A Case of Canine Disseminated Protothecosis

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History and Presentation

Duke is a 5-year-old male neutered Pomeranian who presented to MSU-AHC Necropsy service on March 23rd, 2018. He originally presented to MSU-AHC Emergency on February 12th for a 2-day history of ataxia and dull mentation and a 24-hour history of 2 seizures and a right head turn. After transfer to the MSU-AHC Neurology service a complete neurological examination and MRI were performed. On neurological examination, he had a right head turn, a right head tilt, inducible rotational nystagmus, and right sided hemiparesis more severe in the pelvic limb. An MRI was performed and revealed obstructive hydrocephalus. Serum was submitted for infectious disease titers. He was discharged with doxycycline, clindamycin, fluconazole, omeprazole, and levetiracetam and scheduled for a recheck in 2-4 weeks.

He re-presented to MSU-AHC Emergency on February 19th for cluster seizures. On neurologic examination, he had an absent menace OD, decreased right nasocortical and lateral canthal sensation (impaired maxillary branch of the trigeminal nerve), right head turn, right head tilt, inducible horizontal nystagmus with fast phase to the left, and right thoracic monoparesis. Previous medications were continued with an increased levetiracetam dose. Complete blood cell count revealed moderate mature neutrophilia and mild lymphopenia. Infectious disease titers submitted from the previous visit were negative. Doxycycline, clindamycin, fluconazole, and were discontinued. Prednisolone was added to the treatment plan and cytarabine therapy was instituted.

After cytarabine administration, neurologic signs initially improved. The head turn resolved, a normal menace and palpebral response, normal conscious proprioception, and no spontaneous nor inducible nystagmus were present. On March 16th, Duke returned to MSU-AHC for a second cytarabine treatment. He had been doing well at home with only episodes of mild

ataxia noted. On neurologic examination, he had mild vestibular ataxia, right head tilt, absent menace OD, positional ventrolateral strabismus OD, and inducible horizontal nystagmus with fast phase to the left.

On March 22nd, Duke returned to MSU-AHC Emergency for seizures, tachypnea, and inappetence. He had had two seizures since discharge on March 16th, a tonic-clonic seizure on the 18th and a mild seizure on the 22nd. He had been tachypneic since discharge on the 16th and falling when standing only for a short period of time. On neurologic examination, he had dull mentation, mild vestibular ataxia, right head tilt, absent menace OD, positional ventrolateral strabismus OD, miosis OD, impaired right maxillary branch of the trigeminal nerve function, facial twitching, and right thoracic monoparesis. His mentation improved after administration of hypertonic saline and maropitant.

Antemortem diagnostics on March 23rd revealed mild regenerative anemia, marked panleukopenia with toxic neutrophils and a left shift, moderately elevated ALT and ALP, moderate hypocalcemia, mild hypoalbuminemia, and mild hypernatremia. A canine SNAP cPLI was abnormal. No abnormalities were noted on urinalysis or urine culture or sensitivity. Shortly thereafter Duke underwent cardiac arrest and was unable to be resuscitated.

Pathological Findings

Gross Pathologic Findings

Multifocal to coalescing white pinpoint foci are disseminated throughout multiple organs, including the heart, liver, kidneys, and mesentery. Along the endocardium, the foci are slightly raised. The foci on the surface of the kidney are slightly raised and on cross-sectioning of the cortex and medulla, the foci bulge slightly. The ventricular systems are moderately dilated and

accompanied by mild cerebellar herniation. The ventricular surface is roughened and has multifocal small 1 – 3 mm raised tan plaques. The mesentery and mesenteric fat adjacent to the pancreas has multifocal white firm 1 – 2 mm nodules.

Histopathologic Findings

Disseminated throughout many organs, including liver, kidneys, pancreas, skeletal muscle, myocardium, brain, thyroid, and eyes are many algal organisms. They are often extracellular, rarely intrahistiocytic, and round to oval, 8-20 um wide with a clear 2-4 um thick wall, and basophilic to amphophilic material or multiple (2-8) wedge-shaped endospores. Inflammation associated with the organisms is pronounced within the brain and liver, but only minimally present in the remaining affected organs.

In all brain sections examined (cerebrum, cerebellum, midbrain through brain stem and cervical spinal cord), often effacing the periventricular neuropil, ependyma, choroid plexus, leptomeninges, and randomly throughout the neuropil are variably sized often coalescing granulomas. They are composed of epithelioid macrophages with a necrotic center, occasionally peppered with small numbers of eosinophils and fewer lymphocytes. Multifocally, these granulomas are accompanied by large clusters of algal organisms (sporangia 15 um wide, with many progeny cells) and necrosis of the surrounding neuropil. There are scattered perivascular cuffs of epithelioid macrophages, peppered with fewer lymphocytes. The associated neuropil is hypercellular with microgliosis, astrogliosis with frequent neuronal satellitosis. There are scattered small glial nodules randomly throughout cerebral neuropil. All ventricular spaces are moderately dilated, with multifocal disruption of the ependymal cells by the aforementioned

granulomatous inflammation. The histopathologic changes to the brain explain much of the patient's antemortem neurological signs observed clinically.

Discussion

Biology and Pathogenesis

Prototheca spp. are achlorophyllic, obligate saprophytic, algal organisms. As obligate saprophytic organisms, they are commonly found in environments with plentiful decomposing organic matter, such as sewage or manure. The organisms are found worldwide but are more common in warm, humid climates such as the southeastern United States, Japan, and southern European countries which border the Mediterranean sea[7]. Reproduction occurs via asexual endosporulation with eventual rupture and release of 2 – 20 daughter cells. Diameter of the organism varies from 1.5um to 30um. Variation in size is dependent upon species, time since sporulation, and culture conditions[3,7].

Protothecosis is an uncommon disease reported to affect people, dogs, cattle, and cats as well as other mammals. The most commonly isolated species of *Prototheca spp.* in both dogs and cattle is *P. zopfii.* Dogs often develop a disseminated infection that may cause a multitude of clinical signs relating to the affected organs. Commonly affected sites include the distal gastrointestinal (GI) tract, central nervous system (CNS), and eyes. Gastrointestinal signs are often the first to be noticed and include large bowel diarrhea, hematochezia, weight loss, and vomiting. Approximately half of dogs may have clinical signs attributable to CNS infection with or without GI signs. Neurologic signs may include seizures, central vestibular disease, altered mentation, blindness, deafness, ataxia, or lateralized or generalized lower motor neuron deficits.

Cutaneous lesions can occur independently or in conjunction with signs of disseminated protothecosis[3].

Protothecal mastitis in cattle is the most commonly reported protothecal disease as cattle often live in an environment rich with decomposing organic matter that is ideal for the maintenance of protothecal populations. Protothecal mastitis often results in granulomatous inflammation of the mammary tissue[2]. Mastitis caused by *Prototheca spp.* causes the dairy industry significant economic losses due to difficulty in treatment. Empirical treatment of mastitis with antibiotics will almost always fail in cases of protothecosis. This failure of treatment will eventually lead to removal from the herd due to chronic, unresolved mastitis. Meanwhile, any milk produced during treatment must be discarded due to antibiotic residues.

In contrast to the disseminated disease often found in dogs, protothecosis in cats and humans most often presents as a cutaneous infection caused by *P. wickerhamii*[3]. It is thought that cutaneous infection occurs secondarily to wound contamination and subsequent colonization by *Prototheca spp*. In people, the more uncommon disseminated infection has been linked to immunosuppression from disease or medication. However, in dogs a link between disseminated infection and immunosuppression has not been established. There is some evidence that protothecosis occurs concurrently with neutrophil dysfunction, but it is unknown if this is a predisposing factor of the disease or a consequence of the disease[3].

Due to the rarity of protothecosis, there is little definitive knowledge of the pathophysiology of the disease. As colitis is often the first clinical sign and the most common clinical sign among cases of disseminated protothecosis, it has been proposed that infection usually occurs via ingestion of *Prototheca spp.* and subsequent infection of the lower gastrointestinal tract [8,10]. It is then thought to spread via hematogenous and/or lymphatic routes[7].

However, there are several case reports of atypical presentation, including complete absence of GI infection in the presence of disseminated infection, to infection only affecting nervous tissues with no pathway to explain how the organism reached the CNS without affecting other body systems[1,5,6]. From the reported cases, *Prototheca* appears to have tropism for well vascularized tissues (i.e. eye, CNS, myocardium, and kidney) in disseminated infection. Host response to infection is most commonly granulomatous or pyogranulomatous inflammation[7].

Diagnostic Approach and Considerations

Delays in the diagnosis of protothecosis is one of the main contributing factors to its grave prognosis. There is no established gold standard test for diagnosis. There are multiple ways in which a diagnosis may be reached, all based upon identification of the organisms within samples from the patient or through culture of samples. Samples in which *Prototheca spp.* has been identified upon cytologic examination include urine sediment, colonic scraping, lymph node biopsy, CSF, and vitreous humor[3,4,9]. Selection of samples to examine should be based upon the patient's clinical signs. *Prototheca spp.* is easily cultured with standard media, including blood agar. In patients in which *Prototheca spp.* may be suspected, culture of urine, CSF, or vitreous humor could potentially be diagnostic for protothecosis[7,10].

Treatment and Management

As previously mentioned, the prognosis for disseminated protothecosis is grave. In part due to difficulty in early diagnosis, but also due to the lack of effective treatments. The most promising treatment thus far has been combination therapy using amphotericin B and itraconazole. This combination may have some ability to slow progression of disease[5]. If

protothecosis is identified prior to dissemination from the colon, the implementation of local therapies, such as amphotericin B retention enemas, may be considered but their use has not been evaluated[10].

Case Summary

In this case, it is speculated that the initial infection likely began within the brain. There was minimal to no inflammation associated with the algal organisms in other organs histologically besides the brain. The dissemination of the organisms subsequently followed the course of immunosuppressive therapy, leading to the clinical derangements and death of this dog. Regardless of the site of the primary infection and the cause of dissemination, protothecal meningoencephalitis and disseminated protothecosis both carry a grave prognosis.

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