Fungal pneumonia and air sacculitis in a Maximilian pionus parrot *(Pionus maximiliani)*

Introduction

This report describes a case of fungal pneumonia and air sacculitis in a 13-year old female Maximilian pionus parrot (*Pionus maximiliani).* The bird was also diagnosed with a soft tissue sarcoma on the foot, which will be briefly discussed. The bird initially presented for weight loss despite a normal appetite. Nodular air sacculitis and granulomatous pneumonia was diagnosed on radiographs and the bird was treated with itraconazole for presumptive aspergillosis. The lesions did not resolve and endoscopy was performed. Histopathology on biopsies collected during endoscopy confirmed a diagnosis of fungal pneumonia and air sacculitis. Treatment continued but the bird was not improving, so she was hospitalized for IV therapy and nebulization. While hospitalized the bird developed a severe respiratory distress and was euthanized. Necropsy confirmed granulomatous pneumonia and air sacculitis with intralesional fungal hyphae that were consistent with *Aspergillus* spp. A tracheitis with partial occlusion of the tracheal lumen was also present. This case demonstrates the challenges faced in diagnosing and treating fungal air sacculitis and pneumonia in avian .

Aspergillosis, defined as disease caused by infection with *Aspergillus* spp, is a common condition in avian species.[1] It is a debilitating disease and affects a variety of avian species including poultry, waterfowl, raptors, penguins, and psittacines.[2-8] Diagnosis presents many challenges, and often requires histopathology for definitive diagnosis.[1] Treatment involves local or systemic antifungals but is often unrewarding. Prognosis for birds with aspergillosis is often guarded.

Aspergillosis in birds is most often caused by *Aspergillus fumigatus*, however *A. flavus, A. niger* and other *Aspergillus* species are less commonly reported.[3,9-11] Aspergillosis is the most common cause of respiratory mycosis, but other mycotic causes of pneumonia and air sacculitis are infrequently reported, including penicillium, zygomycosis (mucormycosis) and cryptococcosis.[1,12-16] *Aspergillus* is ubiquitous in the environment and causes opportunistic infections. It is commonly found in soil and decaying plant material.[17] *Aspergillus* spores have been found worldwide with the exception of Antartica.[17] Infection in a bird requires an overwhelming exposure to *Aspergillus* spores or a predisposing condition such as long term antibiotic administration, stress, exogenous corticosteroid administration, or concurrent disease such as viral illness or heavy metal toxicosis.[1,9,11,17,18] Common sources of stress in birds include overcrowding, recent capture and transport, poor nutrition, forced production, migration, and improper temperature or humidity.[6,10,11,17,19,20] There are also species predispositions among birds. Species that are reported to have a high incidence of Aspergillosis include mynah birds, penguins, waterfowl, Goshawks, Gyrfalcons, juvenile red-tailed hawks, and eagles.[1,3-5,8,21,22] Among psittacine species, Amazon parrots, pionus parrots, and African grey parrots have been reported to be particularly sensitive to *Aspergillus* infections.[1,7,8]

The primary route of infection in aspergillosis is respiratory.[17] In addition to the respiratory route of infection, there are other possible routes of infection with *Aspergillus* spp in birds, including oral transmission, penetrating trauma, and invasion of egg shells.[17] Aspergillosis most commonly develops when a susceptible bird inhales fungal spores, referred to as conidia. The *Aspergillus* conidia pass into the respiratory tract of the bird and invade the tertiary bronchioles where they are able to germinate in the aerobic environment. As the conidia germinate they swell to form globose- or oval-celled hyphal branches.[23] Four to six days

following infection these large celled hyphal branches then form straight to spiraling unbranched hyphae.[23] This leads to the production of plaques of hyphae, which in turn induce a strong inflammatory response by the bird. The inflammatory response is primarily characterized by macrophages, multinucleate giant cells, and heterophils.[17] These inflammatory cells surround the fungal plaques and form nodules in the lungs or on the surface of air sacs and other airways. Some of the fungal hyphae asexually produce new conidia, which may be carried to other locations in the respiratory tract and germinate to form additional lesions.[17] Once respiratory lesions have formed, the fungus is able to affect other organs in the body either by direct extension through the peritoneum or air sacs or by invasion of blood vessels and hematogenous spread.[24]

The penetrating trauma and oral routes of infection with *Aspergillus* spp are additional methods by which birds may become infected. Penetrating trauma, in which puncture wounds are contaminated with *Aspergillus* spp spores, may lead to local or respiratory aspergillosis, especially if the wound involves the air sacs.[17] In addition, *Aspergillus* spores have been reported to penetrate egg shells and cause either embryonic death or birds to be hatched with aspergillosis.[a] The oral route of exposure is hypothesized but unlikely. In one experiment involving the feeding of *Aspergillus flavus* and *Aspergillus fumigatus* in wheat cultures to broiler chicks the birds did not develop lesions of respiratory aspergillosis, although lesions of mycotoxicosis were observed.[25]

There are two forms of systemic aspergillosis, acute and chronic. The acute form is less common and is generally seen in raptors, waterfowl and poultry.[7,17,26] This form is thought to result from exposure to an overwhelming number of *Aspergillus* spores.[5,27] Clinical signs of acute aspergillosis include anorexia and severe respiratory distress with a rapid course of disease

and death within one to seven days.[26] Differential diagnoses for anorexia, severe respiratory distress and rapid disease progression would include tracheal stenosis, tracheal or bronchial foreign body, tracheal trauma, paramyxovirus-1 infection, orthomyxovirus infection, bacterial pneumonia, *Chlamydophila psittaci* infection, mycobacteriosis, chronic obstructive/ hypersensitivity syndrome, or inhaled airborne toxins such as cigarette smoke, air fresheners, or polytetrafluoroethylene (PTFE). In cases of acute aspergillosis the fungal spores are spread either through the air passages or through the blood stream, leading to the formation of micronodules in the lungs, air sacs, viscera, and coelomic cavity. A white mucoid exudate may also be present in the air sacs, along with congestion of the air sacs and lungs.[1]

The more common, chronic, form of aspergillosis causes either a localized or disseminated disease that tends to be progressively debilitating with high mortality.[7] Common locations of granuloma formation in chronic aspergillosis include the caudal thoracic and abdominal air sacs and the syrinx.[5,7] Clinical signs of chronic aspergillosis range from mild, non-specific findings such as decreased appetite, lethargy, weight loss (often in spite of a normal appetite), and exercise intolerance to severe respiratory signs such as change in voice, open beak breathing and cyanosis.[7] The presence and severity of the respiratory signs depends on the location of the fungal granulomas, and may not be evident until late in the course of disease. Other clinical signs such as neurologic abnormalities, hepatic signs, or renal signs may be present depending on the location of mycotic invasion.[1,7] Because the clinical signs of chronic aspergillosis are vague and may involve multiple organ systems the differential diagnosis list is extensive, including infectious disease such as bacterial, mycotic, viral, and parasitic disease, toxins, neoplasia, and many more. Additional diagnostics such as hematology, biochemistry, radiology, and endoscopy would be needed to narrow the differential diagnostic list. Localized

infections with *Aspergillus* spp have been reported in the form of chronic rhinitis and sinusitis and ocular infection.[1,19,28,29] Clinical signs of *Aspergillus* rhinitis include distention of the sinuses, serous to purulent nasal discharge, and wheezing respiratory sounds. Differential diagnoses for these upper airway signs would include bacterial sinusitis, *Chlamydophila psittaci,* nasal foreign body, choanal atresia, or obstruction secondary to hypovitaminosis A. Signs of ocular *Aspergillus* infection include blepharospasm, photophobia, conjunctivitis, periorbital swelling, corneal edema, and elevated, dry fluffy lesions on the cornea.[30] Differential diagnoses for these ocular signs include corneal trauma, ocular foreign body, bacterial granuloma, and anterior uveitis.

Obtaining a diagnosis of aspergillosis in birds presents many challenges because the clinical signs of disease, the clinical pathologic findings and the radiographic findings are often vague and non-specific. Birds with aspergillosis often have abnormalities of hematology and plasma biochemistry. These clinical pathology changes are non-specific for aspergillosis but may increase the clinician's suspicion of disease. Hematologic findings in birds with aspergillosis may include a moderate to severe leukocytosis characterized by a heterophilia, lymphopenia, and monocytosis, and a nonregenerative anemia.[1,31] While a monocytosis is common in aspergillosis cases it may also be observed with many other chronic diseases such as chlamydophilosis, mycobacteriosis, bacterial granulomas, or chronic dermatitis.[32] Eosinophilia is not commonly reported in cases of aspergillosis, but it can be observed with inflammation of many causes in avian species, including secondary to intestinal parasites, trauma, abdominal surgery and dermatitis.[32,33] On plasma biochemistry a hyperproteinemia and a hypergammaglobulinemia may be present.[1,8,31] If the fungal organisms have invaded the kidneys or liver, associated changes may be observed on plasma biochemistry such as elevations in uric

acid, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), or bile acids.[1,31] Plasma protein electrophoresis may reveal an elevated β-globulin concentration during the acute stage of infection and an elevation in β-globulin fraction, γ-globulin fraction, or both during the chronic stage of infection.[31,34] A decreased albumin concentration and a decreased A/G ratio may also be seen in birds with aspergillosis.[31] However, affected birds may have normal electrophoretograms.[34,35] Differential diagnoses for birds with a leukocytosis characterized by a heterophilia and monocytosis, and a hypergammaglobulinemia would include fungal disease, mycobacteriosis, *Chlamydophila psittaci*, and leukemic disease.

Radiographic findings in birds with aspergillosis include bronchopneumonia, often with the presence of "ring shadows" in the hilum and midportion of the lungs.[7] These ring shadows are a result of infiltration of the parabronchial walls with heterophilic, histiocytic, or fungal infiltrates. Discrete nodules may also be seen in the lungs in cases of aspergillosis. Nodular air sacculitis, predominately in the abdominal or caudal thoracic air sacs is also common and generally represents advanced disease.[36,37] Other radiographic findings may include air sac hyperinflation, aerophagia, and poor coelomic detail.[7] Again, these radiologic findings are not specific for aspergillosis and other disease processes must be considered. Differential diagnoses for infiltrates in the lungs and nodular air sacculitis include bacterial pneumonia with air sacculitis, *Chlamydophila psittaci*, although this is unlikely to cause granulomatous lesions, mycobacteriosis, hemorrhage, and neoplasia. Types of neoplasia reported to affect the air sacs and lungs include air sac carcinoma, pulmonary carcinoma, leiomyosarcoma or any metastatic neoplasia.[38] The presence of radiographic changes in birds with aspergillosis is considered a poor prognostic sign.[31]

There is no specific blood test available to definitively diagnose aspergillosis in birds. There are *Aspergillus* ELISA tests available to test for the presence of antibodies or antigen in serum.[21,39,b-e] The indirect ELISA test is a standard test for identifying the presence of antibodies in a serum sample. This ELISA test at the Raptor Center at the University of Minnesota has been shown to detect antibodies to *Aspergillus* spp in a wide range of avian species, such as raptors, penguins, waterfowl, and psittacines, including pionus parrots.[b] However, the presence of antibodies in a bird does not indicate disease, but merely exposure to antigen and the ability to produce antibodies. The absence of antibodies may occur even in the presence of disease.[31,35] This may occur early in the course of disease before the bird has made antibodies, if the bird is immune compromised and unable to mount an antibody response, or if the *Aspergillus* spp infection is in a location that induces limited antigenic stimulation.[31,b] Therefore, both false positives and false negatives may occur with ELISA antibody tests. A failure to produce antibodies suggests severe disease and the presence of immune suppression, either due to another cause or to aspergillosis, and carries a poor prognosis for the patient.[b] There is also an ELISA antigen test available for the detection of galactomannan, which is a major cell wall component of *Aspergillus* spp.[21] However, this test was found to have very poor sensitivity in the detection of aspergillosis in falcons, and no other avian species have been evaluated to date.[21] The sensitivity of these blood tests may be moderately improved by performing electrophoresis, antibody ELISA and galactomannan antigen ELISA in conjunction, but both false positives and negatives still occur.[f] Therefore, serology is generally not a useful test in confirming or ruling out a diagnosis of aspergillosis. However, in some cases it is useful to perform *Aspergillus* spp ELISA tests to aid in treating patients with aspergillosis. When birds test positive on serology and are confirmed to have aspergillosis by other diagnostic methods, serology may be a useful

tool for monitoring progress of treatment.[31,c,e] Antibody titers should be interpreted with caution, however, because the level of the titer does not always correspond with the severity of clinical disease.[31,40]

Because non-invasive tests do not provide a definitive diagnosis of aspergillosis, more invasive tests are necessary. Samples of lesions must be obtained and submitted for cytology, culture and histopathology. If there are granulomas present in the trachea a tracheal swab, tracheal wash or tracheal endoscopy may be performed and the sample may be submitted for cytology and bacterial and fungal culture. The presence of *Aspergillus* organisms in the absence of a heterophilic or granulomatous reaction is not diagnostic for the disease because of the ubiquitous nature of the organism. Special stains such as Grocott's methanamine silver (GMS) may be needed to identify the presence of fungal hyphae on cytology or histopathology. When lesions are present in the air sacs and lungs, coelomic endoscopy should be performed. Biopsies should be taken of fungal plaques and submitted for cytology, culture, and histopathology. Histopathology provides the best opportunity for definitive diagnosis of aspergillosis, since both the fungal organisms and the associated granulomatous inflammation can be observed, thus confirming disease. Aspergillosis can be diagnosed histopathologically based on the presence of inflammatory lesions containing GMS positive fungal organisms with septate hyphae that are 3-5μm across and have parallel walls with dichotomous branching.[1,41] Conidia and conidiophores observed in the lesions may help identify the species of *Aspergillus* involved, but there is a large amount of variation in conidial morphology both within and between species making identification difficult.[42] The inflammatory lesions of aspergillosis are generally granulomatous, consisting of a central casseous mass with radiating fungal hyphae bordered by multinucleated giant cells, epithelioid cells, lymphocytes, and fibroblasts.[11] Acute lesions of aspergillosis may

contain masses of fungal hyphae and necrotic debris with little or no associated inflammatory response.[11]

Treatment of Aspergillosis generally involves administration of antifungal medications either locally or systemically. In addition, adjunctive therapy such as surgical or endoscopic debulking of granulomas may be performed. There are two main classes of antifungal drugs currently employed in the treatment of avian aspergillosis, the polyenes, such as amphotericin B[g], and the azoles, including fluconazole[h], enilconazole[i], itraconazole[j], and voriconazolek. An additional class of drugs, the echinocandins, is now being used for refractory invasive aspergillosis in humans, but to the author's knowledge has not yet been evaluated in avian species.

Amphotericin B is an amphoteric polyene macrolide.[43] Amphotericin B has poor absorption across the GI mucosa, and thus must be administered intravenously or topically via nebulization or local application to a lesion. Its mechanism of action involves intercalation of the drug into fungal membranes to form a barrel pore.[43] Formation of pores in the cell membranes disrupts ion gradients and osmotic balance, and leads to lysis and death of the cell. Amphotericin B has a greater affinity for ergosterol, the main sterol found in fungal cell membranes, than it does for cholesterol, the main sterol found in mammalian and avian cell membranes, and thus preferentially affects fungal cells.[43] There are additional biologic effects of amphotericin B on fungal cells, including lipid peroxidation, inhibition of membrane enzymes, and blockade of endocytosis.[43] Amphotericin is nephrotoxic and animals receiving it must be diuresed during therapy.[44-46] The nephrotoxicity has been well documented in dogs and humans, however, there is a question of whether nephrotoxicity occurs in avian species.[45,46] In one study there was no evidence of nephrotoxicity in birds following administration of amphotericin B

once daily for 3 days based on body weight, food intake, consumption of water, excretion of weight and gross examination of the kidneys at post-mortem examination.[47] However, this study did not examine the kidneys histopathologically to rule out microscopic damage. Therefore, it is still generally recommended that birds be diuresed during administration of amphotericin B. Because of the need for diuresis and intravenous administration, animals usually must be hospitalized for treatment with amphotericin B.

In addition to intravenous administration, amphotericin B may be administered locally to the site of infection. This includes administration intratracheally, via nebulization, topically to infected wounds, or topically to granulomas via coelomic endoscpy.[5,8,48,l,m] Amphotericin B is not well absorbed systemically via any of these routes of administration, therefore the risk of nephrotoxicity is minimal. The local methods of application also allow for high levels of drug at the site of infection, which is especially advantageous in areas of poor perfusion such as the air sacs and trachea. However, caution should be used in applying amphotericin B topically because there is a report of a severe, fatal granulomatous reaction to a sinus flush with amphotericin B in an African gray parrot.[49]

In an effort to reduce the nephrotoxicity of amphotericin B, newer formulations of the drug, in which the drug is encapsulated in liposomes or complexed with lipid carriers, have been developed. These formulations are referred to as amphotericin B lipid formulations. These formulations greatly reduce the nephrotoxicity of the amphotericin B.[43] The three formulations of this drug that are available are a lipid complex[n], a unilamellar liposome[o], and a colloidal dispersion[p]. The lipid formulations of amphotericin B work to reduce toxicity by two mechanisms. The first mechanism of reduced toxicity is decreased uptake of amphotericin B to mammalian cell membranes via modulation of the rate of transfer from the lipid carrier to the

cholesterol-containing membranes.[43,50] Because the rate of drug transfer is reduced, the concentration of drug at the fungal cell wall is decreased; therefore, higher drug dosages of lipid formulations of amphotericin B than with traditional formulations must generally be used.[43] For example, in dogs the recommended dosage of amphotericin B desoxycholate, the traditional form of the drug, is 0.5mg/kg, while the dosage of the lipid formulations is 1-2.5mg/kg.[51] The second mechanism of reduced toxicity is a modulation of the peak plasma concentration of amphotericin B. This occurs because the lipid complex and the colloid dispersion (but not the liposomal formulation) are rapidly engulfed by the mononuclear phagocyte system.[43,50] The lipid forms of amphotericin B may be internalized by macrophages and carried to the site of infection, thus acting as sustained release forms of the drug.[43] This aspect of the lipid formulations of amphotericin B allows for the potential of accumulation of the drug in the fungal granuloma and may provide increased efficacy in addition to the reduced toxicity. However, at this time there is no pharmacokinetic data available for the lipid formulation of amphotericin B in birds. There is one report of topical use of liposomal amphotericin B in the form of the unilamellar liposome formulation for wound aspergillosis in a heron.[52] The wound aspergillosis completely resolved following treatment in this bird. Liposomal amphotericin B has also been investigated for use via nebulization in laboratory mice. In one study the unilamellar liposome formulation of amphotericin B was found to reach lung concentrations that were 8 times higher than concentrations reached with the traditional formulation of amphotericin B.[53] Based on these findings, lipid formulations of amphotericin B may be useful in systemic or local application in avian species. Additional research is needed in the use of liposomal formulations of amphotericin B in avian species to determine effective dosages and dosing intervals.

Another class of drugs that is important in treatment of aspergillosis is the azoles. Members of this group of antifungals are synthetic compounds that contain one or more five-membered azole rings.[54] The azoles are generally fungistatic. The mechanism of action for this class of drugs involves inhibition of cytochrome P450-dependent ergosterol synthesis.[54] The nitrogen atom in the azole ring binds to the heme moiety of the enzyme 14α demethylase.[55] 14α demethylase is the enzyme responsible for conversion of lanosterol to ergosterol, which is the primary sterol in fungal cell membranes.[55] Binding of nitrogen atoms to the enzyme leads to an accumulation of ergosterol precursors in the cell membrane, which leads to membrane disorganization and inhibition of cell growth.[55] It is also believed that azoles inhibit cytochrome C oxidative and peroxidative enzymes.[50]

There are two main categories of azoles, the imidazoles and the triazoles. Imidazoles, which include ketoconazole[q], clotrimazole[r], and miconazole[s], contain two nitrogen atoms in their five-membered rings. These drugs are less useful for treatment of systemic aspergillosis, although they have a role as adjunctive therapy or for the treatment of local aspergillosis infections. Many *Aspergillus* species are resistant to ketoconazole, and there are concerns about hepatotoxicity with systemic administration of this drug, therefore it has limited use in the treatment of aspergillosis.[55] Clotrimazole is only appropriate for topical use because of its poor absorption from the GI tract and because it induces of hepatic microsomal enzymes, which increases metabolism of the drug and decreases the antifungal activity.[55] Clotrimazole has been used for nebulization in raptors with aspergillosis, but there are concerns that this may lead to toxicity if birds absorb the drug systemically.[55,t] Miconazole is available as IV or topical formulations, but there are adverse effects associated with the IV formulation because of the

carriers with which it is formulated.[55] The topical formultion of miconazole has been used adjunctively topically, intratracheally or via nebulization for the treatment of aspergillosis.[55,56]

The second category of azoles is the triazoles, which have three nitrogen atoms in their five-membered rings. Drugs in this group include fluconazole, enilconazole, itraconazole, and voriconazole. Fluconazole is most active against yeast including *Candida albicans*. Fluconazole is available for both oral and intravenous administration. It has a high bioavailability following oral administration and has good penetration throughout the body.[57] It has poor in vitro activity against filamentous fungi such as *Aspergillus* spp, but clinical improvement has been observed following treatment with fluconazole in some mammals experimentally infected with *Aspergillus* spp.[57-59] Single dose pharmacokinetics have been described for fluconazole in African grey parrots.[57] Reported adverse effects of fluconazole are uncommon.

Enilconazole is available for use in the United States as a poultry house aerosol disinfectant. Enilconazole is not commonly used for treatment of aspergillosis in the United States despite its efficacy against *Aspergillus* spp due to its limited availability and the fact that it must be used topically or as a nebulization agent, with the safety in those methods of administration unknown. It is stable in the environment and active in the vapor phase, therefore it is useful for treating contamination of poultry houses with *Aspergillus* spp spores.[60] This fumigant form of eniconazole has been used to treat respiratory aspergillosis in avian species. Enilconazole fumigation of day old chicks has been evaluated for safety and efficacy in the treatment of *Aspergillus fumigatus* infections.[60] No adverse effects associated with the fumigation of the birds were reported and the treatment was found to decrease morbidity and mortality in the chicks.[60] Enilconazole has been recommended as a treatment for aspergillosis in wildlife and companion birds, either as a nebulization agent or as a topical solution.[u]

Itraconazole is commonly used to treat systemic aspergillosis in avian species. It is effective in vitro against *Aspergillus fumigatus* and other *Aspergillus* species. It is fungistatic to yeasts but fungicidal to *Aspergillus* species, particularly if high plasma concentrations are reached.[31] In humans itraconazole has been used in patients who are not tolerant to amphotericin B, and relative to amphotericin B there is much less toxicity.[55] Itraconazole is lipophilic and therefore poorly soluble in aqueous solutions. However, it is ionized at low pH, which enhances absorption.[50] To facilitate absorption itraconazole can be acidified with hydrochloric acid or orange juice prior to administration.[61] Itraconazole is commercially available as a liquid in which the drug is bound to hydroxypropyl-β-cyclodextrin, which enhances the absorption of lipophilic drugs by encapsulating the drug to form inclusion complexes.[v] Oral pharmacokinetics of itraconazole have been described in pigeons, mallard ducks, blue-fronted Amazon parrots, and red-tailed hawks.[61-63] Dosage recommendations range from 5-10mg/kg once daily, although it has been suggested that higher dosages may be required to reach therapeutic concentrations in tissues that are poorly perfused.[62] The most significant adverse effect of itraconazole in mammals is hepatotoxicity, however, there are few reports of toxicity in birds.[64] The exception to this appears to be African grey parrots, which have been reported to be sensitive to itraconazole, exhibiting depression, inappetance, hepatotoxicity and death following treatment with itraconazole.[55,l] The most common adverse effect in all avian patients is inappetance, with eagles particularly susceptible to this effect.

Voriconazole is an extended spectrum, second generation triazole that has shown promise for the treatment of aspergillosis in humans.[65] In one study directly comparing voriconazole to amphotericin B in humans with invasive aspergillosis, treatment with voriconazole was reported to improve survival and result in fewer severe adverse effects.[66] Voriconazole is structurally

similar to fluconazole and has the same mechanism of action as the other azoles. It is fungicidal for *Aspergillus* spp but fungistatic for yeasts.[65] In one study evaluating *Aspergillus* spp isolated from the air sac of falcons, 95-100% of the species tested were susceptible to voriconazole.[67] Voriconazole is not dependant on gastric acid for absorption and has a high bioavailability in humans.[65] Adverse effects are similar to other azoles, although in humans visual disturbance is also reported.[65] It is unclear whether this occurs in avian patients. Recently, investigators have begun to perform pharmacokinetic studies of voriconazole in various species. Dosage recommendations have been made for falcons and African grey parrots.[68,l] However, due to certain unique pharmacokinetic properties of voriconazole these dosages cannot be applied across species. There are species differences due to the saturable, non-linear pharmacokinetics of the drug.[69,l] Kinetics are linear until the enzyme system in the liver is saturated, at which point the plasma concentrations of the drug may be prolonged.[l] In addition, voriconazole induces its own metabolism enzymes after repeated dosing in some species but not in others.[l] Therefore single dose studies may not accurately predict multiple dose behavior of the drug. Because of the paucity of information available, dosing of voriconazole across species is often based on anecdotal reports. There are few reports of clinical use of voriconazole at this time, including one case of administration to a cockatiel with aspergillosis, but no adverse effects have been reported.[w] Additional investigations with voriconazole are needed before it will have wide application in avian species.

The echinocandins, including caspofungin[x], are another group of antifungal drugs. The echinocandins are fungicidal against yeast and fungistatic against molds such as *Aspergillus* spp.[70] The mechanism of action involves inhibition of synthesis of β1,3 glucan, which is a major component of fungal cell walls.[50,70] The instability of the cell walls leads to osmotic fragility of

the cells resulting in lysis, especially at the tips of the emerging hyphae.[50] Caspofungin has been demonstrated to be effective against *Candida* spp and *Aspergillus* spp, including strains resistant to other antifungal drugs. Caspofungin is only available as an IV infusion, which will limit its use in avian patients. Adverse effects are uncommon and include elevation of hepatic enzymes and hepatitis in patients receiving concomitant medications.[71] Caspofungin is currently used in immunocompromised human patients with invasive aspergillosis that is refractory to traditional therapy with amphotericin B. This drug holds promise for use in avian species and investigations into safety and efficacy are warranted.

In addition to systemic treatment of aspergillosis with antifungal medications, endoscopic debridement of granulomas has been reported. In one report granulomas were debrided using forceps and scissors followed by endosurgical ablation of the lesions.[48] This was combined with topical application of amphotericin B in confirmed aspergillosis cases. Improvement was seen in two out of four aspergillosis cases, while one bird died during the procedure and one bird died four days following the procedure.[48] Another option for treatment of severe aspergillosis granulomas involves spraying the granulomas topically with amphotericin B endoscopically for several procedures in order to kill the fungal organisms and allow the granuloma to become smaller in size.[72] The granuloma may then be debulked or removed using endosurgery. The advantage of this technique is that the fungal organisms are killed prior to removal of the granuloma, therefore minimizing the risk of rupture of the granuloma and spread of fungal spores throughout the respiratory tract. In addition, because birds may be unable to resolve granulomatous inflammation in the air sacs due to the poor perfusion in that area, endoscopically removing the granulomas may speed the healing process. Disadvantages of this technique

include the risk of multiple anesthetic procedures, high cost, and advanced training and equipment required for endosurgical procedures.[72]

In addition to specific treatment for *Aspergillus* spp infections, supportive care is an important component of treatment of aspergillosis. Patients that are presented in severe respiratory distress should be handled minimally and provided with supplemental oxygen. Fluid therapy using an isotonic crystalloid solution administered intravenously, intraosseously, or subcutaneously should be administered in birds that are clinically dehydrated. Birds with a poor appetite should be gavage fed a high quality gavage feeding formula to maintain a good plane of nutrition.

An important aspect of supportive care for avian patients with respiratory disease is nebulization. Nebulization with saline alone is also beneficial as supportive care to help hydrate the respiratory epithelium, soften necrotic debris, and improve the efficiency of the mucociliary blanket.[73] Nebulization with medications such as antifungals and antibiotics allows for topical application of medication to lesions in the airways. If medications are administered via nebulization the particle size must be 3 micrometers in diameter or smaller to reach the lower airways.[74]

Antibiotics that are broad spectrum, including activity against gram negatives that commonly affect the respiratory tract such as *Escherichia coli* and *Klebsiella pneumoniae* may also be indicated in some birds with aspergillosis.[73] Birds with aspergillosis may develop secondary bacterial infections due to immune suppression or may develop aspiration pneumonia secondary to debilitation and frequent administration of oral medications or gavage feeding. Enrofloxican[y] is one example of a broad spectrum antibiotic with good activity against gram negative aerobic organisms. Enrofloxacin is a fluoroquinolone antibiotic that works by

inhibiting two enzymes, topoisomerase II (DNA gyrase) and topoisomerase IV.[51] DNA gyrase is responsible for producing negative supercoils in the DNA double helix to allow the DNA to become condensed inside the cell.[75] By inhibiting DNA gyrase, fluoroquinolones interrupt the supercoiling process and lead to irreversible DNA damage and cell death.[75] The method by which inhibition of topoisomerase IV leads to bacterial cell death is not understood. Enrofloxacin is a concentration dependent antibiotic with a post-antibiotic effect. A single high dose of antibiotic will continue to be effective against bacteria for a period of 24 hours.

One of the important differential diagnoses for aspergillosis in avian patients is mycobacteriosis. Mycobacteriosis refers to disease caused by the *Mycobacterium avium* complex of organisms, which includes *Mycobacterium avium* subspecies *avium* and *Mycobacterium genevense*. *Mycobacterium* spp most commonly affect the gastrointestinal tract and liver in birds.[76] However, granulomatous lesions in the air sacs and lungs have been reported in a blue-headed pionus parrot with mycobacteriosis.[77] Organisms are usually shed in the feces of affected birds, although birds with respiratory mycobacteriosis may shed organisms in respiratory secrections.[76] Clinical signs of mycobacteriosis include weight loss, poor feathering, polyurea, diarrhea, abdominal distention and less commonly respiratory compromise.[76] Respiratory distress in birds may be caused by mycobacteriosis if mycobacterial granulomas in the respiratory tract or in the GI tract compress the upper air ways or air sacs.[37,78] The hematologic changes observed in mycobacteriosis are similar to the changes that occur with aspergillosis. These include moderate to marked leukocytosis characterized by heterophilia and monocytosis, reactive lymphocytosis, and anemia.[76] Pionus parrots have been reported to be sensitive to development of mycobacteriosis.[76,77,79]

Another differential diagnosis for aspergillosis is primary or metastatic neoplasia. Neoplastic disease can produce nodular lesions in the air sacs or lungs that could resemble *Aspergillus* spp granulomas. Neoplasia could also lead to weight loss and a heterophilia similar to aspergillosis.[8] Types of neoplasia that have been reported in the air sacs of birds include air sac carcinoma, pulmonary carcinoma, fibrosarcoma, leiomyosarcoma or metastatic neoplasms.[38] Fibrosarcoma, a common tumor in pet birds, is a neoplasm arising from fibrous connective tissue. Fibrosarcomas are locally invasive, have a high rate of recurrence, and rarely metastasize.[38] Treatment options for fibrosarcomas include surgical removal, radiation therapy and intralesional cisplatin.[38,80,81] Synovial cell sarcoma is an uncommon tumor in pet birds. Synovial cell sarcomas usually involve a large soft tissue mass with gross destruction of the bone and joint.[38] Amputation has been reported to be successful in one case of synovial cell sarcoma, but the metastatic potential and prognosis for this type of neoplasm is unknown.[38]

Birds with advanced systemic aspergillosis or with aspergillosis lesions in the trachea may present to the clinician in respiratory distress. It is important for the practitioner to recognize clinical signs of tracheal disorders so that appropriate therapy such as placement of an air sac cannula can be instituted. Signs of tracheal disorders in birds may include open-mouth breathing, respiratory noises including a high pitched wheeze on inspiration, change in vocalization, and increased respiratory effort.[82] Differential diagnoses for these signs include tracheal foreign body (i.e. seed), tracheal trauma, fungal granuloma, bacterial granuloma, squamous metaplasia secondary to hypovitaminosis A, or tracheal stenosis. Tracheal stenosis has been reported in birds following tracheal trauma including anesthetic intubation, and generally occurs approximately 2-4 weeks following the traumatic incident.[z] Reported histopathologic changes in avian tracheal stenosis involve damage to the superficial layers of the

tracheal mucosa with proliferation of vascular granulation tissue, giant cell macrophage infiltration of tracheal mucosa and lamina propria, fibroplasia, fibrosis, heterophilic inflammation, and hyperplastic squamous epithelium.[83,z] Treatment options for tracheal stenosis include manual breakdown of tracheal membranes, topical or systemic corticosteroids, and tracheal resection and anastamosis. Diagnosis of tracheal lesions can be aided by radiography, but tracheal endoscopy provides the best method of visualization and diagnostic sampling of lesions.

Clinical Report

A 13-year old female Maximilian pionus parrot was presented for evaluation of weight loss of three weeks duration and cloudy appearance to the eyes. The bird was acquired by her current owner 6 years prior to presentation, with an unknown history prior to acquisition. The bird was housed indoors in a large powder coated parrot cage with another female pionus parrot. The other bird was not showing any clinical signs of illness. The bird's diet consisted of a mixture of commercial parrot pellets supplemented with fresh vegetables and fruits. Nuts and seeds were provided as occasional treats. A commercial avian vitamin supplement[aa] was placed on the fresh foods daily. The bird had a history of egg laying in the past but had not laid any eggs in the previous two years. The owner weighed the bird daily and had noted a weight loss from 239 grams to 222 grams over the prior three weeks. Her appetite and attitude were considered by the owner to be normal. According to the owner, the bird had been tested for polyomavirus, *Chlamydophila psittaci*, and Psittacine circovirus when she was acquired 6 years prior, but the records were not available for review. The bird had been seen by another veterinarian for weight loss one week prior to presentation. The owner reported that the local veterinarian performed a complete blood count (CBC) and plasma biochemistry panel at that

visit. However, the owner refused to provide the identity of the primary veterinarian, therefore medical records were not available for review. The owner stated that the CBC had revealed a leukocytosis, which prompted the veterinarian to start the bird on antibiotics. The bird was administered enrofloxacin at 2.5mg/kg PO q 12 h for 7 days, however, the weight loss continued. The bird was brought to the author's hospital for additional diagnostics.

On physical examination the bird was bright and alert and appeared hydrated based on skin turgor of the upper eyelid and of the skin over the keel and refill of the basilic vein. The parrot was in thin body condition with a body weight of 218 grams and moderate atrophy of the pectoral musculature. There was bilateral white opacity to the lens consistent with cataracts but the eyes appeared comfortable with no evidence of blepharospasm, ocular discharge, or photophobia. There was decreased range of motion associated with the left stifle but there was no crepitus palpated and the joint did not appear painful. A 1cm by 0.5cm firm soft tissue swelling was noted in the subcutaneous tissues dorsal and lateral to the tarsometatarsus of the right pelvic limb. The heart rate was 340 beats per minute and there were no arrhythmias or murmurs ausculted. The respiratory rate was 18 breaths per minute and respiratory auscultation of the lungs, air sacs, and sinuses was considered normal, with no wheezes or crackling sounds ausculted. Feces, urates and urine appeared within normal limits. No other abnormalities were noted on physical examination.

The problem list for this bird included weight loss despite a good appetite, bilateral cataracts, decreased range of motion in the left stifle and a soft tissue mass associated with the right pelvic limb. The important differential diagnoses for the weight loss included neoplasia, fungal disease (aspergillosis), bacterial disease (including *Chlamydophila psittaci*, *Mycobacterium avium*, bacterial pneumonia, or bacterial enteritis), viral disease (including

psittacid herpes virus, adenovirus, or paramyxovirus), intestinal parasites such as Giardia or Capillaria, proventricular dilatation disease, pain from musculoskeletal disease, and cataract related pain, inflammation or decreased vision. Differentials for the mass on the foot included neoplasia such as fibrosarcoma, lipoma, or articular gout. Given the non-specific signs and extensive differential list, a minimum database consisting of CBC, plasma chemistry, and whole body radiographs was performed. The bird was anesthetized for the radiographs. The bird was induced using isoflurane at 3% in 95-100% oxygen administered via a facemask. She was then intubated with a number 3.0 ID uncuffed endotracheal tube and placed on a Bain nonrebreathing circuit. Anesthesia was maintained at 2-3% isoflurane. While under anesthesia the heart rate was monitored constantly with a stethoscope and the spontaneous respiratory rate was constantly monitored. The heart rate, heart rhythm, and respiratory rate remained unchanged during the anesthetic procedure. The duration of anesthesia was approximately 30 minutes. Ventrodorsal and right lateral radiographs of the whole body, and dorsoventral and lateral radiographs of the pelvic extremities were performed under anesthesia (Figures1,2, and 3a,b). Positioning of the lateral radiograph was moderately rotated based on the lack of overlap of the coracoids and coxofermoral joints, therefore the radiograph should be interpreted with caution. VD radiographs revealed a soft tissue density in the right caudal lung lobe and a soft tissue density in the region of the right caudal thoracic or abdominal air sac. There was widening of the cardiohepatic silhouette on the left side of the coelom based on an imaginary line between the shoulders and the coxofemoral joints. This widening could represent organomegaly (left liver lobe, reproductive organs or proventriculus) or could represent displacement of coelomic contents by the space occupying lesion in the right coelom. Bony remodeling was evident in the left stifle joint, consistent with osteoarthritis, likely secondary to prior trauma. On the lateral

Figure 1. Ventrodorsal radiographic view of a 13-year-old female Maximilian pionus parrot (*Pionus maximiliani)* that was presented for weight loss despite a normal appetite and a lateral tarsometatarsal soft tissue swelling. Note the increased soft tissue density in the region of the right caudal thoracic and/or abdominal air sac and the caudal portion of the right lung. There is widening of the cardiohepatic silhouette in the left coelom in the region of the left liver lobe, salpinx/ovary and proventriculus. Bony remodeling is evident in the left stifle joint. There is a soft tissue mass lateral to the right tarsometatarsus. Mineral density consistent with grit is present in the ventriculus. R = right.

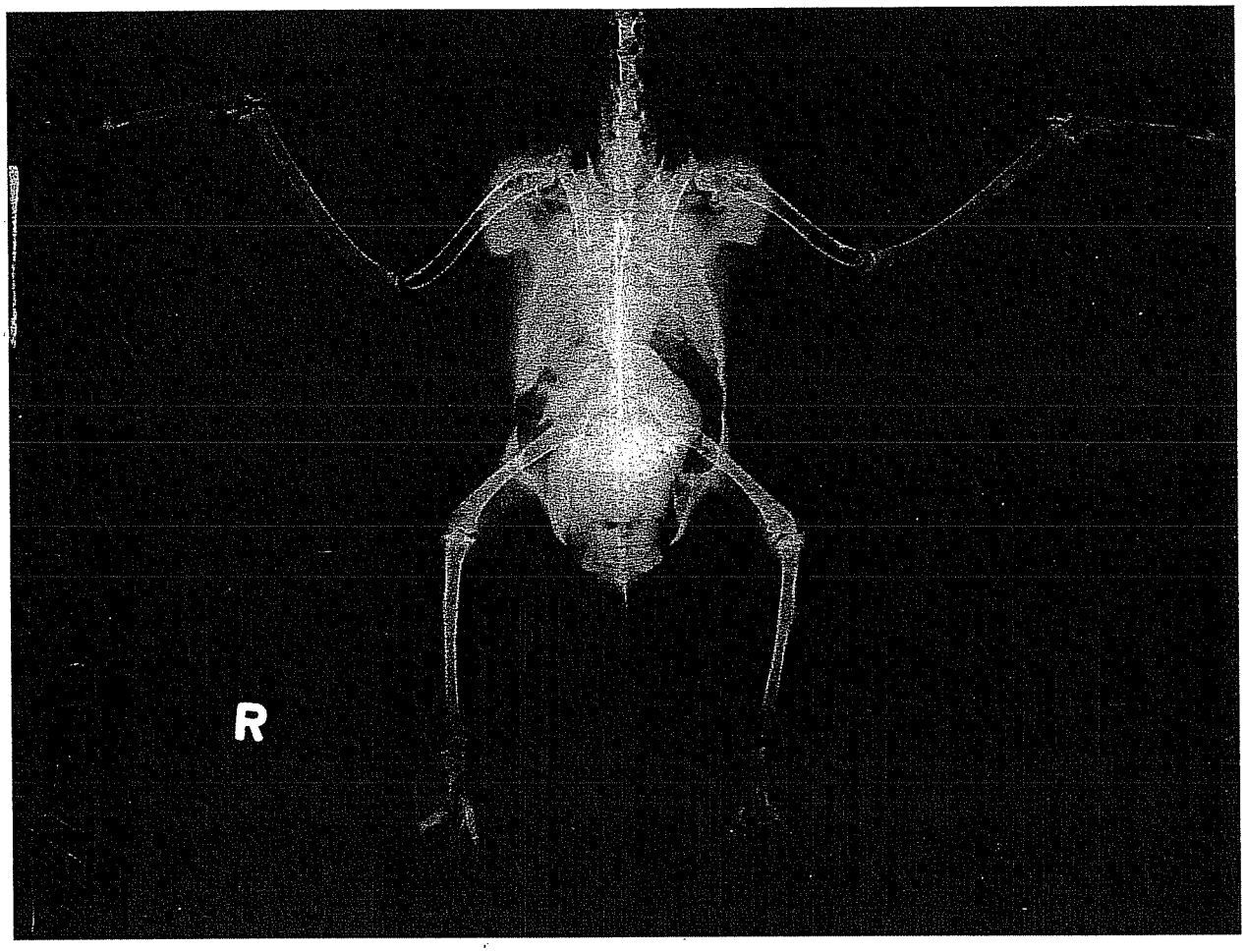


Figure 2 Right lateral radiographic view of a 13-year-old female Maximilian pionus parrot (*Pionus maximiliani)* that was presented for weight loss despite a normal appetite. The positioning of the bird is moderately rotated. Multiple soft tissue densities were identified in the region of the caudal thoracic and/or abdominal air sacs located dorsal to the proventriculus (green arrows). A linear mineral opacity was also noted ventral to the lung fields (white arrows). Mineral dense material consistent with grit is located in the vicinity of the ventriculus. The marker indicating that the bird is in right lateral recumbency was cropped from the image in order to increase the image size.

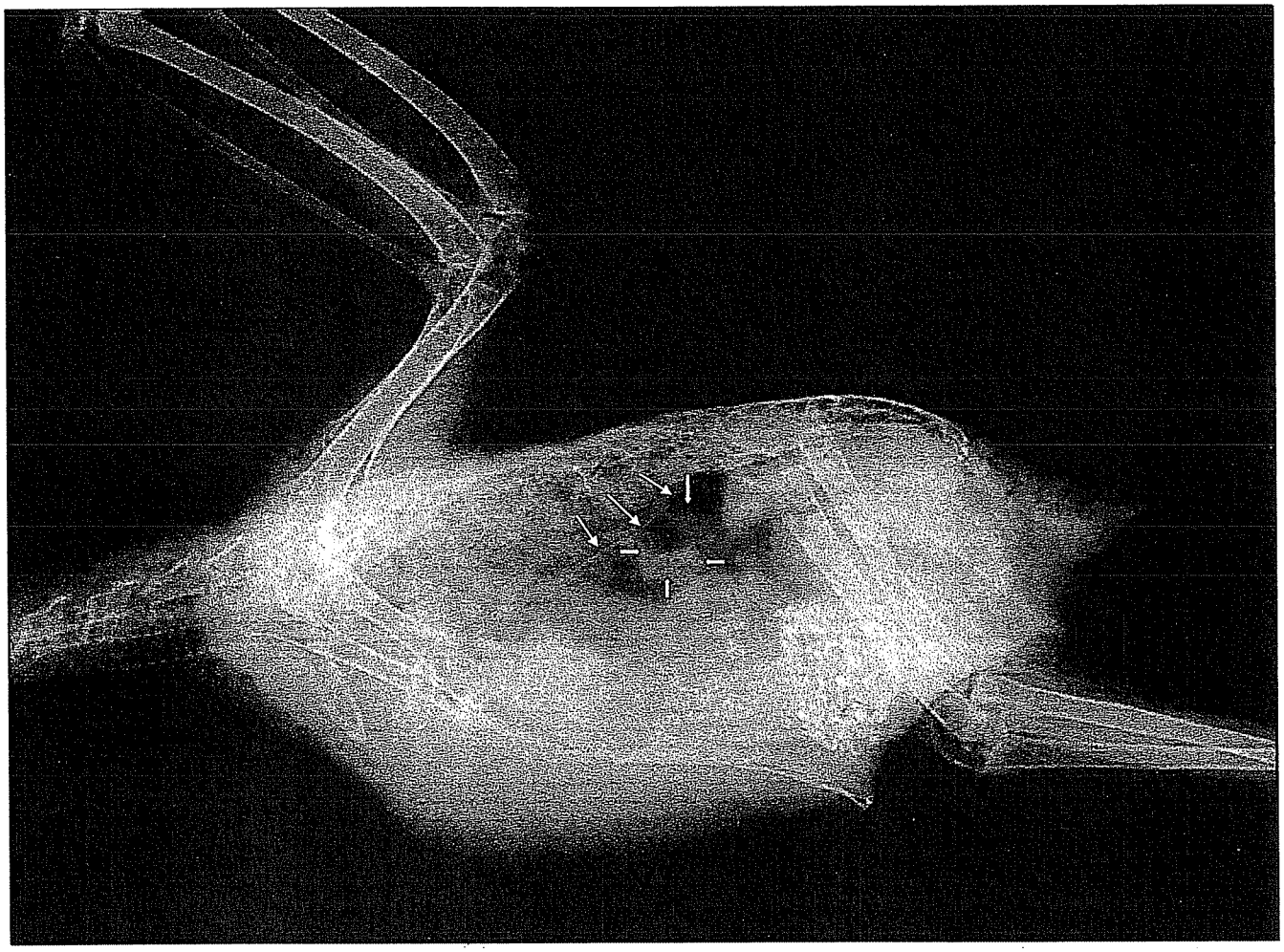
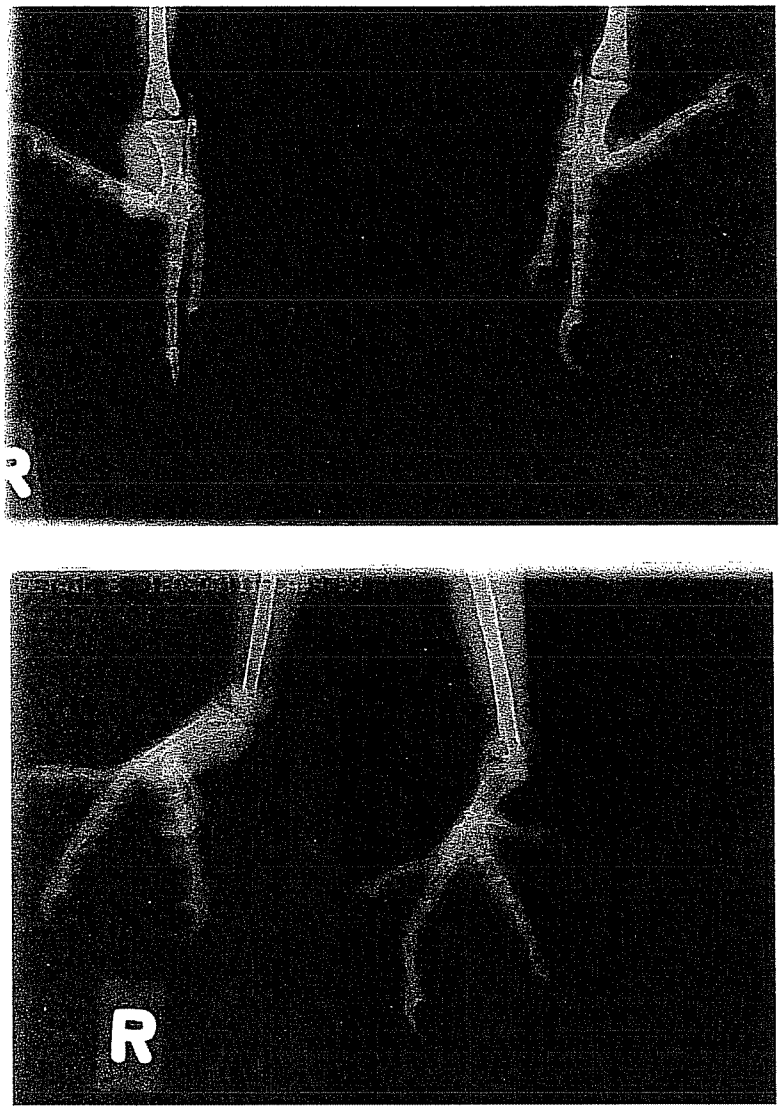


Figure 3. a. Dorsoventral (DV) and **b.** lateral radiographic views of the pelvic extremities of a Maximilian pionus parrot (*Pionus maximiliani)* that was presented for weight loss despite a good appetite and a soft tissue mass lateral to the right tarsometatarsus. There is soft tissue swelling associated with the right tarsometatarsus evident in both views, which appears lateral in the DV view and extends proximally to the intertarsal joint. No evidence of bony proliferation or lysis was identified in either view. R = right.



view multiple soft tissue densities could be seen in the air sac dorsal to the proventriculus. There was also a mineral dense line at the border of the lungs and the air sacs, which was thought to represent a thickened air sac. Another differential would be atherosclerosis or mineralization of a great vessel. On both views mineral dense material was present in the region of the ventriculus, consistent with grit. Radiographs of the pelvic extremities revealed a soft tissue swelling with no evidence of bony involvement on the right pelvic limb, located lateral and dorsal to the tarsometatarsus and the fourth digit (Figure 3a,b). Jugular venipuncture was performed for a CBC and plasma biochemistry (Table 1). CBC and chemistry were performed at an in-house laboratory with reference ranges based on published data.[84,85,c] Abnormalities on hematology included a moderate leukocytosis of 17,800/uL (5000-13,000) with a moderate heterophilia of 14,418/uL (2,750-9620), a mild monocytosis of 356/uL (0-130), and a severe eosinophilia of 1246/uL (0-130). The creatine kinase (CK) was elevated at 1223IU/L (116-408). All other values were within reported reference ranges. Serum was collected for Aspergillus antibody ELISA and submitted to the Raptor Center at the University of Minnesota (Table 1). The ELISA was negative (Optical density <0.121 negative). Fine needle aspirate of the mass on the right foot was collected using a 22 gauge needle and submitted to an in-house laboratory for cytological evaluation (Table 2). The cytology of the foot lesion was inconclusive due to low cellularity of the aspirate. A swab of the proximal trachea and the mucus located on the endotracheal tube was collected and submitted to an in-house laboratory for aerobic, anaerobic, and fungal culture and cytology (Table 2). No cytological abnormalities were observed and there was no growth on aerobic, anaerobic, or fungal culture. The leukocytosis, heterophilia, monocytosis, and eosinophilia were indicative of inflammation, likely chronic in nature due to the absence of band neutrophils, toxic change to the neutrophilia, or elevation in fibrinogen. The

Table 1. Hematology and plasma biochemistry results for a 13-year-old female Maximilian pionus (*Pionus maximiliani*) parrot with weight loss and nodular air sacculitis.

Parameter	Day 1	Day 22	Day 27	Reference Interval[84,85,c]
Hematology				
Hematocrit	47	49	46	45-54%
RBC	3.14	3.28	3.03	2.4-4.0 X 10^3/uL
WBC	17800	37500	9600	5000-13000/uL
Heterophils	14418	19125	7008	2750-9620/uL
Lymphocytes	1602	15750	1536	1000-9100/uL
Monocytes	356	1500	576	0-130/uL
Eosinophils	1246	750	480	0-130/uL
Basophils	0	0	0	0-130/uL
Thrombocytes	Adequate	Adequate	Adequate	Adequate
Fibrinogen	200	300	400	≤200 mg/dL
Clinical Pathologist's Comment	Slight anisocytosis	Slight anisocytosis; Many small lymphocytes. Difficult to differentiate lymphocytes from thrombocytes.	Slight anisocytosis	Slight anisocytosis considered normal
Plasma Chemistry				
Sodium	151	-	-	145-155 mmol/L
Potassium	2.5	-	-	3.5-4.6 mmol/L
Chloride	112	-	-	
Uric Acid	4.5	4.9	-	2-12 mg/dL
Calcium	9.7	9.4	-	8.2-10.4 mg/dL
Phosphorous	2.0	2.6	-	2.9-6.6 mg/dL
Glucose	285	299	-	212-368 mg/dL
Total Protein	3.8	4.1	-	2.3-4.3 g/dL
Albumin	1.2	1.3	-	1.1-2.0 g/dL
Globulin	2.6	2.8	-	1.1-2.7 g/dL
AST	233	641	-	135-358 IU/L
Creatine Kinase	1223	2934	-	116-408 IU/L
Urea Nitrogen	2	2	-	<2 mg/dL
Cholesterol	253	289	-	176-258 mg/dl
Glutamate dehydrogenase	1	7	-	<10 IU/L
Bile Acids	-	27	-	15-92 umol/L
***Aspergillus* spp antibody ELISA**	Negative	-	-	Optical density: <0.120 negative, 0.121-0.250 low positive, 0.251-0.500 medium positive, >0.51 high positive

Table 2. Microbiological and cytological test results for a 13-year-old female Maximilian pionus (*Pionus maximiliani*) parrot that was presented for weight loss and nodular air sacculitis.

Diagnostic Test	Day 1	Day 22	Day 27	Day 42 (Necropsy)
Gram's Stain	Small numbers gram (+) coccobacilli, small numbers large gram (+) rods, no spores			
Acid fast stain		Feces: No acid fast bacilli observed	Feces: No acid fast bacilli observed; Air sac: No acid fast bacilli observed	
Microbial culture	Tracheal swab culture: No growth of aerobic, anaerobic, or fungal organisms		Air sac culture: No growth of aerobic, anaerobic, or fungal organisms	Fungal culture of lung granuloma and air sac: No fungal growth
Aspiration cytology	Tracheal swab: No cytologic abnormalities; Foot mass: Inconclusive due to low cellularity		Impression smear of air sac granuloma: Likely granulomatous inflammation, possible (respiratory) epithelial hyperplasia; GMS stain reveals low numbers of hyphal elements, occasionally branching. Consistent with fungal infection.	

elevated eosinophil count was an unusual finding in this psittacine, but may have indicated inflammation due to intestinal parasites, dermatitis, fungal infection, or any other cause of inflammation. The elevated creatine kinase indicated muscle damage. This could have been a result of trauma during transport to the hospital, muscle damage during handling, or inflammation of muscle due to the granulomatous process observed in the coelom. The bird was administered lactated Ringers solution[bb] subcutaneously at a volume of 60ml/kg prior to recovery from anesthesia to help maintain perfusion and treat any sub-clinical dehydration that might be present. She recovered from anesthesia uneventfully. An ophthalmologic examination was performed and revealed bilateral immature cataracts in the anterior cortex occupying less than 30% of lens. There was no evidence of inflammation and a limited fundic examination was normal. The bird appeared visual. No treatment was indicated for the cataracts. Differential diagnoses for the development of cataracts included hereditary, nutritional (maternal Vitamin E deficiency), blunt or penetrating trauma, infectious disease (paramyxovirus, *Salmonella*, toxoplasmosis) or age related change.

The differential diagnoses for a bird with weight loss, moderate leukocytosis, and fungal pneumonia with nodular air sacculitis included aspergillosis, other fungal infection such as penicillium, metastatic neoplasia, mycobacteriosis, chlamydophilosis (although this was unlikely due to the nodular nature of the lesions on the radiographs), other bacterial pneumonia with granulomatous air sacculitis, walled off foreign body, or a mass associated with the liver. Aspergillosis was considered the most likely differential because of the radiographic appearance of the lesions, the leukogram indicating chronic inflammation, and the species predisposition. However, metastatic neoplasia was a likely possibility given the mass on the foot. Although mycobacteriosis more commonly affects the GI tract of birds, pionus parrots are considered to be

very susceptible to the disease and it has occasionally been reported to cause granulomas in the respiratory tract.[78] Therefore mycobacteriosis was also considered an important differential. While awaiting the results of the Aspergillus ELISA, empiric treatment was initiated with itraconazole at a dosage of 10mg/kg PO q 12 h until the next recheck in 3 weeks. The owner was informed that this dose was very high and that the bird may become anorexic while receiving the medication and was instructed to call for adjustment of medication should the bird become anorexic or regurgitate. Endoscopy was offered to the owner at the initial visit, but she elected to try empirical treatment and determine whether the nodules in the lungs improved with therapy. The enrofloxacin was discontinued at that time since there had been no improvement during the seven day course of therapy. One week after discharge the owner called to report ongoing weight loss and a slightly decreased appetite. This was suspected to be an adverse effect of the itraconazole rather than a progression of the disease process, therefore the dosage of itraconazole was decreased to 5mg/kg po q 12 h.

The bird returned for a recheck 22 days following initial presentation for a physical examination, blood work, and repeat radiographs. The owner reported that the bird was doing well at home and that her appetite appeared normal. Itraconazole at 5mg/kg po q 12 h had been continued for the 22 days between visits and the owner reported that the bird's appetite and weight had improved following the decreased dose. Physical exam revealed the bird to be bright, alert and hydrated based on eyelid turgor, skin turgor over the keel, and basilic vein refill. Her body weight had increased since the previous visit to 234 grams, an increase of 16 grams, although she appeared in slightly thin body condition with mild atrophy of the pectoral muscles. Respiratory rate was normal at 20 breaths per minute and no crackling sounds or wheezes were ausculted. The remainder of physical examination was unchanged from the previous visit.

Jugular venipuncture was utilized to collect blood, which was submitted to an in-house laboratory for a CBC, plasma biochemistry panel, and bile acids to evaluate liver function given that itraconazole has a rare potential for causing liver toxicity (Table 1). The hematocrit was normal but there was a marked leukocytosis of 37,500/uL (5000-13000) with a moderate heterophilia of 19125/uL (2750-9620), a moderate lymphocytosis of 15750/uL (1000-9100), and a marked monocytosis of 1500 (0-130). The clinical pathologist commented that the lymphocytes were small and difficult to distinguish from thrombocytes, therefore the lymphocyte percentage may have been an overestimate. The fibrinogen was mildly elevated at 300mg/dL (≤200). On the biochemistry panel there was a mild elevation of AST at 941IU/L (135-358) and a moderate elevation of creatine kinase at 2934IU/L (116-408). Bile acids were normal at 27umol/L (15-92umol/L). The globulins were elevated at 2.8g/dL (1.1-2.7) and the cholesterol was elevated at 289mg/dL (176-258). A Ziehl-Neelsen acid fast stain of the feces was performed in-house to assess for shedding of mycobacterial organisms (Table 2). No acid fast organisms were evident. The severe leukocytosis, characterized by a heterophilia, lymphocytosis, and monocytosis indicated ongoing and likely progressive inflammation. The fibrinogen, which is an acute phase protein, was also mildly elevated suggesting acute inflammation in addition to the chronic inflammation. The elevated globulins were also suggestive of inflammation and possibly an immune response to antigenic stimulation. The elevated CK indicated muscle damage, while the elevated AST could have been a result of either liver or muscle damage. However, the normal GLDH and bile acids made hepatic damage unlikely. The mild elevation in cholesterol was likely dietary in origin and was not considered a significant finding.

The bird was again anesthetized for repeat whole body radiographs. She was induced using isoflurane at 3% in 95-100% oxygen administered via a facemask. She was then

maintained on a facemask connected to a Bain nonrebreathing circuit with isoflurane at 2-3%. While under anesthesia the heart rate was monitored constantly with a stethoscope and the spontaneous respiratory rate was continuously monitored. The heart rate, heart rhythm, and respiratory rate remained unchanged during the anesthetic procedure. The duration of anesthesia was approximately 15 minutes. The ventrodorsal radiograph was mildly rotated to the left based on positioning of the keel and the spine, and therefore must be cautiously interpreted (Figure 4). The lateral radiograph was mildly rotated at the shoulders based on a lack of overlap of the coracoids (Figure 5). VD and right lateral whole body radiographs revealed that the there was persistence and likely progression of the soft tissue densities in the right caudal lung and caudal thoracic/abdominal air sac (Figures 4,5). The mass visible in the air sacs in the right coelom appeared larger and the air sacs appeared more radio-opaque than in the previous radiographs. The cardiohepatic silhouette was still mildly widened in the left coelom. The densities visible in the air sac on the lateral view were more numerous and additional densities could be seen overlying the lungs. The mineral dense line between the air sacs and lungs was still evident and appeared consistent with thickened air sacs. The mineral density in the ventriculus and the bony proliferation in the left stifle remained unchanged. Differentials for the mass in the air sac remained the same and a recommendation was made to perform endoscopy to obtain samples of the mass for definitive diagnosis. In addition, the mass on the foot would be biopsied while the bird was anesthetized for endoscopy. The bird was discharged with instructions to continue the itraconazole at the 5mg/kg PO q 12 h until returning for the diagnostic procedures.

The bird returned 5 days later (27 days following initial presentation) for coelomic endoscopy. Physical examination was unchanged from the previous visit and the bird's body weight was 226 grams, which was decreased by 8 grams from the previous visit. The owner

Figure 4. Ventrodorsal radiographic view of a 13-year-old female Maximilian pionus parrot (*Pionųs maximiliani)* that was presented for a recheck of nodular air sacculitis and pneumonia. The bird is rotated slightly to the left based on positioning of the keel and the spinal column. Note the soft tissue densities in the region of the right caudal thoracic and/or abdominal air sac and the caudal portion of the right lung. The air sacs appear more radio-opaque and the opacities appear larger than in the previous radiographs. There is persistent mild widening of the cardiohepatic silhouette in the region of the left liver, salpinx/ovary or proventriculus. Bony remodeling is evident in the left stifle joint. Mineral density consistent with grit is present in the ventriculus. R = right.

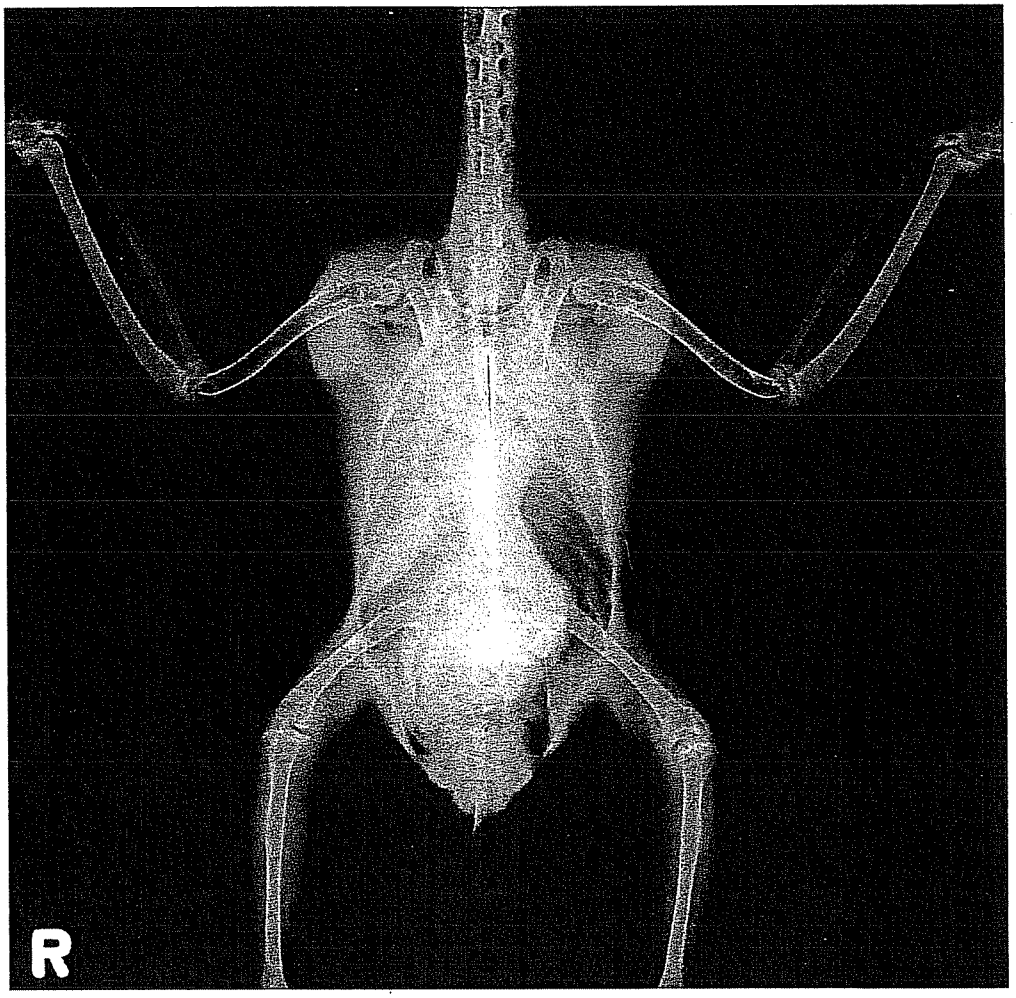
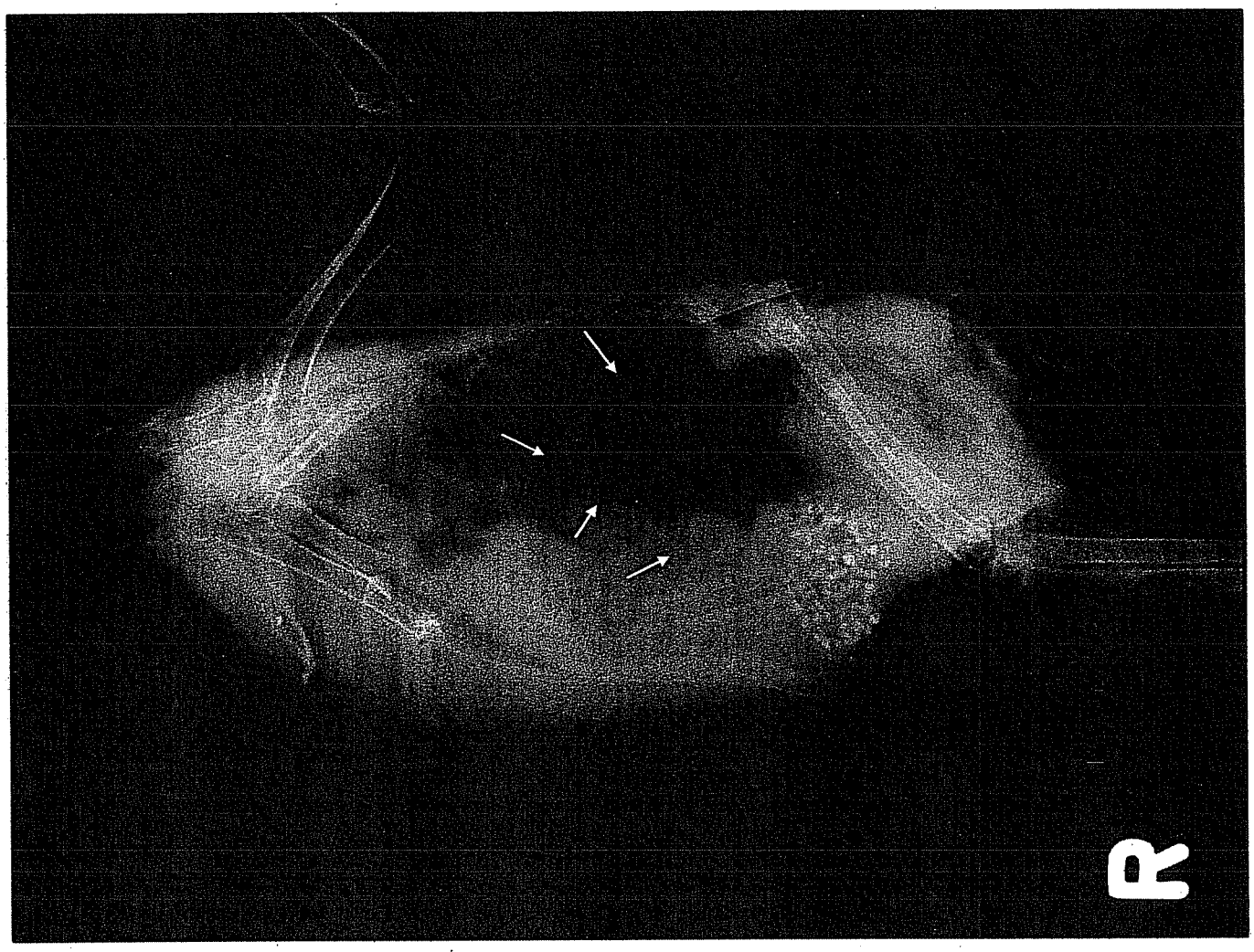


Figure 5. Right lateral radiographic view of a 13-year-old female Maximilian pionus parrot (*Pionus maximiliani)* that was presented for a recheck of nodular air sacculitis and pneumonia. The multiple, coelomic soft tissue densities in the region of the caudal thoracic and/or abdominal air sac dorsal to the proventriculus appear to have increased in size and opacity and now opacities can also be identified over the lung parenchyma and cranial air sacs. The linear mineral opacity extending ventral to the lung fields on the previous films is still evident, and the opacity can be followed cranially and ventrally, suggesting most likely a cranial thoracic or caudal thoracic air sac opacity. Mineral dense material consistent with grit is located in the region of the ventriculus. **R** = right.



reported that the appetite had appeared normal at home in the interim, therefore the weight loss was presumed to be due to variation in time of day and food consumption prior to the appointment, although it may have been secondary to the illness. Because there were several abnormalities on the CBC performed 5 days prior, blood was collected from the right jugular vein for CBC and performed at an in-house lab (Table 1). The leukocytosis had decreased to 9600 (5000-13000) although a mild monocytosis of 576/uL (0-130) was still present. There was also an ongoing mild hyperfibrinogenemia of 400mg/dL (0-200). Plasma biochemistry was not repeated at this visit due to the short interval of time since the prior blood work was performed and the expectation that the values would be unchanged. The decrease in the leukocyte count suggested that the inflammation was resolving, although there was still evidence of acute on chronic inflammation with the elevated fibrinogen and monocyte count. It is possible that the underlying disease process was causing immune suppression therefore the bird did not have a good ability to react with an elevation of white cells.

The bird was premedicated with butorphanol[cc] (0.3mg/kg IM) and midazolam[dd] (0.3mg/kg IM) and then mask induced using isoflurane at 3%. She was intubated with a 3.0mm ID uncuffed endotracheal tube and maintained at 2-2.5% isoflurane throughout the procedure. A mechanical ventilator[ee] was used with the ventilation rate set at 10 breaths per minute. Gas humidification within the respiratory tract was maintained using a heat and moisture exchanger[ff] positioned on the endotracheal tube between the patient and the Bain circuit. Heart rate was monitored with a Doppler unit placed over the dorsal pedal artery. A warm air underbody heater[gg] was used to maintain body heat throughout the procedure. Lactated Ringer's solution was administered intravenously via a 24 gauge IV catheter in the right basilic vein at a rate of 10ml/kg/hr, which is a standard anesthetic fluid rate, throughout the procedure.

A decision was made to initially perform coelomic endoscopy using a left flank approach despite the fact that the radiographic lesions were evident on the right side of the body. The left sided approach allows superior visualization of many of the coelomic structures such as the reproductive tract, spleen, GI tract, kidney, air sacs and liver.[86] Depending on what was observed endoscopically and the condition of the patient under anesthesia, a right sided approach to endoscopy was a possibility to follow. The bird was placed in right lateral recumbency and the left leg was extended cranially in preparation for a standard left flank approach to the coelom. The feathers over the left flank area were plucked and the area was aseptically scrubbed in preparation for the procedure. The area was draped using a sterile fenestrated drape. The 8th rib, the flexor cruris medialis muscle, and the pubic bone were palpated to identify the landmarks for entry at the junction of the flexor cruris medialis muscle and the 8th rib. A 1cm incision was made in the appropriate location and the subcutaneous tissues were bluntly dissected with curved hemostats. The forceps were then inserted through the body wall and into the coelom. A 2.7mm rigid endoscope[hh] was inserted into the coelom, entering the caudal thoracic air sac. This location was confirmed because upon entry the proventriculus was observed ventral to the endoscope and the air sac separating the caudal thoracic and abdominal air sacs was visualized caudally. Upon entering the coelom, the air sacs were observed to be thickened and covered in white mucus, and several yellow lobulated masses consistent with granulomas were observed in the caudal thoracic and abdominal air sac (Figure 6). The spleen appeared enlarged with an enhanced reticular pattern. The remainder of the left sided coelomic contents (kidney, ovary, oviduct, adrenal, lung, GI tract and liver) appeared within normal limits. Biopsies were taken of the air sac lesions and of the spleen using 5 Fr double spoon flexible biopsy forceps with oval jaws and submitted to an in-house laboratory for histopathology (Table 3). Samples of the

Figure 6. Endoscopic image of the caudal thoracic air sac of a Maximilian pionus parrot (*Pionus maximiliani)* with weight loss, nodular air sacculitis, and pneumonia. A yellow-white lobulated mass at the center of the image is consistent with a granuloma. The air sac in the lower left of the image is thickened and covered with white mucus. The proventriculus is the white structure located to the bottom right of the image. A small amount of hemorrhage is present on the surface of the proventriculus. Air sac, granuloma, and spleen (not shown in this image) were biopsied.

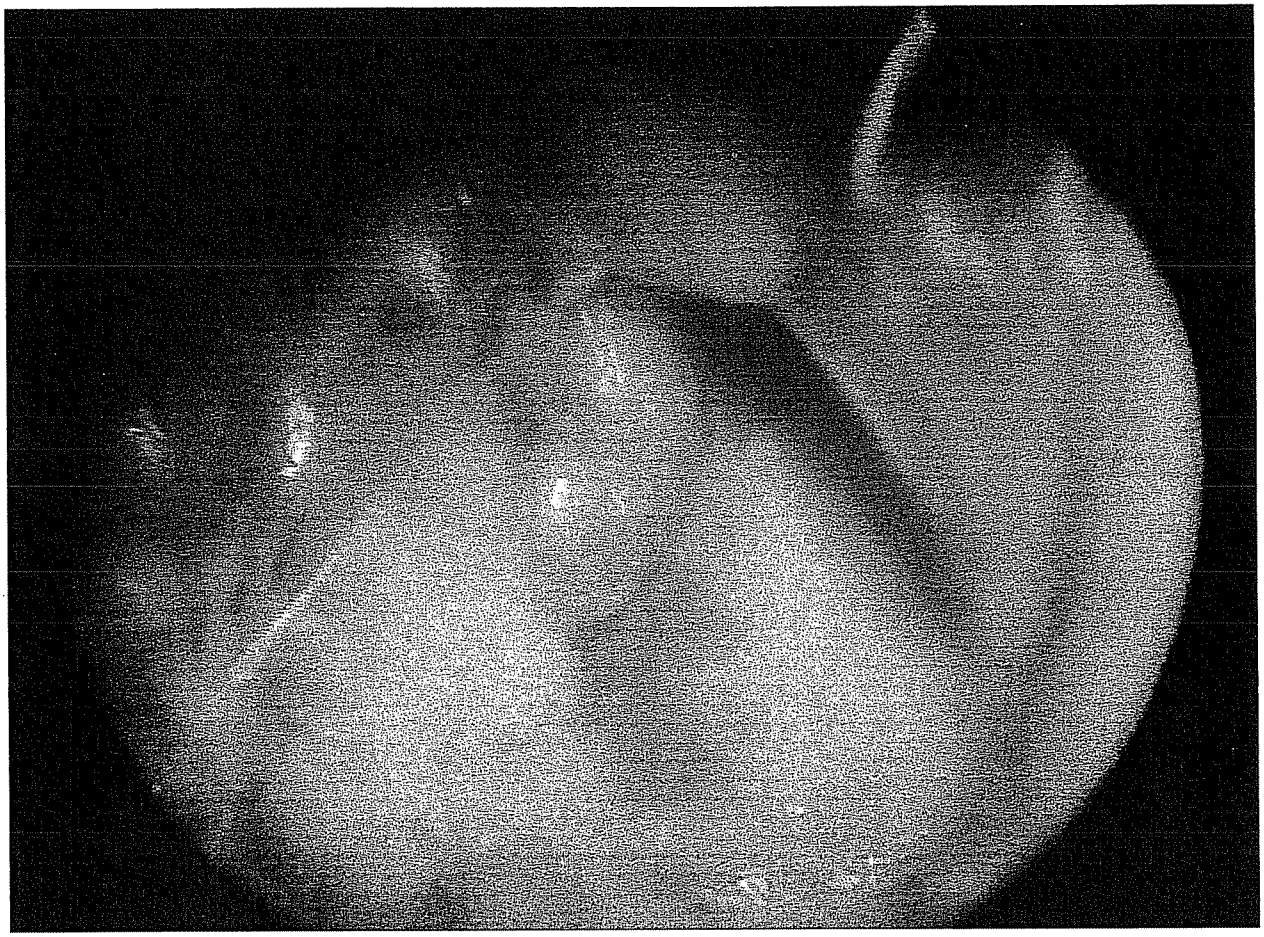


Table 3. Summary of histopathologic findings for a 13-year-old female Maximilian pionus (*Pionus maximiliani*) parrot with weight loss and nodular air sacculitis.

Date of sample collection	Tissue of origin	Histopathologic diagnosis
Day 27	Mass associated with right tarsometatarsus	Sarcoma
Day 27	Air sac	Mild multifocal chronic air sacculitis with fibrosis and intralesional hyaline material with embedded pigmented fungal hyphae, conidiophores and conidia (consistent with *Aspergillus* spp)
Day 27	Spleen	Marked multifocal arteriolar amyloidosis
Day 42 (Necropsy)	Right caudal lung, thoracic air sacs, abdominal air sacs	Extensive granuloma with fungal hyphae (consistent with *Aspergillus* spp)
Day 42 (Necropsy)	Coelom	Fibrinous coelomitis
Day 42 (Necropsy)	Trachea	(Distal) Squamous metaplasia and submucosal fibrosis with partial occlusion; (Middle): mild chronic heterophilic tracheitis with mucosal hyperplasia and multifocal squamous metaplasia
Day 42 (Necropsy)	Muscle in region of right tarsometatarsus	Mild multifocal myodegeneration (No evidence of remnant neoplasia)
Day 42 (Necropsy)	Liver	Mild multifocal heterophilic and lymphoplasmacytic portal hepatitis
Day 42 (Necropsy)	Spleen	Marked multifocal arteriolar amyloidosis
Day 42 (Necropsy)	Kidney	Marked multifocal arteriolar amyloidosis

air sac lesions were also aseptically removed and submitted to an in house lab for acid fast stain as well as for aerobic, anaerobic, and fungal culture (Table 2). Impression smears of the biopsies of the air sac lesions were also submitted in-house for cytology and special stains such as GMS (Table 2). There were no complications of the procedure such as excessive hemorrhage or anesthetic difficulty. The body wall and skin were closed in two layers using 4-0 poliglecaprone 25 suture[ii] in a simple interrupted pattern. Because the left sided approach to the coelomic endoscopy had yielded excellent samples that appeared representative of the disease process, a right sided coelomic endoscopy was not performed. During the same anesthetic episode the mass on the left pelvic limb was biopsied. A 1cm skin incision was made over the mass. The mass was bluntly dissected from the surrounding tissues and partially removed. The mass appeared firm and white-tan in color and extended both proximally toward the tarsometatarsus and caudally toward distal phalanges. It also appeared to possibly involve the intertarsal joint. An attempt was made to remove the entire mass but a distinction between mass and normal muscle and tendon could not be made. Therefore as much of the mass as possible was removed but wide surgical margins were not obtained. There was very little hemorrhage associated with the procedure. The skin was closed with 4-0 poliglecaprone 25 suture using a horizontal mattress pattern. The foot was lightly bandaged to prevent fecal contamination of the incision. The mass was placed in formalin and submitted to an in-house lab for histopathology (Table 3).

Cytology of the impression smears from the air sac lesions revealed granulomatous inflammation, and GMS stain revealed low numbers hyphal elements that were occasionally branching (Table 2). These findings were consistent with a fungal granuloma. Histopathology of the air sac revealed chronic air sacculitis with fibrosis and intralesional pigmented fungal

hyphae, conidiophores, and conidia (Table 3). The fungal hyphae were golden brown and thick walled with 75um wide conidiophores and apical conidia-bearing structures radiating from the terminal vesicle. This morphology was consistent with *Aspergillus* spp, potentially *A. fumigatus,* although the golden brown pigmentation to the hyphae may be seen with *A. niger.*[42] There was no evidence of neoplasia in the air sac lesions. Acid fast stains of the air sac granuloma were negative and there was no growth on aerobic, anaerobic, or fungal culture (Table 2). The histopathology of the spleen indicated marked multifocal arteriolar amyloidosis (Table 3). The most common cause of amyloidosis in avian species is chronic inflammation. Other differential diagnoses for amyloidosis include hereditary, auto-immune, or neoplasia (multiple myeloma). Given the bird's history of chronic inflammatory changes on bloodwork and the lack of evidence of neoplasia, chronic inflammation was the most likely explanation for the amyloidosis. Histopathology of the foot mass was consistent with a soft tissue sarcoma (Table 3). Immunohistochemical staining of the foot mass was performed and the mass stained positive for vimentin but negative for neural markers, S100 and myelin basic protein (MBP). This pattern of staining confirmed that the mass was a sarcoma but did not indicate the tissue of origin. The two primary differentials were fibrosarcoma or synovial cell sarcoma. However, given that there was no evidence of underlying destruction of the bone on radiographs, synovial cell sarcoma seemed less likely.

The bird recovered uneventfully from the anesthesia and was discharged the following day with instructions to continue the itraconazole at the same dosage and have the bandage removed and the foot incision rechecked in 2 days. This recheck was performed by the local veterinarian and the biopsy site appeared to be healing well. Once all of the diagnostic results were obtained and fungal air sacculitis was confirmed, a decision was made to hospitalize the

bird for IV amphotericin B therapy with concurrent fluid support. While she was in the hospital an attempt would also be made to explore the site of the neoplasia on the foot to determine whether additional gross tissue remained behind that could be removed prior to adjunctive therapy such as radiation therapy. Because the foot mass was a sarcoma it was expected to be locally aggressive but unlikely to metastasize. Removal of gross tumor followed by radiation therapy would be the best therapy given that amputation was considered undesirable due to the presence of arthritis in the opposite stifle. If the fungal pneumonia could be controlled than additional therapy on the foot mass could be pursued. No evidence of metastasis was observed on radiographs or endoscopy to this point, but radiographs, ultrasound, and possibly coelomic computerized tomography would be recommended to look for evidence of metastasis prior to initiating radiation therapy in this bird.

The bird returned for hospitalization 40 days after initial presentation (13 days following the endoscopy procedure). This was a longer recheck interval than would have been ideal but was based on the length of time required to obtain all of the diagnostic results including special stains on the foot mass. The owner reported that initially following the procedure the bird had been doing well at home, although she had been sneezing more frequently since the endoscopy. However, for the 2 days prior to presentation she had been exhibiting occasional episodes of increased respiratory rate and tail bobbing. She was still being treated with itraconazole 5mg/kg PO q 12 h. Visual examination of the bird revealed that she was in respiratory distress in her carrier. She was open-beak breathing with an increased respiratory effort and a mild tail bob. Flow by oxygen was provided to the bird during a brief physical examination. The respiratory rate was increased at 120-140 breaths per minute. Auscultation of the lungs and nasal sinuses revealed harsh, occasionally moist sounds and inspiratory and expiratory wheezes. Body

condition was still slightly thin although the body weight was increased by 34 grams to 260 grams. This increase in body weight was likely an error in measurement since the following day the bird's weight was decreased to 220 grams. The remainder of physical examination was unremarkable. The bird was placed in a cage for monitoring and the respiratory distress resolved without treatment. It appeared that the stress of travel and handling exacerbated her respiratory disease but that the bird was stable with no evidence of respiratory distress when she was relaxed. Based on the clinical signs we suspected worsening of the air sac and lung granulomas and possible development of tracheal aspergillosis. Other differentials for the respiratory distress included tracheitis, post-intubation tracheal stenosis, inhaled foreign body, or the development of aspiration pneumonia. It was felt that continuing with the plan of administering IV amphotericin B was important to improving the bird's condition. Because the respiratory distress had resolved, a decision was made to proceed with the planned procedures of IV administration of amphotericin B and surgical exploratory of the site of the previously excised foot mass to look for remaining tumor.

The bird was premedicated with midazolam (0.3mg/kg IM) and butorphanol (0.5mg/kg IM). The bird was induced using isoflurane at 3% in 95-100% oxygen administered via a facemask. She was then intubated with a number 3.0 ID uncuffed endotracheal tube and placed on a Bain nonrebreathing circuit. There was no difficulty intubating the bird and there was no evidence of inflammation around the glottis or the proximal trachea. Anesthesia was maintained at 2-3% isoflurane. While under anesthesia the heart rate was monitored constantly with a stethoscope and the spontaneous respiratory rate was constantly monitored. The respiratory rate was 12-15 breaths per minute during the procedure. While anesthetized the bird experienced periods of bradycardia during which the heart rate dropped from 260 bpm to 180bpm, but these

resolved with stimulation. The total anesthetic time was 35 minutes. The surgical site from the previous biopsy on the left pelvic limb was opened and the area was explored in an attempt to remove any remaining gross tumor, however, no tissue could be identified that was obviously neoplastic, therefore the incision was closed with 4-0 poliglecaprone 25 suture in a simple interrupted pattern. While the bird was under anesthesia she was administered 1mg/kg of the 5mg/ml amphotericin B lipid complex diluted according to the package directions 1:50 in 5% dextrose in water for administration. The drug was administered intravenously in the basilic vein using a 25 gauge butterfly catheter. The amphotericin B was administered over a 20 minute period. The lipid complex formulation of amphotericin B was chosen because of its reduced risks of nephrotoxicity. However, no data is available regarding pharmacokinetics of this drug in avian species. Therefore a conservative dosage of 1mg/kg was chosen. The bird was also administered 60ml/kg of lactated Ringer's solution subcutaneously during the anesthetic procedure for diuresis. The plan was to administer fluids at a rate of one and one half times maintenance over 24 hours on the days that the amphotericin B was administered. A fluid volume of 60ml/kg is considered a maintenance volume for birds, therefore the bird would receive an additional 30ml/kg as fluid therapy later in the day once the initial dose of fluids was absorbed. The subcutaneous route was chosen for fluid administration because the bird was bright despite its respiratory problems and would be unlikely to tolerate an IV or IO catheter. Subcutaneous fluids are slowly absorbed over several hours and therefore provide good diuresis. The bird recovered uneventfully from anesthesia. There was a moderate amount of mucus on the distal end of the endotracheal tube when the bird was extubated.

The bird continued to have intermittent increased respiratory effort and open beak breathing following her recovery from anesthesia, but appeared stable on room air. The

following day the bird began exhibiting more severe respiratory distress, with open-beak breathing and a tail bob. She was placed in an oxygen cage with the oxygen at an FiO2 of 40%. Handling was minimized to reduce stress. Because she was not considered a good candidate for anesthesia and IV therapy, she was nebulized with amphotericin B lipid complex at 1mg/kg diluted in 4ml of water, administered using a nebulizer[jj] with a particle size less than 3μm for 15 minutes. Based on previous studies performed in mice, the lipid complex formulation of the amphotericin B was expected to be soluble and appropriate to administer via nebulization.[53] Nebulization was also expected to have therapeutic benefits in providing humidity to the respiratory tract. The bird continued to exhibit respiratory distress and her appetite decreased over the following 12 hours. The bird was no longer considered to be a good anesthetic candidate, therefore the IV amphotericin B was discontinued. The following day she was treated with oxygen support, lactated Ringer's solution at 30ml/kg SC q 12 h as a maintenance volume, itraconazole at 5mg/kg PO q 12 h, nebulization with sterile saline q 24 h, nebulization with amphotericin B lipid complex at 1mg/kg diluted in 4ml water q 12 h, and gavage feeding with an avian critical care feeding formula[kk] at 3% of body weight twice a day. This volume was felt to be safe in this bird with respiratory difficulty, in which regurgitation and aspiration pneumonia were concerns. Antibiotic therapy (enrofloxacin 10mg/kg diluted in LRS SC q 24 h) was added to the treatment regimen in case aspiration pneumonia was a component of the respiratory disease. The enrofloxacin was administered every 24 hours because it is a concentration dependent antibiotic that has a post-antibiotic effect to continue killing bacteria for several hours following administration. Meloxicam[ll] (0.3mg/kg PO q 12 h) was administered to treat inflammation that may be present in the trachea. Her condition continued to deteriorate throughout the day and the owner elected euthanasia that evening. Euthanasia was performed 42

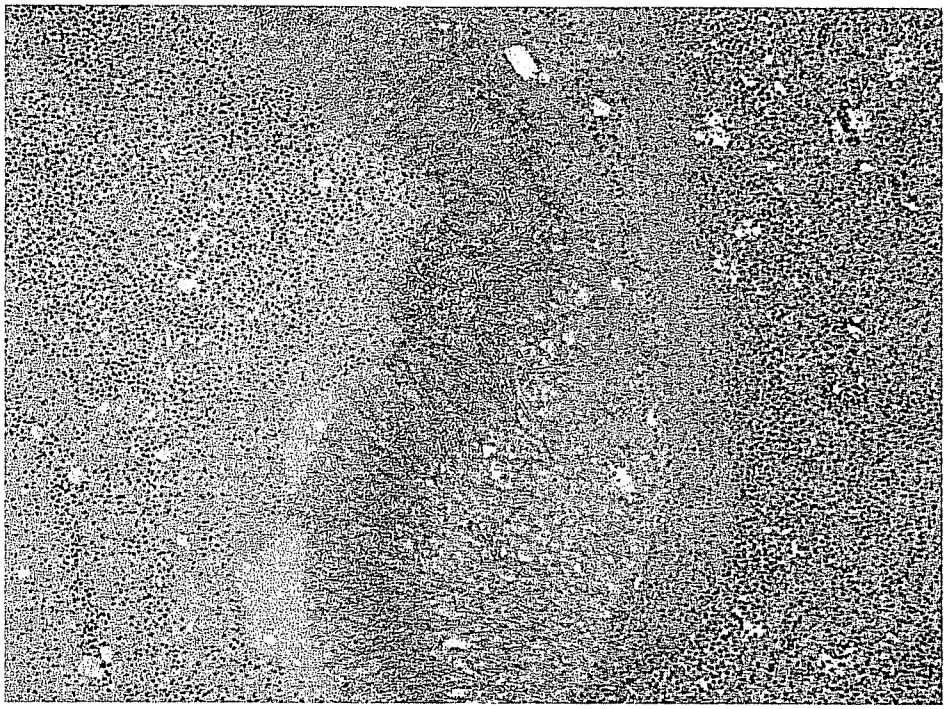
days after initial presentation, using 0.5mL of euthanasia solution[ll] intravenously in the right jugular vein. A complete necropsy was performed.

On gross necropsy the caudal third of the right lung lobe was expanded by a hard, yellow granulomatous mass that was adhered to the adjacent thoracic wall. The thoracic and abdominal air sacs were thickened, opaque, and adhered to the wall of the coelom and the serosal surface of the coelomic viscera. There were adhesions between the small and large intestinal loops. There was no evidence of gross tumor at the site of the previously biopsied sarcoma. Samples of the lung and air sac were submitted for fungal culture, however there was no growth of organisms.

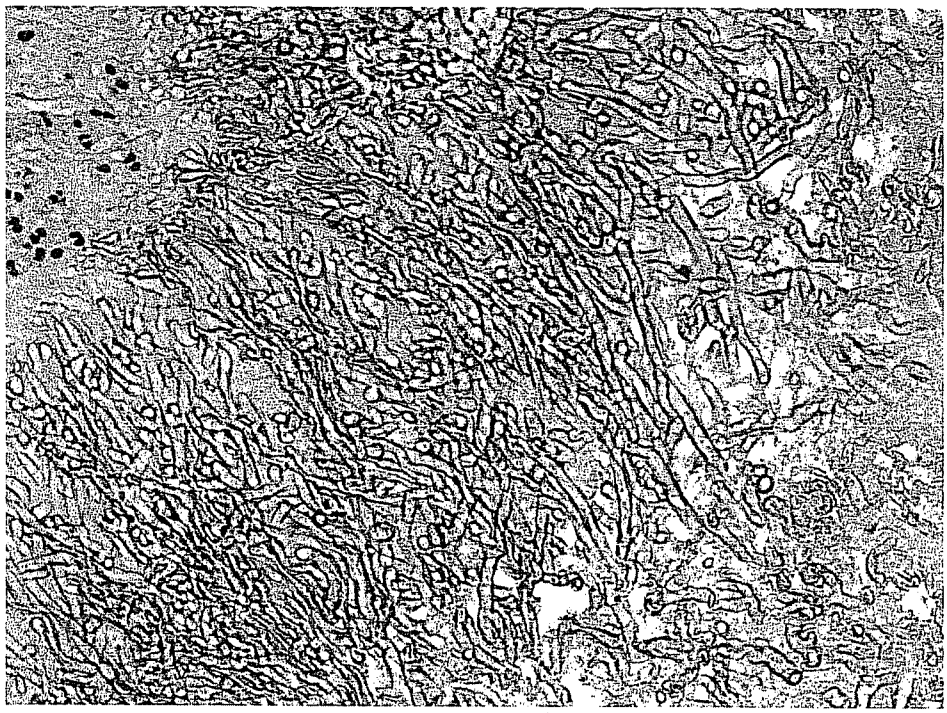
Histopathologic findings are summarized in table 3. Sections of lung, lung with granuloma, liver, spleen, kidneys, ovary, oviduct, adrenal glands, thyroids, heart, skeletal muscle, sciatic nerve, tongue, esophagus, trachea, crop, proventriculus, ventriculus, small intestine, large intestine, pancreas, cloaca, muscle from the site of the sarcoma, and brain were formalin-fixed and routinely prepared for histologic examination with hematoxylin and eosin staining (Table 3). Histologic examination of the tissues revealed extensive granuloma formation with intralesional fungal hyphae in the caudal right lung and the thoracic and abdominal air sacs (Figure 7). A fibrinous coelomitis was also present. There was squamous metaplasia and submucosal fibrosis within the lumen of the distal trachea, with partial occlusion of the tracheal lumen. The middle aspect of the trachea was less severely affected by squamous metaplasia and had evidence of a chronic heterophilic tracheitis. No fungal elements were observed in the samples from the trachea. There was marked amyloidosis in the kidneys and spleen. There was no evidence of neoplasia associated with the previously excised sarcoma on the foot. The muscle in the area where the mass was removed exhibited mild multifocal

Figure 7a. Photomicrograph of an air sac granuloma identified on necropsy of a Maximilian pionus parrot (*Pionus maximiliani*) with fungal pneumonia and air sacculitis. There is necrotic debris in the center of the image bordered by radiating fungal hyphae (dark pink). A dense conglomeration of basophilic inflammatory cells surrounds the lesion. **b.** High power view of the same air sac granuloma from a pionus parrot shown in a. Note the parallel walled, septate, branching fungal hyphae characteristic of *Aspergillus* spp.

a.



b.



myodegeneration that may have been a results of pressure from the mass. There was a mild hepatitis that was considered incidental and unlikely to have affected hepatic function. The necropsy diagnosis was severe granulomatous fungal pneumonia and air sacculitis. Although *Aspergillus* was not grown on fungal culture, the morphology of the organisms within the granulomas was consistent with *Aspergillus* species. There was also tracheal stenosis, believed to be secondary to heterophilic tracheitis. The amyloidosis in the spleen and kidneys was likely secondary to chronic inflammation. There was no evidence of the previously diagnosed sarcoma.

Discussion

Fungal air sacculitis and pneumonia in this pionus parrot were diagnosed based on a combination of hematologic, radiographic, cytologic, and histopathologic findings. The organisms causing the fungal air sacculitis and pneumonia were morphologically consistent with *Aspergillus* spp, but multiple fungal cultures failed to grow the organism. The bird did not improve with treatment with systemic and nebulized antifungal medications, and was euthanized. Necropsy confirmed fungal pneumonia and air sacculitis consistent with aspergillosis. The bird had a soft tissue sarcoma on the right foot, but this was considered to be an unrelated finding and did not contribute to the clinical signs or cause of death in this parrot.

This case demonstrates the difficulty in obtaining a diagnosis of aspergillosis in avian patients. Often other disease must be ruled out concurrently in order to reach a diagnosis. Two important differentials that had to be considered in this case were mycobacteriosis and neoplasia.

Mycobacterium was an important differential for weight loss, leukocytosis, monocytosis, hyperfibrinogenemia, and nodular air sacculitis in this pionus parrot, since this species is reported to be sensitive to mycobacterial infections. Mycobacterium most commonly affects the

gastrointestinal tract and liver in birds. However, lesions in the air sacs and lungs have been reported in a blue-headed pionus parrot. Acid fast staining of feces was performed on day 22 as a non-invasive test to evaluate whether acid fast organisms were being shed. This diagnostic test has poor sensitivity since birds with mycobacteriosis often shed organisms intermittently. In addition, if the primary infection were located in the lungs it is possible that a bird would not shed organisms in the GI tract. However, a positive result on an acid fast stain of the feces would be highly suggestive of mycobacterial infection and would warrant additional diagnostics such as biopsy of the lesion with mycobacterial PCR or culture. When the air sac lesions were biopsied on day 27, acid fast stains of the lesions were performed to look for intralesional organisms, since mycobacterium may be difficult to identify with traditional stains such as Wright's stain. This acid fast test was also negative, and given the presence of intralesional fungal hyphae, mycobacterium as a differential diagnosis was considered much lower on the list. There was no evidence of mycobacterial infection on post-mortem examination.

Given the presence of a mass on the foot, neoplasia, either primary or metastatic, in the air sac was also considered a possibility in this bird. Neoplasia could have explained the weight loss despite a good appetite and could also have resulted in a leukocytosis. Differentials for neoplasia in this bird were broad but based on the appearance of a solitary mass located in the air sacs air sac carcinoma, pulmonary carcinoma, leiomyosarcoma or any metastatic neoplasia were differentials. Metastatic neoplasia was considered most likely initially given the presence of a mass on the foot. Biopsy of the foot mass diagnosed the mass as a sarcoma, with the primary differentials being fibrosarcoma and synovial cell sarcoma. An attempt to further classify the tumor type was made but was unsuccessful due to negative expression of several cell markers (neural markers, S100 and myelin basic protein (MBP)). It is impossible to know if these

markers were negative because the stain is not effective in this avian species or because the tumor was truly negative. Therefore, the tumor could not be further classified. Fibrosarcomas in birds are common neoplasms and tend to be slow growing, locally invasive and rarely metastatic. On the other hand, synovial cell sarcomas are rarely reported in birds and tend to involve proliferative masses with destruction of the joint and bone. Based on the radiographic findings fibrosarcoma was the more likely tumor type, but this could not be confirmed.

It is unclear what predisposed this bird to the development of fungal pneumonia and air sacculitis. In general, an underlying condition or stressor is associated with the disease. The bird's history was only known for the six years since the owner had acquired her. The bird did have a history of reproductive activity, which could have placed physiologic stress on her system. It is possible that the bird was on a poor diet, in an overcrowded environment, kept at an inappropriate temperature, or treated with exogenous corticosteroids prior to acquisition by the owner and that the chronic fungal pneumonia and air sacculitis infection had been progressing during that entire time, only becoming evident when it was already very severe. Chronic aspergillosis in psittacines can be present for months or even years. Pionus parrots are reported to be susceptible to aspergillosis. Again, the mechanism for this is unknown but could represent an increased sensitivity to environmental stressors or a genetic difference in host defenses leading to a poor ability to fight infection when exposed to *Aspergillus* spores. It is also possible that this bird was older than the owner believed and that immune suppression due to age played a role in the development of fungal pneumonia and air sacculitis. If the bird was truly 13 it would be considered young to middle aged for a species that could conceivably live to be 30 years old or more. The fact that there cataracts and neoplasia on the foot were present could support the idea that this bird was older than reported.

The blood work findings in this case were generally supportive of a diagnosis of aspergillosis, although there were some unusual findings. The bird in this report never developed an anemia despite the presence of a chronic fungal infection. It is possible that the normal hematocrit for this bird is higher than the reference range for pionus parrots and that she was in fact anemic. A reticulocyte count may have provided useful information as to the degree of regeneration of red blood cells to suggest whether a mild non-regenerative anemia was in fact present. However, reticulocyte counts are not commonly available for birds. There was slight anisocytosis present at all time points, suggesting regeneration, and therefore the absence of anemia of chronic disease. A leukocytosis with a heterophilia was present on day 1 and day 22 as would be expected with aspergillosis. However, the leukocyte count on day 1 was only moderately elevated and the leukocyte count was normal on day 27. This is an unusual finding in a bird with aspergillosis, in which a moderate to severe leukocytosis would be anticipated. The minimal white blood cell response may have represented a lack of an ability to respond due to the immune suppressive nature of aspergillosis infection. The minimal response may also have represented the fact that the granulomas were walled off and the fungal organisms were not inducing a systemic inflammatory response. There was an elevation in leukocyte count on day 22 that may have represented a sudden worsening of the disease with hematogenous or respiratory spread of the disease and a concurrent systemic inflammatory response. However, given the difficulty distinguishing thrombocytes from lymphocytes in that sample it is also possible that the total white cell count or the lymphocyte percentage was an overestimate. There was a persistent mild to severe monocytosis in this bird, as would be expected in a case of aspergillosis. The eosinophilia on the first blood sample was an unusual finding. In mammals eosinophils increase due to intestinal parasitism, allergy, or less commonly fungal disease.

However, eosinophilia in birds can be due to inflammation of any cause, therefore this finding was non-specific. There was no evidence of GI parasitism on necropsy to explain the eosinophilia. The fibrinogen gradually increased over the course of several visits. This protein is an indicator of bacterial or other types of inflammation and the elevation indicated that acute inflammation was present in addition to the chronic inflammation indicated by the leukocytosis and monocytosis.

Plasma biochemistry results in this bird were not considered unusual. CK was moderately elevated on two different dates, suggesting muscle damage. This may have been a result of trauma either during transport or handling. It is also possible that the coelomic granulomas or the sarcoma on the foot were causing muscle damage to adjacent tissue. Blood work was monitored to evaluate the liver while the bird received itraconazole, since hepatotoxicity is a potential adverse effect. There was no evidence of liver damage based on normal bile acids and GLDH. The elevation in AST observed on day 22 was most likely a result of muscle damage. The lack of hepatic damage was confirmed at necropsy, at which time a mild hepatitis thought to be an incidental findings was reported. There was no evidence of hepatic necrosis or fibrosis that would suggest a toxic insult. The globulin was normal on day 1 but elevated on day 22, which may have represented immune response to antigenic stimulation if there was spread of fungal organisms either throughout the respiratory tract or hematogenously. The cause for the elevation in globulins could have been more clearly defined by performing an EPH. In hindsight, it would have been advisable to perform a plasma biochemistry at the visit on day 40. This would have allowed continued monitoring of hepatic function as well as provide baseline uric acid and BUN to monitor renal function during the course of amphotericin B therapy. This is considered an oversight.

The aspergillosis titer for this bird was negative. This is not an unusual finding in avian species. Both false positives and false negatives are reported for aspergillosis in avian species. Despite the problems with interpreting *Aspergillus* serology, it was felt that it was worthwhile to determine the titer can help determine prognosis and since the efficacy of therapy can be evaluated by monitoring changes in the titer over time. Possible explanations for the negative titer result in this bird include a lack of an antibody response by the bird due to immune suppression, failure of the test due to lack of validation in a given species, or infection caused by a fungal species other than *Aspergillus*. Animals with aspergillosis may be immune suppressed either due to an underlying disease process or to the aspergillosis itself, which may prevent the bird from mounting an antibody response. The inability to produce antibody and the resulting negative titer is therefore considered a poor prognostic indicator in avian species with aspergillosis. Spurious laboratory results were an unlikely cause for the negative titer. The serology was performed at the University of Minnesota, and has been validated in psittacines including pionus parrots. Another possible explanation for the negative titer is that the fungal granuloma was caused by a fungal species other than *Aspergillus*. The fungal granuloma was cultured on two separate occasions, at the time of endoscopy and at the necropsy. Both of these cultures were negative for fungal growth, therefore we were unable to confirm the species responsible for causing the fungal infection. However, the morphology of the fungal organisms was consistent with *Aspergillus* species. The biopsies and the necropsy were interpreted by two different board certified pathologists, both of whom stated that the lesions were consistent with aspergillosis. Fungal pneumonias in birds have been reported to be caused by other species of fungi including penicillium, zygomyces and cryptococcus. However, the morphology of these organisms is different from that of *Aspergillus* and it is unlikely that they would be confused on

histopathology. It is interesting to note, however, that one report of penicillium involving the air sacs was in a pionus parrot. It is not clear why the fungal cultures did not yield growth. In general *Aspergillus* is considered to be relatively easy to grow on standard fungal media, as was used in this case. It is possible that the high doses of itraconazole, which is fungicidal to *Aspergillus* spp at high concentrations, that were used to treat the bird prior to culture had already affected the fungus and therefore there was no growth. It is also possible that the samples that were cultured were obtained from areas with a large amount of granulomatous inflammation but with no fungal spores present. Histopathology is considered a definitive diagnosis for aspergillosis even in the absence of positive culture. However, to identify the exact species of *Aspergillus* polymerase chain reaction (PCR) or immunohistochemistry of the tissues could have been performed. Unfortunately, PCR and immunohistochemistry were not performed in this case.

An additional diagnostic test that could have been performed in the bird is protein electrophoresis (EPH). EPH can be beneficial in determining whether a disease process is acute or chronic and can identify whether infection or inflammation is present in a bird. However, there are no EPH findings that are specific for aspergillosis. A beta globulinopathy may be seen with aspergillosis, but may also be seen with many other diseases including Chlamydophila, mycobacteriosis, and parasitic infections. Conversely, some birds have normal globulin levels despite chronic disease because they are anergic or immune suppressed. In addition, EPH must be interpreted based on species specific reference ranges, which may be difficult with less common species. Therefore, EPH was not pursued in this bird given the fact that it would not change the diagnostic and treatment plan.

Radiographic findings in this bird were consistent with granulomatous air sacculitis, as is commonly seen with fungal infections. However, this finding is not specific for aspergillosis and other causes of soft tissue density in the air sacs had to be considered prior to endoscopy and histopathology. The radiographs were reviewed at a later date to look for any lesions that may have been missed during the initial management of the case. No additional findings were noted. The linear opacity that was observed lying between the lungs and the air sacs likely represented thickened air sacs since histopathologic evaluation of the great vessels did not show any evidence of mineralization or atherosclerosis. The radiographs could have been improved with better positioning, but it does not appear that the diagnosis would have changed. It is interesting to note that lesions that were present in the air sacs on the left side of the coelom at the time of endoscopy were not evident radiographically. This is not an unusual finding in cases of aspergillosis and often lesions are not visible radiographically until they are very severe. The presence of severe radiographic changes on the first visit was a poor prognostic indicator for this bird.

Because the bird only had minimal clinical signs of disease despite the granulomatous pneumonia observed on radiographs indicating severe disease, she was treated initially with oral medications on an outpatient basis. Itraconazole was chosen as the first line antifungal medication. Itraconazole is fungistatic against yeasts but at high doses it is fungicidal against *Aspergillus* spp. It can be given orally and therefore may be administered by owners at home. There is pharmacokinetic data available for itraconazole in psittacines and therefore an appropriate dosage may be chosen. Although itraconazole causes anorexia in some birds at higher doses, as was observed in this bird at a dosage of 10mg/kg q 12 h, it is generally well tolerated by most species of psittacines. However, treatment must be continued for an extended

period of time due to the granulomatous nature of the disease. Also, itraconazole is best absorbed when the carrier has an acidic pH. Care must therefore be taken to avoid aspiration, which can lead to a tracheitis due to the low pH of the oral formulation. This is an important consideration given the fact that the bird developed a tracheitis with partial occlusion of the trachea later in the course of therapy. Birds must also be monitored closely for loss of appetite and weight loss, and the dosage adjusted as needed.

The bird was treated as an out-patient for approximately three weeks and did well at home with an improvement in body weight. However, repeat radiographs revealed worsening of the granulomatous air sacculitis and pneumonia, and the leukocytosis and monocytosis worsened despite therapy. Because the diagnosis had not yet been confirmed, endoscopy was performed to obtain samples for cytology, culture and histopathology. Once the diagnosis was confirmed as fungal pneumonia and air sacculitis a decision was made to treat with amphotericin B both systemically and via nebulization, since the itraconazole did not appear to be improving the bird's condition. When the bird re-presented for IV therapy it had begun showing signs of respiratory distress. The bird was administered one dose of intravenous amphotericin B lipid complex intravenously and was nebulized twice a day with the same lipid complex formulation. This formulation was chosen because of the reduced risk of nephrotoxicity associated with administration. However, there is no data available regarding the pharmacokinetics of this drug in psittacine species. A higher dosage of the lipid formulation than the traditional formulation is required in other species. The IV dosage that we used was the same dosage as is recommended for the traditional formulation in this bird, and was therefore probably too low. The dose that was administered via nebulization was more appropriate, however, because with nebulization the

drug is acting locally. In mice, nebulization with a lipid formulation of amphotericin B resulted in high tissue levels of the drug in the lungs.

We assumed that the worsening of the clinical signs at the visit on day 40 was a result of progression of the fungal pneumonia and air sacculitis or development of additional lesions in the trachea. Another possibility we considered was that the bird had aspirated food material at some point and developed aspiration pneumonia. For this reason enrofloxacin was added to the treatment regimen on the last 2 days in the hospital. However, based on necropsy results it appears that the bird's signs were actually a result of the tracheitis with partial occlusion of the lumen. The respiratory pattern in this bird included increased respiratory rate, tail bob, intermittent open mouth breathing, and both inspiratory and expiratory effort and noises. This made identifying the location of the respiratory problem challenging. Signs of tracheal disorders in birds may include open-mouth breathing, respiratory noises including a high pitched wheeze on inspiration, change in vocalization, and increased respiratory effort. There was no change in voice observed in this bird nor was there a high pitched wheeze present to suggest tracheal disease. The remaining signs observed in this bird can be seen with tracheal disorders or with disorders of the lower airways. In retrospect, additional diagnostics such as a tracheal wash with cytology to look for evidence of inflammatory cells or fungal organisms and with bacterial or fungal culture, or tracheoscopy to visualize and sample any lesions present would have been beneficial in this case. It was questionable whether the bird would have been stable enough to survive these procedures, but they could have been offered to the owner had euthanasia not been elected.

The cause of the heterophilic tracheitis and tracheal stenosis is not clear. It may have represented spread of the Aspergillus infection to the trachea, although fungal organisms were

not observed on histopathology. There may be small numbers of fungal hyphae associated with fungal tracheitis, therefore it is possible that fungal organisms were missed on histopathology. Unfortunately the trachea was not cultured for fungal organisms at necropsy. Aspiration of itraconazole must be considered as a possible cause for tracheitis given the acidic pH of the drug and the long course of treatment in this bird. However, the owner reported that there was no difficulty in administering the drug, but aspiration of drug cannot be ruled out. Another possibility is that the tracheal lesions were secondary to intubation during the endoscopic procedure. The interval between the endoscopic procedure and the onset of clinical signs was about 12 days. Tracheal stenosis has been reported in birds following tracheal trauma such as anesthetic intubation. The post-intubation tracheal stenosis usually occurs 2-4 weeks following the procedure in which the bird was intubated. The histopathologic changes observed in avian tracheal stenosis involve damage to the superficial layers of the tracheal mucosa with proliferation of vascular granulation tissue, giant cell macrophage infiltration of tracheal mucosa and lamina propria, fibroplasia, fibrosis, heterophilic inflammation, and hyperplastic squamous epithelium. The squamous metaplasia, submucosal fibrosis, and chronic heterophilic tracheitis observed in this bird on necropsy were consistent with the findings observed in reported cases of tracheal stenosis. An uncuffed endotracheal tube was used during the anesthetic procedure to minimize the possibility of pressure necrosis on the tracheal lumen. In addition, a Humid-vent was used to keep the gas in the respiratory tract moist during the procedure. However, little is known about why tracheal membranes develop in birds so it is unknown how to prevent them from forming. Hypovitaminosis A is another possible explanation for the changes observed in the trachea. Squamous metaplasia can be seen in birds with hypovitaminosis A, but there were no other lesions suggesting hypovitaminosis A on physical examination or histopathology such

as blunted choanal papillae or squamous metaplasia of the oral cavity, air sacs or urinary tract epithelium. Because of this and the history of an appropriate diet for several years hypovitaminosis A was considered unlikely. Tracheoscopy was indicated in this case to collect biopsies and cultures, to identify a narrowing of the tracheal lumen and to allow for treatment such as manual breakdown of the stenotic tissue or application of topical anti-inflammatories. It was not performed initially because the respiratory pattern on presentation on day 40 did not definitively point to tracheal disease. After the respiratory distress worsened the bird was considered a poor candidate for anesthesia so the procedure was not performed. .

One challenge of treatment of aspergillosis granulomas with itraconazole or amphotericin B is the fact that even if the fungal organisms are killed the bird is left with granulomas that are difficult for the bird's immune system to resolve. The newer technique of endoscopic debulking of granulomas may have been useful in this case. An course of therapy that could have been performed as an alternative to the course that was chosen would have involved local application of amphotericin B to the air sac granulomas endoscopically over multiple visits, followed by surgical debulking of granulomas using endoscopy or endosurgery. In addition to the air sac granulomas this bird did have a granulomatous pneumonia in the right caudal lung that would have been difficult to treat. It is unclear whether local treatment of the air sac granulomas with debulking would have improved this bird's outcome.

Despite the mild presenting clinical signs, this bird had several diagnostic results that were considered poor prognostic indicators. There were severe radiographic changes present in the lungs and air sacs at the first visit, indicating that the disease was already advanced. The antibody titer was negative, suggesting that the disease was chronic and that the bird was anergic. In addition, concurrent neoplasia meant that the bird would require therapy that was

potentially immune suppressive. Due to all of these factors the birds prognosis was ppor from the outset and it was unlikely that the fungal pneumonia and air sacculitis in this bird could have been completely resolved.

Summary

A 13-year old female Maximilian pionus parrot was evaluated for weight loss. Bloodwork revealed chronic inflammation and radiographs revealed pneumonia and nodular air sacculitis. Aspergillosis was suspected because of the weight loss, bloodwork, and radiographic findings. Empiric treatment with itraconazole was initiated. After three weeks of therapy the disease appeared to have progressed radiographically. Endoscopy was performed and air sac granulomas were observed. Fungal air sacculitis and pneumonia consistent with aspergillosis was diagnosed based on histopathology. A sarcoma was diagnosed on the bird's foot, which was unrelated but confounding to the diagnosis of the air sac lesions. The bird started showing signs of respiratory distress two weeks later and amphotericin B therapy was initiated. The respiratory distress worsened despite therapy and the owner elected euthanasia. Necropsy confirmed the diagnosis of severe fungal pneumonia and air sacculitis. A severe tracheitis with tracheal stenosis was also diagnosed, explaining the progressive respiratory distress.

Endnotes

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dd. Versed, Roche Labs, Basel, Switzerland

ee. Mark 7 Respirator, Bird Co., Palm Springs, CA, USA

ff. Humid-Vent Mini, Hudson RCI/Teleflex Medical, Research Triangle Park, NC, USA

gg. BairHugger Pediatric Underbody Blanket, Arizant Healthcare Inc, Eden Prairie, MN, USA

hh. Karl Storz Veterinary Endoscopy, Goleta, CA, USA

ii. Monocryl, Ethicon Inc division of Johnson and Johnson, Piscataway, NJ, USA

jj. Acorn II, ACORN, Marquest Medical Products Inc, Englewood, CO, USA

kk. Emeraid Nutri-Support, Lefeber Company, Cornell, IL, USA

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