Sweet Girl's Sour Diagnosis

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Introduction

Histoplasmosis is a chronic, non-contagious fungal infection caused by variations within the *Histoplasma capsulatum* species. This saprophytic, dimorphic fungus is found globally and has an affinity for soils rich in bat and/or bird droppings. In the United States, *Histoplasma* is most prevalent in soils along the Mississippi, Missouri, and Ohio River Valleys, but can be present in other locations. Within the soil *Histoplasma* exists in the mycelial or mold form. When the soil is disturbed the fungal spores aerosolize, allowing them to be inhaled or less commonly, ingested. The mammalian body temperature then promotes the conversion from the mold form to the pathogenic yeast form. Once established within the body the infection can remain localized or spread to various organ systems.[3]

History and Presentation

Sweet Girl, an approximately 3.5-year-old, female, spayed, domestic shorthair, presented to the Animal Emergency and Referral Center (AERC) in Flowood, Mississippi on January 7th, 2020 for generalized lethargy. She was initially found as a stray cat in May 2019 at which point she was estimated to be approximately 3 years old. Sweet Girl tested negative for feline immunodeficiency virus (FIV) and feline leukemia virus (FELV) so she was vaccinated, spayed, placed on preventatives, and allowed back outdoors. On October 31st, 2019, Sweet Girl was the victim of a dog attack. She sustained a laceration on her right thorax as well as puncture wounds near her rectum, on her ventral abdomen, and on her dorsal neck. Radiographs revealed possible rib fractures along with subcutaneous emphysema of her hind limbs. A complete blood count and chemistry were performed and revealed no significant abnormalities. The laceration on her right

thorax had a deep dorsal pocket so a Penrose drain was placed. The laceration as well as the puncture wounds near her rectum were closed, while the remainder of her wounds were left to heal by second intention. Sweet Girl was discharged on November 6th, 2019, at which point she became an indoor only cat and recovered uneventfully.

Upon presentation to AERC on January 7th, 2020, Sweet Girl was bright, alert, and responsive. Her vital parameters were within normal limits with a heart rate of 192 beats per minute, a respiratory rate of 36 breaths per minute, and a temperature of 101.8 degrees Fahrenheit. Her mucous membranes were pink with a capillary refill time of less than two seconds. Cardiothoracic auscultation revealed normal heart and lungs sounds, and abdominal palpation yielded a soft, non-painful abdomen. A brown exudate was noted in both ears that appeared very pruritic. The remainder of her physical examination was unremarkable.

A complete blood count revealed a hematocrit of 28.4% (30.3-52.3), which had decreased from 32.5% on October 31st, 2019. Her hemoglobin was mildly decreased at 9.1g/dl (9.8-16.2), as well as her platelet count at 133 K/µl (151-600). Serum chemistry indicated a mild increase of globulins at 5.3 g/dL (2.8-5.1). An ear cytology yielded too numerous to count cocci in both ears. A FELV/FIV/Heartworm SNAP test was repeated and was negative on all accounts. Thoracic and abdominal radiographs highlighted a diffuse patchiness of the lungs with no significant findings in the abdomen. It was recommended that the radiographs be sent in for consultation. The consultation report stated that patchy, mixed alveolar, and interstitial infiltrates were noted throughout the ventral lung fields in both the left and right thorax. A moderate diffuse increase in bronchial and interstitial lung markings were also noted. The remainder of the thorax and abdomen were deemed unremarkable. The radiologist concluded that the distribution of the pulmonary changes was most compatible with bacterial bronchopneumonia. Aspiration

pneumonia could not be ruled out but was considered less likely due to the lack of reported vomiting, dysphagia, or regurgitation. Based on these findings Sweet Girl was placed on Veraflox 2.5% oral suspension for 7 days to treat the suspected bacterial bronchopneumonia. Her owner was instructed to return her in 7 days to repeat thoracic radiographs. She was also diagnosed with otitis externa and treated with one dose of Claro in each ear. Sweet Girl was discharged on January 8th, 2020.

On January 13th, 2020 Sweet Girl returned to AERC for lack of clinical improvement and a decreased appetite. On presentation she was bright, alert, and responsive. She had a body condition score of 4/9 and was regarded to be slightly underweight. Mild epiphora was noted bilaterally. While no nasal discharge was noted during her physical examination, her owner stated that she had been seeing intermittent nasal discharge at home. Her mucous membranes were pink with a capillary refill time of less than two seconds. Her heart rate, rhythm, and sound were recorded to be within normal limits, but she was tachypneic with increased lung sounds. No crackles or wheezes were heard on auscultation. The remainder of her physical exam was unremarkable

A complete blood count was repeated revealing a further decrease in hematocrit at 24.4% (30.3-52.3), and hemoglobin at 7.8 g/dL (9.8-16.2). Her red blood cells were also decreased at 6.11 M/µl (6.54-12.2). Sweet Girl's platelets remained static at 133 K/µl (151-600). Her monocytes were mildly elevated at 0.7 K/µl (0.05-0.67). Thoracic radiographs were repeated and submitted for consultation. The interstitial component of the previously noted mixed pulmonary pattern had worsened considerably. A focal alveolar pattern persisted in the caudal subsegment of the left cranial lung lobe. Several ill-defined pulmonary nodules could be seen that were not present in the previous radiographs. Based on the findings, the radiologist concluded that there

was a progressive interstitial pattern with static alveolar and bronchial component. It was recommended that histoplasmosis, blastomycosis, inflammation secondary to parasites, idiopathic inflammation, or diffuse neoplasia be considered. Sweet Girl was hospitalized overnight for supportive care. Although she was not dyspneic, she was placed in an oxygen cage and maintained on 2L/min for additional support.

Diagnostic Approach/Considerations

Due to Sweet Girl's worsening clinical picture, she was referred to the internal medicine department at AERC on January 14th, 2020. Upon physical examination Sweet Girl had a heart rate of 200 beats per minute, a respiratory rate of 72 breaths per minute, and a temperature of 101.5 degrees Fahrenheit. Cardiothoracic auscultation revealed no significant changes. Her respiratory rate ranged from 48-78 breaths per minute despite being in the oxygen cage. She was anorexic with a quiet mentation.

Abdominal ultrasound was performed and revealed a small amount of abdominal effusion. Abdominocentesis was performed and the obtained sample of fluid appeared pink and cloudy. It had a glucose of 278 mg/dL, a lactate of 1.5 mmol/L, a packed cell volume of 1%, and total solids of 2.6 g/dL. Sweet Girl was anesthetized and bronchoalveolar lavage was performed. Saline at a volume of 14mls was infused into the airway of which 6.5mls was recovered and submitted for cytology. The fluid recovered was thick and mucous-like.

Cytology revealed marked chronic neutrophilic inflammation. Poorly preserved to degenerate neutrophils and many macrophages were seen with both intra-and extracellular yeast. The yeast observed were compatible with a *Histoplasma* species. *Histoplasma* antigen by

Enzyme Immunoassay (EIA) was also performed, and results were reported as 17.94 ng/mL, indicating a positive result. A Baermann fecal and *Cryptococcus* antigen test were also performed, and both were negative. An aerobic bacterial culture, fungal culture, and feline upper respiratory PCR panel were also submitted, but were canceled when the positive results from the fluid cytology and *Histoplasma* antigen EIA were received.

Pathophysiology

Within the soil, at approximately 25 degrees Celsius (77°F), *Histoplasma capsulatum* exists as a mold. As a mold it generates two types of spores: macroconidia and microconidia. These asexual, ovoid structures are produced at the tips of hyphae and are easily aerosolized with any disturbance to the soil. Once inhaled, some of the fungal inoculum may remain within the nasal mucosa while the majority of the microconidia, which are 2-5 μm in size travel to the level of the alveoli and terminal bronchioles of the lung. The mammalian body, at roughly 37 degrees Celsius (98.6°F), then drives the conversion from mold to the pathogenic yeast form.[9]

The mammalian lungs are challenged with microbes as well as microbial products with every breath. To combat this extremely high pathogenic load the body relies on the immunological mechanism of the lungs. Epithelial cells provide an initial defense while mucus and antimicrobial peptides prevent further establishment. When the early defense mechanisms fail which can be due to a diverse number of factors including fungal load, virulence, and host immune status a coordinated immune response ensues. Macrophages and dendritic cells as well as other myeloid populations infiltrate the airways to combat the infection.[8] Once *H. capsulatum* enters a host cell, multiple outcomes can be seen. *H. capsulatum* can replicate itself rapidly, thus leading to death of the host cell and spread of yeast to additional cells. Additionally, fungal

antigens can be transported to various lymph nodes and presented to T-lymphocytes to aid in the development of cell-mediated immunity. From this point the activated phagocytes can contain the infection or *H. capsulatum* can establish a persistent infection.[4]

The way in which *H. capsulatum* utilizes antigen presenting cells to its benefit is not completely characterized, and multiple mechanisms have been proposed. Studies have shown that once *H. capsulatum* has been internalized by a host phagocyte it creates a membrane bound vacuole. As fusion of the phagosome and lysosome occurs to create the phagolysosome the pH drops dramatically creating a hostile environment for the internalized pathogen. While *H. capsulatum* can be damaged by the acidic pH and other lysosomal degradation products, some species contain genes to guard against this and allow for their continued propagation.[4]

Histoplasmosis can manifest as a pulmonary, primary gastrointestinal, or disseminated disease. The infection is considered to be disseminated when an organ system outside of the thoracic cavity is affected. Disseminated infection is the most common form of histoplasmosis reported.[1] Cats of any age, sex, or breed can be infected with *Histoplasma capsulatum*, but adult and female cats appear overrepresented in the literature.[3,5,13] While this disease was originally thought to primarily affect outdoor cats, a retrospective study involving thirty cats revealed that 38% of infected cats were reported to be strictly indoors. These findings suggest that cats can be exposed to *Histoplasma* microconidia by routes other than direct outdoor exposure to soil.[3,11]

The clinical presentation of this disease is often non-specific. The most commonly associated signs are weight loss, decreased appetite, and lethargy.[1,3] Upon physical examination the most common findings are reported to be tachypnea or dyspnea, increased respiratory sounds, and fever.[3] Diagnostics, such as hematology commonly reveal either a normocytic/microcytic, normochromic, non-regenerative anemia, and a thrombocytopenia.

Pancytopenia is also a common finding with this disease. On biochemistry, hypoalbuminemia is the most commonly reported abnormality. In cases where thoracic radiographs are utilized interstitial, diffuse miliary, nodular-interstitial, and alveolar infiltrates have been described. Hepatomegaly and splenomegaly are common findings on both abdominal radiographs as well as abdominal ultrasound. Lymphadenopathy and peritoneal effusion were also seen less commonly.[1]

Definitive diagnosis of histoplasmosis is primarily made through demonstration of fungal organisms in circulating monocytes or within phagocytes on cytology and/or histopathology.[1,6] A newer method employed to diagnose histoplasmosis is the measurement of *Histoplasma* spp. antigen in urine and/or serum samples. In patients with an ongoing infection, *Histoplasma* spp. antigens are reported to be shed into affected tissues as well as bodily fluids. In a retrospective study of confirmed cases the overall sensitivity of the urine antigen assay was reported to be 94.4%. Studies in humans revealed that testing both urine and serum increased the sensitivity for diagnosis over testing urine alone. While more testing may be needed, the urine antigen assay could pose as a less invasive method to achieve diagnosis as compared to traditional methods. It could also be used adjunctively to further support a positive diagnosis.[6] Serology is also a method of diagnosis that is available; however, the high rate of false positive as well as false negative test results significantly limits the clinical use.[3]

Treatment and Prognosis

Histoplasmosis has historically been treated with azole antifungal drugs. Ketoconazole, itraconazole, and fluconazole have all been reported to have successful therapeutic outcomes in

cats. These drugs target the fungal cell membrane leading to an increase in permeability that ultimately causes lysis of the cell. Ketoconazole was initially the drug of choice for histoplasmosis, but the high risk of hypersensitivity and hepatotoxicity seen in cats led to its disfavor. Itraconazole and fluconazole are reported to be as efficacious and cause less severe side effects.[10] While effective, the use of itraconazole may be cost prohibitive for some owners. For histoplasmosis infections involving the central nervous system, the use of fluconazole is advocated. Fluconazole is smaller, less protein bound, and more soluble in fluids as compared to itraconazole, which allows it to cross the blood brain barrier more readily.[13] Amphotericin B is a broad-spectrum antibiotic that also has activity against systemic fungi such as *Histoplasma capsulatum.* Like the azole antifungals, amphotericin B also increases cellular permeability leading to cell lysis. While effective, this drug can cause nephrotoxicity and thus should only be considered for progressive and potentially fatal infections.[10]

Successful treatment of histoplasmosis often requires antifungal therapy for at least 4-6 months or 2 months past resolution of clinical signs.[3,13] Concurrent glucocorticoid therapy is often initiated at an anti-inflammatory dose due to the inflammation caused by the dying fungal organisms. Bloodwork as well as behavior should be monitored regularly in cats receiving azole antifungals to monitor for elevations in liver enzymes, as well as gastrointestinal signs.

While long term antifungal treatment is required, it is possible for remission to occur. Remission may be determined by the resolution of clinical signs, physical examination findings, imaging studies, and cytologic examination.[11] The *Histoplasma* antigen EIA has been proposed as an additional method to aid in both monitoring the success of treatment as well as confirming remission. In a study involving fifteen cats diagnosed with histoplasmosis, urine and serum *Histoplasma* antigen concentrations were shown to decrease significantly over the treatment

period. Both urine and serum antigens were eliminated after a median of 13 weeks, which was significantly shorter than the time to clinical remission. Based on this study the sensitivity of detecting disease remission was measured to be 90% with a specificity of 64.6% indicating it could be helpful adjunctively.[11]

At the time of diagnosis, cats that are not severely affected by this disease are said to have a fair to good prognosis with long term treatment. If disseminated histoplasmosis has been diagnosed prognosis becomes guarded to poor even with treatment. Severe respiratory disease, dyspnea, and/or adventitial lung sounds at presentation are reported to be negative prognostic indicators of outcome. Severe liver, hematologic, and/or neurologic disease were also reported as negative prognostic indicators.[5] Recrudescence of disease in cats is presumptively caused by choice of therapeutic agent, length of therapy, or virulence of the *Histoplasma* species.[3] Drug resistance has not been reported as of yet in feline histoplasmosis but theoretically could be possible. Proper anti-fungal choice in the event of relapse is highly debated. Disease-free interval and median survival time of cats treated for histoplasmosis has not been well characterized due to the small sample sizes as well as the retrospective nature of the various studies.

Case Outcome

Following the bronchoalveolar lavage Sweet Girl was maintained in an oxygen cage at 2L/min and received Plasmalyte at 12 mL/hr. She was started on an albuterol inhaler, clindamycin for a possible toxoplasmosis infection, and fenbendazole for a possible lungworm infection. When histoplasmosis was confirmed the clindamycin was discontinued and itraconazole was initiated. Mirataz was also implemented as Sweet Girl was still anorexic. Due

to her anorexia and difficulty administering medication an esophagostomy tube was placed on January 16th, 2020. Radiographs were used to ensure the proper placement of the esophagostomy tube. Sweet Girl recovered uneventfully.

Overnight on January 16th, 2020, Sweet Girl's tachypnea worsened, and she became dyspneic when the oxygen cage was opened for examinations. The oxygen was increased to 4 L/min. She became increasingly depressed in the early morning of January 17th, 2020 and was unwilling to stand. Her rectal temperature was 96 degrees Fahrenheit, so a warming pad was placed under her cage. Within an hour she became apneic and passed away. The owner elected to not have CPR performed.

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