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Case Number: N22-0194

Signalment: 8-year-old spayed female Black Mouth Cur dog

History: This patient presented to the Texas A&M University Emergency service due to seizure recurrence. She was first seen in the Emergency service three days prior to presentation also due to seizures and transferred to the Neurology service, where she was noted to be non-ambulatory with left lateralizing tetraparesis and proprioceptive ataxia. The dog was medicated and discharged. On the day of presentation, the owners reported the dog was shaking her ears, chattering her jaw, and drooling, despite the medication. The episodes first lasted for 10 seconds with 90 seconds intervals and then progressed to up to 40 seconds long with a minute interval. Given the guarded prognosis, euthanasia was elected.

Gross Findings: On necropsy, the leptomeninges were congested, and petechiae were evident on the right occipital lobe. Post-fixation sectioning of the brain revealed a poorly demarcated, 2x1x1 cm, soft, brown, partially cavitated area in the right cerebrum that extended from the thalamus towards the occipital lobe. The lesion affected primarily the white matter and multifocally extended into the gray matter. Other changes observed during necropsy were two areas of ecchymosis, one in the right lateral aspect of the middle third of the trachea and the other in the subcutaneous tissue of the ventral neck, cranial to the scapula.

Histologic Findings: Right cerebrum. In approximately 80% of the section, the white and gray matter, the meninges, and the Virchow-Robbins space are multifocally

expanded and replaced by numerous coalescent aggregates of epithelioid macrophages and multinucleated giant cells mixed with degenerate and viable neutrophils and eosinophils, lymphocytes, and plasma cells. Affected areas also have proliferation of glial cells (gliosis). Within the center of the inflammatory aggregates are frequent accumulations of eosinophilic amorphous material and cellular debris (necrosis). Surrounding these areas are a large number of concentrically arranged reticular fibers, and occasional immature, loose fibrous connective tissue (fibrosis). Within the necrotic areas and within multinucleated giant cell are numerous 7 to 10 µm in diameter, non-parallel walled, septate hyphae with bulbous projections and 45° (acute angle) branching. The blood vessels in the affected areas are moderately infiltrated by inflammatory cells that obscure the vascular wall (vasculitis). The neuroparenchyma is rarified and lost and infiltrated by numerous foamy gitter cells. The surrounding neuroparenchyma is moderately vacuolated and contains numerous polygonal to elongated astrocytes with abundant eosinophilic cytoplasm (gemistocytes).

Histologic Diagnosis: Right cerebrum: Marked, multifocal to coalescing, chronic, granulomatous meningoencephalitis with vasculitis, necrosis, and numerous fungal hyphae.

Comments: Granulomatous inflammation with fungal hyphae were also identified in the heart, spleen, liver, kidney, and peritracheal tissue. Grocott methenamine silver (GMS) stain was performed in the cerebrum, confirming the presence of fungal hyphae. The presence of melanin was confirmed using Fontana Masson stain. Additional histochemical stains included Masson's trichrome and reticulin. Based on these results, the etiologic diagnosis for this case was disseminated phaeohyphomycosis.

DNA from formalin-fixed paraffin-embedded (FFPE) samples was extracted and submitted for panfungal PCR targeting the internal transcribed spacer (ITS) region. The PCR product yielded a band of DNA at approximately 350 bp. After purification, the resulting 323 bp contig sequence was analyzed with the NCBI BLAST database. The sequence matched *Cladosporium* sp. with 100% identity. A second match identified *Penicillium* sp. with 100% identity. However, given the fungal morphology and positive Fontana Masson stain, this second match is considered a contaminant.

Phaeohyphomycosis is a disease caused by dematiaceous (pigmented) fungi. It is commonly associated with immunosuppressive underlying conditions, although cases in healthy individuals are also described. Dermatitis is the main clinical manifestation in veterinary species, but the infection can become systemic in rare cases.

Cerebral phaeophyphomycosis in veterinary species is usually associated with *Cladophialophora bantiana*, formerly called *Cladosporium bantianum*, a dematiaceous fungus that has tropism for nervous tissue. Recently, *Cladosporium cladosporioides* and *Cladosporium halotolerans* were also described as causes of mycotic encephalitis in dogs. *C. halotolerans* infection in a dog was associated with a coinfection by canine adenovirus-1 and canine parvovirus-2, the latter being a common cause of immunosuppression. Our patient had no clinical history of an underlying systemic disease and no concurrent disease was detected during postmortem examination.

Systemic infections by *Cladosporium* sp. are reported to affect the kidneys, liver, spleen, and brain. In the brain, it can cause ventriculitis and choroid plexitis, with secondary obstructive hydrocephalus. In our case, the lesions in the brain were restricted to the right cerebrum, extending from the thalamus to the occipital lobe. Other affected organs included the heart, spleen, liver, kidney, and peritracheal tissue.

The route of infection in this case is not clear. The larger primary lesion was the brain, and no lesions were found in the skin, lung, or intestine. During necropsy, an area of hemorrhage was found in the subcutaneous tissue of the ventral neck, proximal to the scapula. At the time, the hemorrhage was attributed to trauma due to seizures, and unfortunately, samples for histopathology were not collected. Given the presence of hemorrhage in the peritracheal tissue that later was associated with fungal infection, we speculate that the hemorrhage in the ventral neck could have also been associated with fungi and potentially be the primary site.

Interestingly, the fungal organisms in our case were not pigmented on routine H&E staining. Even though the mechanism is not clear, dematiaceous fungi do not always exhibit brown pigmentation in histologic sections, requiring additional stains such as Fontana Masson. The characteristic brown pigmentation is due to production of melanin, an important virulence factor in fungi. Melanin contributes to fungal survival in the environment, protecting from solar and gamma radiation. In the host, melanin alters phagocytosis of macrophages and neutrophils, delays recruitment of CD4+ lymphocytes, and increases resistance to oxidative damage, among other effects. However, non-dematiaceous fungi also can produce melanin, and therefore stain positive to Fontana Masson, requiring some caution when interpreting this stain in fungal infections. Panfungal PCR and sequencing can assist in fungal identification from FFPE samples. In this case, the fungal morphology, panfungal PCR results, and strong staining with Fontana Masson implicate *Cladosporium* sp. as the etiology.

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