Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

Figure 1—Postmortem images of the right eye and the surface of a kidney of a 1-year-old 1.72-kg castrated male domestic rabbit that was euthanized because of weight loss, lethargy, dehydration, and respiratory distress. A—Injected scleral and conjunctival vasculature are evident and indicative of active conjunctivitis. B—There are multifocal capsular depressions in the renal cortex.

History

A 1-year-old 1.72-kg castrated male domestic rabbit was presented to the University of Georgia Veterinary Teaching Hospital for evaluation of weight loss, lethargy, dehydration, and respiratory distress. Upon admission, severe ocular inflammation, including scleral injection and conjunctivitis with iriditis, was noted in the right eye. The rabbit lived in a herd of 19, which had a previous history of diarrhea and eimeriosis. Along with the submitted rabbit, 2 other rabbits in the herd reportedly had similar clinical signs but died without confirmatory diagnosis.

Clinical and Gross Findings

The rabbit was euthanized for diagnostic purposes to direct treatment for the rest of the herd. On necropsy, the rabbit had a body condition score of 2/5, weighed 1.72 kg, and had moderate postmortem autolysis. Evidence of diarrhea was present in the form of abundant thick, yellow to brown, opaque material covering the anal and preputial orifices,

although well-formed, desiccated fecal pellets were present in the descending colon. Scleral injection and conjunctivitis were noted on the right eye (**Figure 1**), and abundant dark brown waxy debris was present in both vertical ear canals.

Complete necropsy of the rabbit revealed numerous irregular depressions (1 to 3 mm in diameter) throughout the renal cortex (**Figure 1**) and a diffuse lack of corticomedullary distinction bilaterally. Between the pericardial sac and the visceral surfaces of the lung lobes were numerous pale tan fibrinous adhesions. There was a diffuse mahogany to tan reticular pattern in the liver. No other major macroscopic changes were identified during the necropsy.

Formulate differential diagnoses, then continue reading.

Histopathologic Findings

Within the brain, the meninges were variably expanded by coalescing aggregates of lymphocytes, macrophages, and plasma cells. Multifocally surrounding cortical blood vessels in the cerebrum and brain stem were large perivascular cuffs comprised of lymphocytes and plasma cells (**Figure 2**). The endothelium of surrounding vessels was plump or swollen. Glial nodules comprised of haphazardly arranged rod-shaped microglia and astrocytes, admixed with necrotic foci, were scattered throughout the cortical gray matter, and the neuroparenchyma was rarefied. Within the central core of glial nodules were

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Figure 2—Photomicrographs of tissue sections of brain (A) and kidney (B) from the rabbit described in Figure 1. A—Multifocal perivascular cuffs (arrowhead) comprised of lymphocytes and plasma cells and a focal glial nodule (arrow) are seen. H&E stain; bar = 100 μ m. B—There is coalescing lymphoplasmacytic inflammation in the renal cortex, with replacement of tubules. H&E stain; bar = 100 μ m.

Figure 3—Photomicrographs of sections of brain tissue from the rabbit described in Figure 1. A—There is a focal area of necrosis surrounded by abundant inflammatory cells with numerous free, gram-positive microsporidian organisms (arrow) that are up to 3 μ m in length. Gram stain; bar = 20 μ m. B—There is scant acid-fast reactivity of the free microsporidian organisms (arrows). Ziehl-Neelsen (acid fast) stain; bar = 20 μ m.

occasional intra- and extracellular, 1 μ m in width and 3 μ m in length, gram-positive, and variably acid-fast oval microsporidia (**Figure 3**). On histochemical staining of brain tissue sections, abundant numbers of the organism were visualized.

Radiating throughout the cortex and medulla of the kidney were large, multifocal aggregates of lymphocytes, plasma cells, and macrophages, which replaced approximately 25% of the interstitium (Figure 2). Fibrous stroma multifocally replaced the interstitium, and there were multifocal areas of necrosis with karyorrhectic debris. There was multifocal renal tubule degeneration and necrosis with multifocal tubule regeneration. The renal pelvis was expanded by coalescing lymphoplasmacytic inflammation (pyelitis).

Periportal areas of the liver and perivascular and interstitial spaces of the lung and heart had similar inflammatory infiltrates. Within the right eye, the equatorial and posterior cortical lens fibers were degenerative, characterized by swollen or hyperosinophilic fibers with occasional fragmentation. The limbal portions of the left cornea had scattered heterophils, lymphocytes, and plasma cells with neovascularization.

Morphologic Diagnosis and Case Summary

Histologic and gross findings of necrotizing and lymphohistiocytic meningoencephalitis with gram-positive microsporidia in the cerebrum; interstitial lymphohistiocytic and plasmacytic inflammation of

the kidney, lung, heart, and liver; and cortical lenticular degeneration (cataract) in the right eye and heterophilic and lymphoplasmacytic limbal keratitis of the left eye were suggestive of chronic, systemic microsporidiosis, most likely caused by *Encephalitozoon cuniculi*.

Comments

Encephalitozoon cuniculi is an obligate intracellular microsporidian organism that forms parasitophorous vacuoles and propagates within host cells.^{1,2} Four non-host-specific genotypes are known to exist, with genotype I typically associated with rabbits.³ This genotype has the potential for zoonotic transmission as an opportunistic pathogen, which suggests that fundamental knowledge of the clinical presentation and diagnosis of the disease may be beneficial for veterinary practitioners as well as their clients.⁴⁻⁶

Pathogen transmission occurs horizontally via ingestion of *E cuniculi* spores in contaminated urine and vertically in utero with infected does. Once the parasite is ingested, the organism is transported by spores in monocytes to highly vascularized organs including the brain, kidneys, lungs, heart, and liver.² Infection begins within gastrointestinal tissues, spreading first to abdominal organs, and then to thoracic organs.³ Chronic interstitial nephritis and fibrosis causes capsular retraction with occasional atrophy of glomeruli.^{6,7} Although rare, visualization of spores may be observed in areas of inflammation throughout the cortical interstitium with routine staining with H&E stain.⁶ Gram and modified trichrome

stains (MTS), in conjunction with H&E stain, have been suggested as the best option for detecting *E. cuniculi* histologically.⁶ To determine whether active, environmental shedding was occurring, renal tissue from the this rabbit was submitted for PCR assay and tested negative for *E. cuniculi*. The negative PCR assay result may have reflected a low number of organisms present within the kidney at the time of necropsy. Additionally, *E. cuniculi* primer specificity may have contributed to a negative PCR assay result. Inflammation of the eye resulting from systemic *E. cuniculi* infection also frequently manifests as cataract formation, potentially progressing to hypopyon and uveitis in many cases. Lens rupture following *E. cuniculi*-induced inflammation leads to phacoclastic uveitis in a high percentage of lenses testing positive for *E. cuniculi* via PCR assay. Although urine and cerebrospinal fluid samples are unreliable for PCR assay detection of *E. cuniculi*, testing samples of phaco-emulsified lens material has proven to be sensitive.^{2,8} Recent research suggests that quantitative PCR assessment of affected tissues may be more likely to reveal presence of microsporidian DNA in affected tissues, even in the absence of clinical signs of infection.³

Brain tissue is commonly the last organ to be affected, with *E. cuniculi* reaching the tissue approximately 3 months after infection.^{3,7} Granulomatous meningoencephalitis is a typical lesion, and brain tissue in deceased rabbits that had neurologic signs may contain lymphoplasmacytic perivascular cuffs. Histologically, granulomatous lesions were scattered throughout the cerebrum with perivascular macrophages containing cyst-like microsporidia within a parasitophorous vacuole.⁷ Subclinical infection to sudden death may occur in infected rabbits, and diagnosis at necropsy may be challenging due to low numbers of organisms present in affected tissues.²

In the rabbit of the present report, abundant numbers of the organism were visualized using histochemical stains on histologic brain sections. This can be used to approximate the length of time *E. cuniculi* has been present in the herd, and in the absence of appropriate biosecurity measures, it was likely that other rabbits in the herd had been exposed and should be tested for evidence of subclinical disease. Although prevalence of *E. cuniculi* is thought to be widespread within colonies, the disease process is often chronic and subclinical, with only a low percentage of infected animals developing clinical disease.⁹ Treatment is considered challenging; however, individual fenbendazole administration has shown promise in rabbits with microsporidian infections.¹⁰ Albendazole administration in human patients with microsporidiosis has been reported to have similar success; however, treatment does not reverse tissue damage. It should be noted that zoonosis is a concern, with *E. cuniculi* infections rarely occurring in immunocompetent individuals; however, infection in immunosuppressed individuals (eg, young, elderly, and AIDS patients) may be severe.¹¹

In this case, herd-level recommendations included separation/isolation and supportive care of rabbits exhibiting clinical signs, limiting introduction of immune-naïve or new rabbits into the population, and routine cleaning and disinfection of bedding and housing, along with proper use of personal protective equipment. Combined with proper sanitation methods, sequential serology testing of all animals in the herd may provide useful clinical information to veterinarians, relating to the clinical stage of infection. Elevations in both anti-*E. cuniculi* IgM and IgG antibodies have shown to correlate with active infection in rabbits. This research suggests antiparasitic treatment may be indicated in rabbits with anti-*E. cuniculi* IgM antibodies.^{12,13} There is not currently a standard treatment protocol for *E. cuniculi* infection in rabbits, and further study of this disease and associated human health implications may be warranted.⁸

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