Blastomycosis in Dogs

1. Introduction

Blastomycosis is a systemic fungal infection of dogs that can be fatal if it is not diagnosed early. Most cases may be diagnosed by identification of the yeast in cytologic or histologic samples. Early diagnosis may also be aided by detection of fungal antigen in urine or serum. This article will review the clinical features of blastomycosis in dogs, and discuss the current diagnostic and treatment recommendations.

2. Epidemiology

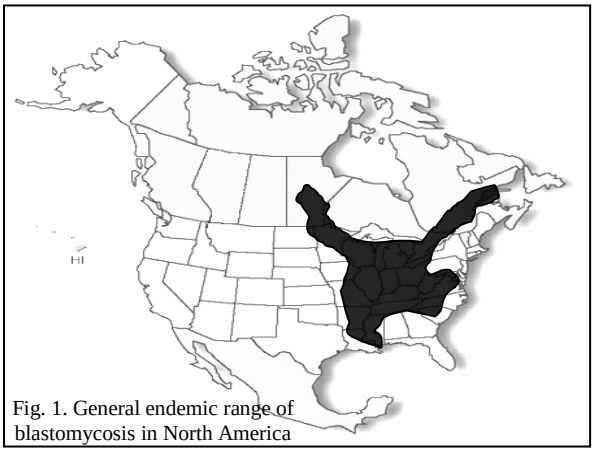

Fig. 1. General endemic range of blastomycosis in North America

While blastomycosis may occur in a wide variety of animals, most diagnosed cases are in dogs. Endemic areas for blastomycosis include the Mississippi, Ohio, and Missouri river valleys, the Eastern Seaboard, Southern Canada, and areas adjacent to the Great Lakes (see Fig. 1). The states with areas of highest endemicity are Wisconsin, Minnesota, Missouri, Illinois, Michigan, Kentucky, West Virginia, Arkansas, Tennessee, North Carolina, South Carolina, Louisiana, and Mississippi. Other endemic states include Indiana, Iowa, Ohio, Virginia, Georgia, and Alabama. Cases, however, may occur outside the endemic area (1). The annual incidence was 1420 cases per 100,000 dogs in a highly-endemic area (2). Proximity to waterways and exposure to excavation are significant risk factors but age, sex, and activities such as hunting, swimming and exposure to beavers are not. While most cases occur in dogs with extensive outdoor exposure, cases also may be seen in indoor pets (1). Cases occur most often in the fall (3;4), but may occur any time of the year. Blastomycosis occurs mainly in young, large-breed dogs (3), with the highest rates in Coonhounds, Pointers and Weimaraners (4). Doberman Pinschers and Retrievers also may be at increased risk for blastomycosis, but any breed is susceptible if exposed to the organism. In some reports, the prevalence was higher in males than females (4;5). Higher rates in sexually intact male dogs was thought to be caused by roaming behavior or selective use in hunting (4).

3. Pathogenesis and Clinical Findings

Blastomycosis is acquired by inhaling fungal spores, and causes a respiratory and/or disseminated infection. If the inoculum is small and the animal is not immunocompromised, the infection may be limited to the respiratory tract and may have few or no clinical signs. The most

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common clinical findings are nonspecific and include loss of appetite, weight loss, and fever. Respiratory abnormalities also are common, and radiographs show nodular or interstitial infiltrates, often referred to as a "snowstorm pattern" (3). Less frequently thoracic radiographs show tracheal bronchial lymphadenopathy, masses, or cavitary lesions (3). Draining skin tracts and lymphadenopathy are commonly present. Among fatal cases, the organs most often involved are the lungs, eyes and skin (4). Ocular lesions occur in about one third of cases (6). Other less common sites of dissemination include the central nervous system and genitourinary tract (3).

Early detection of the ocular lesions is important for saving vision and for diagnosing the systemic nature of the disease. In a review of cases with ocular involvement, endophthalmitis was most common, followed by posterior segment disease, and anterior segment disease (6). Lens rupture is a potential complication (7). In an earlier report, the most common ocular lesion was uveitis, and other manifestations included retinal detachment, panophthalmitis, and glaucoma (8). The most common ocular findings include photophobia, conjunctival hyperemia, meiosis, blepharospasm, and aqueous flare. Most of the patients also exhibited pneumonia and many had skin lesions or enlarged lymph nodes. The presence of ocular disease in patients from areas endemic for blastomycosis should prompt careful evaluation for the condition.

4. Diagnosis

In a review of the Veterinary Medical Database, in over 90% of cases the diagnosis was validated by laboratory tests, while in <5% the diagnosis was based solely on clinical findings (4). A variety of methods are useful for diagnosis.

Identification of the organism. Cytology and/or histopathology is considered the gold standard method for diagnosis. *Blastomyces* organisms appear in cytologic preparations stained with Romanowsky-type stains as 8-20 µm, blue, spherical, thick-walled yeasts (see Fig. 2). Broad-based budding is commonly observed. Cytology was positive in 71% of cases in one report, including mostly skin and lymph node samples, and occasionally transtracheal washes (3). In some cases, cytology of subretinal aspirates has been positive (8). Culture was the basis for diagnosis in only 12% of cases (3), and is not commonly used in veterinary cases due to risk of infection of laboratory personnel when handling the mycelial form of the fungus.

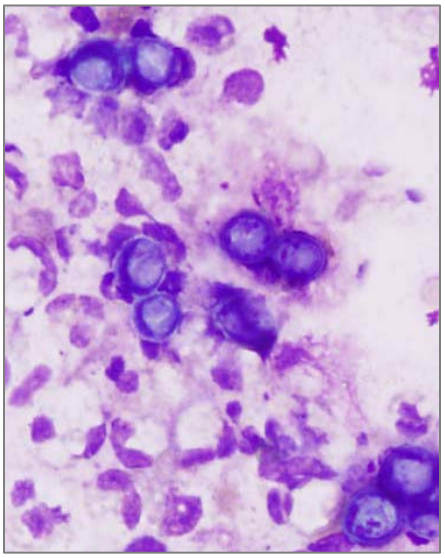

Fig. 2. *B. dermatitidis* yeasts mixed with degenerate neutrophils (Diff-Quik stain)

Antigen detection. The presence of antigens can be detected in urine and/or serum (as well as bronchoalveolar lavage fluid and cerebrospinal fluid), and was first reported in 2006 (9). Spector *et al.* demonstrated 94% sensitivity for antigen detection in urine, and 87% sensitivity in serum of dogs with either histopathology or cytology

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proven blastomycosis (10). Antigen concentration correlates with the severity of the infection, and results are almost always positive in moderately-severe or severe blastomycosis. Antigen tests may initially be negative in mild cases of blastomycosis; therefore, a negative result does not exclude the diagnosis. In addition, nearly complete cross-reactivity occurs between antigen detection in histoplasmosis and blastomycosis, and current antigen tests cannot differentiate the two systemic fungal infections.

Antibody detection. Tests for antibody to *Blastomyces* using immunodiffusion (ID) methods are available. Sensitivity is variable among published reports, ranging from only 17% (11) to 83% (3). Antibody detection is infrequently used as a basis for diagnosis (3). Enzyme immunoassay(EIA) methods of antibody detection reportedly have higher sensitivity (76%) compared to ID (17%) [11]; however, no commercial *Blastomyces* antibody EIA tests are currently available. Antibody testing may be useful for diagnosis of mild cases in which antigen is not detected.

Molecular techniques. Real-time polymerase chain reaction (RT-PCR) assays for *Blastomyces* and other fungal organisms are available from several diagnostic laboratories. Molecular methods are highly variable, and no peer-reviewed published studies are currently available to support the use of PCR in the diagnosis of canine blastomycosis. The role of PCR in fungal diagnosis remains to be established.

5. Treatment

Although effective therapy is available, one quarter of dogs with blastomycosis die, usually during the first week of treatment, and most often due to respiratory failure (12). There is a strong correlation between the extent of lung involvement and survival time. Outcome was especially poor in cases with brain, spinal cord (1) or ocular involvement (6). In another report over half of patients with ocular blastomycosis were euthanized or died, while some dogs did respond to amphotericin B (8). A high concentration of antigen in the urine or blood correlates with clinical severity in dogs. In an unpublished study, 75% of dogs with antigen concentrations > 14.7 ng/mL required oxygen treatment and 86% were euthanized, compared with 8% and 15% of dogs, respectively, that had antigen concentrations <14.7 ng/mL (A. Mourning, presented at 2010 ACVIM forum, Anaheim, CA).

Itraconazole. Itraconazole is currently the treatment of choice for blastomycosis in dogs. The usual dosage is 5 mg/kg/d. Legendre *et al.* showed that response to a two month course of itraconazole at 10 mg/kg/d was 74%, while a lower dose of 5 mg/kg/d given with food was nearly as effective (12). Dogs receiving the higher dosage had significantly more adverse effects.

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When possible, brand-name itraconazole capsules or commercially available oral suspension (Sporanox®, Janssen Pharmaceuticals) should be used, as compounded preparations may have poor bioavailability (23). Itraconazole capsules require an acid pH for maximum absorption, and should be taken with food. The suspension does not require an acidic environment, and should be taken on an empty stomach.

Itraconazole is eliminated by hepatic metabolism through cytochrome P450 3A4, and blood levels may be affected by medications that interact with that enzyme. Itraconazole blood level measurement is encouraged at day 14 of treatment, and if treatment failure or drug toxicity is suspected. Levels may be sub-therapeutic in up to half of dogs receiving the recommended dosage. Trough blood levels of at least 1µg/ml by HPLC, and 3µg/ml by bioassay, are recommended. A review of the bioassay testing of veterinary specimens at MiraVista Diagnostics from July 2011 to 2012 showed that levels were undetectable or below 0.3 µg/ml in 20%, between 0.3 and 0.9 µg/ml in 11%, 1.0 and 2.9 µg/ml in 16%, and 3.0 µg/ml or more in 53% of cases. In 20% of cases levels were above 10 µg/ml, and potentially toxic.

Itraconazole may cause a variety of adverse effects, most commonly loss of appetite, anorexia, vomiting, or diarrhea, which may be related to high blood levels (24). Serum liver enzymes should be monitored during therapy. Activity of serum alanine aminotransferase (ALT) greater than 200 U/L may warrant discontinuation of itraconazole until appetite returns and ALT activity returns to <100 U/L (25). Itraconazole may be restarted at half of the former dose. Ulcerative dermatitis was also observed in 7.5% of dogs receiving itraconazole at 10 mg/kg/d (12).

Fluconazole. Fluconazole is often used for treatment of blastomycosis because of its lower cost, and in some cases because of its central nervous system penetration. Response to itraconazole and fluconazole was compared in a retrospective study (15). Of note is that severity of disease, namely respiratory difficulty, was greater in dogs treated with itraconazole. Ninety percent of dogs treated with itraconazole at an average dose of 5 mg/kg/d for an average of 4 months achieved a clinical remission, but 18% relapsed. Seventy-five percent of dogs treated with fluconazole 10 mg/kg/d for six months responded to therapy, but 22% relapsed. There was a 10% mortality rate with itraconazole and a 25% rate with fluconazole. While the differences in mortality and in response between itraconazole and fluconazole were not statistically significant, they suggest that itraconazole may be slightly more efficacious than fluconazole, which is the case in humans (14). In cases where itraconazole cannot be used (due to high cost or intolerance of the medication), fluconazole is another treatment option. Fluconazole blood level monitoring is usually unnecessary because levels are generally predictable if the recommended dosage is administered as prescribed.

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Other azoles. Other treatment options include posaconazole and voriconazole. Both are active in vitro (16) and effective in animal models of blastomycosis (17;18). However, neither has been adequately studied for treatment of blastomycosis. There are case reports of patients with CNS blastomycosis treated successfully with voriconazole (19;20). These newer azoles are more expensive than itraconazole, and require monitoring to assure adequate blood levels. Ketoconazole is less effective than other azoles, but may be used if the cost of itraconazole or fluconazole is prohibitive (21).

Amphotericin B. Amphotericin B was historically recommended for treatment of blastomycosis in dogs and cats (26-28), as in humans; however, the risk of nephrotoxicity has limited its use at the current time. Itraconazole has largely replaced amphotericin B as the recommended therapy. In severe or refractory cases of disseminated blastomycosis, the use of liposomal or lipid-complexed forms of amphotericin B should be considered initially, before transitioning to itraconazole. Lipid-complexed amphotericin B (Abelcet®, Enzon Pharmaceuticals; Bridgewater, NJ) may be used at a dosage of 1-2.5 mg/kg, every 48 hours or three times a week, until a cumulative dose of 12-15 mg/kg is reached (29). Despite a lower incidence of nephrotoxicity as compared to unaltered amphotericin B, renal function and serum electrolytes should be monitored during treatment.

Duration of therapy. The optimal duration of therapy has not been determined. Plumb recommends two months for fluconazole but two to three months for itraconazole (13). Among cases in dogs that survive the initial illness, relapse occurs in about 25% of cases (Appendix), usually within the first year following therapy. Mazepa treated for a median of six months if fluconazole was used and 4.5 months if itraconazole was used, and relapse occurred in 21% of dogs that survived initial therapy (15). Legendre, using itraconazole, treated for two months in about 90% of cases, but relapse occurred in 28% of dogs that responded to the initial therapy, supporting a statement that longer treatment may reduce relapse (12). Bromel recommends that at least four to six months of itraconazole should be given to reduce the likelihood of relapse (1). Life-long suppressive therapy, given two or three times weekly, may prevent recurrence in patients that have relapsed more than once despite appropriate durations of therapy with documentation of itraconazole blood levels of at least 3 µg/mL.

Adjunctive therapy. Lung infiltrates in 23% of dogs worsened in the initial week of therapy with antifungals, likely attributed to an inflammatory response to dying organisms (30). Fifty percent of dogs with severe lung disease die during the first week of therapy. Dexamethasone (0.25-0.5 mg/kg IV for 2-3 days) may be given to dogs that develop life-threatening respiratory signs (25). Concurrent antifungal therapy is recommended in order to reduce the risk for progressive dissemination caused by corticosteroid-induced immunosuppression.

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Monitoring therapy. Antigen concentration declines with effective therapy (10), and some veterinarians have used it to decide when to stop therapy. In an unpublished study monitoring antigen every three months during and for six months after treatment in 31 dogs treated with fluconazole for an average of six months, antigen levels at the end of therapy were negative in serum at a rate of 97% and in urine of 39% (D. Foy et al., presented at 2010 ACVIM Forum, Anaheim, CA). Among the dogs with persistent antigenuria at the end of therapy, the concentration ranged from 0.2-1.4 ng/mL. Five of the 31 (16%) dogs relapsed, including the one with the highest concentration of 1.4 ng/mL.

These findings indicate that antigenuria should become negative or drop to below 1 ng/mL with successful therapy, offering a marker to assist in deciding when to stop therapy. Failure of the antigen concentration to decline may also raise concern about the effectiveness of treatment, which may be caused by inadequate itraconazole blood levels or development of resistance to fluconazole (22). If itraconazole levels are low the dosage should be increased and blood levels should be rechecked at 14 days. Inability to achieve concentrations above 3 µg/ml would be a reason to change to fluconazole 10 mg/kg/d. Increase in antigen concentration after stopping treatment suggests relapse, which occurs in about 15-20% of cases treated with either itraconazole (12;15) or fluconazole (15). Testing for antigenuria about every three months during and for six months after stopping therapy, and any time that clinical signs suggest recurrence, is recommended.

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