

Histoplasmosis

Introduction

Histoplasmosis is common in animals from endemic areas and causes similar clinical syndromes to those found in humans (1). In a review of systemic mycoses in dogs, histoplasmosis was 2.5 times more as common as blastomycosis (2). While histoplasmosis is not transmissible from animal to human, concurrent infection is common because of shared exposure (3). Familiarity with the clinical manifestations may alert a veterinarian to consider the diagnosis. Antigen detection in urine and serum may provide a rapid diagnosis, precluding the need for invasive procedures to obtain specimens for histopathology and culture in many patients. Serology may be useful in cases with negative results by antigen testing. Treatment with amphotericin B for more severe cases and itraconazole for mild cases are highly effective, and may be monitored by antigen testing. Itraconazole absorption and metabolism vary considerably, at times causing undetectable or toxic blood levels, and blood level measurement is encouraged to assure adequate drug exposure.

Epidemiology

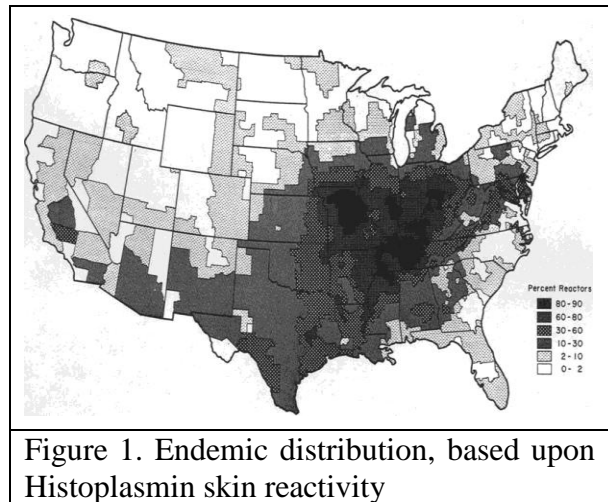
Histoplasmosis caused by *Histoplasma capsulatum* variety *capsulatum* is endemic in certain parts of North and South America. In some endemic areas, histoplasmosis was the most common systemic mycosis in animals. Between 1964 and 1976, 14 schools of veterinary medicine in the United States and Canada participated in a study of systemic mycoses and noted rates per hundred thousand patient years of 62 for histoplasmosis, 25 for blastomycosis and 17 for coccidioidomycosis (2).

In a study of necropsy findings in dogs from Kentucky, 36.1% had positive cultures for *H. capsulatum* compared to 0.7% for *Blastomyces dermatitidis* (4). In a similar study from Loudoun County, Virginia *H. capsulatum* was isolated from 44% of healthy appearing dogs and cats (5). In Kentucky, 47% of dogs (6) and 50% of Thoroughbred horses (7) exhibited *Histoplasma* skin test reactivity, while only 7.3% of horses demonstrated *Blastomyces* skin test reactivity (7). Histoplasmosis was twice as common in animals from rural than from urban areas (4). Cases have also been acquired outside of the endemic area. Johnson reported cases in cats from central California(8).

Bats may play a role in spreading the organism in the environment (9). Bats develop chronic intestinal dissemination and shed the yeast in the feces (10;11), potentially depositing the organism in sites outside the endemic area (12).

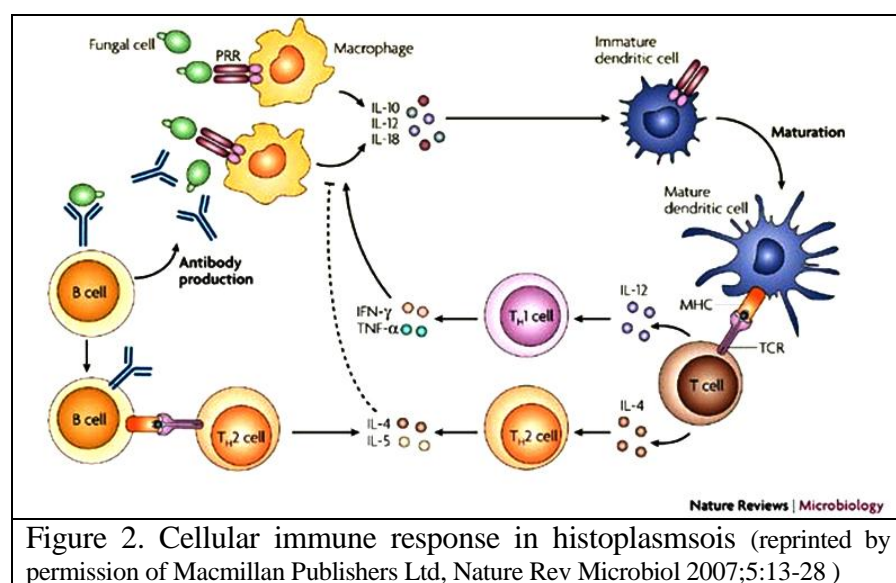
Pathogenesis

Histoplasmosis is caused by inhalation of microconidia or hyphal fragments. Although intestinal lesions are prominent in dogs with disseminated histoplasmosis, experimental infection by gastric inoculation failed to induce disease in dogs (13). All mammals are susceptible to histoplasmosis, but cases have been reported most often in dogs, cats, and horses. Birds, because of



their higher body temperature, are not susceptible to natural infection (14) but may be infected experimentally, causing infection localized to their feathers (15). Histoplasmosis is not transmissible from patient to patient, or from animals to man, but disease in an animal may be a harbinger of infection in humans who were exposed at the same time (3).

Cellular immunity is critical in defense against *H. capsulatum*, based on analysis of risk factors for severe disease (16) and studies in murine models of histoplasmosis. The microconidia are inhaled and in the lungs they attract dendritic cells, neutrophils and macrophages, which phagocytose the organism (17;18). Within the macrophage the conidia transform into yeasts in 4-6 days (19), which multiply unchecked in the non-immune subject. During the first two weeks, the infection progresses and disseminates hematogenously throughout the reticuloendothelial system. By day 14 of infection, specific T cell immunity develops, halting proliferation of the yeast and progression of the infection (20). Susceptibility to progressive disease following experimental infection appears to differ among species of inbred mice (21-23). Knowledge learned from murine models of experimental histoplasmosis may not apply to other animal species, in which immunity has not been studied. Evidence for self-limited dissemination in humans includes demonstration of calcified granulomas in the spleen and liver, in which non-viable organisms may be seen by fungal stain (24), and occasional isolation of *H. capsulatum* from extrapulmonary specimens in patients with acute pulmonary histoplasmosis (24).



Cytokines that are most important in immunity to *H. capsulatum* include IL-12 (25-27), IL-18, TNF- α (28) and interferon- γ (29;30). A successful T cell response requires dendritic cells, CD4 and CD8 T cells (31-34); interferon- γ , tumor necrosis factor- α , interleukin 12 and IL-18; and activated macrophages (35) (Figure 2). T cells produce interferon- γ and tumor necrosis factor- α , which activate macrophages to kill *Histoplasma* yeast

(35). If these elements are impaired, the yeast proliferates unchecked in the reticuloendothelial tissues, causing progressive disseminated histoplasmosis, and death if untreated (16). The importance of TNF- α in humans is highlighted by the emerging recognition of histoplasmosis as a major opportunistic infection in patients treated with TNF inhibitors (36).

While histoplasmosis is self-limited in humans, and following experimental infection in mice, spontaneous resolution may not occur in other animal species. In an interesting experiment, healthy dogs from a non-endemic area (Cheyenne, Wyoming) were transported to Lexington, Kentucky and exposed to *Blastomyces dermatitidis*, which had been introduced into the soil in a tightly closed wooden shed, where the dogs were housed in cages for two days (37). Soil known to be positive for *H. capsulatum* was located within 10 m of the cages. Two years later, 18 of 27 healthy-appearing dogs were immunosuppressed for eight weeks and nine were observed as controls, to determine if immunosuppression caused reactivation of blastomycosis. When the dogs

were euthanized one month later, none had positive cultures for *B. dermatitidis* but 52% had positive cultures for *H. capsulatum*. Thus, while blastomycosis cleared spontaneously, histoplasmosis persisted, without clinical findings in most cases.

Chronic infection was also identified in 44% of euthanized dogs and cats in Loudin county, Virginia (38), 31% of dogs from Cincinnati, Ohio (24), and 34% from Lexington, Kentucky (39). Chronic infection also is common in bats (10;11). Interestingly the tissue reaction in bats was minimal or absent, possibly explaining their inability to eradicate the organism (10;11;40;41). The infection rate varied markedly in different genera of bats, suggesting genetic differences in susceptibility (42). *Histoplasma* was not isolated from wild-caught mice, suggesting that their immune response was able to kill the organism (14). Studies in inbred mice infected by intratracheal injection of *Histoplasma* yeast suggest that chronic infection may occur with some of strains of *H. capsulatum*, and/or some strains of inbred mice, however (43;44).

Chronic infection is rare in humans, almost exclusively occurring in patients with underlying conditions that impair cellular immunity (16) or those with emphysema (45). In contrast the findings in dogs, which exhibit chronic subclinical infection, yeasts seen in calcified lung lesions in humans are not viable (46).

Clinical Presentation

The severity of clinical manifestations correlates with the intensity of exposure and the underlying health of the exposed individual. Cole described rapidly progressive fatal course over two to four weeks in 10% of dogs with histoplasmosis, any chronic progressive course over two to 20 months in 90% (47). Demonstration of positive cultures of pulmonary and extrapulmonary tissues of healthy appearing dogs and cats from endemic areas (24;37;38) suggest that the clinical findings may be overlooked in many cases. Syndromes most commonly identified include pneumonia, mediastinal lymphadenitis, and progressive disseminated histoplasmosis. Two manifestations identified in humans, chronic pulmonary histoplasmosis and fibrosing mediastinitis (48), have not been reported in animals. Chronic pulmonary histoplasmosis occurs mostly in humans emphysema (45) and may not occur in animals. Fibrosing mediastinitis, which is characterized by obstruction of airways, pulmonary and bronchial vessels, or the superior vena cava is caused by exuberant fibrosis surrounding densely calcified lymph nodes, may have been overlooked in animals.



Figure 3. Radiograph diffuse pulmonary histoplasmosis

Pulmonary. Pneumonia is the most common manifestation in humans, and probably in animals. Patients usually present with symptoms of malaise, loss of appetite, weight loss, fever, nonproductive cough, and labored breathing. Radiographic findings characteristically include diffuse nodules, referred to as "cotton tuft" lesions (49) (Figure 3) or diffuse interstitial infiltrates, often accompanied by mediastinal lymphadenopathy (50). Alveolar infiltrates or nodules also may be seen (50).

Mediastinal lymphadenitis. Enlarged hilar or mediastinal lymph nodes may impinge upon the airways and cause cough and respiratory distress (51;52). Radiographs show tracheobronchial lymphadenopathy usually accompanied by interstitial pneumonia. The outcome has ranged from spontaneous resolution to progressive obstruction of the airways and death. Whether some of these cases may represent fibrosing mediastinitis requires investigation. Concurrent dissemination may occur (52).

Progressive disseminated histoplasmosis. Fever, weight loss, reduced activity, anemia, and interstitial lung disease are the most common manifestations in cats (53), while diarrhea, intestinal blood loss, anemia and reduced activity predominate in dogs (47;54) (Table 1), but any tissue may be involved. Bone lesions are common in cats (53). Central nervous system and ocular lesions may be found in all animal species. Endocarditis also has been reported, noted in seven of 17 (41%) autopsied cases in dogs (56). Other tissues commonly involved at autopsies include liver, spleen, abdominal lymph nodes, and less frequently adrenal glands, kidneys, pancreas (47).

Pulmonary involvement occurs in most cases and is usually manifested as labored breathing (53;54). Radiographs typically show diffuse interstitial, miliary or nodular infiltrates (53). Abnormal physical findings include hepatomegaly, splenomegaly, eye lesions or discharge, subcutaneous nodules, and skin lesions (53;54). The common laboratory abnormalities are anemia, leukopenia, thrombocytopenia, hypoalbuminemia, liver enzyme elevation, creatinine elevation, and hypercalcemia (57). The untreated course ranges from asymptomatic chronic infection to a rapidly fatal illness.

Equine abortion. Infections in the fetus or newly born foal may occur, causing the mare to abort or the foal to die soon after birth (58-60). Pulmonary and disseminated involvement usually are present in the fetus or newborn (59). In most cases the mare appears healthy but the placenta is involved.

Diagnosis

Balows noted that "successful recovery from histoplasmosis was achieved but only because

| Test | APH N=29 | SPH N=18 | PDH N=140 |
|------------------------------------|-------------|------------------|--------------|
| Antigen | 83% | 78% | 99% |
| Pathology | 35% | 75% ¹ | 68% |
| Culture | 41% | 50% ² | 56% |
| Serology | 64% | 100% | 68% |
| ¹ N=4; ² N=6 | | | |

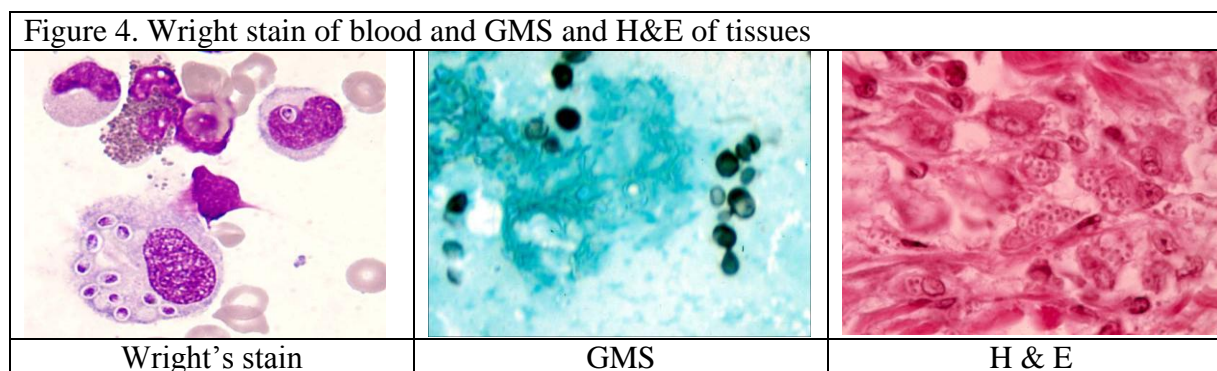
diagnosis was made and therapy instituted promptly" (61). Several tests are helpful for diagnosis of histoplasmosis in humans (53;54;57;62). In humans with acute pulmonary and progressive disseminated histoplasmosis the highest sensitivity is with antigen detection, which is most sensitive in patients with acute or severe disease (Table 3). Serology may aid in the diagnosis of mild cases, in which antigen tests may be negative.

In veterinary cases, the diagnosis was usually based on fungal stains or cultures of tissues (54;57;59;63), which often required invasive procedures to obtain the specimens. Some authors have indicated that identification of the organism is necessary for diagnosis of histoplasmosis (53;54;64-66), and that serology is not useful (64-66). However, the most sensitive approach would include *Histoplasma* antigen detection in urine and serum in all cases, noting that the test also is cross reactive in patients

| Finding | Dogs (54) N=12 % | Cats (53;55)** % |
|--------------------------------------|------------------------|------------------------|
| Fever | 25 | 58 |
| Weight loss | 42 | 83 |
| Respiratory symptoms | 50 | 39-42 |
| Chest radiograph abnormal | NS* | 87-88 |
| Lethargy/depression | 58 | 66 |
| Intestinal-diarrhea, bleeding | 83 | NS |
| Hepatomegaly | NS | 33 |
| Splenomegaly | NS | 17 |
| Lymphadenopathy | 33 | 33 |
| Eye lesions or discharge | NS | 24-42 |
| Bone lesions | NS | 18 |
| Skin lesions | NS | 8 |
| Anemia | 58 | 100 |
| Hepatic enzyme elevation | 50 | 40 |
| *NS=not stated. N=12 (53); N=96 (55) | | |

with blastomycosis. If the antigen test is negative but lesions or pulmonary infiltrates are present, cytology or histopathology of lesions or bronchial washings may be necessary to establish a diagnosis: bronchial washings also should be tested for *Histoplasma* antigen. Available data in animals supports the findings in man that serology may be useful if the diagnosis cannot be made by antigen detection or pathology.

Fungal stains. *Histoplasma* yeast measure 2-3 μm in diameter and exhibit narrow-necked budding (Figure 4). Fungal stain was positive all 12 cats (53) and dogs (54) with disseminated histoplasmosis. Requirement of demonstration of the organism by fungal stain or culture for diagnosis biases these reports for a high sensitivity for fungal stain, however. In humans, fungal stain is less sensitive than antigen detection for diagnosis of disseminated and acute pulmonary histoplasmosis (62). Mitchell reported diagnosis of disseminated histoplasmosis by demonstration of yeasts by Wright's stain of blood smears or hematoxylin and eosin or fungal stain of rectal scrapings or biopsies, but these cases appeared to have advanced disease, as mortality was 80% (56). One drawback of fungal stain is the requirement to perform invasive procedures to obtain specimens for evaluation. Also, the accuracy may be poor unless the pathologist is experienced with recognition of *Histoplasma* yeasts, as other yeasts or staining artifacts may be mistaken as *Histoplasma* and small numbers of yeasts may be easily overlooked.



Culture. Cultures are positive in most cases of disseminated histoplasmosis (24), but growth may be slow, requiring up to four weeks. The highest yield is from the lung, skin or mucosal lesions, or bone marrow, thus requiring invasive procedures to obtain specimens.

Antigen detection. A galactomannan antigen in the cell wall of proliferating *Histoplasma* yeasts is released into the tissues and blood, and excreted in the urine. This antigen can be detected in an enzyme immunoassay (62). Antigen can be detected in 95-99% of humans with disseminated histoplasmosis (67), and in 83% of those with acute pneumonia (68). The greatest sensitivity for diagnosis required testing both urine and serum (62). Although antigen detection is used often for diagnosis of histoplasmosis in animals, and has been reported in blastomycosis (69), diagnosis by antigen detection has been reported in only one case in animals, a bottlenose dolphin with proven histoplasmosis (70). Prospective studies are needed to define sensitivity of *Histoplasma* antigen detection in animals. Also, a negative result does not exclude the diagnosis. In cases with negative results, follow-up specimens may be positive in patients exhibiting progressive illness. Also, serology or culture may be positive in patients with negative antigen results.

Antigen may be detected in the respiratory secretions in patients with pulmonary histoplasmosis (71), occasionally permitting diagnosis in patients with negative results in urine and serum. Antigen also may be detected in the cerebrospinal fluid of patients with meningitis, offering a helpful method to diagnose this elusive manifestation (72).

Antigen levels decline during treatment and increase with relapse, providing a tool for monitoring therapy.

The galactomannan antigen found in histoplasmosis cross reacts with that found in blastomycosis (69). Furthermore, the clinical findings and endemic distribution overlap. Thus, differentiation of the two mycoses may be difficult, but treatment is the same, reducing the need to distinguish histoplasmosis and blastomycosis.

Serology. In humans, serologic tests are positive in over 90% of cases of pulmonary histoplasmosis and 70% of cases of disseminated histoplasmosis (62). Some experts are of the opinion that serology is not a reliable method for diagnosis of histoplasmosis in animals, however (39;50;56;64;65). The most information is from dogs with histoplasmosis. Ackerman described seven dogs with acute pulmonary histoplasmosis that was diagnosed based upon demonstration of high titers of complement fixing antibodies (52). Balows reported positive results of complement fixation in eight of nine (89%) dogs with histoplasmosis, and concluded that serology was useful for diagnosis (61). Mitchell reported a positive serology, using both immunodiffusion and complement fixation, in only one of nine (11%) dogs with disseminated histoplasmosis (56). In the only study of a large number of animals (N=27), the complement fixation test was positive in 90% of dogs with culture proven histoplasmosis (37).

The immunodiffusion test may be less useful. In one study, only 25.2% of dogs with culture proven histoplasmosis exhibited precipitating antibodies by immunodiffusion (4). Results of the complement fixation test were not reported in that publication, but in a separate publication describing the same dogs, the sensitivity of the complement fixation test was about twice that of the immunodiffusion test (39).

In these studies serology was also positive in dogs with negative cultures (4;39). Whether these represent culture negative cases of active histoplasmosis or seropositivity following recovery from histoplasmosis was not determined, however. In humans serology often is positive in non-immunocompromised patients with pulmonary histoplasmosis in which cultures are negative. Potentially serology may be useful for diagnosis of milder cases of histoplasmosis in animals.

Less is known about the use of serology in other animals. Kabli reported positive results in three of four cats with histoplasmosis (49). Sharbaugh demonstrated a potential role for serology in calves that were injected intravenously with *Histoplasma* mould, showing that all five animals developed H precipitins and high titers of complement fixing antibodies, and four developed M precipitins (73). The immunodiffusion test was positive in five of six horses tested in another report (59).

Cross reactions by complement fixation may occur in animals with other endemic mycoses, and serum may be anti-complementary, preventing measurement of antibodies by complement fixation. H and M precipitin bands are specific for histoplasmosis and a precipitin bands for blastomycosis, assisting in differentiation of the two mycoses. However, the sensitivity of immunodiffusion is low (<20%) in dogs with blastomycosis (69) and has not been studied in a large number of dogs with histoplasmosis (52). Sensitivity may be improved using an enzyme immunoassay, as described in blastomycosis (69). Of note, however, serology may be negative during the first month following exposure. Studies of larger numbers of animals are needed to establish the accuracy of serology in histoplasmosis.

Molecular. The published studies have not demonstrated superiority of PCR over other rapid methods. PCR was falsely-negative in 31% of tissues in which yeast resembling *H. capsulatum* were seen by histopathology (74). Tang evaluated PCR on urine specimens from patients with histoplasmosis, noting positive results in only 8% of specimens, occurring only in those from which *Histoplasma* was isolated (75). The role of PCR remains to be established.

Treatment

Guidelines for treatment are provided, but textbooks and other reviews should be used for more thorough instruction on antifungal treatment in animals.

Amphotericin B. Amphotericin B is recommended for the first week or two in severe cases (64-66), as in humans (76). Balows described a protocol administering amphotericin B intravenously in escalating doses (2.5 mg/lb to 5 mg/lb) on alternate days for 10 injections, observing a clinical response in all cases, two of which relapsed following discontinuation of treatment (61). Mitchell described the results in seven dogs treated with amphotericin B 0.5 mg/kg daily for a cumulative dose of 11 mg/kg, of which two died and four of the five survivors relapsed (56). Marx, taking advantage of the high incidence of culture proven histoplasmosis in apparently healthy dogs central Kentucky, randomized dogs from an experimental colony to receive amphotericin B 1.85 mg/pound given over 19 days or observation (6). One month later the dogs were euthanized and mediastinal lymph nodes, lungs, and spleen were cultured. 24% of controls had positive cultures compared to 2% of treated animals. Amphotericin B is usually given at 0.7-1 mg/kg/d intravenously in small animals. Instructions for use of amphotericin B were provided in a review on treatment in veterinary practice (77). Renal function, serum potassium and magnesium should be monitored during treatment.

The high rate for relapse following 10 to 20 days of amphotericin B treatment supports the need for "step down" treatment with itraconazole for several months following discontinuation of amphotericin B, as recommended in humans (76).

Itraconazole. Itraconazole is recommended in mild cases and after clinical improvement with amphotericin B (64-66), as in humans (76). Hodges reported eight cats that responded to itraconazole 5 mg/kg/day given for 60-130 days, but two relapsed, requiring a second course of treatment (57). The usual dosage is 5 (66) to 10 mg/kg (64;65) given once or twice daily. 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 during the second week of treatment, and if treatment failure or drug toxicity is suspected. Target blood levels are 1.0-10 µg/mL. 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 (78). Bilirubin and hepatic enzymes also may be elevated, in association with clinical evidence for hepatitis in some cases; and blood levels should be monitored during therapy (76).

Other azoles. Several other azoles are active in histoplasmosis and provide alternatives in patients unable to take or who have failed itraconazole. Ketoconazole is infrequently used because it is less effective and causes more adverse effects than itraconazole. One of five cats responded to ketoconazole (49). Ketoconazole's lower cost may be a reason to use it in some cases. Fluconazole is also less effective but may be used because of lower cost or reduced adverse effects than with itraconazole. To prevent failure due to emergence of resistance to fluconazole (79), doses of at least 10 mg/kg/day are recommended.

Posaconazole and voriconazole are more active than fluconazole and have been used successfully in humans with histoplasmosis (76), but have not been evaluated in animals and are expensive. These agents are reserved for patients unable to take or who have failed itraconazole and fluconazole. *H. capsulatum* also may become resistant to voriconazole (80).

Adjunctive therapy. Patients with mediastinal lymphadenitis causing airway obstruction may benefit from corticosteroid therapy. Schulman noted rapid clinical improvement in ten dogs, five of which also received antifungal treatment (51). Six other dogs received antifungal treatment alone and appeared to improve clinically, but more slowly, and one continued to cough four years

later. While none of the dogs developed evidence for disseminated disease, the authors caution that owners should be advised of the potential risk for dissemination. Disseminated disease was present in a fatal case in another report, however (52). Concurrent antifungal therapy is recommended in humans (76), and may reduce the risk for progressive dissemination caused by corticosteroid-induced immunosuppression.

| Table 4. Treatment recommendations |
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| Severe cases: Amphotericin B 4-14 days followed by itraconazole 4-6 months* Monitor antigen every 3 months and if suspect relapse Itraconazole blood level** |
| Mild cases: Itraconazole 4-6 months* Monitor antigen every 3 months and if suspect relapse Itraconazole blood level** |
| *Some cases may require more than 6 months of treatment. **During 2 nd week of therapy to ensure levels between 1.0 and 10 µg/mL and if suspect relapse or itraconazole toxicity. |

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