ZOO AND WILDLIFE DISEASES

MYCOBACTERIUM TUBERCULOSIS INFECTION IN CAPTIVE WHITE RHINOCEROSES *(CERATOTHERIUM SIMUM SIMUM)*

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Introduction: An outbreak of *Mycobacterium tuberculosis* affected several species in a Swedish zoo. Three white rhinoceroses (*Ceratotherium simum simum*) tested positive inconsistently on serology and tuberculin skin tests. *M. tuberculosis* was cultured once from a nasal wash from one of the rhinoceroses.

Materials and Methods: Necropsy, histopathology and real-time PCR on formalin-fixed, paraffin wax-embedded tissues were conducted.

Results: At necropsy examination, two rhinoceroses had one nodule (0.4 cm and 1 cm, respectively) in the lungs. These were composed of clusters of solid granulomas, formed by epithelioid macrophages, multinucleated giant cells, lymphocytes, fibrosis and neovascularization. Initially, no acid-fast bacteria (AFB) were detected histologically and tuberculosis was not confirmed by culture from numerous tissues. Retrospectively, in-depth studies were done. A few AFB were observed on serial sections of the granulomas and *M. tuberculosis complex* infection was confirmed in the two rhinoceroses by real-time PCR.

Conclusions: Post-mortem confirmation of tuberculosis, in particular in exotic species, can be a challenge. No visible tuberculosis lesion (or minimal lesion, as in this case) is a frequent presentation of infected, but clinically healthy animals. A thorough systematic necropsy examination, including thin slicing of lungs and lymph nodes, increases the likelihood of detecting small lesions, which is key to the confirmation of tuberculosis. A further difficulty is that the histomorphology of the lesions differs in different taxonomic groups. This study describes the pathology of minimal-lesion tuberculosis in rhinoceroses, presents post-mortem tools and discusses diagnostic challenges. A successful pathology-based confirmation of tuberculosis is of particular importance for valuable individuals humanely destroyed due to positive reactivity in live tests.

CHLAMYDIA PNEUMONIAE IN SNAKES: A CASE STUDY

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Introduction: Chlamydial infections in reptiles and amphibians have been associated with granulomatous inflammation in the inner organs. *Chlamydia pneumoniae* has been repeatedly detected in these lesions. However, limited knowledge is available concerning the clinical significance of chlamydial infections in snakes.

Materials and Methods: Mortality cases ($n = 6$) during the winter rest (2010–2013) were reported in a captive snake population. Dead snakes were investigated by histopathology and molecular tests for Chlamydiaceae. The total remaining population ($n = 47$) was tested for Chlamydiaceae by PCR on swabs of the choana and cloaca. *C. pneumoniae*-positive animals ($n = 7$) were treated with marbofloxacin and chlamydial shedding was monitored by PCR. Concurrent viral infections (i.e. adenoviruses and paramyxoviruses) were tested for by analysis of swab samples.

Results: Infection with *C. pneumoniae* was confirmed in a horned viper (*Vipera ammodytes*) with histiocytic granulomas in the heart and liver. The other mortality cases were negative for *Chlamydia*. By PCR, seven out of 47 snakes were positive for *C. pneumoniae*. All positive snakes were either horned or Caucasus vipers *(Vipera kaznakovi)*, were clinically unremarkable and were positive either for swabs from the choana or the cloaca or both. Marbofloxacin treatment was associated with negative swab samples after 5–10 days of treatment in five snakes. One snake remained positive up to 4 months after initial testing. Investigations for viral agents were negative ($n = 47$).

Conclusions: *C. pneumoniae* has been detected in granulomas in reptiles, but might also represent an opportunistic pathogen in snakes. Chlamydial shedding via the choana and the cloaca, or both, is likely to be the source of infection.

CHLAMYDIALES IN THE KOALA (*PHASCOLARCTOS CINEREUS*): ORGAN DISTRIBUTION AND RELATED HISTOPATHOLOGICAL FINDINGS

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Introduction: Chlamydial infections in koalas can cause chronic diseases leading to blindness and sterility. However, knowledge about the systemic spread of chlamydiae in the inner organs of the koala and related pathological organ lesions is limited.

Materials and Methods: In this study, a thorough investigation of organs from 23 koalas was performed and their histopathological lesions were correlated with molecular chlamydial detection. To reach this goal, 246 formalin-fixed and paraffin wax-embedded organ samples from 23 koalas were investigated by histopathology, Chlamydiaceae real-time PCR and immunohistochemistry (IHC), ArrayTube microarray for Chlamydiaceae species identification as well as Chlamydiales real-time PCR and sequencing.

Results: By PCR, two koalas were positive for *Chlamydia pecorum*. By IHC, *Chlamydiaceae* were detected in 10 tissues out of nine koalas. The majority of these ($n = 6$) had positive labelling in the urogenital tract related to histopathological lesions such as cystitis, endometritis, pyelonephritis and prostatitis. Somewhat unexpected was the positive immunohistochemical labelling in the gastrointestinal tract including the cloaca. Immunoreactivity in lung and spleen indicated systemic spread of infection. Uncultured Chlamydiales were detected in several organs of seven koalas by PCR, and four of these suffered from plasmacytic enteritis of unknown aetiology.

Conclusions: Chlamydiales might be associated with plasmacytic enteritis. Chlamydiaceae can cause systemic infections, but can be also detected in the intestine without association with histopathological lesions. The gastrointestinal tract might be a site of persistent chlamydial infection and a source for re-infection of the genital tract, as recently shown in a mouse model.

HIGH INCIDENCE OF NEOPLASIA IN A GROUP OF CAPTIVE RICHARDSON'S GROUND SQUIRRELS (*UROCITELLUS RICHARDSONII*)

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Introduction: Richardson's ground squirrels (RGSs) are kept infrequently as exotic pets and there is a lack of pathological studies on captive populations. Cancer is regarded as uncommon, but hepadnavirus-associated hepatocellular carcinoma has been documented in a number of individuals. This study demonstrates the high incidence of neoplasia in a group of rescue animals ($n = 13$).

Materials and Methods: From February 2011 to May 2014 nine animals were examined utilizing combinations of radiography/computed tomography, incisional/excisional biopsies and post-mortem examination. Histology ($n = 6$) and transmission electron microscopy (TEM) ($n = 1$) was carried out to investigate the cause of death and presence of a viral agent.

Results: Identified neoplasms comprised hepatocellular carcinoma ($n = 3$), lipoma ($n = 3$), elodontoma ($n = 3$), leiomyosarcoma ($n = 1$), hepatic adenoma ($n = 1$), squamous cell carcinoma (SCC) ($n = 1$), myelolipoma ($n = 1$) and renal papillary adenocarcinoma (RPA) ($n = 1$). The mean age at diagnosis ranged from 25 to 47 months of age. Seven females and three males were affected. Elodontoma was the only tumour diagnosed in males. Mean survival was 1 to 14 months. TEM could not identify virus particles.

Conclusions: Contrary to previous findings, this study indicates a high incidence of neoplasia in RGSs and includes the first reports of elodontoma, leiomyosarcoma, SCC, myelolipoma and RPA. The