Cryptococcosis of the central nervous system in a dog

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- Central nervous system cryptococcosis is an uncommon but potentially fatal disease.
- In contrast to humans, disease caused by cryptococcal infection in dogs occurs in the immunocompetent host.
- Recent advances in magnetic resonance imaging may assist in diagnosis of cryptococcal infection in the brain.
- Administration of fluconazole may resolve clinical disease, but long-term treatment may be required to prevent recurrence of clinical signs.

A 4-year-old 33-kg (73-lb) female German Shepherd Dog was referred to the Emergency Service at **Tufts University School of Veterinary Medicine (TUSVM)** for evaluation of signs of progressive neurologic disease. The dog had been acquired by its owner as a puppy from a breeder and had lived only in Vermont. The dog was vaccinated against rabies and canine distemper and had been receiving a preventative for heartworm disease monthly.

Three weeks prior to referral, the dog had been evaluated by the referring veterinarian because of right-sided peripheral vestibular signs. The dog had been treated with amoxicillin (15 mg/kg [6.8 mg/lb], PO, q 12 h) and a tapering course of prednisone (0.2 mg/kg [0.1 mg/lb], PO, q 12 h). Despite initial improvement, the dog's neurologic signs progressed during a 3-week period; at that time, the dog was examined for mental obtundation and vestibular ataxia of all limbs, as well as vomiting and urinary incontinence. Prednisone treatment was reinstituted (0.6 mg/kg [0.3 mg/lb], PO, q 24 h), and the dog seemed improved within 1 day but was urinating with increased frequency and continuing to vomit. Another antimicrobial (amoxicillin-clavulanic acid,[a] 8 mg/kg [3.6 mg/lb], PO, q 12 h) was prescribed, and the dog was referred to TUSVM for diagnostic evaluation and continued treatment.

At the time of referral, the dog appeared mentally obtunded but responsive and could stand and ambulate only with assistance. The dog was normothermic (101.6°F [38.7°C]) and tachycardic (132 beats/min) and was panting. The dog had mydriasis and appeared to be blind bilaterally with no pupillary light responses; the head was tilted to the right, and spontaneous vertical nystagmus was observed bilaterally. A right ventrolateral strabismus was present. Severe vestibular ataxia was identified during evaluation of the dog's gait. Findings of the cranial nerve examination were otherwise normal. Results of fundic examination confirmed marked chorioretinitis bilaterally with multiple raised granulomatous lesions. There was no known history of seizures. The dog was admitted by the TUSVM Emergency Service. Oral administration of amoxicillin-clavulanic acid and prednisone was discontinued, and the dog received treatment with ampicillin[b] (22 mg/kg [10 mg/lb], IV, q 8 h). Flurbiprofen ophthalmic solution[c] was administered topically to both eyes (1 drop each eye, q 4 h). The dog drank water when it was offered but would not eat food.

A CBC and serum biochemical profile were performed. The WBC concentration was within reference range, except for monocytosis (3,427 monocytes/µL; reference range, 150 to 1,350 monocytes/µL). Results of serum biochemical analyses were unremarkable, except for hypercalcemia (12.9 mg of calcium/dL; reference range, 8.5 to 11.3 mg of calcium/dL); slightly high activities of alkaline phosphatase (231 U/L; reference range, 10 to 150 U/L) and alanine transferase (135 U/L; reference range, 5 to 60 U/L) were also detected. Thoracic radiography revealed no abnormalities.

Within 6 hours of admission, the dog became agitated; it began to vocalize and paddle its limbs with decreasing responsiveness. A single dose of mannitol[d] (0.5 g/kg [0.23 g/lb], IV) was administered, and the dog appeared more alert and less agitated. The paddling and vocalization decreased. However, by the following morning (6 hours after mannitol treatment), the dog had become obtunded and was unable to support its weight even with assistance. Supportive treatments, including IV administration of fluids (lactated Ringer's solution[e]; 150 mL/h) and a gastric protectant (famotidine[f]; 0.5 mg/kg, q 12 h), were begun. A generalized seizure of 40 seconds' duration was observed.

The dog was anesthetized, and **magnetic resonance imaging (MRI)** of the brain was performed. On the T1-weighted spin echo sequence, a 0.8-cm focal area of hypointensity in the left cerebral hemisphere at the junction of the cruciate and splenial sulci was observed; this focus extended a distance of 2 cm caudally along the splenial sulcus and was hyperintense on the T2-weighted spin echo, **fluid-attenuated inversion recovery (FLAIR)**, and gradient-echo susceptibility-weighted sequences (**Fig 1**). On the T1-weighted sequences obtained after IV administration of gadopentetate dimeglumine[g] (0.1 mmol/kg [0.05 mmol/lb]), faint ring-like enhancement of the focal lesion was noted. In addition, 2 pinpoint, ill-defined areas of abnormal contrast enhancement were also seen in the left side of the cerebellum and left ventral cerebral hemisphere; these lesions were not observed on the other sequences.

Following the MRI, a sample of CSF was obtained from the cerebellomedullary cistern. The fluid was characterized by high protein concentration (74.1 mg/dL; reference limit, < 25 mg/dL), and the nucleated cell count was increased at 12 nucleated cells/mL (reference range, 0 to 4 nucleated cells/mL) with 54% mildly degenerate neutrophils, 24% macrophages, 13% mature lymphocytes or plasma cells, and 9% eosinophils. No blood contamination was evident (0 RBCs; reference limit, < 30 RBCs/mL).

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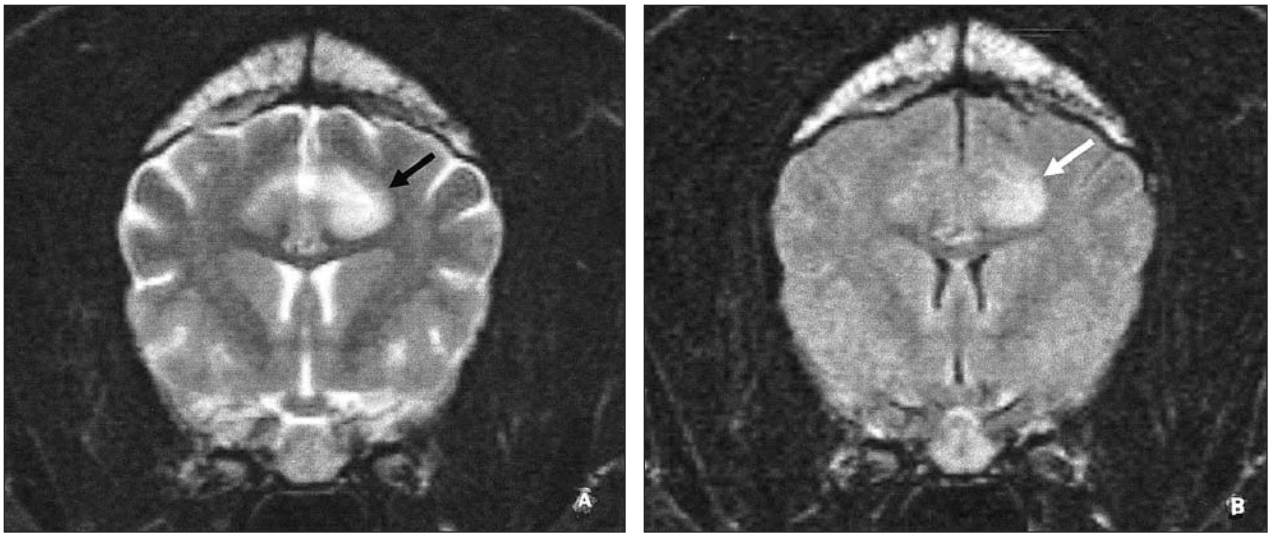

Figure 1—Magnetic resonance images of the brain at the level of the head of the caudate nucleus in a dog with cryptococcosis. A—Transverse T2-weighted image. Notice the hyperintense focus surrounding the left splenial sulcus (black arrow). B—Fluid-attenuated inversion recovery image. Notice the increased conspicuity of the same lesion (white arrow) resulting from attenuation of the CSF signal.

Numerous small (5 µm) yeast organisms that were stripped of their capsules were identified, as well as some macrophages that had engulfed fungal organisms with large, clear capsules. An India ink preparation confirmed the presence of these organisms. A fungal culture of CSF yielded growth (2+) of *Cryptococcus neoformans* within 6 days. *Cryptococcosis neoformans* capsular antigen titers in CSF and serum were assessed via **latex agglutination tests (LCATs)**[h]; the results were each markedly high at 1:262,144 and 1:1,048,576, respectively.

Antifungal treatment with fluconazole[i] (10 mg/kg [4.5 mg/lb], IV, q 12 h on day 1, then 5 mg/kg [2.3 mg/lb], PO, q 12 h) was started soon after recovery from anesthesia. Within 12 to 24 hours, the dog began to drink water and ate a small amount of canned food. The dog's condition continued to improve during a 48-hour period, such that the dog was discharged on day 5 of hospitalization. The dog was nonambulatory and blind but responded to its name. Oral administration of fluconazole was continued.

Within 10 days of discharge, the owner reported that the dog could stand and walk normally, but at times would vocalize and pace in circles. These events were episodic and followed by more appropriate behavior. Administration of an antiepileptic medication for treatment of suspected partial seizure activity was suggested, and gabapentin[j] (9 mg/kg [4.1 mg/lb], PO, q 8 h) was chosen after discussion of options with the owner. Other choices (phenobarbital or potassium bromide) were considered less desirable because of potentially greater sedative effects; with such sedative effects, interpretation of the dog's progression or deterioration would be made more difficult, because many of the monitoring updates were assessments made by the owner (obtained via telephone) and not via examination of the dog (the journey to the referral hospital was 5 hours' duration). Within 3 days, vocalization and circling had resolved.

After 3 weeks, the owner reported that the dog was improving steadily and responding appropriately to commands. The vestibular ataxia was still detectable after 8 weeks of treatment, but the dog was otherwise ambulating well. The dog responded to visual stimuli via the left eye but not via the right eye. The right-sided head tilt persisted, and the nystagmus was now characterized as horizontal with the fast phase to the left. The pupils were less dilated and responsive to light bilaterally; raised granulomatous retinal lesions were still evident bilaterally. The dog was eating and drinking normally. Because of the progressive improvement, the owner had discontinued administration of gabapentin. At this time, results of a CBC were within reference ranges, but slightly high serum activities of liver enzymes were still noted. The dog continued to receive fluconazole without dosage change. The result of an LCAT to assess *C neoformans* capsular antigen titers performed on a serum sample was improved, compared with the previous LCAT result, but remained high (1:4,096).

The dog was reevaluated 16 weeks later. At this time, the dog was ambulating well with no signs indicative of a balance deficit. Mentation was appropriate, and no seizure activity was reported. The head remained tilted to the right, and positional horizontal nystagmus was noticeable. The dog had loss of vision in the right eye, and ophthalmic examination confirmed the persistence of active retinal granulomatous lesions bilaterally. The result of a serum LCAT indicated a high (1:8,192) *C neoformans* capsular antigen titer. The dog was alert and had gained 3 kg since its initial evaluation. Fluconazole was continued at the dosage prescribed previously.

Sixteen weeks later (48 weeks from initial diagnosis), the dog was continuing to improve neurologically, was bright, and behaved appropriately. Vestibular signs such as right head tilt and positional horizontal nystagmus were still evident, but the dog was ambulating well with no proprioceptive deficits or ataxia. The dog responded to visual stimuli bilaterally. In the right eye, the retinal lesions were inactive and smaller than previously noted, and approximately 10% of the retina was detached; the left eye had an active granuloma on the retina. The dog's weight was stable, and it had a

good appetite. The *C neoformans* capsular antigen titer obtained from a serum LCAT was 1:128. Treatment of the dog with fluconazole was continued.

Twelve weeks later (ie, after 60 weeks of treatment with fluconazole), the *C neoformans* capsular antigen titer obtained via serum LCAT remained constant at 1:128 despite continued clinical improvement. A mild right head tilt persisted, and no nystagmus was elicited. Because the retinal granulomas had not resolved bilaterally, fluconazole treatment was continued.

Cryptococcus neoformans is a saprophytic, yeast-like fungus that has worldwide distribution.[1] Infection in animals and humans may result in death, yet *C neoformans* is an uncommon cause of disease. Findings of a retrospective study[2] on the incidence of cryptococcosis in dogs indicated a likelihood of infection of < 13 in 100,000. Of those dogs, Cocker Spaniels had a slightly increased risk for infection. *Cryptococcus neoformans* var *neoformans* has been found in soil and some foods (milk and fruit), but its main reservoir is probably pigeon droppings. In the environment, the fungus is primarily in an unencapsulated form. In tissues, however, the organism forms a thick capsule that promotes virulence. The capsule interferes with antigen presentation and contributes to the organism's evasion of the cell-mediated immune response, thereby reducing clearance from the body.[1]

The diagnosis of CNS cryptococcosis should be made on the basis of results from 1 of 3 tests performed on CSF samples; these tests include identification of the organism via direct microscopy, detection of high *C neoformans* capsular antigen titer via LCATs, or growth of the organism in fungal culture.[3] In the retrospective study,[2] the investigators categorized dogs with an infection with *C neoformans* in an extraneural site and simultaneous CNS signs as dogs likely to have CNS cryptococcosis. The LCAT measures cryptococcal capsular polysaccharide antigen and has a sensitivity of 98%.[4] Compared with low *C neoformans* capsular antigen titers, high titers detected via LCATs have been associated with increased severity of disease, but such values are not reliable prognostic indicators for death. Low titers are often indicative of localized infections or those of short duration.[4]

The accepted mode of infection in humans is via inhalation of airborne unencapsulated organisms. Healthy individuals are able to respond with an appropriate cell-mediated immune reaction; thus, widespread dissemination of the fungal organisms throughout the body is avoided. Signs of infection in humans are usually associated with some degree of immunocompromise. In dogs, inhalation appears to be the route of infection, and the primary site of disease is the nasal cavity.[5] In contrast to humans, however, disease caused by cryptococcal infection in dogs occurs in the immunocompetent host.[2] As the organism forms a polysaccharide capsule, its size increases, which thereby decreases the likelihood of it passing into the smaller airways and alveoli. Presumably, the organisms spread by evading local defenses and can enter the CNS by either local invasion or via a hematogenous route.[2,5-7] In a study[7] of randomly selected dogs and cats that were clinically normal, a substantial colonization of *C neoformans* organisms was demonstrated in the nasopharynx in 14% of the dogs and 7% of the cats. Results of LCATs were negative for all of those animals.

Predilection for spread of cryptococcal infection to the CNS has been described.[1,2,6] Caudal fossa signs, including vestibular and cranial nerve deficits as well as seizures, have been documented as a result of infection. Retinal lesions associated with cryptococcosis, including granulomas and retinal detachment, can develop as a result of hematogenous spread of infection[8] or via local invasion from the meninges along the optic nerve.[9]

Magnetic resonance imaging features of humans with CNS cryptococcosis include meningeal enhancement; parenchymal mass lesions (cryptococcomas) with variable contrast enhancement; nonenhancing pseudocystic lesions of the basal ganglia and periventricular areas (the former considered to represent distended perivascular spaces filled with fungal organisms and mucoid material); and contrast-enhancing parenchymal, leptomeningeal, and intraventricular granulomas.[10-12] Hydrocephalus and mineralization are occasionally observed.[10-12] As cryptococcal mass lesions may be isointense to CSF on T1- and T2-weighted sequences, FLAIR is useful to differentiate cryptococcal lesions from surrounding CSF or arachnoid cysts.[13] Granulomas have been reported to remain detectable on MRIs for > 5 years after clinical cure.[14]

Data regarding the MRI features of CNS cryptococcal infections in animals are limited; however, the spectrum of lesions appears similar to that of humans.[15-17] Foster et al[15] reported a cryptococcal granuloma in a cat; the granuloma was in the left cerebral hemisphere, and compared with brain tissue, it appeared hypointense on T1-weighted images, hyperintense on T2-weighted images, and displayed lobular peripheral enhancement. In a dog, cryptococcal meningoencephalitis with multiple enhancing cortical foci compatible with granulomas and nonenhancing lesions of the basal ganglia that may have represented gelatinous pseudocysts was reported by Tiches et al.[16] Magnetic resonance imaging of 2 dogs with cryptococcal meningitis and meningoencephalitis revealed dural enhancement, as well as other contrast-enhancing lesions.[17] In the dog of this report, the pinpoint foci were compatible with granuloma formation, and the poorly enhancing focus adjacent to the splenial sulcus may have been a granuloma. The usefulness of IV administration of contrast medium and FLAIR images to increase conspicuity of lesions is highlighted in this dog; the contrast-enhanced foci were not apparent on any of the precontrast sequences. Furthermore, the poorly enhancing focus adjacent to the splenial sulcus might have been mistaken for a cystic lesion or partial volume averaging of the normal CSF space if only T1- and T2-weighted images had been obtained.

Treatment of CNS cryptococcosis with antimicrobial agents, such as amphotericin B, 5-flurocytosine, and ketoconazole (administered either as single agents or in combination), has been unrewarding.[1,6,18,19] These agents had limited ability to reach effective therapeutic concentrations within the CNS without adverse effects[6,7,20-22] but have been shown to work synergistically to reach the CNS.[23] Some of the triazoles that have become available more recently have provided a more effective means of treatment of cryptococcosis.[24] Itraconazole and fluconazole both have high bioavailability when orally administered. Fluconazole has minimal protein-binding properties and can attain high

plasma concentration. In addition, fluconazole has good penetration into the CNS, reaching concentrations that are 60 to 80% of that attained in serum. It is metabolically stable and excreted unchanged in the urine. In contrast, itraconazole and ketoconazole are both extensively metabolized in the liver, and concentrations within the CNS are often < 10% of serum concentrations.[17,18,20,21] In 1 study[25] of cats with cryptococcosis, an excellent response to fluconazole was observed. Also, Tiches et al[16] reported the successful treatment with fluconazole of a dog with CNS cryptococcosis.

Because of limited experience in the treatment of CNS cryptococcosis in dogs, the optimal duration of treatment is unclear. For infection in humans, it has been recommended to continue treatment until 3 consecutive CSF samples (obtained at weekly intervals) yield no growth of the organism on fungal culture.[3] Because this may be impractical in animals, results of sequential LCATs in serum may provide a safe, less invasive means to monitor treatment progress. At the time of diagnosis of CNS cryptococcosis, the serum antigen titer may be greater than that in CSF, because the nasal cavity is usually the initial site of infection. Serum antigen titers may lag behind clinical improvement or even remain high, because cryptococcal polysaccharide antigen can persist in circulation.[4,26] Because recurrences have been reported years after resolution of signs, it has been recommended to periodically evaluate CSF via antigen titer tests, cytologic examinations, and fungal cultures for up to 1 year following resolution of signs.[19]

In the dog of this report, clinical findings of CNS cryptococcosis were correlated with a conclusive diagnosis; in addition, the usefulness of FLAIR imaging was highlighted. Depending on the severity of disease and response to treatment, long-term treatment and monitoring may be necessary. The dog of this report had no prior signs of upper respiratory tract disease, but the owner had a mulberry tree that was frequented by a large population of pigeons. The dog had played about the tree, chewing on branches and digging in the dirt; it is likely that the dog inhaled the organism or traumatized the nasopharynx with subsequent inoculation of the organism during play. The high serum antigen titer was probably indicative of a primary site of infection outside the CNS, and spread of the organism to the brain may have been either hematogenous or by local invasion. The former seemed more likely, because there was no MRI evidence that trauma introduced infection to the brain. Despite the dog's clinical improvement, serum antigen titers remained high, which probably represented persistence of infection. The active retinal granuloma may also have been a site of persistent infection.

[a]Clavamox, SmithKline Beecham Animal Health, Philadelphia, Pa.
[b]Ampicillin, Bristol Myers Squibb Co, Princeton, NJ.
[c]Flurbiprofen, Bausch & Lomb, Tampa, Fla.
[d]Mannitol, Abbott Laboratories, North Chicago, Ill.
[e]Lactated Ringer's solution, Baxter Healthcare Corp, Deerfield, Ill.
[f]Pepcid, Bedford Laboratories, Bedford, Ohio.
[g]Magnevist, Berlex Laboratories Inc, Wayne, NJ.
[h]Premier cryptococcal antigen kit, Meridian Biosciences Inc, Cincinnati, Ohio.
[i]Fluconazole, Pfizer Inc, Deerfield, Ill.
[j]Neurontin, Parke Davis, Vega Baja, Puerto Rico.

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