Clinical signs, treatment, and prognostic factors for dogs with histoplasmosis

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OBJECTIVE
To determine the clinical manifestations of histoplasmosis in a large sample of dogs, compare outcomes achieved with fluconazole versus itraconazole, and identify variables available at the time of diagnosis with prognostic value.

DESIGN
Retrospective case series with nested cohort study.

ANIMALS
79 dogs with confirmed histoplasmosis evaluated at 2 veterinary teaching hospitals from 1999 through 2015.

PROCEDURES
Medical records were reviewed and data extracted regarding clinical signs at evaluation, physical examination findings, clinical laboratory values, other diagnostic test results, treatments, and outcomes. Data were compared between antifungal agents used (fluconazole or itraconazole) and between other variables.

RESULTS
Various breeds were represented. Working and herding breeds had mostly disseminated histoplasmosis, and toy breeds had mostly the gastrointestinal form. The diagnosis was often achieved with noninvasive techniques, such as cytologic evaluation of rectal scrape samples (n = 24) or blood films (15). Clinical remission was achieved in 16 of 25 (64%) dogs receiving fluconazole and 17 of 24 (71%) dogs receiving itraconazole. No differences were identified between antifungal agents in survival, clinical remission, or disease relapse rates. Identified negative prognostic factors included Great Pyrenees breed, dyspnea, need for oxygen supplementation, icterus, palpable abdominal organomegaly, anemia, thrombocytopenia, hypercalcemia, high serum alkaline phosphatase activity, and hyperbilirubinemia, whereas diarrhea was a positive prognostic factor.

CONCLUSIONS AND CLINICAL RELEVANCE
Findings suggested that histoplasmosis should be considered in a sick dog of any breed in an endemic area. Clinical signs may be nonspecific. Diagnosis may often be possible with noninvasive and inexpensive tests. Either fluconazole or itraconazole may be an effective treatment option. (J Am Vet Med Assoc 2018;252:201–209)

Histoplasma capsulatum is a dimorphic soil-borne fungus that is endemic in the Midwest and Southern United States. Dogs inhale H capsulatum microconidia found in the environment. At body temperature, within the lungs, microconidia convert to the yeast form. Clinical illness can be isolated to the lungs, or H capsulatum yeast can disseminate within macrophages via the lymphatics and bloodstream to other organs or systems, including the liver, spleen, bone marrow, gastrointestinal tract, eye, lymph nodes, skin, bone, oral cavity, and CNS.1–10 Ingestion is another possible route of infection, given that experimental oral inoculation of dogs with the organism results in disseminated histoplasmosis, and this route might explain infections in dogs isolated to the gastrointestinal tract.3,11,12

Although dogs of all ages can become infected, most dogs are <5 years old at the time of diagnosis.3,5,13 Both sexes are susceptible. Sporting, terrier, and hound breed groups as well as Brittany, English Pointer, and Weimaraner breeds might be at increased risk of histoplasmosis relative to other groups or breeds.1,7,13 Affected dogs typically have chronic nonspecific signs such as pyrexia, lethargy, decrease in appetite, and weight loss, although many have additional specific signs related to infected organs, such as cough or increased respiratory effort, diarrhea, or lymphadenopathy.3,5

The gold standard for diagnosis is cytologic or histologic confirmation of H capsulatum organisms.6 A urine Histoplasma EIA, which detects the fungal

ABBREVIATIONS
ALP Alkaline phosphatase
ALT Alanine transaminase
EIA Enzyme immunoassay
PT Prothrombin time
PTT Partial thromboplastin time

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antigen galactomannan, is highly specific and sensitive for diagnosing histoplasmosis in dogs, and this assay might aid in diagnosis when more invasive testing (such as cytologic evaluation of a lung aspirate sample) is deemed unsafe or results are considered nondiagnostic.\textsuperscript{14}

The treatment of choice for dogs with histoplasmosis is currently itraconazole, although no studies have been conducted to compare the efficacy of various antifungal agents for this purpose.\textsuperscript{6,7} Fluconazole is suggested for ocular or CNS involvement owing to its excellent penetration into these tissues.\textsuperscript{15,16} In addition, fluconazole might be more cost effective than itraconazole, averaging a third of the cost of itraconazole in a recent blastomycosis study.\textsuperscript{17} Duration of treatment with azole medication is typically at least 4 to 6 months; adverse effects can include nausea, vomiting, anorexia, and hepatotoxicosis; and drug interactions need to be considered such as with gastric acid suppression treatment (itraconazole) and cytochrome P450 metabolism (both itraconazole and fluconazole).\textsuperscript{7,8,17,18}

Prognosis for dogs with pulmonary histoplasmosis is reported to be fair to excellent, whereas prognosis for dogs with disseminated or gastrointestinal disease varies from guarded to good, depending on organ involvement and severity.\textsuperscript{6-10} Research regarding histoplasmosis in humans has allowed antifungal treatment and supportive care to be carefully tailored on the basis of specific prognostic factors, including organ involvement, disease chronicity, and host immunocompetence\textsuperscript{19-22}; the identification of more specific prognostic factors could help target treatment in dogs as well.

The objectives of the study reported here were 3-fold. The first objective was to describe the clinical manifestations of histoplasmosis in a large number of dogs treated at 2 veterinary teaching hospitals. The second objective was to compare outcomes of dogs treated with fluconazole versus itraconazole. Our hypothesis was that there would be no significant difference in survival or clinical remission rates. The third objective was to evaluate variables for which information was available at the time of diagnosis for prognostic value. Our hypothesis was that the need for oxygen supplementation and the presence of disseminated disease would be associated with lower survival and clinical remission rates than in dogs without these characteristics.

Materials and Methods

Case selection criteria

Dogs with a diagnosis of histoplasmosis between 1999 and 2015 were identified from the medical records of Oklahoma State University Veterinary Medical Teaching Hospital and Kansas State University Veterinary Health Center by use of the search terms “canine and fungal” or “canine and histoplasmosis.” The case definition of histoplasmosis was identification of \textit{H capsulatum} organisms on blood film examination or cytologic or histologic evaluation. Additionally, dogs were included if they had positive results of urine or serum \textit{Histoplasma} EIA\textsuperscript{2} testing with concurrent consistent ocular findings (chorioretinitis or uveitis) or tachypnea or dyspnea with thoracic radiographic changes consistent with histoplasmosis. Dogs were excluded if they had positive results of serologic \textit{Histoplasma} antibody testing without confirmatory results of cytologic, histologic, or \textit{Histoplasma} EIA testing.

Medical records review

Data were collected from the medical records of included dogs regarding signalment, body weight, clinical signs, and physical examination findings on initial evaluation; results of initial CBC and serum biochemical analysis; serum ALT activity at 1, 3, and 6 months after antifungal treatment began; results of serum or urine \textit{Histoplasma} EIA testing; information from thoracic radiography reports; method of diagnosis; and organs known to be infected. Complete blood count and serum biochemical data were included if they were obtained within 7 days before or within 72 hours after the initial hospital visit.

On the basis of known organ involvement, histoplasmosis was classified as disseminated, gastrointestinal, or pulmonary.\textsuperscript{5,6,9} Dogs were included in the disseminated group if they had evidence of infection involving ≥ 2 organ systems or single organ infection outside the gastrointestinal or respiratory tracts, such as infection of the eye, spleen, or peripheral blood. Dogs were included in the gastrointestinal group if \textit{H capsulatum} organisms had been identified on cytologic examination of a rectal scrape specimen or gastrointestinal histologic examination and lacked clinical findings of disease in another organ or body system. Dogs were included in the pulmonary group if they had evidence of pulmonary disease and lacked clinical findings of disease in another organ or body system.

Collected treatment information included initial antifungal medication administered, including dosage and treatment duration; changes in antifungal treatment during the first 30 days and first 6 months; need for oxygen supplementation; and concurrent administration of glucocorticoid drugs. Duration of hospitalization was recorded as well as whether the dog survived to discharge from the hospital and for 1 and 6 months after treatment began. Survival information was obtained by review of recheck examination data in the medical record; if the dog did not return to the hospital where it was initially evaluated, survival information was obtained from the medical records kept by its primary veterinarian.

Because the study was retrospective in nature, no standardized recheck schedule or specific time to clinical remission was evaluated. Dogs were considered to have achieved clinical remission if they had resolution of all clinical signs of disease recognizable by their owners at home (eg, dyspnea or diarrhea) and, as determined by recheck examinations, had resolution...
of any physical examination abnormalities and resolution of radiographic evidence of disease. In addition, dogs with resolution of clinical signs and remaining inactive ocular lesions or radiographic lesions that remained unchanged on serially (monthly) obtained radiographs were considered to have achieved remission.17,25 A negative result of antigen testing was not required for determination of remission, but if such testing was performed, the result was required to be negative. Dogs were considered to have had a relapse if clinical signs recurred after clinical remission and if either H capsulatum organisms were identified via cytologic or histologic examination or a positive Histoplasma EIA result was obtained.

Statistical analysis

Continuous data were summarized as median and range. The magnitude of increase in serum ALT activity above the upper reference limit was calculated for dogs receiving antifungal treatment. Dogs that received no antifungal treatment or received antifungal treatment for ≥7 days in the month prior to diagnosis of histoplasmosis were excluded from the statistical analysis. Clinical signs, examination abnormalities, diagnostic abnormalities, and treatment were evaluated for associations with outcome. Only dogs that received itraconazole or fluconazole alone as initial treatment were included in outcome comparisons; dogs that had a drug change during treatment were excluded from analyses regarding treatment duration or dosage. Evaluated outcome data included whether (yes or no) the dog had survived to hospital discharge, survived to 1 month, survived to 6 months, achieved clinical remission, or relapsed.

The 2-sided Fisher exact test was used to test for associations between binomial variables (eg, initial antifungal medication used, need for oxygen supplementation, or concurrent corticosteroid administration) and outcomes; the χ² test was used for non–2 × 2 comparisons. For continuous variables (eg, rectal temperature and laboratory values), univariate logistic regression was used to identify associations with outcome. Statistical software was used for analysis; values of P < 0.05 were considered significant.

Results

Animals

Eighty-one dogs were initially identified as having a diagnosis of histoplasmosis. For 2 (2%) dogs, the diagnosis had been based solely on positive results of serologic testing and therefore these dogs were excluded. Overall, 79 dogs were included in the descriptive statistics and 61 in the statistical analysis.

Of the 79 dogs included in the descriptive analysis, median age was 3 years (range, 0.3 to 12 years) and median body weight was 14.4 kg (31.6 lb; range, 1.1 to 51.0 kg [2.4 to 112.4 lb]). Forty-eight (61%) dogs were female (33 [42%] spayed and 15 [19%] sexually intact) and 31 (39%) were male (17 [22%] castrated and 14 [18%] sexually intact). Dogs were recorded as mixed breed (n = 19 [24%]), Miniature Schnauzer (11 [14%]), Great Pyrenees (7 [9%]), Boxer (4 [5%]), Labrador Retriever (4 [5%]), Boston Terrier (3 [4%]), Pug (3 [4%]), Yorkshire Terrier (3 [4%]), Australian Cattle Dog (2 [3%]), Shih Tzu (2 [3%]), Border Collie (2 [3%]), Golden Retriever (2 [3%]), and Basset Hound (2 [3%] as well as Dachshund, Jack Russell Terrier, Papillon, English Setter, Australian Shepherd, Akita, Samoyed, Mastiff, Pomeranian, Standard Poodle, West Highland White Terrier, Bluetick Coonhound, Cocker Spaniel, Belgian Malinois, Schipperke, and Brittany (1 [1%] each). Represented breed groups as defined by the American Kennel Club included working (14 [18%]), terrier (13 [16%]), toy (10 [13%]), sporting (9 [11%]), herding (6 [8%]), nonsporting (5 [6%]), and hound (4 [5%]).

Clinical signs

Median duration of clinical signs for the 79 dogs prior to initial evaluation was 4.2 weeks (range, 0.2 to 156 weeks). The most common clinical signs were those of lethargy (53/79 [67%]), decrease in appetite (47/79 [59%]), weight loss (45/79 [57%]), diarrhea (41/79 [52%]), pale mucous membranes (31/69 [45%]), pyrexia (ie, rectal temperature ≥39.4°C [103°F]; 23/74 [31%]), hematochezia (21/79 [27%]), palpable abdominal organomegaly (17/74 [23%]), dyspnea (16/79 [20%]), peripheral lymphadenomegaly (14/77 [18%]), dehydration (10/73 [14%]), icterus (10/78 [13%]), vomiting (10/79 [13%]), and cough (8/79 [10%]). Additional clinical signs included epistaxis (n = 4), cutaneous lesions (4), signs of ocular disease (3), and melena (2). Cutaneous lesions included undiagnosed alopecia and crustings (3) and generalized demodicosis (1). Signs of ocular disease included loss of vision (2), blepharospasm (1), ocular discharge (1), and scleral injection (1); further characterization led to the diagnosis of anterior uveitis (3), fundic cotton-spot lesions (1), glaucoma (1), retinal detachment (1), and chorioretinal scar (1).

Three dogs had signs of neurologic disease at initial evaluation, including central blindness (n = 1), seizures (1), and circling (1). For one of these dogs, necropsy results confirmed histoplasmosis of the CNS. The dog with seizures had hypoglycemia, which resolved with dextrose administration, and was found to have gastrointestinal histoplasmosis, was treated successfully with itraconazole, and lived to clinical remission beyond 6 months after treatment began. An additional dog had no neurologic signs at initial evaluation but developed seizures during hospitalization prior to diagnosis and treatment; this dog was subsequently euthanized. Necropsy revealed Histoplasma infection of the CNS in addition to involvement of the lungs, gastrointestinal tract, liver, spleen, pancreas, kidneys, and lymph nodes. Two dogs newly developed seizures after discharge from the hospital during initial antifungal treatment. One of these 2 dogs had disseminated histoplasmosis (diagnosed on the basis of blood film and ascites fluid examination)
and had been treated with fluconazole (9.6 mg/kg [4.4 mg/lb], PO, q 12 h) for 48 hours prior to the first seizure. The second dog had gastrointestinal histoplasmosis and had been treated with fluconazole (10 mg/kg, PO, q 12 h) for 20 days and with metronidazole (dosage unknown) 6 days prior to seizures. Both dogs were euthanized by their primary veterinarian, and no necropsy was performed.

Clinicopathologic findings

Complete blood count data were available for 75 (95%) dogs, and serum biochemical data were available for 74 (94%) dogs (Table 1). Twenty-five of 75 (33%) dogs had an Hct < 30%; of these dogs, 21 (84%) had normocytic normochromic anemia, 2 (8%) had microcytic hypochromic anemia, 1 (4%) had microcytic normochromic anemia, and 1 (4%) had macrocytic normochromic anemia. Reticulocyte counts were available for 13 of the 25 (52%) dogs with an Hct < 30%, and the median absolute reticulocyte count was 90.6 X 10³ cells/µL (range, 34.4 X 10³ cells/µL to 259.0 X 10³ cells/µL).

Seven of 75 (9%) dogs had thrombocytopenia with evidence of clumping (median thrombocyte count, 91.5 X 10³ cells/µL; range, 1 X 10³ cells/µL to 152 X 10³ cells/µL). The CBC results for 3 additional dogs lacked a platelet count. Platelets counts for these 10 dogs were excluded from summary data and statistical analysis. Ten dogs had coagulation profiles consisting of PT (median, 22.9 seconds; range, 15.5 to > 239 seconds) and PTT (median, 8.8 seconds; range, 6.4 to 42.9 seconds). Three dogs had a prolonged PT; 4 dogs had a prolonged PTT; no dog had the combination of prolonged PT and PTT plus thrombocytopenia.

Results of serum biochemical analysis were available for 74 dogs, revealing hypoalbuminemia in 57 (77%) dogs and hypocalemia in 23 (31%) dogs. Twenty-one hypocalemic dogs were also hypoalbuminemic, and when findings were corrected for the hypoalbuminemia, 20 of 21 (95%) dogs were considered normocalcemic.

Radiographic findings

Thoracic radiographs were obtained for 50 of 79 (63%) dogs, and 19 (38%) of these were interpreted as unremarkable. Twenty-two (44%) dogs had thoracic findings consistent with interstitial disease, 6 with alveolar disease, and 5 with bronchial disease; 8 had a mixed pulmonary pattern. Of the 22 dogs with interstitial disease, 15 were characterized as having diffuse disease; 3 of these were further characterized as having a miliary pattern and 3 others were characterized as having a structured nodular pattern. Five dogs had no evidence of lung lesions but had other radiographic abnormalities. Sternal lymphadenopathy was identified in 6 of 50 (12%) dogs, pleural effusion in 4 (8%) dogs, tracheobronchial lymphadenopathy in 3 (6%) dogs, pneumomediastinum in 2 (4%) dogs, cranial mediastinal lymphadenopathy in 1 (2%) dog, pneumothorax in 1 (2%) dog, and subcutaneous emphysema in 1 (2%) dog. No thoracocentesis had preceded pneumothorax or pneumomediastinum in the dogs in which these radiographic abnormalities were noted.

Other diagnostic tests

Initial diagnosis was made by antemortem cytologic (n = 57 [72%]) or histologic (7 [9%]) examination,
positive results of urine Histoplasma EIA testing without concurrent cytologic or histologic examination (6 [8%]), or necropsy (9 [11%]). Various diagnostic samples allowed confirmation of H capsulatum infection, with 23 dogs having multiple diagnostic tests performed or organ specimens obtained. Diagnostic procedures included cytologic examination of rectal scrape specimens (n = 24), urine Histoplasma EIA testing (21; including 1 dog that also received serum Histoplasma EIA testing), blood film analysis (15), necropsy (10), gastrointestinal histologic examination (8), lymph node cytologic examination (7), ascites fluid analysis (5), lung cytologic examination (5), hepatic cytologic examination (4), bone marrow aspirate examination (3), bronchoalveolar lavage fluid analysis (2), splenic cytologic examination (2), nasal tissue histologic examination (1), and arthrocentesis fluid analysis (1).

Of the 21 dogs that received urine Histoplasma EIA testing as part of their diagnostic workup, 2 had results above the limit of quantification, 4 had positive results below the limit of quantification, and the median value for the remaining 15 dogs was 9.1 ng of Histoplasma galactomannan antigen/mL (range, 0.87 to 24.04 ng/mL). For the 1 dog that received serum EIA testing, the value was 8.91 ng/mL.

**Disease characterization**

Most dogs (45/79 [57%]) were categorized as having disseminated disease, whereas 27 (34%) dogs had the gastrointestinal form and 7 (9%) had the respiratory form. This disease categorization did not differ significantly (P = 0.51) between the 2 hospitals. Considering the combination of available clinical signs and thoracic radiographic findings, 19 dogs in the disseminated category were suspected to have pulmonary involvement. Although many dogs had only 1 organ evaluated despite multiorgan involvement, the distribution of organ involvement as confirmed via cytologic or histologic examination included the gastrointestinal tract (n = 42 [52 in the large intestine, 8 in the small intestine, and 2 in the stomach]), peripheral blood or bone marrow (22), lungs (14), lymph node (14), liver (11), spleen (7), peritoneal cavity or ascites (5), kidney (3), brain (3), nasal cavity (1), pancreas (1), bone (1), heart (1), and joint (1).

**Treatment**

Prior to referral, 31 of 79 (39%) dogs had received systemic corticosteroid treatment, and an additional 2 of 79 (3%) dogs had received topical ophthalmic steroid treatment. Additional agents administered prior to referral included various antimicrobials, antiparasitics, analgesics, gastrointestinal supportive care (eg, antacids, antiemetics, and probiotics), antioxidants, diuretics, and additional topical ophthalmic treatments. Two dogs had been receiving phenobarbital for well-controlled epilepsy. Antifungal treatment had been administered to 4 dogs prior to histoplasmosis confirmation; 1 dog received itraconazole (for 28 days prior to diagnosis), 2 dogs received fluconazole (for 1 and 46 days prior to diagnosis), and 1 dog received ketoconazole (for 14 days prior to diagnosis). Sixty-seven (85%) dogs began treatment for histoplasmosis, and 4 (5%) dogs received an antemortem diagnosis of the disease but their owners elected to decline treatment.

Three dogs were removed from statistical analyses regarding treatment, outcome, and prognosis because they received antifungal treatment for > 7 days in the month prior to diagnosis. Three additional dogs were excluded from further analysis because they initially received amphotericin B alone (n = 2) or a combination of amphotericin B and itraconazole (1). Of the 61 remaining dogs, 34 (56%) were treated initially with itraconazole alone (median dosage, 10.1 mg/kg/d [4.6 mg/lb/d]; range, 4.3 to 35.8 mg/kg/d [2.0 to 15.3 mg/lb/d]), and 27 (44%) were treated initially with fluconazole alone (median dosage, 11.4 mg/kg/d [5.2 mg/lb/d]; range, 2.7 to 19.2 mg/kg/d [1.2 to 8.7 mg/lb/d]). Clinicians at Oklahoma State University predominantly prescribed itraconazole (27/32 dogs), whereas clinicians at Kansas State University predominantly prescribed fluconazole (22/29 dogs; P < 0.001).

Two of the 61 (3%) treated dogs included in the analysis received compounded formulations (both itraconazole), 51 (84%) dogs received generic or trade formulations, and the formulation was unclear for 8 (13%) dogs. Twenty-five (41%) dogs received concurrent corticosteroid treatment during initial antifungal treatment; of these, 14 were in the fluconazole group and 11 in the itraconazole group. Nine of 61 (15%) dogs required hospitalization (median duration, 2 days; range, 1 to 26 days).

Of the 61 dogs that received treatment for histoplasmosis, 3 had a change in antifungal treatment during the first 30 days of treatment, including from fluconazole to itraconazole (n = 1), addition of terbinafine to fluconazole (1), and addition of amphotericin B to itraconazole (1). Four additional dogs had antifungal treatment changes during the first 6 months of treatment. Two dogs initially received fluconazole, but then this treatment was changed to itraconazole for one dog and amphotericin B was added for the other dog. Two dogs initially received itraconazole, but then this treatment was changed to fluconazole for one dog and terbinafine was added for the other dog.

Data regarding serum ALT activity 1 month after antifungal treatment began were available for 25 treated dogs (12 treated initially with itraconazole, 12 treated initially with fluconazole, and 1 treated initially with itraconazole and amphotericin B), and values were within the reference interval for all but 2 dogs. Neither dog had documented hepatic involvement of histoplasmosis. Both dogs were initially treated with fluconazole. One dog had concurrently received prednisone and long-term phenobarbital administration (serum ALT activity, 145 U/L). The other dog had initially received fluconazole, topically applied prednisolone acetate, doxycycline, and meloxicam and had unremarkable serum ALT activity (116 U/L; reference interval, 12.6 to
itraconazole; clinical remission (and survival to 6 months) was identified between final dosage of fluconazole treatment was 182 days (range, 50 to 339 days; median duration of fluconazole treatment was 125 days (range, 17 to 214 days) and of itraconazole (80%) dogs, and clinical remission was achieved for 33 (67%) of these dogs, including 16 of 25 (64%) dogs treated with itraconazole and 24 of 30 (80%) dogs treated with fluconazole (median, 0 days; range, 0 to 26 days) than those treated with fluconazole (median, 0 days; range, 0 to 7 days). No association was detected between initial antifungal medication administered and histoplasmosis form (respiratory, gastrointestinal, or disseminated; \( P = 0.69 \)). By 1 month after antifungal treatment began, 19 of 26 (73%) dogs treated with fluconazole and 24 of 30 (80%) dogs treated with itraconazole remained alive (\( P = 0.75 \)). By 6 months, 16 of 24 (67%) dogs treated with fluconazole and 21 of 28 (75%) of dogs treated with itraconazole remained alive (\( P = 0.55 \)). Clinical remission status was available for 49 of 61 (80%) dogs, and clinical remission was achieved for 33 (67%) of these dogs, including 16 of 25 (64%) dogs treated with fluconazole and 17 of 24 (71%) dogs treated with itraconazole (\( P = 0.76 \)). For dogs that achieved clinical remission, median duration of fluconazole treatment was 125 days (range, 17 to 214 days) and of itraconazole treatment was 182 days (range, 50 to 339 days; \( P = 0.38 \)). For dogs that survived to hospital discharge, no association was identified between final dosage of fluconazole and survival to 6 months (\( P = 0.72 \)) or achievement of clinical remission (\( P = 0.86 \)). Findings were similar for itraconazole (\( P = 0.26 \) and \( P = 0.28 \), respectively). Six of 28 (21%) dogs with sufficient follow-up information available had a relapse of histoplasmosis (2 of 12 treated with fluconazole and 4 of 16 treated with itraconazole; \( P = 0.67 \)). No significant difference was detected between the 2 hospitals in rates of survival to hospital discharge (\( P = 0.10 \)), 1 month of treatment (\( P = 0.21 \)), and 6 months of treatment (\( P = 0.23 \)) and achievement of clinical remission (\( P = 0.38 \)). Of the 9 dogs that required oxygen supplementation, 2 failed to survive to hospital discharge and 2 additional dogs did not survive to 1 month of treatment. Oxygen supplementation was not associated with survival to hospital discharge (\( P = 0.054 \)), survival to 1 month of treatment (\( P = 0.19 \)), or survival to 6 months (\( P = 0.17 \)). However, for dogs with available data, 3 of 9 dogs requiring oxygen supplementation achieved clinical remission, compared with 30 of 40 dogs not requiring oxygen supplementation (\( P = 0.04 \)). Twenty-five dogs received systemic corticosteroid treatment after diagnosis and concurrent with antifungal treatment (14 treated with fluconazole and 11 treated with itraconazole). Twenty-three of 25 (92%) dogs survived to hospital discharge, 19 of 25 (76%) survived to 1 month of treatment, 15 of 23 (65%) survived to 6 months, and 13 of 22 (59%) achieved clinical remission. No association was identified between corticosteroid treatment and these outcomes (\( P = 0.56, P = 1.00, P = 0.54 \), and \( P = 0.36 \), respectively). Similarly, no association was identified between corticosteroid administration prior to diagnosis and outcomes (\( P = 0.25, P = 1.00, P = 1.00 \), and \( P = 0.55 \), respectively).

Breed group was not associated with any outcome, nor was it associated with histoplasmosis form (\( P = 0.052 \)). However, 5 of 6 toy-breed dogs had gastrointestinal histoplasmosis, whereas 10 of 11 working-breed dogs and 3 of 4 herding/breed dogs had disseminated histoplasmosis. When compared with other breeds, Great Pyrenees were less likely to survive to 1 month (\( P = 0.008 \)) or 6 months (\( P = 0.02 \)) of treatment and less likely to achieve clinical remission (\( P = 0.03 \)). Rates of survival to hospital discharge or 1 month or 6 months of treatment and rate of achievement of clinical remission did not differ significantly among the 3 histoplasmosis forms (\( P = 0.20, P = 0.07, P = 0.12, \) and \( P = 0.10 \), respectively). Dyspnea (\( P = 0.003 \) for survival to discharge), palpable abdominal organomegaly (\( P < 0.001 \) for survival to 1 month and 6 months and \( P = 0.003 \) for achievement of clinical remission), and icterus (\( P = 0.01 \) for survival to 1 month, \( P = 0.001 \) for survival to 6 months, and \( P = 0.009 \) for achievement of clinical remission) at initial evaluation were associated with a lower survival rate or clinical remission rate, whereas diarrhea at initial evaluation was associated with a higher rate of survival to 6 months (\( P = 0.05 \)), compared with respective rates for dogs without these characteristics. No clinical signs were associated with disease relapse.

Anemia (\( P = 0.004 \) for survival to 1 month, \( P = 0.005 \) for survival to 6 months, and \( P = 0.004 \) for achievement of clinical remission) and thrombocytopenia (\( P = 0.01, P = 0.008, \) and \( P = 0.008 \), respectively) were associated with lower survival and clinical remission rates, compared with rates for dogs without these characteristics. For dogs with data available on remission status, 6 of 14 dogs with initial Hct value ≤ 30% achieved remission, whereas 27 of 35 dogs with a value > 30% achieved remission. No association was detected between anemia and hematochezia (\( P = 0.41 \)).
Also for dogs with data available on remission status, 7 of 16 dogs with thrombocytopenia (≤ 175 × 10³ platelets/µL) achieved remission, whereas 25 of 29 dogs without thrombocytopenia achieved remission. Hypercalcemia (P = 0.02 for survival to hospital discharge), high serum ALP activity (P = 0.03 for survival to 1 month and P = 0.02 for achievement of clinical remission), and hyperbilirubinemia (P = 0.006 for survival to 6 months and P = 0.005 for achievement of clinical remission) were also associated with a lower survival or clinical remission rate, compared with rates for dogs without these characteristics. No laboratory variable was associated with disease relapse.

**Discussion**

Findings of the present study regarding histoplasmosis in dogs add considerable descriptive data to previously reported findings from smaller studies.⁵,¹³ No significant differences were identified between itraconazole and fluconazole in rates of survival to hospital discharge, survival to 1 or 6 months of antifungal treatment, achievement of clinical remission, or disease relapse. Negative prognostic factors at diagnosis included Great Pyrenees breed, dyspnea, need for oxygen supplementation, icterus, and palpable abdominal organomegaly, whereas diarrhea was a positive prognostic factor. Anemia, thrombocytopenia, hypercalcemia, high serum ALP activity, and hyperbilirubinemia were also negative prognostic factors. This information could assist clinicians in the recognition and diagnosis of histoplasmosis in dogs as well as with treatment options, prognosis determination, and outcomes.

Consistent with the pre-existing literature, dogs of the study reported here were of a wide age range but were generally young adults (median age, 3 years) and were of both sexes. Working, terrier, and sporting breeds were commonly represented, and Miniature Schnauzer (a terrier breed) and Great Pyrenees (a working breed) were the most common pure breeds. However, 13% of dogs were of toy breeds, which was an important new finding and a reminder to consider toy breeds in endemic areas at risk for histoplasmosis if clinical signs are consistent. A comparison of breed distributions between the included dogs with histoplasmosis and the overall hospital populations from which they were selected would have provided additional information on risk by factoring in breed popularity at each hospital.

Working and herding breeds most often had disseminated histoplasmosis in the present study, whereas toy breeds most often had gastrointestinal histoplasmosis. Dogs with versus without extended outdoor exposure are likely at increased risk of inhalation of spores, and disease may go unrecognized for a longer period, allowing dissemination. Compared with other breeds, Great Pyrenees had a worse outcome, which has not been reported previously. This finding may have been attributable to a true breed difference (eg, in hereditary immune function or drug metabolism) or husbandry factor (eg, later detection of weight loss or other insidious signs owing to the long hair coat or less direct interaction with handlers owing to long periods spent guarding livestock). Additionally, this was the only giant breed represented in the study by > 1 dog. No attempt was made to identify the reason for euthanasia, and it was possible that Great Pyrenees were more likely to be euthanized for financial reasons. The reason toy breed dogs primarily had the gastrointestinal form of histoplasmosis remains unknown and may have involved differences in immune response, environmental factors, or inoculum exposure.

Clinical signs at initial evaluation, findings of physical examination, and abnormalities on CBC and serum biochemical analysis were nonspecific, reinforcing the importance of considering histoplasmosis as a differential diagnosis for a clinically ill dog in an endemic area. An inflammatory leukogram and monocytosis were common. Anemia (Hct < 30%) was identified in 33% of dogs and was likely due to anemia of chronic inflammatory disease as well as bone marrow involvement and gastrointestinal hemorrhage in some dogs.⁷ Thrombocytopenia was detected in 38% of dogs, which is consistent with findings of one study (6/12 dogs) but not of another study (0/24 dogs). Serum biochemical findings were nonspecific, with hypoalbuminemia most likely from intestinal loss, hyperglobulinemia from chronic infection, and mildly high liver enzyme activity and hyperbilirubinemia from hepatic involvement. Diffuse interstitial disease was the most common abnormality identified via thoracic radiography; as has been traditionally expected with fungal pneumonia, but the variety of identified radiographic changes suggested that consideration of histoplasmosis as a differential diagnosis should not be limited to dogs in which a classic miliary or nodular radiographic pattern is noted.¹³ Various diagnostic procedures were used to confirm the diagnosis of histoplasmosis in the study reported here. Diagnosis was most commonly confirmed with cytologic examination of a rectal scrape (30%) or blood film (19%) specimen, both of which are noninvasive and inexpensive tests that should be performed by a veterinary clinical pathologist when a dog is suspected to have gastrointestinal or disseminated histoplasmosis. Cytologic or histologic examination of specimens from other organs (eg, lymph node, liver, or lung) and fluid analysis for various effusions were also useful to confirm the diagnosis.³,⁴,²⁴ Traditionally, itraconazole has been considered the treatment of choice for dogs with histoplasmosis, largely on the basis of research findings for humans and mice.⁵,²⁶ No previous studies have assessed the effectiveness of fluconazole or compared it with that of itraconazole for treatment of histoplasmosis in dogs. However, research failed to demonstrate a significant difference between fluconazole and itraconazole in treatment effectiveness or relapse rate when fluconazole was compared with itraconazole for cats with histoplasmosis or dogs with blastomycosis.³⁷ The present study revealed that fluconazole can be an effective treatment for histoplasmosis in dogs, given that no significant difference in survival, remission, or disease relapse rates was identified between fluconazole and itraconazole. Mild to moderate increases in serum ALT activity were observed, as has been reported for dogs receiving itraconazole or fluconazole.³⁷ but resulted in no reported clinical signs and had minimal impact on therapeutic decisions.

All 3 dogs in the present study that died prior to hospital discharge were categorized as having disseminated disease with pulmonary involvement. Dyspnea and need for oxygen supplementation were identified as negative

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prognostic factors, suggesting that pulmonary involvement of histoplasmosis may not have a fair to excellent prognosis as previously reported.9-10 Although no statistical differences were identified in outcome among the 3 forms of histoplasmosis as defined, many (19/45) dogs with disseminated disease also had evidence of pulmonary disease, which confounded this analysis.

Corticosteroid treatment has been used in dogs with histoplasmosis to decrease airway obstruction secondary to hilar lymphadenomegaly and is recommended for humans with moderately severe to severe acute pulmonary histoplasmosis who develop respiratory complications, such as hypoxemia or respiratory distress.19,27,28 Concern exists about causing immunosuppression and potential for dissemination of fungal disease.9 In the present study, corticosteroid treatment prior to diagnosis or in conjunction with antifungal treatment had no association with outcome. Additional research is warranted into the benefits and risks of corticosteroid treatment in dogs with histoplasmosis.

Classification of histoplasmosis by respiratory, gastrointestinal, or disseminated forms was not associated with outcome for the dogs of the present report. However, several negative prognostic factors were identified, suggesting that the presence or severity of pulmonary, bone marrow, and hepatic involvement may have prognostic implications. Alternatively, diarrhea was identified as a positive prognostic factor, suggesting that dogs with gastrointestinal histoplasmosis may have a more optimistic outlook. Studies8,9,20 involving AIDS patients with histoplasmosis have revealed that diarrhea, neurologic manifestations, dyspnea, anemia, thrombocytopenia, prolonged PTT, hypoalbuminemia, high serum aspartate aminotransferase activity, hyperbilirubinemia, azotemia, acute renal failure, and sepsis are associated with adverse outcomes or death, and these factors have influenced treatment recommendations for affected patients. Treatment guidelines for humans with histoplasmosis also consider specific organ involvement, severity, chronicity of infection, and immune status.9,20 A prospective study is warranted to determine whether changes in treatment strategies, such as treatment with amphotericin B or combination treatment, for dogs with the identified prognostic characteristics might improve their outcome.

The present study had limitations inherent to a retrospective study. For example, the Histoplasma EIA result was used in combination with consistent clinical findings for the diagnosis of histoplasmosis in 6 dogs. Although this test has a reported specificity of 100% for the diagnosis of histoplasmosis in dogs, cross-reactions are possible with Blastomyces spp and other rare fungal infections.14,29 None of the 6 dogs with an EIA-based diagnosis had a reported history of travel to a Blastomyces-endemic area, and 2 of these dogs had a concurrent negative result of Blastomyces EIA testing. However, it is possible that disease was misdiagnosed in these dogs. Categorization of histoplasmosis by organ involvement and by pulmonary, disseminated, or gastrointestinal form may also have been inaccurate in some instances given that no extensive imaging and organ sample collection was performed for many dogs beyond confirmation of the diagnosis. Therefore, caution should be used when interpreting the results associated with this categorization.

Other limitations included the fact that selection of antifungal treatment strategy was made by the attending clinician, without use of a consistent dosage or protocol, nor was consistent follow-up information available at predetermined times. At one hospital, clinicians appeared to prefer fluconazole, whereas at the other hospital, they appeared to prefer itraconazole; however, no differences in classification of disease or any outcome existed between the hospitals. The few dogs with drug changes during treatment may have also affected outcome comparisons; however, such changes were uncommon and occurred similarly in both antifungal groups. Two dogs received compounded itraconazole, which is not recommended because of low bioavailability in the species and a lack of bioequivalence.30 Although both of these dogs survived to 6 months of antifungal treatment, the retrospective nature of the study made it unclear whether a compounded product had been used exclusively or whether the product was compounded from bulk or reformulated from an FDA-approved product.

Overall, the study reported here showed that histoplasmosis affected a variety of dog breeds, including working, terrier, toy, sporting, and herding breeds. Clinical signs at initial evaluation varied owing to the many organs that can be involved. Diagnosis was commonly made by noninvasive and inexpensive tests, such as cytologic examination of rectal scrape and blood film samples. Findings suggested that either itraconazole or fluconazole can be used to achieve clinical remission, and a remission rate of approximately 67% may be possible. The identified prognostic factors can aid clinicians when discussing prognosis with owners of affected dogs.

Footnotes
a. Histoplasma quantitative EIA test, Miravista Diagnostics, Indianapolis, Ind.

References


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**Comparative pharmacokinetics of two florfenicol formulations following intramuscular and subcutaneous administration to sheep**

Christie C. Balcomb et al

**OBJECTIVE**

To compare the pharmacokinetics of 2 commercial florfenicol formulations following IM and SC administration to sheep.

**ANIMALS**

16 healthy adult mixed-breed sheep.

**PROCEDURES**

In a crossover study, sheep were randomly assigned to receive florfenicol formulation A or B at a single dose of 20 mg/kg, IM, or 40 mg/kg, SC. After a 2-week washout period, each sheep was administered the opposite formulation at the same dose and administration route as the initial formulation. Blood samples were collected immediately before and at predetermined times for 24 hours after each florfenicol administration. Plasma florfenicol concentrations were determined by high-performance liquid chromatography. Pharmacokinetic parameters were estimated by noncompartmental methods and compared between the 2 formulations at each dose and route of administration.

**RESULTS**

Median maximum plasma concentration, elimination half-life, and area under the concentration-time curve from time 0 to the last quantifiable measurement for florfenicol were 3.76 µg/mL, 13.44 hours, and 24.88 µg·h/mL, respectively, for formulation A and 7.72 µg/mL, 5.98 hours, and 41.53 µg·h/mL, respectively, for formulation B following administration of 20 mg of florfenicol/kg, IM, and 2.63 µg/mL, 12.48 hours, and 31.63 µg·h/mL, respectively, for formulation A and 4.70 µg/mL, 16.60 hours, and 48.32 µg·h/mL, respectively, for formulation B following administration of 40 mg of florfenicol/kg, SC.

**CONCLUSIONS AND CLINICAL RELEVANCE**

Results indicated that both formulations achieved plasma florfenicol concentrations expected to be therapeutic for respiratory tract disease caused by Mannheimia haemolytica or Pasteurella spp at both doses and administration routes evaluated. (Am J Vet Res 2018;79:107–114)