

dorsal hoof wall and laminae, solar wall and third phalanx. Sections were stained with haematoxylin and eosin and Warthin-Starry.

Results: The early stage of the disease is represented by dermatitis of the digital skin and coronary band and is predominantly lymphoplasmacytic. In the most severe stage, the cornified layer of the dorsal hoof wall exhibits severe suppurative inflammation, haemorrhage and intralesional bacteria, and there is separation of the hoof wall from the underlying laminae. In the later, healing stages of the disease, the hoof wall re-grows, but is often deformed. Milder histological lesions of lymphoplasmacytic dermatitis of the coronary band and suppurative inflammation of the horn remain. The dorsal aspect of the third phalanx exhibits a moderate degree of periosteal activation and osteophyte formation. Warthin-Starry staining reveals the presence of spirochaetal organisms morphologically consistent with *Treponema* spp.

Conclusions: This is the first description of the pathological changes of CODD, and demonstrates the presence of what is considered the most likely aetiological agent (i.e. *Treponema* spp.).

RETROSPECTIVE MOLECULAR STUDY OF CANINE INFECTIOUS HAEMOLYTIC ANAEMIAS

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Introduction: The most common infectious agents of haemolytic anaemia in Croatian dogs are *Babesia canis* and *Leptospira interrogans*. Despite varied causes, post-mortem findings include anaemia, icterus, splenomegaly and haemoglobinuric nephrosis. The aim of this study was to genotype pathogens in archival samples from dogs that died from haemolytic anaemia.

Materials and Methods: Slices (30 µm) from formalin-fixed and paraffin wax-embedded spleen, lung, myocardium and kidney samples from 19 dogs were selected for molecular analyses. After incubation with xylene followed by ethanol washes, DNA was extracted with a commercial kit. All samples positive for mammalian cytochrome-C were screened for the presence of *Leptospira* spp., *Babesia* and *Theileria* spp., *Anaplasma* and *Ehrlichia* spp., *Hepatozoon canis* and *Bartonella* spp. Amplified samples were purified and sequenced. Results were compared with patho-anatomical, histopathological, cytological and serological findings.

Results: *Babesia* spp. found by post-mortem cytology in seven cases was confirmed in four dogs by PCR from lung and myocardium, but not spleen. Sequencing revealed *B. canis* in three dogs and *Theileria* spp. in a single dog. Co-infection of *B. canis* and *A. phagocytophilum* was found in one myocardium. *Candidatus* *Neoehrlichia mikurensis* was detected in the kidneys from a dog and *A. phagocytophilum* in the kidneys and lung from another.

Conclusions: *Babesia canis* was the most frequent pathogen, as expected. The presence of *A. phagocytophilum*, *Candidatus* *N. mikurensis* and *Theileria* spp. presents an unexpected finding in organs from dogs who died from haemolytic anaemia. These findings suggest a complex aetiology of haemolytic anaemia in dogs without pathognomonic lesions characteristic for any of the detected pathogens.

MERS CORONAVIRUS INFECTION OF RABBITS

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Introduction: A new coronavirus (CoV), Middle East respiratory syndrome CoV (MERS-CoV), is causing an ongoing outbreak in people, sometimes resulting in severe or even fatal pneumonia. MERS-CoV uses dipeptidyl peptidase 4 (DPP4) as a functional receptor and is able to infect cells of a limited number of animal species including bats, camels, goats and non-human primates *in vitro*, but so far there is no good animal model for human disease. Because the virus binding region in rabbit DPP4 closely resembles that in human DPP4, we tested whether rabbits can be infected with MERS-CoV as an animal model for MERS-CoV infection in man.

Materials and Methods: Sixteen rabbits, serologically negative for MERS-CoV, were inoculated with MERS-CoV or sham inoculum via nose and trachea and swabs were taken frequently. The rabbits were killed 3, 4 or 21 days ($n = 4$ per day) after inoculation and during necropsy examination samples were taken for pathology, immunohistochemistry, in-situ hybridization and virology.

Results: The rabbits had no clinical signs and 3 or 4 days after inoculation. They had high viral loads as determined by PCR and scattered virus antigen expression in the lungs and nose, associated with mild alveolitis and moderate rhinitis.

Conclusions: The results of this study demonstrate that rabbits can be infected with MERS-CoV with virus replication in the lungs and nose. Therefore, rabbits infected with MERS-CoV may be used as a model to study the pathogenesis of MERS, transmission of MERS-CoV and to test intervention strategies aimed at inhibition of MERS-CoV replication *in vivo*.

NO EVIDENCE OF SARCOCYSTIS CALCHASI INVOLVEMENT IN MENINGOENCEPHALITIS OF UNKNOWN ORIGIN IN MAMMALS

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Introduction: *Sarcocystis calchasi* is an intracellular protozoan parasite belonging to the phylum Apicomplexa. It was identified as the causative agent of pigeon protozoal encephalitis (PPE) during an outbreak in Berlin in 2008. PPE is an ongoing threat as new cases are diagnosed continuously in pigeons in the Berlin area. Birds and mammals usually serve as intermediate hosts of other *Sarcocystis* spp., thus a retrospective study was conducted to determine whether *S. calchasi* may be involved in cases of meningoencephalitis of unknown origin (MUO) in mammals.

Materials and Methods: Formalin-fixed and paraffin wax-embedded (FFPE) samples of 142 brains with MUO from different mammalian species (i.e. dog, cat, pig, cattle, sheep, guinea pig, horse, goat, mouse, raccoon, ferret, hamster, mink and manx wolf) collected between 1989 and 2012 were re-examined histologically using HE-stained sections. DNA was isolated from FFPE material and screened by PCR with primers specific for the *18S rRNA* and the *ITS-1* genes to detect *S. calchasi* or other apicomplexan parasites, respectively.

Results: In all samples the diagnosis of non-suppurative (lymphoplasmacytic and/or granulomatous) meningoencephalitis was confirmed, but no parasitic structures were found. DNA of *S. calchasi* or other apicomplexan parasites could not be detected in any of the samples.

Conclusions: Despite the seemingly high prevalence of PPE and persistent threat of *S. calchasi* in pigeons in the Berlin area, no evidence was found for a role of this parasite in MUO in mammalian species.