

Aspergillosis in birds of prey

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ABSTRACT

Aspergillosis is a common disease in raptors held in captivity, and *Aspergillus fumigatus* is frequently found in affected birds. This disease is divided in acute and chronic. The acute form occurs when birds are exposed to an overwhelming dose of spores. The chronic form affects birds under conditions of immunosuppression. Some species are more susceptible to the disease such as gyrfalcons (*Falco rusticolus*), goshawks (*Accipiter gentilis*) and red-tailed hawks (*Buteo jamaicensis*). The clinical signs are very non-specific and a variety of diagnostic procedures can help, including: hematology, endoscopy, radiology, cytology and serology. Indirect ELISA is a very useful tool for the diagnosis or monitoring the progress or response to treatment. The prognosis for infected birds is poor, especially if treatment is not started early. Prevention is the best way for the control of the disease.

INTRODUCTION

Aspergillosis is an infectious, non-contagious disease, caused by fungus of the genus *Aspergillus* that affect humans, mammals and mainly wild or domestic birds.¹⁻⁵ This genus was described for the first time in a lesion of a bird in 1842 by Rayer and Montagne in the air sacs of a bullfinch, but molds likely belonging to the genus *Aspergillus*, were described in wild birds in the early 1800s.^{3,4,6}

The fungus is a ubiquitous, soil saprophyte and grows in organic matter at temperatures up to 25°C.^{1,2,3,7,8,9} The species frequently isolated are *Aspergillus fumigatus*, *A. flavus*, *A. nidulans*, *A. niger* and *A. terreus*,¹⁰⁻¹⁸ but *A. fumigatus* is isolated from 95% of raptors with aspergillosis.^{8,19} This species was first found in the lungs of a bustard (*Otis tarda*) in 1863 by Fresenius, he also applied the term aspergillosis to this respiratory disease.⁶

Aspergillosis is an opportunistic infection, causing the disease under immunocompromised situations of the host or when the bird is exposed to an overwhelming number of spores.^{1,5,18,20,21,22} Stress seems to be the major predisposing factor in the development of the disease and can be caused by shipping, heat, recently capture, or changes in management. Aspergillosis also occurs associated with prolonged disease or when immunosuppressive doses of corticosteroids are used.^{1,2,4,5,9,10,11,14,15,16,21,23,24,25}

Malnutrition, vitamin deficiencies (especially vitamin A), long term antibiotic use, age (young or old) also contributes to the presentation of the disease, as well as trauma, dusty environments, lead poisoning and irritants of the airways like smoke of cigarette or ammonia.^{1,2,3,4,11,19,21,23}

Environmental factors play an important role in the development of the disease, increasing the number of spores to which the birds are exposed. Poor sanitation in nests as well as food or fecal accumulation promote fungal growth. Poor ventilation, in conjunction with these factors, increase the possibility that airborne spores may invade and infect a bird's respiratory system. Wood shavings used as bedding material have been frequently implicated in outbreaks of the disease.^{1,4,10,19,21}

Some species of the genus *Aspergillus* produce mycotoxins, like aflatoxins, produced by *A. flavus* and *A. parasiticus* and ochratoxins produced by *A. ochraceus*, that affect poultry when they consume contaminated food. Other important aspects of the genus *Aspergillus* is that they may cause the brooder pneumonia, originated by hatcher or brooder contamination with spores of this fungus. These spores get into the egg shell, producing embryo and neonatal death.^{3,5,7,26}

Multiple clinical signs may be seen on presentation. This makes aspergillosis one of the most difficult diseases to treat,^{21,25,28} and a good program of preventive medicine is needed, not just to avoid aspergillosis but any other disease that can affect wildlife. Also, it is important to make a quick diagnosis to have a good prognosis, because the treatment is ineffective in advanced cases.

Aspergillosis occurs frequently in birds of prey held in captivity,^{3,5,13,20,28} i.e. in zoos (for conservancy, research or education), falconry and those that are maintained in rehabilitation centers. This disease is responsible for 15 to 30% of deaths in birds of prey exhibited in zoos, but rarely occurs in free-living birds.¹⁹

Birds of prey include diurnal birds, mainly from the order falconiforms and also nocturnal birds of the order strigiforms. Falconiforms are divided in families that include vultures, condors, eagles, hawks and falcons. Strigiforms are all kind of owls.^{5,19}

The species more susceptible to this disease are: Goshawk (*Accipiter gentilis*), Gyrfalcon (*Falco rusticolus*), Snowy owl (*Nyctea scandiaca*), Rough-legged hawk (*Buteo lagopus*), immature Red-tailed hawk (*Buteo jamaicensis*), Golden eagle (*Aquila chrysaetos*). While the resistant species are: Prairie falcon (*Falco mexicanus*) and Harris hawk (*Parabuteo unicinctus*).^{2,5,9,10,11,19,20,21,22,23,25,28} This disease is one of the principal causes of death in captive penguins.^{3,4,10,11,13,16,19,21,25,26,29,30} The Bald eagle (*Haliaeetus leucocephalus*) is more susceptible in cases of lead poisoning.^{21,25}

ETHIOLOGY

Aspergillus is classified as: division: Eumycota, subdivision: Deuteromycotina (fungi imperfecti), class: Hyphomycetes, order: Moniliales, family: Moniliaceae.^{6,26,31} Most of the *Aspergillus* are classified as fungi imperfecti (asexual reproduction), but the perfect state has been found in *A. nidulans* that can produce ascospores.^{8,26,32} Genus *Aspergillus* was classified in 1729 by Micheli and many species have been described.³²

Species of the genus *Aspergillus* are molds formed by septate hyphae that are tubular branched structures from 2 to 10 μ . when the growth starts, hyphae get together forming a mycelia. Vegetative mycelia consists of superficial hyphae and hyphae that are on the surface are the aerial mycelia. These structures produce conidiophores.^{7,29} Conidiophores are formed in foot cells and end in a vesicle. In this structure grow one line of phialides or one line of metulae and over these structures one line of phialides that produce chains of conidia or spores. Conidia are uni- or multi-nucleated, but always one-celled. The shape can be globose, elliptical or oval, and the surface can be smooth, finely rough or echinulated. The color of the conidia determines the color of the conidial head, which also usually determines the color of the colony. The function of the conidia is air dissemination of the fungus.^{26,29,32,33}

Vesicle and chains of conidia are called conidial head and the shape can be columnar or spherical. If phialides are produced just in the vesicle surface, the shape of the head is columnar as in *A. fumigatus* and *A. terreus*, if phialides are produced over the whole surface, conidia chains are radiated and the shape of the head is spherical as in *A. Niger* and *A. flavus*.^{26,32,33}

This fungus has a worldwide distribution; air and soil of almost any part of the world contain conidia of different species.^{15,26} Genus *Aspergillus* is usable as a food in many different substances because of the number of enzymes that it can produce. The only two main requirements of substrates for the fungal growth are organic matter and moisture. If both factors are present, the fungus can grow in almost any substance.²⁶

The species that can cause infections in birds are: *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans* and *A. terreus*, but the most important species is *A. fumigatus* because is isolated in 95% of affected birds.^{3,10,11,12,13,14,15,19}

A. fumigatus produce haemolysins, proteolytic enzymes and other toxic factors but their role in the pathogenesis is not know.⁸ However, it is known that an antibiotic produced by this species named fumagilin, may have poor antibacterial activity but is a potent amoebicide, specially against *Entamoeba histolytica*.^{8,26} *A. flavus* produce aflatoxins that will be described later.

PATHOGENESIS

Infection generally occurs when the bird inhales airborne spores. The organism may then penetrate respiratory tissues, reproducing by simple division of tubular hyphae to form mycelia. Tissue invasion incites an inflammatory response, with heterophils, lymphocytes, monocytes and some giant cells infiltrating the lesion.^{1,2,6,11,34}

Severity of lesions depend on chronicity of infection, organs affected and the number of spores inhaled. This disease is divided in acute and chronic.^{1,3,4,21,35}

ACUTE FORM

Seen most often in wild birds or psittacines under poor sanitary or ventilation conditions. It occurs following the inhalation of an overwhelming number of spores.^{2,10,21,23,35} In this form of the disease, massive, rapid colonization of the lungs occurs, and the lungs become diffusely infiltrated with miliary granulomas. Severe dyspnea is often seen, with rapid progression to death, because treatment is usually ineffective. The course of the acute form is usually less than a week, but anecdotal reports indicate that apparently healthy raptors exposed to moldy hay have died within 48 hours.^{2,10,15,21,23,25} Diagnosis is made at necropsy, where these pulmonic nodules are visible grossly.^{1,3,19,35} Histologically, multiple foci containing fungal hyphae and rimmed by hemorrhage and heterophilic, mononuclear and multinuclear cell infiltrates are seen. The fungus can be isolated from many other tissues like liver, spleen or blood.^{1,10,19}

CHRONIC FORM

This is the most commonly observed form of the disease and follows a stressful event or immunosuppression. Under these circumstances, the bird is unable to effectively eliminate or contain even small numbers of *Aspergillus* organisms.^{1,2,15,21,35} Chronic aspergillosis is divided into focal and generalized. It is possible to find both forms present in one patient. Focal aspergillosis has better response to treatment, but in generalized form, the treatment is prolonged and generally ineffective and the prognosis is poor.^{2,4,6,11,12,21,25}

o **Focal**

NASAL: is presented as a solid mycotic plug or aspergilloma localized in nares or choana. The organism invades the sinus, and nasal bones. It may present as a unilateral lesion. Combined infections of gram-negative bacteria and *Aspergillus* are frequent and the fungus can not be detected if the diagnosis is made based solely on bacterial culture. Histopathological examination of granulomas generally shows a necrotic foci surrounded by macrophages, heterophils and giant cells, sometimes within a connective tissue capsule.^{10,11,21}

TRACHEAL: is characterized by the presence of mycotic colonies above the syrinx or at the bifurcation due to air turbulence which deposits spores in this section of the air ways, in addition, the narrowing in the respiratory tract at this point may predispose it to blockage with necrotic debris and caseous exudate. The lesions reduce air movement through the lower airway, so inspiratory dyspnea exists. Mycelia penetrate walls and in combination with inflammatory cells and connective tissue, granulomatous nodules are formed. Treatment can be successful if dyspnea is corrected and treatment is started immediately.^{1,4,5,10,11,21,23,25}

CUTANEOUS: Necrotic granulomatous dermatitis has been described and *A. fumigatus* was isolated from infected tissue. Skin lesions have been encountered under moisture-retaining bandages. Cutaneous lesions and their manifestations are rare in birds. Atkinson (1998) reports one case in a wing of a Great horn owl (*Bubo virginianus*) and Abrams (2000) on the head of an hybrid peregrine-gyrfalcon (*Falco peregrinus-Falco rusticolus*).^{2,3,6,9,23}

OPHTHALMITIS: exist 2 presentations: one superficial and the affected tissues are conjunctiva and external surfaces of the eye with the development of a cheesy exudate or plaque forming beneath the nictitating membrane.^{3,6,9,17,36} The second presentation is deeper and occurs rarely and probably as a result of hematogenous dissemination of the organism from a primary respiratory infection, reaching the posterior eye. Pathological changes can occur in the vitrous humor and extend into adjacent tissue. Pecten edema can be present with heterophil infiltration and mononuclear cells. Also it is possible to observe fungal hyphae, heterophils, macrophages and cell debris in the retina.^{6,23}

In rare cases, well-encapsulated *Aspergillus* granulomas can be found in the trachea, esophagus or under the skin. These probably represent an effective walling-off of early fungal infection on the part of the bird.¹⁹

o **Generalized**

PULMONARY: in this presentation yellowish granulomas can be seen disseminated throughout the pulmonary tissue (figure 1). Lesions may also be seen in air sacs and their extensions. Characteristic lesions are granulomas that are larger and more diffuse than other presentations possibly indicating the primary site of infection. Granulomatous lesions become caseated as the disease progresses, and it is possible to find suppurative exudate accumulated in the bronchi.^{3,4,8,15,21,24,33}

In chronic cases, there is pulmonary hypertension caused by granulomas and necrosis can occur in the tissue (figure 2) leading to right ventricular dilatation or ascites. In well oxygenated sections of tissue like bronchi the organism can sporulate.^{6,7,11,34}

After inhalation, spores can disseminate through the blood producing lesions in brain, bones, pericardium, pecten and other tissues.^{2,3,6,10,17,23,25,33,36} Cerebral presentation is described by encephalitis or meningoencephalitis in different bird species. Necrotic caseous lesions surrounded by giant cells in the brain or cerebellum may be observed causing granulomatous encephalitis with hyphae can be seen in the central area of some lesions. There are reports in the literature that birds presenting with the infection in their lungs and air sacs in many cases have kidneys and liver involvement. In bones, the infection can cause vertebral deformities resulting in paralysis.^{6,7,17,24} The extension of the fungal colonization dependent upon host immune system integrity. Clinical signs are often not apparent until fungal colonization is largely extended.¹

IN AIR SACS: This presentation is slowly progressive and results from persistent exposure to low levels of spores in immunocompromised birds.^{10,23} Initial lesions are found in zones with high oxygen tension and poor blood circulation, like air sacs. Specifically the caudal thoracic, and the abdominal air sacs where abundant caseous and necrotic debris may be found.^{1,4,6,10,15,21,33}

Hyphae can come together and create plaques over the connective tissue and can completely cover air sacs, In the case of *A. fumigatus* these plaques are grayish-green in color. In some cases, sporulation can occur in air sacs after a growing period and when oxygen conditions are optimal.^{1,2,3,6,8,14,15,19,33} (figure 3)

Fungus dissemination can be the result of direct contact with pneumatic bones, because there are diverticula of various air sacs that connect to lungs, like humerus, coracoids and femur,^{2,11,23} also, lungs, liver (figure 4) and adjacent structures can contain yellowish-white granulomas.^{1,6,8,19,29}

This is the most chronic and debilitating form of the disease and is the least responsive to treatment.¹⁹

AFLATOXINS

Aflatoxins are mycotoxins produced as secondary metabolites of some fungi, they are a group of 18 or more related compounds. *Aspergillus* species that produce aflatoxins are: *A. flavus* and *A. parasiticus*. The 4 main aflatoxins are B₁, B₂, G₁ and G₂ and are the most carcinogenic compounds in the nature. Aflatoxin B₁ is the most toxic and the primary effect is hepatotoxicity.^{26,37,38} *A. flavus* produces aflatoxins B₁ and B₂, and *A. parasiticus* produces the 4 aflatoxins. However, not all strains are able to produce toxins, i.e. just 50% of *A. flavus* strains can produce aflatoxins. Other important variables are that for most of the fungi to produce toxins they need adequate temperature, humidity, oxygen and substrate.^{26,37}

The main effects of aflatoxins include: inhibition for protein formation, altering function and integrity of the liver, carcinogenesis, and blocking immune responses.³⁷

Other mycotoxins produced by genus *Aspergillus* exist i.e. Ochratoxins, produced, by *A. ochraceus* are hepatotoxic and nephrotoxic. Aflatoxins and ochratoxins affect mainly poultry when fed contaminated feed,²⁶ but referring to birds of prey, Heidenreich (1997) mentions that affected birds do not die of respiratory insufficiency, but rather from the effects of aflatoxins produced by the fungus. This explains why birds may die with only a few fungal granulomas present in the lungs or just one or two colonies of mold in the air sacs.¹⁹

CLINICAL SIGNS

Clinical signs in any bird with acute form can include depression, polyuria, diarrhea, anorexia, cyanosis, dyspnea and open beak breathing. Occasionally birds die suddenly with any sign involving respiratory system.^{1,2,3,4,5,9,10,19}

In the Chronic form: clinical signs vary with the location of the infection. Being that the respiratory system is the primary one involved, problems with this system are frequent. The clinical signs depend on the extension and location of the lesions. Change in the voice, inability to vocalize or respiratory noises, can be heard when the lesion involves the airways, especially the syrinx.^{2,4,5,21,24,25} Also, severe dyspnea can be noted if the lesion is big enough to obstruct the trachea or primary bronchi.¹

In a great number of cases, there are no specific signs, and weight loss, muscle wasting, anorexia, diarrhea, vomiting, polyuria and depression or lethargy are observed. Green coloration in urates (biliverdinuria) can be seen and hepatomegaly when the liver is involved.^{1,2,4,5,6,10,11,19,21,23,33,39} Some respiratory signs are seen just before the bird dies.¹⁹

If lungs or air sacs are involved, depression, dyspnea, tachypnea or exercise intolerance can be seen. The first respiratory sign noted is a prolonged tachypnea, following handling or flight. In pulmonary aspergillosis, clinical signs take from days to weeks to be visible, but when air sacs are involved, it is not rare that lesions develop over months and if they invade pneumatic bones such as the humerus, a wing droop can be observed.²² If main airways are not obstructed, respiratory signs can be totally absent, inclusive with an extended lesion and if the lungs remain functional, the birds are capable of surviving for a long time.^{1,2,6,19,21}

Ataxia, torticollis, seizures or loss of equilibrium, can indicate that the CNS is involved. Other signs include head shaking and tail “bobs”.^{1,2,6,10,17}

Granulomatous extension from the caudal air sacs to the spine or sacral plexus cause uni or bilateral paresis or paralysis.^{1,10,11,17,21}

If fungal colonization is limited to upper airways such as the periorbital sinus and nasal cavity, uni or bilateral muroid or mucopurulent nasal discharge may be present with rhinitis. Nostrils may become plugged with inspissated exudate. The common sign in this presentation is open beak breathing. If an extension of the lesion occurs inside the beak or periorbital bones, it can produce severe destruction of the normal architecture, causing beak malformation and nasal or periorbital swelling.^{1,21}

DIAGNOSIS

Ante-mortem aspergillosis diagnosis can be difficult, principally in chronic cases. The clinical signs are nonspecific and fungus mycelia are generally intimately associated with the tissues, making them rarely visible in exudates or body fluids. A careful clinical history can reveal the presence of poor sanitation in the environment. An immunosuppressing factor, chronic weakness history, weight loss, change in voice or exercise intolerance, also species susceptibility to this disease must be considered. Aspergillosis must be suspected in cases of weak animals that do not respond to or get worse with antibiotic treatment.^{1,2,7,11,12,19,21,25}

It is important to mention that to establish a definitive diagnosis, many tests should be made, like hematology, serology, cytology, radiographs and endoscopy, because this fungus is ubiquitous and it is easy to contaminate the sample when the cultures are made which may affect the results.^{1,2,40}

- **Hemogram**

Severe leucocytosis of 20, 000 or more than 100, 000 cells/ μ l is common. The differential count usually reveals heterophilia with a left shift, monocytosis and lymphopenia.^{1,2,4,19,28,35} However, gyrfalcons (*Falco rusticolus*) have poor responses in white cells production, compared with other birds of prey and total white cells count may range as low as 12, 000 to 15, 000 cells/ μ l.^{1,12,19,21,25}

In chronic infection non-regenerative anemia can be present, an increase in total serum proteins and in the globulin portion, and an increase in AST and bilie acids when the liver is involved are noted.^{1,19,21,25}

- **Serology**

Indirect ELISA is a useful tool for the diagnosis and for monitoring the progress and the response to the treatment of aspergillosis in birds of prey. A specific conjugated anti-falcon exists, because falconiforms don't have a good response to anti-turkey conjugated that is useful for other species. This test can detect antibodies against *A. fumigatus*.^{10,19,21,23,25,27}

Experimentally, antibodies can be detected one week after infection, which is much earlier than the clinical signs of the disease are present and the treatment can be started having better results.^{19,25}

The available conjugate for falconiforms do not recognize immunoglobulins from owls and there are any anti-owl conjugated available.^{2,23,27}

Other tests that have been used but with limited diagnostic value are: immunodiffusion in agar gel, indirect hemagglutination, serum electrophoresis and indirect immunofluorescence.^{5,6,8,12,33,41}

- **Endoscopy**

Endoscopy is an invaluable tool for the diagnosis of aspergillosis. In-patients with severe dyspnea, tracheoscopy can reveal a lesion obstructing the trachea or syrinx. In the lumen, plaques or white discharges can be noted, samples for cytology and culture should be taken. Tracheoscopy requires injectable or inhalant anesthesia.^{1,5,25,40}

Air sac endoscopy can reveal diffuse opacity or the presence of white or yellow fungal plaques, these plaques can be covered with a grayish mold. Samples are taken directly or with air sacs washes for cytology and culture.¹ To perform the air sac endoscopy, the bird is put under anesthesia. After surgical preparation of the selected site (The incision is done behind the last rib, just posterior to the dorsal part of the last rib and above the level of the junction of the vertebral and sternal parts of the rib cage and in birds above 1kg between the last two ribs) the skin is picked up with a rat-toothed forceps to form a "tent". A nick is made with scissors and their points used to spread and change the hole to reveal the underlying muscle and ribs. The point of a closed pair of a straight mosquito forceps or round pointed scissors is quickly thrust through the muscle and into the abdominal cavity The points of the instrument used are then opened sufficiently to enable insertion of the endoscope.⁴²

- **Radiographs**

Radiographic changes may not be visible in early cases. However, in advanced cases radiographic abnormalities include a prominent parabronchial pattern, (figure 5) lost of definition and asymmetry in air sacs by consolidation, enlargement of air sacs and/or focal densities in lungs or air sacs. Many of the lesions in air sacs are found in the cranial part of the abdominal air sac, next to the lungs.^{2,21,25} Nephromegaly or hepatomegaly are seen when these organs are involved. When radiographic changes are visible prognosis is poor because of the disease is in an advanced stage.^{1,2,4,9,10,11,19,25}

- **Organism identification**

The organism identification is made by histopathological or cytological study of the lesions and by organism culture from the site of infection, but it must be considered that isolation of *Aspergillus* species by culture is not a definitive diagnosis, because the organism is in the environment and contamination is common.^{1,8,9,10,11}

- **Culture**

To do the culture from the air sacs the procedure is the same as in the endoscopy to access to this structure. 3 to 5 ml/kg of saline are used and the air sac is irrigated using a siryngue and a urinary catheter, then the liquid is recovered and cultured.²³ This procedure should be done in as sterile a fashion as possible.

Trachea culture is done using a sterile nasopharyngeal swab, introducing it deep into the trachea during the inspiratory process, when glottis is open and trying to not make contact with oral cavity to avoid sample contamination.^{19,25,28} This procedure can be done with or without anesthesia. The recovered material in the swab is immediately transfered to an appropriate culture media and incubated at 37°C. Also, a tracheal wash can be performed, using a syringe, a feline urinary catheter and 3ml/kg of saline. The catheter is introduced into the trachea, the liquid is injected and recovered immediately for culture and cytological study.^{19,23}

Aspergillus species grow at temperatures from 25 to 37°C, some species can grow at 45°C in Sabouraud dextrose agar or blood agar and antibiotics like cloramphenicol must be used to avoid bacterial contamination, however, *Aspergillus* species are sensitive to cycloheximides.^{1,6,8,17,24,26,29,33} Following up colony characteristics of some species are described.

Aspergillus fumigatus colonies have a diameter of approximately 3 to 4cm in 7 days. The flat colonies are white at first, then bluish green as conidia begin to mature, especially near to the center of the colony. As the colony matures, conidial masses become gray-green, while the colony edge remains white. A distinctive feature of *A. fumigatus* is the development of columnar masses of chains of conidia arising from the vesicle. These conidial chains can reach a length of up to 400µ. This organism is resistant to high temperatures and can grow well at 45°C. These are the most typical characteristics, but variation occur in colony color, both surface and reverse, and colony morphology.^{1,6,8,31}

A. flavus grows very rapidly, obtaining a colony diameter of 6-7cm in 10 days at 25°C. The colony begins with a white color, turning yellowish to yellow-green with a white edge as conidia develop. Mature colonies may become somewhat olive-green.^{6,8,31}

A. niger begins with a white color, but rapidly develops a black color as the conidia mature.(figure 6) The colony reverse is yellowish.^{8,31}

- **Microscopic exam**

Samples can be seen microscopically, using wet preparations with 20% KOH, (figure 7),lactophenol blue, calcofluor white or new methylene blue. First put some drops of the stain on the slide, and then add the sample and covering with the coverslip. When KOH is used, the slide may be heated over a flame.^{6,7,8,11,29,33}

Organisms can be seen with slides stained with Hematoxilin-Eosin, PAS or Grocott's.^{1,7,8,11,24,29,34} Microscopic identification also, can be made using the culture colonies, using lactophenol blue stain (figure 8) or new methylene blue.^{1,11,14}

Microscopically, *Aspergillus* mycelia are composed by tubular septate hyphae. Occasionally, fruiting bodies are seen when samples come from lungs or air sacs, which are places where the organism can mature.^{1,11,14}

Aspergillus fumigatus presents smooth conidiophores with a length up to 300 μ and a diameter from 5 to 8 μ . Vesicles are from 20 to 30 μ in diameter, with a single series of phialides. Phialides are from 6 to 8 μ in length and are arranged upward paralleling the axis of the conidiophore. Conidia are echinulated, spherical to semispherical and from 2 to 3 μ in diameter.^{6,8,29,31}

A. flavus contains conidiophores of up to 100 μ in length and 10 to 65 μ in diameter. Vesicles are spherical, from 30 to 45 μ in diameter and phialides in 2 series over the vesicle surface, but can be in one series. Conidia are elliptical or spherical, echinulated and from 3 to 6 μ in diameter.^{6,8,29,31}

A. niger is observed with big fruiting bodies. Vesicle is spherical from 50 to 75 μ . Conidia are black, rounded and smooth with phialides in one or two series.^{8,31}

DIFFERENTIAL DIAGNOSIS

Differential diagnosis in a mature bird showing weight loss, and severe heterophilia can include chlamydiophylosis and tuberculosis.^{4,10,11} Neoplasias can some times cause weight loss and heterophilia. Differential for dyspnea include increase in abdominal pressure from a mass, ascites, hepatomegaly, pneumonia and inhalation of foreign bodies.¹¹ Vitamin A deficiency can cause ocular lesions. Some granulomas can be produced by bacteria. Other mycotic infections must be suspected like candidiasis.^{2,10,19}

TREATMENT

Treatment for aspergillosis is complicated, because the drugs used do not reach the fungus that is walled-off by the bird's inflammatory response and therefore isolated of the bloodstream. This disease has a poor prognosis when the infection in the tissues is extensive and when only systemic drugs are used. The best treatment results if the granulomatous lesions are debrided and a topic treatment, in conjunction with a systemic therapy is given.^{1,9,12,19,25} When the patient shows cachexia, dyspnea and vomiting is beyond of the point of treatment.^{19,25}

Options for the treatment of aspergillosis are limited, The drugs used include: itraconazole, flucytosine, fluconazole, clotrimazole, miconazole, ketoconazole and amphotericin B.^{1,2,4,5,6,9,25,28,29}

ITRACONAZOLE

Itraconazole has a high specificity against *Aspergillus*,⁴³ is given orally, using a dose of 5mg/kg twice a day, or 10mg/kg once a day, 15mg/kg twice a day can be given, this depends of the reaction of the bird to the drug, because if it is given in high doses it can cause depression and anorexia and if these signs continue, the use must be suspended. These adverse effects have been related to hepatic toxicity and can vary between different bird species. The absorption of this drug is increased with fat consumption.^{1,12,23,43} Itraconazole is the treatment of choice for this disease.⁹

Jones (2000) describes itraconazole pharmacokinetics in red-tailed hawks (*Buteo jamaicensis*) and he observed that plasma disposition after daily doses of 5 and 10mg/kg for 14 days is minor to the granivorous birds, however, disposition in tissues like liver, lungs, kidneys, small intestine and air sacs is good, except cerebrum who had a low disposition. Also demonstrated was a better concentration when 10mg/kg were used.²⁰

In severe aspergillosis cases, treatment can be combined with amphotericin B IV. Also is used in nebulizations with clotrimazole 1% in 2 or 3ml saline 1.5 hours/day for 4 to 6

weeks or with amphotericin B besides intratracheally if bronchial and tracheal lesions are detected. Long treatment is recommended, in many cases for some months.^{1,11,12,20,21,42}

Abrams (2001) explains the treatment in a hybrid of peregrine falcon - gyrfalcon (*Falcon peregrinus* x *Falco rusticolus*) who presented with blepharitis and dermatitis caused by *Aspergillus* sp. In the beginning the diagnosis of a fungus was obscured because of the more common presentation of trauma and secondary bacterial infection in birds of prey. After antibacterial treatment, condition progressed to severe blepharitis and dermatitis that involved upper and lower eyelids of both eyes and the head. Diagnosis consisted of histopathological exam of the lesions where septated hyphae were seen, and sample culture, recognizing fungus of the genus *Aspergillus*. Treatment started with itraconazole, dose of 15mg/kg once a day, 2 weeks later, head lesions were debrided, the dose was increased to 15mg/kg twice a day and a topic ointment was applied twice a day. A month and a half after the use of the itraconazole, lesions decreased and itraconazole and clotrimazole were still used at the same dose and frequency, at 2 months the skin was normal on the top of the head and around the eyes. Use of itraconazole continued for one more month, and the total time of treatment was 3 months.⁹

AMPHOTERICIN B

Amphotericin B can be used for initial treatment of severe infections. It is fungicidal and rarely resistance occurs for *Aspergillus* species. This drug is not well absorbed when is given orally and causes irritation with intramuscular or subcutaneous injections. In severe cases of aspergillosis affecting respiratory system, amphotericin B, IV can be given simultaneously, intratechal and in nebulizations, followed by itraconazole or flucytosine. Treatment is started with IV applications at a dose of 1.5mg/kg TID for 3 to 5 days.^{1,23,24,25,42} It is worth mentioning that this drug is potentially nephrotoxic in birds, but the prolonged use in birds of prey has not been associated with nephrotoxicity.^{11,43}

When nebulization is used, the drug needs to reach the internal surface of the air sacs, to achieve this, the drop size must be under 5µ in diameter, otherwise, drops do not remain suspended enough in the air stream to reach the intended area. Vaporization of drugs does not work due to the fact that drops are too big and get condensed in the

upper airways. The drug needs to be given from a nebulizer to a chamber big enough to keep the bird comfortable in order to eliminate material loss from dead space effects. When amphotericin B is used for this treatment, 100 mg of the drug in 15 ml of saline, or it is even better if can be used in a vehicle like tyloxapol, because this substance helps the drug to have a better dispersion. Nebulizations are given for 20 minutes, 3 to 4 times a day. Nebulization is useful for the drug to reach the air sacs without systemic absorption.^{19,23,42}

Amphotericin B can be used directly in the air sacs or coelomic cavity using a dose of 0.5mg/ml (50mg/L using saline) when lesions have been identified through endoscopy, and removal of mycotic plaques or granulomas is needed.^{1,19,35,44}

In nasal aspergillosis, treatment depends on successful plug removal or dissolution by nasal, choanal or sinus washes, and systemic fungistatic treatment should be initiated immediately and continued until total remission. Vigorous flushing with saline solution under isoflurane anesthesia is sufficient to initiate breakdown of the plug. Surgical excision by trephination should be considered if mechanical breakdown fails. Once partial patency to the choana is established, a combined fungicidal/proteolytic solution can be used to flush the nares. The solution is made by combining 0.2 to 0.4ml of a commercial neomycin-chymotrypsin-trypsin-hydrocortisone ointment with 1.0mg/kg amphotericin B and then diluting the mix in 20ml of saline solution. The resulting combination has proven to be effective in dissolving and dislodging the caseous plug; 10 ml should be flushed vigorously but in small amounts through each naris. Patency through the upper respiratory tract should be verified by observation of drainage through the choana. Anesthesia and tracheal intubation are seldom necessary once patency to the oral cavity is established. After the third or fourth sinus flush, the plug usually becomes dislodged, and is seen to appear to the choana. Flushes should be continued using only physical restraint. Maintaining the unsedated bird in a vertical position is critical to preventing aspiration. If the solution enters the lower airway, it can cause severe tissue damage. Daily topical treatment of the nares and sinuses should continue until the lesion and signs disappear. Treatment by sinus lavage rarely exceeds 7 days.²¹

The treatment for the tracheal or syrinx form of Aspergillosis may be successful if the lesion can be removed surgically through an incision in the lower portion of the trachea

or removing it endoscopically,^{23,35} or if the growth is controlled using antifungal drugs. When the bird presents with open beak breathing, noticeable neck extension, discomfort, or acute respiratory crisis, air sac cannulation is indicated.²¹ This procedure can be made using a cut-off endotracheal tube as a cannula, the bird is put under anesthesia and the cannula is inserted into the left side of the bird's abdomen, just posterior to the dorsal part of the last rib and above the level of the junction of the vertebral and sternal parts of the rib cage. The purpose is to insert the cannula into the caudal thoracic air sac and not into the abdominal air sac which is deeper in the abdomen. In larger birds (above 1 kg) it may be possible to insert the cannula between the last two ribs. In cases of severe airway obstruction, this procedure is made just with physical restraint of the bird and if done quickly, seems to cause a little discomfort in the bird. After surgical preparation of the selected site the skin is picked up with a rat-toothed forceps to form a "tent". A nick is made with scissors and their points used to spread the hole to reveal the underlying muscle and ribs. The point of a closed pair of a straight mosquito forceps or round pointed scissors is quickly thrust through the muscle and into the abdominal cavity. The point of the instrument used are then opened sufficiently to enable insertion of the plastic cannula which can be sutured in place. The surgeon can check if air is moving freely through the tube by holding a wisp of cotton wool or fine suture material near to the opening and watching for fluctuation during each respiration.^{23,42} This procedure can also be performed by securing a large-bore (18 gauge or larger) flexible Teflon intravenous catheter in one of the abdominal air sacs to relieve any dyspnea by extratracheal airflow. Another air sac that can be used is the clavicular.⁵ Intratracheal injection of amphotericin B (1mg/kg diluted in 2ml/kg saline) twice a day using a feline urinary catheter,^{1,23} may eliminate the granuloma because amphotericin B is a fungicide and direct contact with the lesion can be an effective method of treatment. The granuloma may become larger until it bursts at the center, giving it the appearance of a rosette. This lesion will then rapidly decrease in size and become dislodged. The debris fall as a plug into the syringe or one of the bronchi. Total obstruction of the lower airway may initiate an acute respiratory crisis. Extratracheal respiration through an air sac cannula prevents asphyxia. Complete blockage, if it occurs, is fatal. Once endoscopy reveals no visible lesion, the air sac cannula can be removed. As part of treatment itraconazole can be used orally for 90 to 120 days, but treatment must be suspended if the bird presents with anorexia or depression. Positive results have also been noted using flucytosine.²¹

FLUCYTOSINE

Flucytosine can be given orally using doses of 20 to 60mg/kg twice a day in conjunction with amphotericin B. ^{1,19,25,43} This drug is fungistatic and must be given for a long period, in many cases for 6 or more months. Flucytosine is widely distributed to tissues that are difficult to penetrate such as, the CSF, eye and joints. Resistance develops quickly when the drug is used alone and should be used in combination with itraconazole or amphotericin B. Hemogram monitoring is recommended because this drug can cause bone marrow toxicity. ^{1,43}

FLUCONAZOLE

Fluconazole, in contrast to ketoconazole and itraconazole is highly water soluble and is readily absorbed from GI tract regardless of acidity or food intake. It penetrates the CSF, brain tissue, ocular fluids and sputum and is the drug of choice in situations where penetration into the CFS is desirable. It can be used in combination with itraconazole or ketoconazole. The dose used is 15mg/kg twice a day, orally. ^{21,42,43}

KETOCONAZOLE

Ketoconazole is used orally at doses of 20 to 30mg/kg twice a day for 2 to 6 weeks and can be used in conjunction with other antifungal drugs. It is widely distributed to tissues but is highly protein-bound and does not significantly penetrate into the cerebrospinal or ocular fluids. Ketoconazole is considered less active and potentially more toxic than fluconazole and itraconazole. ^{1,42,43}

There are different antifungal drugs in ointments for the topical treatment of Aspergillosis like amphotericin B, miconazole and enilconazole. ^{9,23,43}

Birds with chronic Aspergillosis show a severe immunosuppression and support therapy is needed, including fluids, force feeding and a warm environment, also it is important to decrease any factor that can cause stress in the birds, in gyrfalcons (*Falco rusticolus*) psychological well-being seems to play an important role. ¹⁹

Many times Aspergillosis treatment must be continued for some months and to monitor the treatment response, white blood cells count and indirect ELISA are useful. ^{23,43}

PREVENTION

Aspergillus genus is an opportunistic pathogen, therefore every attempt should be made to reduce predisposing immunosuppressive factors such as stress and malnutrition, good management of the birds is essential.^{1,7,10,11,19,21,24,25} To avoid inhalation of a large number of spores, birds should be housed in a well-ventilated area, with a bedding changes daily. When treating other illnesses, the benefits of long term or repeated antibiotic usage, or the use of immunosuppressive doses of corticosteroids, must be weighed against the possibility of opportunistic deep mycotic infections.¹

Flucytosine at dose of 50 to 60mg/kg twice a day for 10 days or itraconazole, at dose of 10mg/kg once a day for 10 days (both orally) have been used prophylactically in birds deemed to be at high risk for the development of aspergillosis. This type of prophylaxis is of value for any endangered raptor species during stressful periods such as transport, after injuries and during medical treatment.^{6,12,19,28}

In recent years some breeders have tried to counteract the susceptibility of gyrfalcon to aspergillosis by crossing them with the relatively resistant saker falcon. Indeed, gyrfalcon - saker falcon hybrid (*Falco rusticolus* x *Falco cherrug*) appear to be less vulnerable to fungal infection. As these hybrids are back-crossed to gyrfalcons, their susceptibility seems to rise proportionally to the degree of gyrfalcon genes.¹⁹

IMMUNIZATION

Some autogenous vaccines have been applied and seem to be effective in decreasing cases of aspergillosis, but there is not much information about this vaccine. This measure seems to be the most important for prevention to this disease, but more studies are needed.^{6,10,11,19,25,28}

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FIGURES

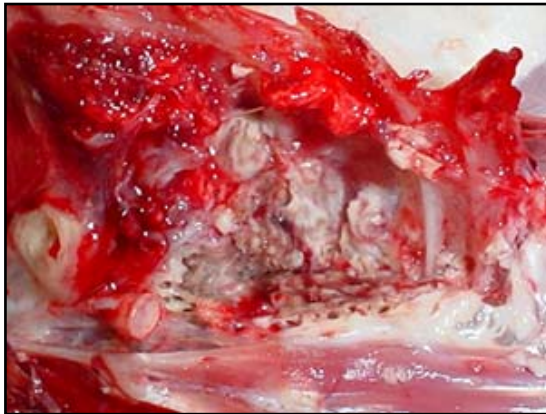


Figure 1: Multiple granulomas in the lungs immature red-tailed hawk (*Buteo jamaicensis*).

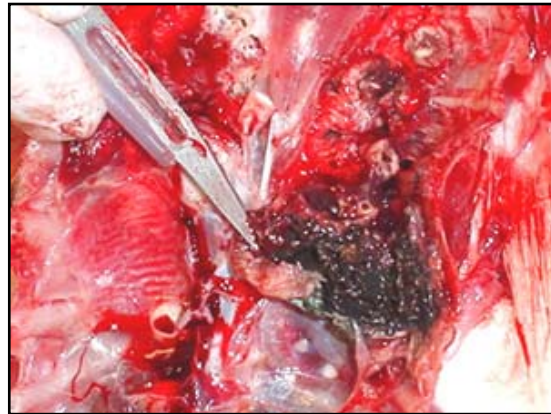


Figure 2: In severe cases of generalized aspergillosis, the granulomas cause necrosis in pulmonary tissue.

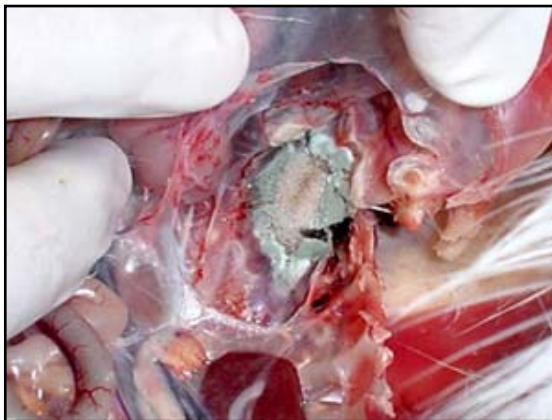


Figure 3: *Aspergillus fumigatus* colony with spores in the caudal thoracic air sac of an immature red-tailed hawk (*Buteo jamaicensis*).

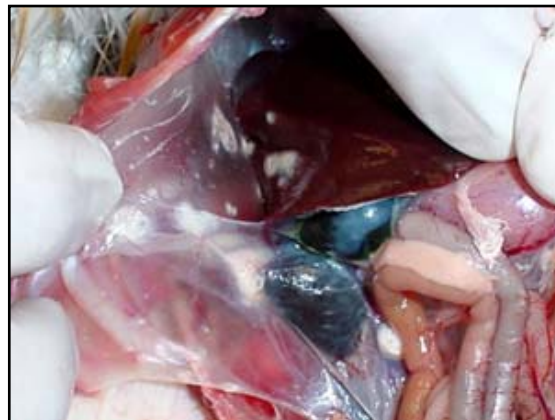


Figure 4: Lesions dissemination by direct contact from the caudal thoracic air sacs to the liver.

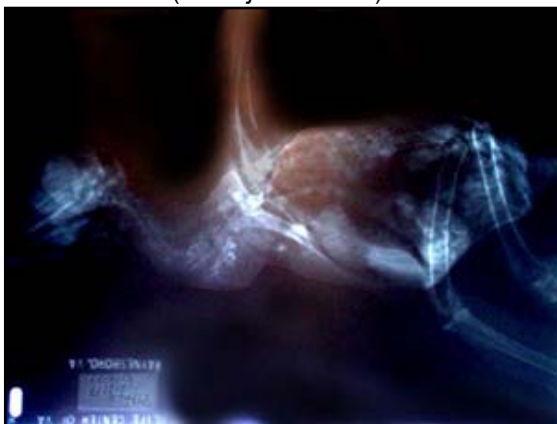


Figure 5: Radiographs of an immature red-tailed hawk (*Buteo jamaicensis*) with a prominent parabronchial pattern.



Figure 6 *A. niger* culture in Sabouraud's dextrose agar.

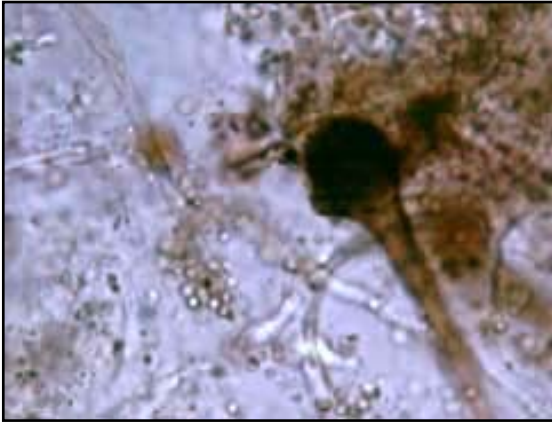


Figure:7: KOH microscopic examination, *A.fumigatus*



Figure 8 *A.fumigatus* lactophenol blue stain, showing conidial heads

Appendix I Indirect ELISA in falconiforms.²³

A positive result indicates either active infection, long term exposition or a high level of antibodies against *Aspergillus fumigatus*, resulting from a previous infection. A negative result indicates no antibodies, either as a result of lack of disease or inability to produce them. This test was developed at the Raptor Center (1929 Fitch Avenue, St Paul, Minnesota, 55108, USA). You can still obtain results from them.

In the test three categories of response are used:

1. **Below cut-off** (optical density below to 0.12), which implies no detectable antibodies, and is a category in which false negatives have been encountered only in circumstances where the patient, in addition to having aspergillosis, had another debilitating condition such as tuberculosis or lead poisoning.
2. **Mid-range** (optical density between 0.13 and 0.30), which implies exposure and low level antibody production either due to low level disease development or poor immune response to severe diseases.
3. **High-range** (optical density between 0.31 up to slightly over 1.0) which is associated with vigorous immune response and may bode well for recovery. An affected bird often yields a mid-range response early in the disease that increases into the high range during the second to fourth week of treatment. Failure to show increasing optical density readings during treatment implies lack of antibody response and may be indicative of a guarded prognosis.