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BLASTOMYCOSIS IN ANIMALS

MVista® *Blastomyces* Antigen Quantitative EIA
Test Code: 316
Specimen Type: Urine, Serum, BAL, CSF & other pipetteable fluid

***Blastomyces* Antibody Immunodiffusion**
Test Code: 322
Specimen Type: Serum

1. Introduction

Blastomycosis, a systemic fungal infection that occurs in various animal species, can be fatal if not diagnosed early. Though seen most commonly in dogs, it may also occur in cats (1), horses (2), and probably any animal species exposed to areas containing the organism. In endemic areas, Blastomycosis is nearly 10 times more common in dogs than in humans (3). 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

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 (4). The annual incidence was 1420 cases per 100,000 dogs in a highly-endemic area (5). 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 (4). Earlier studies indicated that cases occur most often in the fall (6;7) but more recent studies demonstrated a preponderance of cases in the summer (8). However, infections may occur any time of the year (8). Blastomycosis occurs mainly in young, large-breed dogs (6), with the highest rates in Coonhounds, Pointers and Weimaraners (7). 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 (7;9). Higher rates in sexually intact male dogs was thought to be caused by roaming behavior or selective use in hunting (7).

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 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” (6). Less frequently thoracic radiographs show tracheal bronchial lymphadenopathy, masses, or cavitary lesions (6). Draining skin tracts and lymphadenopathy are commonly present. Among fatal cases, the organs most often involved are the lungs, eyes and skin (7). Ocular lesions occur in about one third of cases (10) . Other less common sites of dissemination include the central nervous system (11) and genitourinary tract (6;12).

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 (10). Lens rupture is a potential complication (13). In an earlier report, the most common ocular lesion was uveitis, and other manifestations included retinal detachment, panophthalmitis, and glaucoma (14). 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 (7). A variety of methods are useful for diagnosis.

Identification of the Organism. Cytology and/or histopathology are 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 (6). In some cases, cytology of subretinal aspirates has been positive (14). Culture was the basis for diagnosis in only 12% of cases (6), and is not commonly used in veterinary cases due to risk of infection of laboratory personnel when handling the mycelial form of the fungus.

Antigen Detection. The presence of antigens can be detected in urine and/or serum in dogs with blastomycosis (15), and the assay has been refined to permit quantification (16). The sensitivity is above 90% in urine, but false-negative results do occur, mostly in dogs with mild or localized disease. Thus, a negative result does not exclude the diagnosis. Repeat antigen testing or demonstration of yeast in body fluids or tissues may be positive in such cases. More recently an antibody enzyme immunoassay for antibodies to a cell surface protein antigen has been reported to be positive in 90% of cases, overcoming the limitation of poor sensitivity in the agar gel immunodiffusion (AGID) method (Mourning, in press). Antibody testing using this method should be considered in evaluation of cases with negative antigen results.

Nearly complete cross-reactivity occurs between antigen detection in histoplasmosis and blastomycosis. The two mycoses cannot be differentiated by antigen detection so there is no need to perform the test for both infections. Antibody detection permits differentiation of these mycoses in over 90% of cases (Mourning).

Antigen concentration in serum is higher in dogs with severe blastomycosis (unpublished): antigenemia was greater than 14.7µg/mL in 71% of dogs that required oxygen treatment compared to 15% that did not. A similar trend was observed for antigenuria. Accordingly, antigen concentration above 14.7µg/mL would support more aggressive therapy, including hospitalization for amphotericin B therapy.

Antibody Detection. The sensitivity of the AGID method has ranged from 17% (17) to 83% (6) and experience in clinical practice has been unfavorable. The sensitivity of the EIA method is superior to AGID, supporting its use as

an aid to the diagnosis of blastomycosis. The EIA is also specific, exhibiting false-positivity in less than 10% of human patients with histoplasmosis (18). These findings support the use of the antibody EIA for diagnosis in antigen-negative cases and differentiation between histoplasmosis and blastomycosis.

Molecular Techniques. Real-time polymerase chain reaction (RT-PCR) assays for *Blastomyces* and other fungal organisms are available from several diagnostic laboratories (19). Two reports describe use of PCR for diagnosis in veterinary cases (20;21). The role of PCR for diagnosis of blastomycosis in veterinary patients remains to be determined.

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 (22). There is a strong correlation between the extent of lung involvement and survival time. Outcome was especially poor in cases with brain, spinal cord (4) or ocular involvement (10). In another report over half of patients with ocular blastomycosis were euthanized or died, while some dogs did respond to amphotericin B (14). 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).

Amphotericin B. Amphotericin B has been used for treatment of blastomycosis in dogs and cats (4;23;24) and may still be the treatment of choice in severe cases. Amphotericin B induces a clinical response more rapidly than itraconazole in humans with histoplasmosis (25), because of its fungicidal mode of action and immediate achievement of therapeutic blood concentrations. Itraconazole is fungistatic and requires 2 weeks to achieve steady state blood levels in dogs. Administration of a lipid form of amphotericin B for the first 3 to 7 days of therapy may improve early survival, after which treatment could be changed to itraconazole or fluconazole. Lipid-complex amphotericin B (Abelcet®, Enzon Pharmaceuticals; Bridgewater, NJ) may be used at a dosage of 1-2.5 mg/kg given daily for 3-7 day (26). Renal function and serum electrolytes should be monitored during treatment.

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 (22). Dogs receiving the higher dosage had significantly more adverse effects.

Brand-name itraconazole (Sporanox®, Janssen Pharmaceuticals) or generic itraconazole capsules should be used, as compounded powder formulations have poor bioavailability (27). Generic itraconazole contains the same formulation as in Sporanox and achieves blood levels and effectiveness comparable to brand name Sporanox and is less expensive. Itraconazole capsules require an acid pH for maximum absorption, and should be taken with food. Generic and brand name itraconazole are “pelletized” and contain cyclodextrin, which improves solubility and enhances GI absorption of itraconazole, and both produce therapeutic blood concentrations. The “compounded” powder formulations of itraconazole do not contain cyclodextrin and do not achieve therapeutic blood levels. Sub-therapeutic levels may also occur in 10 to 20% of dogs treated with generic and brand name

itraconazole, due to inherent variability in absorption and/or metabolism. Blood levels should be measured 14 (dogs) to 21 (cats) days after beginning therapy, and the preferred range is 3.0 to 10.0 µg/mL as measured by bioassay, and at least 1.0 µg/mL by HPLC. As itraconazole has a long half-life, timing of the specimen after dosing is not critical but trough levels are preferred. If itraconazole levels using generic or brand-name pelletized itraconazole are below3 µg/mL by bioassay, or 1 µg/mL by HPLC, the dosage should be increased and blood levels should be rechecked at 14 (dogs) to 21 (cats) days later. 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 (16;22;28;29) or fluconazole (28) .

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 (30). 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 (31). 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 (22).

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 (28). 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 (32). 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.

Other Agents. Other treatment options include posaconazole and voriconazole (29). Both are active in vitro (33;34) and effective in animal models of blastomycosis (35;36) . However, neither has been adequately studied for treatment of blastomycosis. There are case reports of patients with CNS blastomycosis treated successfully with voriconazole (37-39). 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 (40). While some veterinarians have used terbinafine for treatment of blastomycosis no studies have been conducted to establish efficacy. Studies have, however, demonstrated potentially effective concentrations in the blood following a single dose in dogs; mean maximal concentration of 3.5µg/mL and AUC of 15.4µg.h/mL (41).

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 (42)). 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 (28). 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 (22). Bromel recommends that at least four to six months of itraconazole should be given to reduce the likelihood of relapse (4). 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 (43)). 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 (44). Concurrent antifungal therapy is recommended in order to reduce the risk for progressive dissemination caused by corticosteroid-induced immunosuppression.

Monitoring Therapy. The antigen test is also used in deciding when to stop therapy and to diagnose relapse (16) (the publication can be obtained at (http://onlinelibrary.wiley.com/doi/10.1111/jvim.12306/abstract). Among 27 dogs, most which were treated with fluconazole for an average of six months, antigenuria was detected in 8 (30%) at discontinuation of treatment, but all at low levels (<1 ng/mL in 7 and 1.4 ng/mL in the 8th dog). Relapse occurred in 7 dogs (26%), 2 of which had a positive urine antigen at treatment discontinuation, including the dog with a result of 1.4ng/mL. Relapse was associated with a rise in antigenuria in 5 of 7 dogs (71%). The authors recommended continuing therapy until the clinical findings, including eye exam, resolved, chest radiographs were normal or stable, and urinary antigen was negative. A reasonable approach would be to test for antigenuria every 3 months during therapy, at 3 and 6 months after stopping therapy, and if relapse is suspected.

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Appendix: Summary of treatment studies in canine blastomycosis

Drug	Cure	Death	Relapse	Reference
Keto 10 mg/kg/d for 60 days (N=9)	3 (33%)	3 (33%)	3 (33%)	Legendre 1984 (40)
AmB 1 mg/kd/dose for 8-9 mg/kg (N=35)	20 (57%)	8 (23%)	5 (20%)	Legendre 1984 (40)
AmB 1 mg/kg/d for 4 doses then Keto 10 mg/kg/d for 60 days (N=18)	11 (61%)	4 (22%)	3 (17%)	Legendre 1984 (40)
AmB and Keto 10 mg/kg/d for 60 days (N=19)	12 (63%)	7 (37%)	Not stated	Arceneaux 1998 (6)
Itra10 mg/kg/d for 60 days (N=56)	30 (54%)	14 (25%)	12 (21%)	Legendre 1996 (22)
Itra 5 mg/kg/d with food for 60 days (N=35)	19 (54%)	9 (26%)	7 (20%)	Legendre 1996 (22)
Itra 10 mg/kg/d for 60-90 days (N=31)	16 (52%)	10 (32%)	5 (16%)	Arceneaux 1998 (6)
Itra 5 mg/kg/d for 120 days (N=31)	23 (74%)	3 (10%)	5 (16%)	Mazepa 2011 (28)
Flu 10 mg/kg/d for 180 days (N=36)	21 (58%)	9 (25%)	6 (17%)	Mazepa 2011 (28)
Overall (N=262)	152 (58%)	64 (24%	46 (18%)	

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