# Diagnostic Challenge

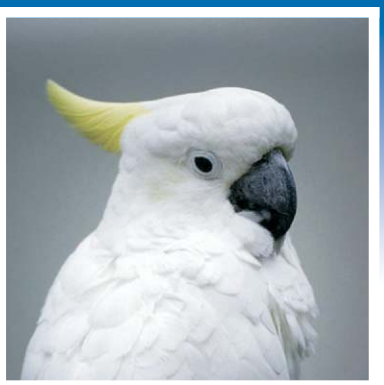

**Figure 1.** Lateral survey radiograph of a red-shouldered hawk (*Buteo lineatus*).


**Figure 2.** Ventrodorsal survey radiograph of a red-shouldered hawk.

## History

An adult red-shouldered hawk (RSH) (*Buteo lineatus*) was presented to the Louisiana State University School of Veterinary Medicine for evaluation of a 4-day history of anorexia and possible weight loss. The hawk was being trained as a possible resident for the veterinary school's raptor education program and had been in the program for 6 weeks. The bird was being housed in a mew that measured 12 ft × 12 ft × 6 ft (3.6 m × 3.6 m × 1.8 m). The floor of the mew was concrete. Three perches were placed in the mew, and all were covered with AstroTurf. The hawk was fed pre-killed rat (45 g) daily.

On physical examination, the hawk was deemed to be in adequate body condition (body condition score, 2.5/5). The bird was stoic and did not vocalize during the restraint process. Primary feathers 1 through 4 were missing from the left wing. The only other abnormality noted on physical examination was a previously diagnosed injury to the left wing.

The initial diagnostic plan included collecting blood for a complete blood count (CBC) and taking survey radiographs (Figs 1 and 2). Abnormal findings on the CBC were: total white blood cell count, 6.2 × $10^3$ cell/μl (reference range, 7.7-16.8 × $10^3$ cell/μl) and lymphocytes, 0.4 × $10^3$ cell/μl (reference range, 1.2-12.6 × $10^3$ cell/μl).

The hawk was hospitalized and, although not considered dehydrated at the time of physical examination, maintenance fluids (100 mL/kg/d subcutaneously) (Normasol R; Hospira, Inc., Lake Forest, IL USA) were provided because of the history of anorexia. In addition to the fluid therapy, the bird was force-fed pre-killed rat prey (at a rate of 5% of the bird's body weight). No other therapeutics were initiated before receiving the results of the complete blood count and radiographs.

*At this time, please evaluate the CBC and Figures 1 and 2. From these preliminary diagnostics, develop a differential list and plan for additional diagnostics.*

## Diagnosis

Because there is no published CBC reference range for the RSH, this bird's CBC was interpreted with a reference range for sharp-shinned hawks (*Accipiter striatus*).[1] According to the sharp-shinned hawk reference range (white blood cell count, 7.7-16.8 $\times 10^3$ cell/$\mu$l), the RSH was leukopenic ($6.2 \times 10^3$ cell/$\mu$l). The leucopenia was attributed to a lymphopenia ($0.4 \times 10^3$ cell/$\mu$l; reference range, 1.2-12.6 $\times 10^3$ cell/$\mu$l). In this case, the lymphopenia was attributed to stress, although viral diseases in birds can also result in a severe lymphopenia. However, the absence of eosinophils also suggested a stress leukogram.

On the radiographs, the normal honeycomb pattern of the lung was not visible in the lateral view, and there was a generalized increased opacity of the lung and air sac fields. Differential diagnoses for these findings included pneumonia and air saculitis due to either bacterial or mycotic infection.

Because of the changes suggested by the radiographic findings, an exploratory endoscopic examination of the coelomic cavity was performed. The hawk was anesthetized with isoflourane (IsoFlo; Abbott Laboratories, North Chicago, IL USA). The bird was masked, and the inhalant was delivered at a rate of 5% (oxygen, 1 L/min). Once anesthetized, the bird was intubated with a 2.5 (outside diameter) endotracheal tube and maintained during the procedure on isoflurane at a rate of 3% (oxygen, 0.5 L/min). The endoscopic procedure was performed with the technique described by Hernandez-Divers.[2] Numerous white plaques were found coating the walls of the air sacs and surface of the lung. The walls of the air sacs appeared thickened. The liver appeared swollen and had poorly defined margins. Based on the degree of infiltration of the lungs and air sacs and the grave prognosis for treatment in a clinically ill patient, the decision was made to euthanize (2 mL, basioccipital sinus, Beuthanasia; Schering-Plough Animal Health, Corp., Union, NJ USA) the bird. The body was submitted for necropsy. Gross examination and histopathology confirmed a diagnosis of aspergillosis. Culture was done to confirm a diagnosis of *Aspergillus fumigatus*.

## Comments

Fungal air sacculitis is a common ailment of captive wild birds, especially raptors. There are a number of risk factors that have been associated with the development of this disease in captive raptors, including confinement in small enclosures with poor ventilation, contaminated soil, stress, and species predisposition.[3-9] *Aspergillus fumigatus* is the fungal agent that is most commonly associated with fungal air sacculitis. *Aspergillus flavus* and *Aspergillus niger* have also been implicated.[3-5] Aspergillosis spores are found commonly in the soil and contaminating moldy feed and hay. Birds of prey are often exposed by inhaling spores while eating infected prey.[3] This particular bird was fed frozen (thawed) rats, as are the other 9 birds in the education program at the university.

Although exposure to *Aspergillus* spp. is fairly common, infections causing disease are relatively rare.[3-5] Birds presenting with clinical aspergillosis often suffer from stress, malnutrition, or concurrent infection with another disease leading to an immunocompromised state.[3-5] Clinical aspergillosis can be classified in many different ways. Some authors simply divide the disease into an acute form and a chronic form, whereas others use a system based on the location and invasiveness of the fungal lesions.[3,4] With the latter system, there are 4 categories for characterizing aspergillosis: acute, tracheal, localized, and invasive. Both systems describe the acute form as occurring as a result of a single exposure to a high number of spores. The clinical signs most frequently associated with acute aspergillosis include anorexia, polydipsia, polyuria, and respiratory distress. This form can be fatal within 1 to 7 days without appropriate therapy. With the tracheal form, the lesion is generally found within the trachea and near the syrinx. Vocalization changes are common with this form. The localized form is generally characterized by granulomatous lesions found throughout the lungs and air sacs.

The RSH in this case had severe granulomatous disease. Invasive aspergillosis occurs when localized lesions spread hematogenously or via air sacs to other organ systems.[4] This form is most commonly seen when the disease has been ongoing for a prolonged period of time. The clinical signs observed in chronic cases are often related to the organ systems involved and may include lethargy, inappetence or vomiting, and respiratory distress.

Diagnosis of respiratory aspergillosis is often complicated by its variable presentations.[3-9] Therefore, diagnosis is often based on a combination of clinical signs, hematology, serology, radiography, endoscopy, histopathology, and culture. It is important to realize that the sensitivity of these different tests can vary depending on the form of aspergillosis. The most common CBC findings with aspergillosis are a marked leukocyto-

**Figure 3.** Note the reduced radiolucent opacity (*arrow*) of the air sacs in this lateral survey radiograph of a red-shouldered hawk.

sis with a monocytosis.[3-9] In this case, the CBC was not elevated, but instead suggested a stress leukogram. Based on the extent of the lesions from the radiographs, endoscopic examination, and necropsy, the authors would have expected an elevated CBC. This case reinforces the importance of using multiple diagnostic tests to confirm a diagnosis. Aspergillosis remained on our differential list after receiving the CBC results, but it was not a top disease consideration.

Radiography can be used to assist in diagnosing aspergillosis, but radiographic changes to the respiratory tract should not be considered pathognomonic for this disease. Radiographically, aspergillosis may present as homogenous, inhomogeneous, or ill-defined pulmonary opacities. It is difficult to differentiate bacterial, mycobacterial, and mycotic infections radiographically, but the presence of inhomogenous or ill-defined opacities increases the likelihood of mycotic infection.[10] Involvement of other organ systems, such as the liver, spleen, or bone, may also indicate a mycobacterial or mycotic infection. Mineralized opacities in the lung fields may also be detected, and their presence may indicate mycotic infection. In this particular case, the loss of detail in the air sacs redirected us to respiratory disease. Although it would be expected that the air sacs would be radiolucent, the RSH in this case had increased diffuse radioopacities in the air sacs (Figs 3 and 4). It was based on these findings that we pursued the endoscopic examination.

Although not used in this case, serologic testing for aspergillosis is also possible.[7] One of the limitations associated with these diagnostic tests is that it is difficult to design a serologic assay that can be used to confirm a diagnosis in a wide variety of avian species. Cross reaction of antibodies between species is uncommon, resulting in the need to develop different assays for each species.[6,7] In recent years, newer assays have been developed which provide cross detection between species. The Raptor Center at the University of Minnesota (Minneapolis, MN USA) has developed an enzyme-linked immunosorbent assay that can detect antibodies across a range of avian species, including raptors, ratites, penguins, waterfowl, and gallinaceous species.[6] The assay appears sensitive to detecting chronic infections, but may not be sensitive enough (eg, false negatives) to identify early infections or animals too debilitated to mount an immune response. The results of any serologic test should be interpreted in light of the clinical signs and findings of other diagnostic tests (eg, CBC and radiographs). Evaluating serial titers in these animals is useful both for establishing a diagnosis and response to treatment.[6,7]

Definitive diagnosis of aspergillosis is based on fungal culture. Because this can take several days to weeks to confirm, a presumptive diagnosis may be made from histopathology that shows fungal elements in the tissues. In this particular case, histology and culture were used to confirm the diagnosis. The decision to euthanize the bird during the endoscopic procedure was made before confirmation of the disease because of the

**Figure 4.** Note the reduced radiolucent opacity (*arrows*) of the air sacs in this ventrodorsal survey radiograph of a red-shouldered hawk.

extensive lesions noted during the examination.

Treatment was not pursued in this case for several reasons, one of which was the extent of the lesions. Cost is also often prohibitive for many clients. Treating aspergillosis may require several months and is not always successful.[3-9,11,12] Prognosis improves when the treatment is early and aggressive, including debulking fungal plaques and concurrent long-term antifungal medications. Antifungal medications that have been used successfully to treat aspergillosis include amphotericin B, clotrimazole, enilconazole, fluconazole, 5-flucytosine, itraconazole, ketoconazole, miconazole, and terbinafine. Often, combination therapy with multiple drugs is required. The Raptor Center at the University of Minnesota has found some success using clotrimazole nebulization in combination with itraconazole for slowing and reducing lesions from respiratory aspergillosis.[12] At Louisiana State University, combination therapy with itraconazole (orally) and amphotercin B (nebulized) has proven successful.

Because treatment for aspergillosis is not always successful, and because the disease can be fairly advanced before it is detected, prevention is a more optimal solution for defeating this disease. Housing birds in clean, stress-free environments with good ventilation is strongly recommended, especially for susceptible species.[3] In this case, the bird was housed in an appropriate environment, and it was believed that this infection occurred because of species susceptibility. In those cases where a species is considered to be high risk for developing the disease, prophylactic treatment with antifungals may be used.[3,9] Although this would be useful when dealing with transient raptors, long-term treatment with antifungals may prove problematic. This was the second RSH in the Louisiana State University School of Veterinary Medicine program to develop aspergillosis. The other bird presented with a similar clinical presentation, including a leukopenia. To prevent this problem in the future, RSH hawks are not going to be incorporated into the education program.

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