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Aspergillosis in Aviary and Wild Birds

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Introduction

Aspergillosis is a non-contagious, infectious fungal disease caused by saprophytic fungi in the genus *Aspergillus,* which are associated with soil, decomposing organic matter, agricultural waste grains, silage, litter and mouldy feed.

These fungi are an important cause of mortality in captive aviary birds and under favourable conditions in wild free-living bird populations. Clinical disease is predominantly respiratory, although any organ system may be involved with a variety of clinical manifestations.

Aetiology

Clinical disease is mostly caused by *Aspergillus fumigatus* although *A. flavus*, *A. niger*, *A, glaucus*, *A. nidulans* and mixed infections have also been documented. *A. fumigatus* spores are much smaller than the spores of other *Aspergillus* spp and this is thought to be the reason for the high incidence of *A. fumigatus* associated airborne infections, as the smaller spores can invade deeper into the respiratory tract in narrower airways. Aspergilli can also contribute to disease in various other ways.

They can produce mycotoxins (aflatoxin, ochratoxin) in feed, producing mycotoxicosis when ingested. *Aspergillus niger* and *A.flavus* can produce large quantities of oxalates on feedstuffs resulting in oxalate poisoning if contaminated food is consumed. Inhaled fungal elements can act as allergens in some birds.

Calcium oxalate crystals have also been documented at sites of *Aspergillus* infection with tissue crystal deposition being seen most commonly in the lung and air sacs in conjunction with respiratory aspergillosis.

Epidemiology

Aspergillosis affects a wide variety of avian species and occurs frequently in immunosuppressed birds or birds exposed to high levels of fungal agent. Factors associated with immunosuppression include administration of tetracyclines, long-term steroid therapy, vaccination, metabolic bone disease, malnutrition with hypovitaminosis A, overcrowding, transportation, quarantine or capture, starvation, temperature extremes, migration, in-breeding, immunosuppressive viral infections (circovirus), toxicosis (heavy metals), traumatic injuries and reproductive activity. Poor sanitation and long-term storage of feed can contribute to increased levels of spores in the air. Locations with high temperatures, poor ventilation and high humidity facilitate the growth of the fungus.

Primary transmission is through inhalation of airborne *Aspergillus* spores. Spores are released when fungal fruiting bodies are shattered by bird's movements. Immunosuppressed birds and wild birds appear to require fewer spores to become infected. *Aspergillus* spores are also capable of penetrating eggs, resulting in embryo death before hatching or birds hatching with aspergillosis. Contamination of puncture wounds into the air sacs by fungal hyphae has been documented. Oral exposure however, appears to be unlikely.

Pathogenesis

Infection with *Aspergillus* spp is caused by inhalation of spores and hyphae, but contaminated food and water can also serve as infection sources. Due to their smaller size, *Aspergillus fumigatus* spores are too small to be trapped completely in the nasal cavity or trachea

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and are able to reach the lungs and air sacs. The air sacs are the primary site of infection as inhaled air passes through the air sacs first, prior to making contact with epithelial surfaces of the lung. Large numbers of spores or impaired immune responses result in the formation of plaques of hyphae within the respiratory system, resulting in obstruction in the trachea or bronchi and may fill the air sacs. These hyphae incite a strong inflammatory cell response. Occasionally sporulation occurs within the lungs and air sacs with hyphae and their fruiting bodies filling the lumens and they may penetrate the air sacs causing serositis and superficial necrosis of adjacent organs, because of direct extension. Conidia are also carried to other sites in the respiratory tract, germinating and producing more lesions. *Aspergillus* hyphae are known to be angioinvasive resulting in haematogenous systemic spread.

The first line of defence against *Aspergillus* are alveolar macrophages that ingest, inhibit and kill the spores. The second line of defence are heterophils, which kill the hyphae through oxidative and non-oxidative mechanisms without phagocytosis. Conidia of *Aspergillus fumigatus* are also thought to be able to resist phagocytosis by respiratory macrophages.

Clinical symptoms

The clinical manifestations of disease are dependent on the infective dose, distribution pattern of spores, pre-existing diseases and immune response of the host. Infections may be localised or disseminated, acute or chronic. Acute infections are seen in highly susceptible species or in birds inhaling large numbers of spores, while chronic aspergillosis is generally associated with immunosuppression. Young birds, penguins, waterfowl, raptors, farmed ratites plus captive pelicans, flamingos, loons, pectorals and shorebirds appear to be particularly susceptible.

Acute infections develop rapidly within days with affected birds usually being in good body condition and without other underlying disease problems. Birds die rapidly from severe respiratory compromise due to generalised lung infection. Large epizootics of acute aspergillosis are well documented in wild bird species especially gregarious bird species.

Chronic aspergillosis on the other hand is generally an individual bird problem occurring in debilitated birds, usually with other complicating disease conditions, oiling or with a history of recent capture or rehabilitation.

Respiratory aspergillosis remains the most common form of the disease with other syndromes being documented including nasal aspergillosis, mycotic keratitis, cutaneous aspergillosis, neurological disease and blepharitis/eyelid dermatitis.

Nasal aspergillosis: exudative rhinitis with or without associated turbinate necrosis (Figure 1).

Figure 1. Nasal aspergillosis in in African grey parrot with extensive turbinate necrosis.

Mycotic keratitis: is characterised by swollen eyelids with ocular discharge, blepharospasm, photophobia and periorbital swelling.

Cutaneous aspergillosis: most commonly cutaneous mycotic infections are associated with feather follicles, but a rare and unusual cutaneous form characterised by extensive epidermal necrosis in the absence of feather follicle involvement has been described.

Neurological aspergillosis: neurological signs associated with fungal meningoencephalitis with parenchymal infarcts.

Blepharitis/eyelid dermatitis: swelling of the eyelids with associated crusting dermatitis (Figure 2).

Figure 2. Aspergillus associated blepharitis and eyelid dermatitis with swelling of eyelids and crusty dermatitis of the eyelid skin.

Pathology

Acute aspergillosis is characterised by birds in good body condition with miliary pyogranulomas disseminated throughout the lungs and air sacs associated with pulmonary congestion and oedema (Figure 3). In addition, in some species, flat yellow fungal plaques are sometimes observed in the interclavicular, anterior and posterior thoracic and abdominal air sacs.

Figure 3. Acute aspergillosis with miliary pyogranulomata scattered through the lung associated with pulmonary congestion and oedema.

In chronic aspergillosis, caseo-necrotic plaques in the air sacs dominate the pathology (Figure 4). Plaques may appear blue-green, olive green, brown, or black.

Figure 4. Chronic aspergillosis with caseo-necrotic plaques dominating the pathology.

If conidia develop this imparts a smooth velvet appearance to the plaques (Figure 5).

Figure 5. Smooth velvety appearance to large areas of the fungal plaque confirming conidia production.

Local extension of fungal plaques to involve adjacent visceral organs is sometimes documented in these chronic cases (Figure 6). Even with localised lesions, aspergillosis can be fatal, for example, when a fungal granuloma is encountered at the bifurcation of the major bronchi in the syrinx.

Fungal granulomas have also been reported in the sinuses, trachea, brain, other internal organs or body cavities. In some cases, there may be systemic spread from this primary respiratory focus.

Figure 6. Spleen exhibiting multifocal fungal pyogranuloma formation with amyloidosis.

The pathology of the other forms of aspergillosis are described above under clinical signs.

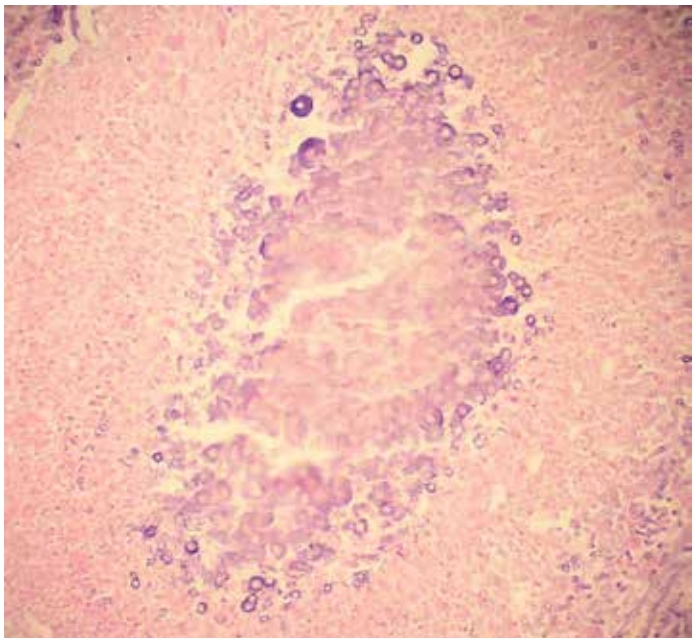

Figure 7. Lung: fungal granuloma with central caseous necrosis rimmed by epithelioid macrophages and giant cells. This PAS stain reveals positive staining fungal elements including fruiting bodies at the periphery of the necrotic zone.

On histopathology, the diagnostic lesions are mycotic granulomas, which usually occur as multiple, varying sized areas of caseous necrosis surrounded by heterophils, macrophages and multinucleated giant cells (Figure 7).

In some instances, noncontagious granulomas composed exclusively of histiocytes and multinucleated giant cells are encountered. At the centre of these granulomas, parallel walled, septate, fungal hyphae are usually encountered. In fungal plaques, conidia with fruiting bodies are sometimes observed (Figure 8). Visualisation of fungal hyphae is enhanced by special stains such as PAS. Routine polarisation of histological sections of fungal infections, especially those involving the respiratory tract should be considered to check for bifringent calcium oxalate crystals

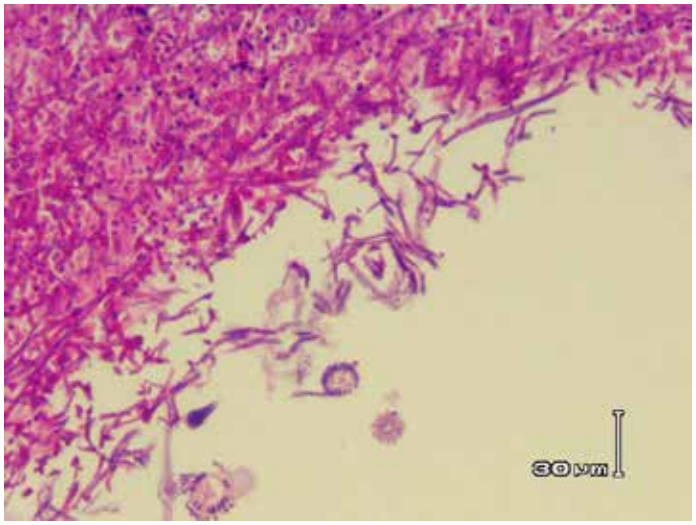

Figure 8. Fungal plaque containing myriad parallel walled, septate, fungal hyphae and multiple fruiting bodies with conidia.

Diagnosis

Clinical diagnosis, including diagnostic imaging, is challenging, as the signs of aspergillosis are nonspecific. Haematology and clinical chemistry are at best indicative. *Aspergillus* serology has limited diagnostic applications. In acute stages, antibody production lags behind antigen exposure by 10-14 days and if the bird is immunosuppressed low antibody production results in false negative results. In general, negative tests do not rule out aspergillosis and positive tests are only diagnostic when backed up by other evidence.

Antifungal agent	Administration route	Dose
Amphotericin B	Intravenous	1.5mg/kg q8h 3 to 5 days (Flammer, 1993; Joseph et al., 1994), 10 to 14 days (Jenkins, 1991)
	Intratracheal	1.5mg/kg q8 to 12h (raptors) (Redig & Duke, 1985)
	Nebulisation	1mg/kg q24h 10 to 14 days (Jenkins, 1991), q12h (raptors) (Joseph et al., 1994)
	Into air sac	1mg/kg 15 min 5 to 7 days every other week (Orosz & Frazier, 1995), q12h (Joseph *et al*., 1994)
	Topical (wound)	Dose not specified (Flammer, 1993)
		1.35mg/kg q24h (liposomally encapsulated amphotericin B) (Bonar & Lewandowski, 2004)
Clotrimazole	Topical	Dose not specified (Flammer, 1993)
	Nebulisation	10mg/ml polyethylene glycol for 30 to 45 min q24h 3 days on/2 days off (1 to 4 months) (Joseph *et al*., 1994; Orosz & Frazier, 1995)
Enilconazole	Topical	Dose not specified (Flammer, 1993)
	Nebulisation	0.1ml/kg for 30 min q24h 5 days on/2 days off (raptors) (Heatly et al., 2007)
	Disinfection	Environment: flush with solutions recommended for use in poultry houses (Flammer, 1993)
Fluconazole	Oral, intravenous	5mg/kg q24h 7 days (Bauck et al., 1992, Flammer 1993)
		15mg/kg q12h (psittacines) (Pericard, 2005)
Itraconazole	Oral	5 to 15mg/kg q12h with food for 7 to 21 days (Bauck et al., 1992)
		10mg/kg q24h 3 weeks (Verstappen & Dorrestein, 2005) (falcons) (Jones et al., 2000)
		15mg/kg per os q12h/q24h (Abrams et al., 2001), 10 – 20mg/kg q12h/q24h (Flammer, 1993)
		5mg/kg q24h 30 days (African grey parrots) (Orosz and Frazier, 1995)
		5 to 10mg/kg q 24h (Amazon parrots) (Orosz et al., 1996), 6mg/kg q12h (pigeons) (Lumeij et al., 1995)
		Preventive: 10mg/kg q24h 10 days (Forbes, 1992), 20mg/kg q24h (Meredith, 1997), 15 to 25mg/kg/day for 1 week (Xavier, 2008)
Ketoconazole	Oral	10 to 30mg/kg q12h 21 days (resuspending in orange juice q5days) Bauck et al., 1992)
		20 to 30mg/kg q12h (Flammer, 1993), 30mg/kg q12h 14 to 30 days (Orosz & Frazier, 1995)
Miconazole	Topical	q12h (Flammer, 1993; Orosz & Frazier, 1995; Abrams *et al*., 2001; Suedmeyer et al., 2002)
	Nebulisation	pH balanced solution, aqueous base, dilute in saline, 15 to 20 min q12h (Orosz & Frazier, 1995)
	Intratracheal	5mg/kg, 10mg/ml. q12h (diluted to maximum 0.5ml with saline) (Westerhof, 1995)
Terbinafine	Oral	10mg/kg q12h to q24h, 15mg/kg q12h (psittacines) (Flammer &Orosz, 2008)
	Nebulisation	Can be combined with itraconazole (Flammer, 2006). Dose not mentioned
Voriconazole	Oral	10mg/kg q12h (pigeon) (Beernaert *et al*., 2009a,b), q24h (chickens) (Burhenne *et al*., 2008) (African grey parrots) (Scope et al., 2007)
		12 to 18mg/kg q12h (African grey parrots) (Flammer & Orosz, 2008)
		12.5mg/kg q12h, 3 days loading dose, then q24h (raptors) (DiSomma *et al*., 2007, Schmidt *et al*., 2007)
		40mg/kg q24h (quails) (Tell *et al*., 2009)
5-fluorocytosine	Oral	50 to 100mg/kg q12h (Flammer, 1993)
		60 to 250mg/kg q12h (cage birds); 40mg/kg q6 to 8h (raptors) (Jenkins, 1991)
		150 to 250mg/kg q12h 21 days (Bauck *et al*., 1992)
		120mg/kg q12h 3 weeks (Westerhof, 1995)
		75 to 120mg/kg q24h (dose/4 q6h) (Redig & Duke, 1985)
		120mg/kg q6h (Joseph *et al*., 1994)

(From: Beernaert LA, *et al*.)

Definitive diagnosis relies on isolation of *Aspergillus* on fungal culture or by the demonstration of fungal elements in cytological preparations and histopathology (in association with diagnostic lesions).

When *Aspergillus* is isolated, it is always advised to run a fungal antibiogram on the isolate as drug resistance is being increasingly reported.

Treatment

Treatment of birds with aspergillosis is complicated due to a number of factors including limited knowledge about the pharmacokinetics of antifungal agents in different avian species, poor penetration of granulomatous lesions by antifungal agents, concurrent disease, immunosuppression and the usual late stage of clinical at presentation. Treatment regimes' involve a combination of topical therapy, systemic therapy and environmental disinfection. Topical therapy is administered via nebulisation, nasal or air sacs flushing, or surgical irrigation of the abdominal air sacs. Systemic therapy is either administered intravenously or orally.

For drug dosages and administration routes the table provided by Beernaert et al – *Avian Pathology* 2010; 39:325-331, should be consulted.

In recent avian studies, voriconazole has shown high effectiveness and wide tissue distribution. In addition, long-term treatment did not cause clinical signs or adverse effects. Advised dose regime is 10mg/kg daily PO for 30 days.

A treatment protocol applied to penguins with suspected aspergillosis at a local marine facility includes treatment with voriconazole on its own or in combination with terbinafine and amphotericin B. Drug dosage rates used are the following

- Voriconazole 10mg/kg once a day orally.
- Turbinafine 10-15 mg/kg once a day orally.
- Amphoteracin B for nebulising, 0.2ml in 10 ml sterile saline solution.

Treatment continues for as long as needed.

Voriconazole can have toxic effects (ataxia, lethargy) and in some instances, the treatment was stopped after 2-3 months (Dr Caryll Knox – personnel communication).

Control

In free-living wild bird populations reducing exposure to mouldy feed sources including grains or silage, needs to be reduced by feed removal, covering of contaminated food sources or ploughing it in. All feed supplies stored for feeding wild bird populations should be stored under temperature and humidity conditions that are not conducive to mould growth.

In captive aviary birds frequent cleaning of and around food and water utensils reduces the risk of fungal growth. Cages and boxes should be frequently cleaned and aviary hygiene should be well maintained. Similar strict hygienic procedures need to be applied in the hatchery with thorough cleaning and disinfection of the incubators between hatches to remove fungi.

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QUESTION 1
Which of the following Aspergillus species have the smallest spores?
a. *Aspergillus flavus*
b. *Aspergillus niger*
c. *Aspergillus nidulans*
d. *Aspergillus fumigatus*
e. *Aspergillus glaucus*

QUESTION 2
Which of the following Aspergillus species have been associated with oxalate production on feedstuffs?
a. *Aspergillus nidulans*
b. *Aspergillus niger*
c. *Aspergillus fumigatus*
d. *Aspergillus glaucus*
e. *Aspergillus terreus*

QUESTION 3
Which of the following crystals are deposited in tissues in some cases of aspergillosis?
a. Urates
b. Struvite
c. Triple phosphate
d. Melamine
e. Oxalate

QUESTION 4
Which is considered the primary route of transmission of aspergillosis in birds?
a. Inhalation of airborne spores
b. Wound contamination by fungal hyphae
c. Oral ingestion of spores
e. Egg contamination by spores
f. Percutaneous through contact with contaminated environmental sources

QUESTION 5
Clinical manifestations of disease on not dependent on which of the following factors?
a. Infective dose
b. Distribution pattern of spores
c. Pre-existing disease
d. Immune response of the host
e. Breed of bird

QUESTION 6
Which is the most common form of clinical aspergillosis in birds?
a. Nasal aspergillosis
b. Respiratory aspergillosis
c. Cutaneous aspergillosis
d. Mycotic keratitis
e. Neurological aspergillosis

QUESTION 7
Which of the following gross post mortal features of fungal plaques indicate the presence of conidia?
a. Blue-green colour
b. Olive green colour
c. Brown colour
d. Velvet appearance
e. Black colour

QUESTION 8
Which of the following diagnostic procedures would confirm a diagnosis of aspergillosis?
a. Diagnostic imaging
b. Haematology
c. Clinical chemistry
d. Serology
e. Fungal culture

QUESTION 9
Which of the following procedures is not used as a method of topical antifungal application in birds?
a. Anti-fungal bathing
b. Nebulisation
c. Nasal flushing
d. Air sacs flushing
e. Surgical irrigation of abdominal air sacs

QUESTION 10
Which of the following antifungal agents have shown high effectiveness, wide tissue distribution and can be used continuously for a prolonged period?
a. Amphotericin B
b. Fluconazole
c. Voriconazole
d. Itraconazole
e. Miconazole

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