Anoplocephala perfoliata* is described as the most prevalent tapeworm infecting horses associated with different types of colic. The parasites frequently attach to the wall of the caecum and ileum, leading to mechanical obstruction of the intestine, damage of the gut mucosa, hypertrophy of the intestinal circular muscle layer as well as ileal impaction or intussusception.

**Materials and methods:** A 6-year-old female horse was sent for post-mortem examination after it was euthanized due to anorexia, depression and progressive weight loss. Blood analysis showed mild anaemia. Full gross and histological examination and parasitological examination of the intestinal parasites was performed.

**Results:** Necropsy of the horse showed severe emaciation and severe tapeworm infestation of the jejunum, with multiple ulcerations and haemorrhages of the intestinal wall but only few tapeworms in the caecum. Parasitological examination of the jejunal specimens identified more than 100 tapeworms belonging to the species *A. perfoliata*. Morphological investigation also revealed serous atrophy of body fat tissue and gelatinous transformation of the femoral bone marrow. Histological examination showed mucosal atrophy of the jejunum and mild lymphocytic enterocolitis without eosinophil infiltration.

**Conclusions:** Pathological findings of the horse included changes not typically associated with *A. perfoliata* infestation, such as severe jejunal infestation instead of the typical localization of the parasite in the ileocaecal region. Mild intestinal inflammatory changes with absence of eosinophils could be explained by the bone marrow atrophy and hence reduced haematopoiesis.

### DOWNREGULATION OF THE MICROBICIDAL PROFILE OF M1 MACROPHAGES BY ENCEPHALITZOON CUNICULI

F.T. Camargo Konno<sup>\*,†</sup>, J.G. Xavier<sup>\*</sup>, A.M. Alvarez-Saraiva<sup>\*</sup>, E.C. Pérez<sup>\*</sup>, D.D. Spadacci-Morena<sup>‡</sup>, M.A. Lallo<sup>\*</sup>. <sup>\*</sup>Patologia Ambiental e Experimental, Universidade Paulista-UNIP, São Paulo, BR; <sup>†</sup>Universidade Paulista, Instituto Butantan, São Paulo, BR; <sup>‡</sup>Departamento de Fisiopatologia, Instituto Butantan, São Paulo, BR

**Introduction:** Microsporidia infect vertebrates and invertebrates and are recognized as opportunistic agents in individuals with immunological deficiencies. Although the activity of CD8<sup>+</sup> T lymphocytes is essential to eliminate microsporidia, macrophages are attributed a fundamental role in innate immunity and in the activation of acquired immunity. For some infectious agents, the polarization of macrophages to the M1 and M2 profiles is fundamental in defining the course that the infection will take. This study aimed to evaluate *in vitro* the activity of macrophages modulated for the M1 and M2 profiles in encephalitozoonosis.

**Materials and methods:** Murine bone marrow macrophages, previously differentiated, were polarized in M1 with recombinant IFN- $\gamma$ +LPS and in M2 with IL-4 for 24 hours and challenged with *E. cuniculi* at a 2:1 ratio for 5, 10 and 24 hours for determination of the phagocytic index, nitric oxide and cytokine production and cell phenotyping.

**Results:** Macrophages polarized to M1 showed high expression of CD40<sup>+</sup>, iNOS, CD80/86 and MHC. However, we observed a decrease in CD40<sup>+</sup> expression in M1 macrophages challenged with *E. cuniculi*. Infection by *E. cuniculi* determined a significant decrease in CD206<sup>+</sup> expression by M2 macrophages, once again indicating the ability to modulate the phenotype of these cells induced by the presence of the pathogen. Additionally, the

phagocytic index of *E. cuniculi* spores was lower in M1 compared with M2 macrophages.

**Conclusions:** The microsporidian *E. cuniculi* was able to modulate the microbicidal phenotype of M1 macrophages by decreasing CD40 and iNOS expression over time of infection.

#### CROTOXIN MODULATES THE M1 PROFILE OF MACROPHAGES INFECTED WITH ENCEPHALITOZOON CUNICULI

C.G. Nascimento de Oliveira\*, J.G. Xavier\*, A.M. Alvares-Saraiva\*, E.C. Pérez\*, D.D. Spadacci-Morena†, S.C. Sampaio†, M.A. Lallo\*. \*Patologia Ambiental e Experimental, Universidade Paulista-UNIP, São Paulo, BR; †Departamento de Fisiopatologia, Instituto Butantan, São Paulo, BR

**Introduction:** Crotoxin (CTX), a bioactive extract from the snake *Crotalus durissus terrificus*, demonstrated the ability to modulate the profile of macrophages infected with *Leishmania amazonensis*, with increased phagocytic capacity and elimination of intracellular parasites. Microsporidia are opportunistic, obligate intracellular fungi that infect vertebrates and invertebrates, having demonstrated the ability to modulate the macrophage profile. The aim of this study was to evaluate the effects of crotoxin on the viability of spores of the microsporidian *Encephalitozoon cuniculi*, as well as on the microbicidal activity of macrophages *in vitro*.

**Materials and methods:** Peritoneal adherent cells, obtained from peritoneal washings of BALB/c mice, were infected with spores of *E. cuniculi* and treated with CTX for 3 h. The profile and viability of macrophages, cytokine production and microbicidal activity were assessed as parameters.

**Results:** Macrophages infected with *E. cuniculi* and treated with CTX showed an increase in the M1 profile, more necrosis and increased production of cytokines TNF-alpha and IL-6. Also, the spores obtained from these macrophages had a reduced proliferative capacity.

**Conclusions:** The results indicate that CTX modulated macrophages infected with *E. cuniculi* differentiate towards the M1 profile, with increased production of pro-inflammatory cytokines and greater microbicidal activity. This suggests the potential of macrophages to modulate fungistatic and/or fungicidal activity against *E. cuniculi*.

#### INTERSTITIAL PNEUMONIA ASSOCIATED WITH EHV-5 IN THREE ADULT DONKEYS: PATHOLOGICAL AND MOLECULAR FINDINGS OF THE FIRST CASES IN ROMANIA

D.L. Hodor\*, A.G. Negoescu\*, C. Toma\*, A. Mureşan†, C. Molnar‡, C. Cătoi\*, M. Taulescu\*. \*Pathology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, RO; †Internal Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, RO; ‡Transylvania Arabians, Cluj, RO

**Introduction:** Equine herpesviruses (EHV) play a significant role as pathogens in both horse and donkey populations, leading to substantial economic losses. EHV-5 is a member of the equine gammaherpesvirus subfamily, along with EHV-2 and EHV-7, and is significantly associated with equine multinodular pulmonary fibrosis (EMPF), a distinct form of progressive interstitial pulmonary fibrosis in horses. This study documented the first occurrence of chronic respiratory disease associated with EHV-5 in donkeys in Romania.

**Materials and methods:** In an ecological donkey farm with a herd of 140 donkeys, eight animals showed respiratory signs including long-term dyspnoea. Three donkeys died and were sent for post-mortem examination. Samples (lung tissue and mediastinal lymph node) were collected for cytological, histopathological and PCR (EHV-1, EHV-4, EHV-5) analyses.

**Results:** The macroscopic lesions in affected donkeys consisted of lungs that failed to collapse and showed a diffuse rubbery-firm texture with visible rib imprints and a micronodular aspect on the cut surface. Histologically, the lungs exhibited severe multifocal to coalescing interstitial pneumonia with multinucleated syncytial cells, mild fibrosis, pneumocyte type 2 hyperplasia and alveolar histiocytosis. Mediastinal lymph nodes showed severe reactive lymphoid hyperplasia and severe infiltration with

macrophages and syncytial cells. PCR identified EHV-5 in all examined animals.

**Conclusions:** EHV-5 infection showed gross and histological lesions consistent with interstitial pneumonia and no distinctive features indicative of other herpesvirus infections. Molecular analysis confirmed EHV-5 infection in all examined cases. To the authors' knowledge, this is the first report in Romania of EHV-5-associated interstitial pneumonia in donkeys.

#### MASS SPECTROMETRY IMAGING (MALDI-MSI) AS A MOLECULAR HISTOLOGY APPLICATION FOR CHARACTERIZING PULMONARY LESIONS CAUSED BY PRRSV-1 STRAINS OF DIFFERENT VIRULENCE

J. Sánchez-Carvajal\*, I. Rodríguez-Gómez\*, I. Ruedas-Torres\*, F. Pallarés\*, L. Carrasco\*, E. Chicano-Gálvez†, J. Gómez-Laguna\*. \*Anatomy and Comparative Pathology and Toxicology, Faculty of Veterinary Science, University of Córdoba, Córdoba, ES; †IMIBIC/UCO/HURS, Córdoba, ES

**Introduction:** Porcine reproductive and respiratory syndrome virus (PRRSV) is a significant viral pathogen that affects swine populations worldwide. This study applied MALDI-MSI as a molecular histology tool to determine the peptide signature of the lungs of piglets infected with PRRSV-1 strains of different virulence.

**Materials and methods:** A total of 52 piglets were divided into three groups: (i) control group (n = 12), (ii) piglets infected with the low-virulence PRRSV-1 strain 3249 (n = 20), and (iii) piglets infected with the virulent PRRSV-1 strain Lena (n = 20). Animals were sacrificed at 1, 3, 6 and 8 days post infection (dpi). For the MALDI-MSI study, each three animals euthanized at 6 and 8 dpi were selected based on clinical signs, macroscopic and microscopic lesions and viral load.

**Results:** We found 190 proteins to be differentially expressed between the control and infected groups, with statistical significance (ANOVA;  $P < 0.025$ ). Using sparse PLS Discriminant Analysis (sPLS-DA) based on the protein intensity profiles, a clear differentiation was observed between the experimental groups and sacrifice dates, confirming the analysis' stability. Ontology analysis revealed that the differentially expressed proteins were associated with signalling pathways related to 'posttranscriptional gene expression regulation', 'mRNA catabolic processes', 'exocytosis' and 'neutrophil degranulation'. Hub proteins, including C3 and C4a complement components, apolipoprotein A and B and ISG15, were identified as central players in protein-protein interaction networks during PRRSV-1 infection at the lung level.

**Conclusions:** MALDI-MSI enables a proteomic approach for the detection of novel peptides potentially associated with different observed lesion patterns in piglets infected with PRRSV-1.

**Acknowledgments:** JMCS and IRT are supported by a "Margarita salas" contract from the Spanish Ministry of Universities. This work was supported by the Spanish Ministry of Economy and Competitiveness (#AGL2016-76111-R and PID2019-109718GB-I00).

#### CHARACTERIZATION OF AQUAPORIN-4 EXPRESSION IN THE BRAINS OF DOGS WITH CANINE LEISHMANIASIS

G.G. Jussiani, E.D. Frigério, R.M.B. de Aquino, M.E.E. Marchioni, M.A.I. Zoccoler, G.F. Machado. School Of Veterinary Medicine, São Paulo State University, UNESP, Araçatuba, BR

**Introduction:** Aquaporin-4 (AQP4) is a water channel protein expressed in astrocytes that has been associated with several neuroinflammatory conditions. The objective of this study was to assess whether AQP4 expression in astrocytes is affected during canine leishmaniasis (CanL).

**Materials and methods:** We used formalin-fixed, paraffin-embedded blocks, cut at 5 µm thickness, of 20 brains of dogs diagnosed with CanL who presented with mild to pronounced inflammatory changes in the nervous tissue, such as meningitis, choroiditis and/or presence of perivascular cuffs, determined in HE-stained sections. Immunohistochemical stain was performed using primary antibodies specific for AQP4, GFAP (glial fibrillary acidic protein) and CD3 (T lymphocytes) in order to