Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

History

A 3-year-old castrated male Rhodesian Ridgeback was presented to the referring veterinarian for a 3-week history of a dry, nonproductive cough. The dog had lived its entire life in California and had no travel history outside of the state. The dog had received a 14-day course of doxycycline (5.7 mg/kg, PO, q 12 h) and 7-day course of hydrocodone (0.19 mg/kg, PO, q 12 h) prior to referral with no clinical improvement. The referring veterinarian had submitted samples for a CBC, serum biochemical profile, and fungal serology to a commercial laboratory; performed in-house canine heartworm antigen testing (SNAP 4Dx Plus Test; Idexx Laboratories Inc); and obtained thoracic radiographic images. The CBC revealed mature neutrophilia (16,432 cells/µL; reference range, 2,060 to 10,600/µL). Results for the biochemical profile were within reference limits, and the dog tested negative for heartworm antigen. Results were also negative for fungal serology testing for antibodies against Histoplasma, Blastomyces, Aspergillus, and Coccidioides. Thoracic radiography revealed a mild diffuse bronchointerstitial pulmonary pattern, with no overt evidence of pneumonia or a pulmonary neoplastic process.

Clinical and Gross Findings

Bronchoscopy was performed due to the chronicity of the cough, lack of improvement with antimicrobials, and no overt causes identified on radiographic evaluation. The proximal portion of the trachea was grossly normal. At the base of the carina, a pale, lobulated mass obstructed greater than 80% of the tracheal lumen (Figure 1). The mass was snared and removed with gentle traction. The area was visualized after removal of the mass, with no defect in the trachea identified. There was a moderate amount of hemorrhage noted in the pulmonary parenchyma, so a bronchoalveolar lavage was not performed. The dog recovered well from the procedure.

Histopathologic Findings

Formalin-fixed samples of the removed mass were routinely processed into paraffin blocks and routinely stained with H&E stain (Figure 2). Histopathologic examination revealed a large mass of inflammatory cells beneath a small amount of stratified squamous epithelium. The mass consisted of large accumulations of foamy vacuolated macrophages forming sheets of cells with smaller numbers of neutrophils scattered throughout and sometimes forming small neutrophilic aggregates. Scattered lymphocytes and plasma cells, occasionally forming small aggregates, were also present throughout the tissue. There was scant fibrous septae dissecting throughout the mass. Throughout the tissue were numerous round yeast-type fungal organisms that were sometimes intra-cytoplasmic within the macrophages. These fungal organisms had a basophilic round central structure.
that was 6 to 7 µm in diameter and surrounded by a basophilic 5-µm-thick wall. This in turn was surrounded by a thick, clear mucinous capsule that was 5 to 30 µm thick.

**Morphologic Diagnosis and Case Summary**

Morphologic diagnosis: intratracheal fungal granuloma with intralesional fungal life stages.

Case summary: cryptococcosis, presenting as a solitary tracheal mass, in a dog.

**Comments**

Cryptococcosis is an opportunistic systemic mycosis resulting from infection by 1 of 2 species of dimorphic yeasts, *Cryptococcus neoformans* (serotypes A, D, and AD) and *C gattii* (serotypes B and C). Other species of *Cryptococcus* have been identified, although these have not been shown to be pathogenic.

As opposed to the other systemic fungal pathogens that are more strictly confined geographically, *Cryptococcus* is prevalent worldwide.\(^1\) Of the 2 species, *C neoformans* makes up the majority of cases in humans, with the exception of in Australia, whereas a study\(^2\) shows that *C gattii* was 1.5 times more frequently reported. Pigeons are considered the most important vector of *C neoformans*, whereas *C gattii* is thought to be associated with bark and leaf litter of certain eucalyptus trees in tropical and subtropical areas.\(^3\) Although both *Cryptococcus* species can cause disease in either the cat or dog, a study\(^4\) shows that *C neoformans* was more likely to be isolated from dogs and *C gattii* was more often isolated in cats.

Most instances of cryptococcosis in dogs are characterized by the presentation of systemic dissemination of infection often resulting in severe illness. The majority of cases are reported in dogs less than 6 years old, with the inhalation of fungal spores believed to be the primary route of infection.\(^4,5,6–8\)

The most common sites of infection include the CNS, eyes, gastrointestinal tract, and adrenal glands with the majority of dogs with disseminated disease having CNS involvement.\(^4,9\) Cryptococcosis typically occurs as an opportunistic infection in immunocompromised people, although it can occur in immunocompetent people as well. The dog in this case report did not have an identifiable preexisting condition or medication that would have impaired its immune system, so the cause of the development of the tracheal Cryptococcus is unclear.

Fungal cytology and histology are the mainstays of diagnosis for *Cryptococcus*, but fungal culture is difficult because prolonged growing time is needed for isolation.\(^9\) Generally, cytology from affected tissues is the most efficacious means of identifying cryptococcal organism, with organisms identified in 55% of canine cases, although the sensitivity of this test is affected by the length of infection and some studies\(^1,10\) show higher sensitivities of cytology when the organism has been incubated in the tissue for longer. In infected tissues, the organism is a variable-sized yeast with a large heteropolysaccharide capsule. If cytology fails to identify organisms, histopathology should be used. Culture can be another useful diagnostic tool, although in 1 study,\(^3\) cryptococcal organisms were cultured from nasal washings in 14% of dogs with no signs of infection and 7% of cats with no signs of infection. As such, positive cultures must be interpreted within the context of clinical signs and degree of clinical suspicion.

Serology is also possible with the cryptococcal antigen latex agglutination system (CALAS). This is a quantitative test that detects the *Cryptococcus* polysaccharide capsule antigen. The fungus capsule serves several functions including reducing the host immune response, depleting complement component, and inhibiting the antigen-presenting capacity of the monocytes.\(^11,12\) CALAS can be used in both serum and CSF samples and has a high specificity and sensitivity.\(^1,4,13,14\) The titer does not correspond to the severity of disease but to the amount of antigen that has gained access to circulation.\(^1,3,15,16\) Titters as low as 1:2 are important as circulating antigen implies tissue invasion. These antigen tests do not differentiate between live and dead organisms, and titters can also increase substantially after treatment due to the release of antigen from disintegrating organisms. Because the titer is just illustrating the amount to the release of antigen from disintegrating organisms, it does not correspond to the severity of disease in individual patients. Typically, titters are recommended 6 to 8 weeks after initiation of treatment as increasing titters at this point could indicate a failure in treatment.\(^1,16\) More recently, several different PCR assays to detect *Cryptococcus* have been developed and can be used on CSF, whole blood, and deep pharyngeal swab samples.\(^17\) PCR assays are advantageous as they allow for identification of the species and genotype involved.\(^18\) Similarly to

**Figure 2**—Photomicrograph of a section of the tracheal mass imaged in Figure 1 showing cryptococcal yeast (black arrow). H&E stain; bar = 80 µm.
serology, a positive diagnosis can be associated with both active disease and colonization.17

Treatment of Cryptococcus involves long-term treatment with antifungal medications including amphotericin B (AMB) and azoles (AZ), although most animals treated early in the course of infection and survive the first 2 weeks of treatment have a good prognosis.16,19 There are 2 classes of antifungals: polyene antimicrobials and AZ antifungals. The former includes AMB. AMB requires parenteral administration because absorption from the gastrointestinal tract is poor, and even in this route, penetration into the CNS is poor.20 Most concerning is that the AMB mechanism of action is to bind to cholesterol within the fungi; however, it also has affinity for cholesterol in the mammalian cells, which can cause cell death. For these reasons, AZ antifungals are more commonly used. AZ antifungals inhibit the fungal P-450 enzyme that is needed for the development of ergosterol in fungal cell walls.21 Patients with granulomas, as with this case, generally require excision of the granuloma to successfully clear the organism in addition to antifungal treatment.22

There are few evaluations of the long-term prognosis of these patients. In a study15 evaluating dogs and cats in Australia, the overall success of treatment was 76% for cats and 55% for dogs. Based on that study,21 the authors found higher success in animals treated initially with AMB followed by oral AZ treatment with dogs tolerating the AMB better than cats and recommended treatment with AMB be continued until the latex cryptococcal antigen agglutination test titer dropped at least 4- to 5-fold. Following this, oral AZ treatment is usually recommended for several months. Difficulties in interpreting this titer occur with the continued presence of nonviable Cryptococcus yeast in the animals’ system, although usually such titers are low.21 Owners should be warned at the onset that many of these animals require antifungal treatment for months to years, which may prove cost prohibitive and even despite this does not preclude from relapses.19 In animals that seroconvert to negative after treatment, it is recommended that they be retested after treatment has been discontinued for 1 month to evaluate for reinfection.19

In conclusion, the present report described an interesting case of an intrathacael fungal granuloma, which, to the authors’ knowledge, has never been previously reported in the literature. The report showed the importance of diagnostic sampling of masses to aid in diagnosis although treatment of fungal disease remains difficult and requires prompt diagnosis to increase therapeutic success.

References