Avian Aspergillosis

Doug Black BVSc (Hons) MACVSc (Avian Health)

61 Meninya Street Moama NSW 2731 Australia

Introduction

Aspergillosis is an opportunistic, non-contagious infectious disease caused by fungus of the genus *Aspergillus* that affects many different animals such as birds, reptiles and mammals including man. It is most commonly seen in birds in which the disease is generally assumed to rely on some compromise to the immune system such as other diseases, stress, malnutrition, concurrent drug therapy or immaturity. Cases of aspergillosis in free-living birds are rare, being mostly linked to poor weather conditions and climate, malnutrition and concurrent disease. Treatment of the disease is difficult due to the chronic nature of the disease, the challenge in ante-mortem diagnosis, the often inaccessibility of the fungal lesions, the debilitated state of the patient and the relative resistance of the fungus to therapeutic agents. Prevention of aspergillosis is based on minimising predisposing immuno-suppressive factors, reduction of levels of *Aspergillus* spore contamination in the environment, increased ventilation and selection for innate resistance to infection.

Causative Agent

The *Aspergillus* genus of fungi was named by Italian priest and biologist Pietro Antonio Micheli in 1720. It is thought that the name derives from *aspergillum*, a tool used to sprinkle holy water which has an image very similar to conidiophores emerging from the bulbous vesicle of the *Aspergillus* hyphae. All of this is derived from the Latin verb asperge (also *asperse*), which means to besprinkle or bespatter. This is also quite apt in that the *Aspergillus* fungus is ubiquitously distributed from the arctics to the tropics. Aspergillosis lesions were first described in a bird in 1842 by Rayer and Montagne in the air sacs of a bullfinch, but fungal lesions likely belonging to the genus Aspergillus, were also described in wild birds in the early 1800s. The first description of human aspergillosis was published in 1847 by Sluyter (Varkey 1998, Abundis-Santamaria 2003).

The *Aspergillus* fungus is a filamentous, ubiquitous fungus found in nature and is commonly isolated from soil, plant debris and the indoor air environment. The species frequently isolated in avian aspergillosis are *A. fumigatus, A. flavus, A. nidulans, A. niger* and *A. terreus* with *A. fumigatus* being, by far, the most common isolate. Aspergillus colonies are downy to powdery in texture and the surface colour may vary depending on the species. *A. fumigatus* is a thermotolerant fungus and grows well at temperatures over 40°C and can grow at a temperature range of 20 to 50°C. This property is unique to *A. fumigatus* among the *Aspergillus* species.

The avian air sacs provide a humid environment of 40-43°C which is ideal for a thermophilic fungus like *A. fumigatus*. In addition to this, the avian respiratory system allows direct inhalation of fungal spores into the caudal air sacs and precludes a mechanism for the ejection of these inhaled fungal spores. The absence of resident macrophages within airway lumens and the dependence on heterophils that use cationic proteins, hydrolase and lysosymes rather than catalase and myeloperoxidase may also be responsible for the increased sensitivity of birds to aspergillosis (Klika et al. 1996, Harmon 1998).

Susceptibility

Aspergillosis is uncommon in free-living birds being mostly linked to poor climatic conditions, inadequate

nutrition or secondary to toxicoses such as lead poisoning in wild birds. However, aspergillosis is a very common and important infectious disease of captive birds. It remains one of the principal causes of death in captive penguins, a species that appears to be particularly susceptible to the disease. The disease is also commonly seen in captive waterfowl, wading birds, raptors, pheasants, ostriches, passerine and psittacine birds (Kearns 2003, Black 1993, German 2000). Young birds appear to be more susceptible than older birds. It can also be higher in incidence in certain strains of birds that may be genetically more susceptible (Black 1993).

Clinical Disease

The type of disease induced is dependent on the source and number of spores contacted and the general condition of the bird involved. Healthy birds can generally withstand exposure to a high concentration of spores.

Clinical signs can result from obstruction of the airways by the mycelium, respiratory embarrassment due to the effects on the respiratory system by the chronic granulomatous lesions and through systemic effects following toxin release (Redig, 1986). Generally, avian aspergillosis involves signs associated with disease in the respiratory tract. The disease can be focal or more generalised or even systemic and may present as an acute or chronic syndrome. Lesions can become established in any area of the avian respiratory tract but are especially common in the posterior thoracic and abdominal air sacs or as a focal granulomatous lesion in the trachea especially at the syrinx. Lesions frequently originate in one system or area and later advance into adjacent organs and systems as the disease progresses.

Acute cases occur when spores germinate in a particularly vital area or when multiple lesions germinate at once (Ritchie et al 1994). Acute aspergillosis involving severe pneumonia is often referred to as brooder pneumonia and is most common in young chicks exposed to high levels of Aspergillus spores during incubation, hatching or immediately after hatching. This is often seen in production species due to poor management and hygiene of commercial incubators, hatchers or brooder areas (Black 1993).

Another less common form of avian aspergillosis is aflatoxicosis due to disease caused by the effects of mycotoxins produced by the few species of Aspergillus fungus that are capable of producing toxins. This form of the disease is generally associated with ingestion of contaminated feed.

Signs

Avian aspergillosis can present as an acute or chronic disease, the latter being by far the more common presentation. However, birds with chronic long-term lesions may also present with acute signs due to sudden obstruction of airways, fulminating germination of fungal spores or haematogenous spread to internal organs or the central nervous signs. The clinical outcome is dependent on the avian host respiratory immune defences and the ability of the organism to germinate and produce an invasive mycelium (Oglesbee 1997). Acute cases can be seen following inhalation of a high dose of spores, due to heavy environmental contamination (Redig 1993).

In *acute* disease, signs are often quite non-specific including inappetence, depression, polyuria, dyspnoea, open-beak breathing, cyanosis or sudden death without any prior clinical signs (German 2000, Abundis-Santamaria 2003).

In the *chronic* form of the disease the signs will depend on the area of invasion of the fungus. The invasion of the spores is initially via the respiratory tract resulting from inhalation of a normal spore load by a stressed or immunosuppressed adult bird. Primary colonisation of the air sacs (usually thoracic and abdominal air sacs) and syrinx occurs (areas of high oxygen tension and poor perfusion), with spread of spores throughout the respiratory system following sporulation. Luminal fungal plaques and necrotic debris may eventually obstruct the airways. Sporulation can be observed grossly as dark fruiting bodies. Haematogenous spread may result in disseminated lesions (generally focal granulomata), involving pneumatic bone, peritoneum, internal organs or the CNS (Redig 1993). The disease progression is slow and onset insidious, hence diagnosis prior to extensive

lesions is difficult (Oglesbee 1997). Non-specific signs such as depression, weight loss, muscle wasting, anorexia, diarrhoea and lethargy are often seen. Hepatic involvement may cause biliverdinuria and hepatomegaly. CNS involvement can lead to signs such as ataxia, torticollis and seizures or hind limb paresis/paralysis (Oglesbee 1997). Ocular lesions, although uncommon, usually present as uveitis.

If chronic respiratory disease is involved the signs may also be non-specific such as weight loss, poor body condition, depression and lethargy as listed above and signs specifically associated with the respiratory tract disease may or may not be apparent. Chronic respiratory disease signs will depend on the region of the respiratory tract involved. Upper respiratory tract involvement may present as mucoid to mucopurulent nasal discharge, beak malformation and nasal or periorbital swelling. Birds with syringeal granuloma may have only a single lesion at the syrinx or they may have concurrent lower respiratory disease as well. The classic sign of a syringeal lesion is a change in the pitch or quality of the voice. Psittacine birds, passerine birds, and waterfowl are the species that most commonly present in this way. If the syringeal granuloma is large enough to substantially obstruct the airway, signs such as dyspnoea, tachypnoea and exercise intolerance will be seen. A bird with diffuse lower respiratory tract disease may present with one or more of the following signs: dyspnoea, open-mouth breathing, bobbing motion of tail, increased respiratory rate or effort at rest, prolonged tachypnoea following manual restraint, wide-based stance on perch and is likely to appear fluffed and less active. This presentation is particularly common in passerine birds, psittacine birds, and raptors. Diffuse lower respiratory tract disease is also common in waterfowl and wading birds, but frequently these species will die suddenly with little or no premonitory signs, although chronic weight loss may be seen (Kearns 2003). Ostriches with chronic air sacculitis will show poor growth rate, significant weight loss, lethargy, sometimes inappetence and will show respiratory signs such as increased respiratory rate (in excess of 12-16 breaths per minute under conditions of cool temperatures and at rest), increased respiratory (usually *inspiratory)* effort, poor exercise tolerance and coughing (can be productive bringing up significant mucus). Additional signs include dyspnoea, cyanosis, open beak breathing - even in cold weather and without stress or exercise, yawning, head shaking, cervical air sac rupture resulting in swollen neck or rupture of other air sacs resulting in subcutaneous emphysema in other locations and collapse under stress, restraint or exercise. Signs in affected ostriches can often be detected from some distance as the birds are seen to lift their wings with some effort during inspiration and then suddenly drop them during expiration. Auscultation of ostriches with significant aspergillosis will commonly reveal an audible squeak, grunt or whistle just at the end of inspiration or expiration (Black 2003). This can sometimes be detected in other smaller avian species with significant air sac disease.

Diagnosis

Ante-mortem diagnosis of aspergillosis in birds can be very difficult due to the often poor specificity of signs or even a lack of signs in the early stages of the disease. A thorough clinical history may reveal the presence of poor sanitation, predisposing environmental conditions, concurrent disease, previous antibiotic treatment or other immunosuppressive factors. All of this may lead to a presumptive diagnosis of aspergillosis especially if treatment with antibiotics has failed to elicit any improvement in the condition of the bird. A definitive diagnosis will rely on further testing such as haematology, serum biochemistry, radiography, endoscopy, cytology, fungal culture, serum protein electrophoresis, serology and polymerase chain reaction (PCR) testing. Of these, endoscopy, cytology and PCR testing are the most useful.

Haematology

Birds with chronic aspergillosis will frequently have a significant leucocytosis with total white blood cell counts often greater than 30,000 cells per microliter. It is even possible to see white blood cell counts in excess of 100,000 cells per microlitre. There is usually also a heterophilia and, especially in more chronic cases, a monocytosis and lymphopaenia. This is not reliable as some of the infected birds can show poor immune response to the disease and total white blood cell counts may be substantially lower.

Serum Biochemistry

Commonly an increase in total serum protein and increased globulin fraction will occur. Other biochemical changes will reflect involvement, if any, of granulomatous lesions in other organs.

Imaging

Radiographs are one of the more helpful diagnostic tools available for detecting aspergillosis in avian species. However, radiographic changes are unlikely to be visible in early cases, but advanced cases may show significant changes. In high anaesthetic risk cases standing or perching lateral views as well as a dorsoventral view can be quite helpful (Jones and Orosz 2000). Radiographic signs consistent with advanced aspergillosis include focal densities in air sacs or lungs, loss of definition, asymmetry, enlargement or hyperinflation in air sacs, especially the thoracic and abdominal air sacs. If the lungs are involved there may be an exaggerated parabronchial pattern and some consolidation. Syringeal granulomas may also be visible on radiographs. Focal soft tissue opacities within the oropharynx or periorbital sinuses may also be seen. If lesions are located in the kidneys or liver then nephromegaly or hepatomegaly may be seen. Contrast radiography and CAT scans have also been reported as diagnostic aids (Redig 1986). If disease is advanced to the point where lesions can be observed on radiographs, particularly in the air sacs or pneumatic bones such as the humerus, prognosis is guarded to poor. Magnetic resonance imaging (MRI) may be useful in cases where focal granulomas of organs such as the brain are suspected

Endoscopy

Endoscopy can provide a definitive diagnosis of aspergillosis in birds but, because it involves the use of a general anaesthetic, it can also be a risky procedure in, particularly in advanced cases. In birds with substantially obstructive syringeal *Aspergillus* granulomas the use of the caudal thoracic air sac cannulation may be required to alleviate dyspnoeic signs. The air sac tube can also be used to deliver the gaseous anaesthesia required for any endoscopic procedure needed for diagnosis or attempted removal of the granuloma. In patients of suitable size, tracheal endoscopy can allow visualisation of the tracheal or syringeal lesions for sampling for cytology or culture or attempted debridement. Avian patients with syringeal *Aspergillus* granulomas should be subjected to air sac endoscopy if safe general anaesthesia can be performed via air sac cannulation. This is needed to identify the existence of concurrent air sac or pulmonary lesions. In fact, if time and the health of the patient permit, endoscopy should be used to evaluate the entire respiratory tract, including the choanal opening, glottis, trachea and syrinx, lung parenchyma, air sacs, and the entire coelomic cavity.

Endoscopy of the air sacs can reveal diffuse opacity or, more commonly, the presence of white or yellow fungal plaques that may have a superficial layer of blue, grey or aqua mould. These lesions can be sampled for cytology or culture, debrided from the air sac surface, excised or treated directly with an anti-fungal therapeutic agent.

Cytology

Samples can be obtained directly via an endoscope, indirectly using a tracheal or air sac wash technique or by using a swab of lower trachea or upper respiratory tract lesions. The material can then be used for culture or immediately smeared on a slide and examined microscopically to identify characteristic Aspergillus morphology. A direct wet preparation smear can be examined with or without 20% KOH to clear the background debris and the organisms may be better visualised with the use of lactophenol blue (Figure 1) or new methylene blue.

Sporulating forms can be seen, especially when samples are taken from air sacs or trachea. These forms have a very characteristic appearance and are referred to as conidiophores. The conidiophore

consists of a vesicle at the terminal end of the hypha. Phialides arise as spherical protuberances from the surface of the vesicle and are bounded by a much thinner wall than that of the vesicle. The development of adjacent phialides is well synchronized but the development of the vesicle as a whole is not precisely co-ordinated. The phialides then produce many conidiospores. When first formed the conidiospores have a cylindrical shape and they only assume their characteristic spherical shape at a later stage in development. Trinci et al (1968) (Figure 2)

Fungal Culture

Material obtained via the methods listed above for cytological examination can be used for culture purposes. Culture can be achieved using Sabouraud dextrose agar (SDA) or blood agar at temperatures from 25 to 37°C. Antibiotics such as chloramphenicol can be added to the agar to inhibit bacterial growth, but *Aspergillus* species are sensitive to cycloheximide. *Aspergillus fumigatus* colonies have a diameter of approximately 3 to 4cm in 7 days. The flat colonies can appear within 18-24 hours and are initially white, then turn bluish green, especially near the centre as, or if, sporulation occurs (usually after 48 hours). As the colony matures, the conidiophore masses become grey-green while the colony perimeter remains white. However, many colonies do not sporulate well. As previously mentioned *A. fumigatus* is resistant to high temperatures and can grow well at 45°C. *A. flavus* grows very rapidly, obtaining a colony diameter of 6-7cm in 10 days at 25°C. The colony begins with a white colour, turning yellowish to yellow-green with a white edge as sporulation occurs. Mature colonies may become somewhat olive-green. *A. niger* colonies begin as a white colour, but rapidly develop a black colour as the conidiophores mature. Staining of samples from the colonies with lactophenol cotton blue or new methylene blue can aid in differentiation of the Aspergillus fungus grown.

Care should be taken with interpretation of a positive culture result on its own. Isolation of Aspergillus species by culture is not a definitive diagnosis, because the organism is ubiquitous in the environment, can be isolated from the avian respiratory tract of non-infected birds and is a common laboratory contaminant (German 2000, Abundis-Santamaria 2003).

Serum Protein Electrophoresis

Serum protein electrophoresis may aid the diagnosis of aspergillosis in birds in confirming the presence of severe inflammation consistent with the disease. Protein electrophoresis has been used as a diagnostic and prognostic tool in aspergillosis in different avian species when used in conjunction with other diagnostic tests. Distinct variation in protein patterns and indices exist between avian species and sometimes within species. Birds affected by mycotic diseases such as aspergillosis may show an increase in the beta globulins in acute stages of the disease. Concomitantly, there is usually a decrease in the albumin concentrations, resulting in a decreased A-G ratio. Chronic aspergillosis infections may show an increase in beta or gamma fractions or both. In psittacine species, a marked increase on the alpha2 and beta globulins indicates acute or severe infection or inflammation and it is generally accepted that an increased beta globulin fraction, along with suggestive clinical data, supports the diagnosis of aspergillosis in birds (Sanchez and Murray 2005, Jones and Orosz 2000).

Serology

Serological diagnosis of avian aspergillosis was originally based on precipitin formation in agar gel. Precipitins form when the optimal concentration of antigen and antibody are reached. Some of these tests are still used to diagnosis as they are relatively inexpensive, simple and can often prove useful to the avian clinician. It is important that the test that is used has been adapted for use with avian sera. The author found that agar gel immunodiffusion testing was a useful ancillary diagnostic tool in some particularly chronic advanced cases of aspergillosis in ostriches. Care was required in interpreting these serological test results as false negative results were common. Also the degree of intensity of the positive reaction did not necessarily reflect the degree of severity or chronicity of the disease in the

bird (Black 1993).

The development of enzyme-linked immunosorbent assays (ELISA) has allowed earlier diagnosis (within 7 days of exposure) and monitoring of response to treatment.

Indirect ELISA testing using specific anti-falcon conjugates has been a useful tool for the diagnosis of aspergillosis in birds of prey and has also been used for monitoring the progress and response to treatment modalities. This test can detect antibodies as early as one week after experimental infection, much earlier than the onset of clinical signs of the disease (Abundis-Santamaria 2003).

Reidarson and McBain (1992) reported the use of ELISA testing in the diagnosis and monitoring of penguins at Sea World, California. Correlation between rise and fall in antibody titre and white cell count was shown with development of disease and subsequent itraconazole treatment, despite continued elevation in globulins. However, they described two Humboldt penguins which had retained high titres despite successful treatment. This report highlighted the treatment of an asymptomatic penguin based on an elevated antibody titre with a subsequent fall in titre after treatment despite continued elevation of globulin levels.

Apart from the difficulties associated with the sensitivity of serological tests, limitations can be met due to the poor antibody responses in immunosuppressed avian cases.

Although the indirect ELISA can detect antibodies as early as 7 to 10 days post-infection, in subacute infections or in patients with a poor humoral response (sequestered granulomatous infections on air sac membranes or immunocompromised patients), testing for antigen may provide more information than testing for antibody response alone. In more chronic cases in which antigen levels may be low, ELISA for the detection of antibodies may be more helpful (Jones and Orosz 2000).

Polymerase Chain Reaction Testing

PCR-based testing for the detection of DNA specific for the Aspergillus fungus is a much more sensitive method to confirm infection and can be suitable for immunocompromised birds since detection of antibodies is not involved.

As early as 1996, Katz *et al* developed a PCR-based assay capable of detecting small amounts of DNA from *Aspergillus fumigatus* sourced from oral swabs of ostriches suffering from clinical aspergillosis. However, the assay lacked both the sensitivity and specificity required to be used as a reliable means for the early detection of aspergillosis in ostriches. The results suggested that the amount of *Aspergillus fumigatus* DNA present in the pharynx was very low, even in birds with extensive *Aspergillus* infections. The assay also gave positive results using some samples from uninfected ostriches likely from contamination or incidental fungus. The study also showed genetic variation between isolates of *Aspergillus fumigatus* from ostriches sometimes even from the same flock. This discovery has positive consequences in that PCR testing could be used to analyse the potential source of infection. However, it also emphasises the possibility of flawed results using only DNA testing to diagnose aspergillosis.

This genetic diversity of *Aspergillus fumigatus* isolates was demonstrated in turkeys by genotyping using a microsatellite polymorphism marker analysis technique. In this study older turkeys harboured several combinations of genotypes whereas day-old chicks carried a unique genotype, suggesting a common source of contamination (Lair-Fulleringer et al 2003).

Recently PCR tests for the *Aspergillus* genus as well as specifically for *Aspergillus fumigatus* have become available through veterinary laboratories in several countries. These PCR tests can be performed on swabs of trachea, air sac or suspected Aspergillus lesions. Blood samples can also be

subjected to PCR testing but, due to the nature of the disease, the risk of false negative results via blood testing is significant. PCR testing of chronic granulomatous lesions may allow aetiological diagnosis in cases where fungal fragments have not been able to be identified by culture or cytology using conventional stains.

Autopsy and Histopathology

Due to the nature of the disease, most acute cases of apergillosis are often only diagnosed post-mortem. Many chronic cases presenting with non-specific signs may also die prior to an ante-mortem diagnosis is established. Obviously an autopsy provides the ultimate diagnosis and, in many cases a diagnosis can be made based entirely on the gross findings of the autopsy.

Lesions are typically confined to the respiratory tract but in some cases, haematogenous spread can lead to disseminated lesions in internal organs such as liver abdominal viscera or the CNS. In systemic cases in gallinaceous chicks, lesions are typically found in lungs, air sacs, heart muscle, liver and abdominal viscera. Grossly, lesions are all similar, appearing as dry, granulomatous, nodular lesions (often cream to yellow-coloured) with superficial fungal growth being also often apparent as variable-coloured (usually grey, blue-green or white) "cotton wool-like" mycelial masses. Tracheal or syringeal aspergillosis lesions usually occur as plugs of creamy white necrotic debris at or near the tracheal bifurcation, but can extend further down the bronchi. Nasal aspergillosis typically presents as a dry, granulomatous, destructive swelling within one nostril. Destruction of adjacent tissue, including bone or beak, may be substantial (Bauck 1994).

The classic autopsy finding in older birds is chronic granulomatous air sacculitis. This can be focal and nodular in appearance or may be widespread throughout one or more air sacs, generally the anterior thoracic air sacs.

Histopathological examination of granulomas generally shows a necrotic foci surrounded by macrophages, heterophils and giant cells, sometimes within a connective tissue capsule. On histopathology, *Aspergillus* spp. have slender, tubular septate hyphae, with parallel-sided walls, dichotomous 45° branching and spherical spores. The organisms often stain poorly with haematoxylin-eosin and periodic acid-Schiff, Grocott or silver stains may be used to aid visualisation (Oglesbee 1997).

Epidemiology

Aspergillus fungi are ubiquitous and the disease can occur given suitable environmental conditions and susceptible birds. Organic matter used as bedding or floor substrate will expose birds to inhalation of high numbers of fungal organisms especially in conditions of high humidity or poor ventilation. The disease is not considered contagious by horizontal or vertical transmission; however, more than one bird in a group is frequently affected due to exposure to the same stressors or other environmental conditions (Black 1993).

Treatment

The successful treatment of avian aspergillosis remains very challenging but the key is an early diagnosis and effective long-term treatment consisting of surgical removal of lesions (where feasible) in conjunction with a combination of systemic and topical (nebulisation, intratracheal, intranasal, intrasinus or surgical air sac flushing) anti-fungal therapeutic agents. Once signs become obvious the disease can be in an advanced state. Also the infected bird's immune system and respiratory reserve can often be compromised, adding to treatment challenges. In many cases the granulomatous changes are severe enough to prevent systemic or local access by the anti-fungal agents. Thus, even with aggressive combination therapy, the prognosis is often guarded to poor.

The treatment regime may vary according to the clinical syndrome, the degree of respiratory distress and the

location of the lesions. Surgical removal of lesions, especially those in the nasal sinuses, trachea, syrinx, and abdominal air sacs is recommended when feasible.

If a bird presents in acute respiratory distress, as frequently occurs in cases of syringeal granuloma, an indwelling air sac catheter may need to be placed before proceeding with further diagnostic measures or treatment. This air sac catheter is generally placed in the caudal thoracic air sac via the bird's abdominal flank and, in many instances, may be placed quickly and safely with just physical restraint of the bird. If a syringeal or tracheal Aspergillus granuloma is to be removed by manual debridement or removal via tracheal endoscopy (if possible) or tracheostomy, general anaesthesia must be delivered via the indwelling air sac catheter. Nasal lesions may be removed by repeated lavage, curettage, or trephination of sinuses.

These removal or debridement procedures are performed in conjunction with treatment using a combination of local and systemic anti-fungal agents. Commonly used antifungal agents can be grouped into three classes based on their mode of action - *azoles*, which inhibit ergosterol synthesis (e.g. clotrimazole, miconazole, ketoconazole, itraconazole, enilconazole, fluconazole and voriconazole); *polyenes*, which interact physiochemically with fungal membrane sterols (amphotericin B and nystatin); and *fluorinated pyrimidines*, which inhibit macromolecule synthesis (5-fluorocytosine). Of these, only amphotericin B and nystatin are fungicidal in activity; the others are fungistatic. Additionally there is terbinafine hydrochloride, a synthetic allyamine-available anti-fungal agent that inhibits squalene epoxidase, a key enzyme in fungal sterol synthesis (Dahlhausen 2000).

The *systemic* anti-fungal agents now used most commonly in birds include itraconazole (5-10 mg/kg PO twice daily for 5 days, then once or twice daily for 60–90 days), fluconazole (15 mg/kg PO twice daily), voriconazole (12.5 mg/kg PO twice daily for 60–90 days), amphotericin B (1.5 mg/kg IV every 8-12 hours for 3-5 days) and terbinafine (10-15mg/kg PO every 12-24 hours) (Kearns 2003, German 2000, Dahlhausen 2000, Orosz 2000). Fluconazole or terbinafine are the systemic drugs of choice for all forms of the disease in African Grey parrots, since this species appears particularly susceptible to liver failure and death following treatment with itraconazole (Kearns 2003).

The anti-fungal agents most commonly used for *nebulisation* include clotrimazole (10 mg/ml for 30 - 60 minutes, 2-4 times daily), terbinafine (1 mg/ml aqueous solution for 20 minutes, 1-2 times daily), enilconazole (up to 1:50 dilution for 45 minutes once daily) and amphotericin B (1 mg/ml sterile water or saline for 15 minutes twice daily) (Kearns 2003, German 2000, Dahlhausen 2000, Sanchez and Murray 2005, Orosz 2000). Amphotericin B can also be directly injected into the trachea at 1mg/kg, diluted to 1 ml volume in sterile water given every 8-12 hours for 1-5 days. (Dahlhausen 2000). Some studies have brought into question the efficacy of nebulisation in delivering antifungal agents into the air sacs, but this may be due to a delivery system that fails to generate droplet sizes less than 5µm in diameter. If the droplets are greater than 5µm in diameter, they do not remain suspended enough in the air stream to reach the air sac cavities and membranes.

Recently the author and many avian practitioners have been using treatment regimes incorporating the nebulisation of F10, a veterinary disinfectant containing benzalkonium chloride, quaternary ammonium, polyhexamethylene biguanide compounds, non-toxic ampholytic surfactants and sequestrants. This has been used at 1:250 dilution for 15-30 minutes 2-3 times daily for up to 90 days and appears to be non-toxic and non-irritant and well tolerated by a range of psittacine birds and raptors. It can also be used for nasal flushing (generally once or twice weekly or up to once daily) or as an intra-tracheal injection.

Itraconazole has a high specificity against *Aspergillus spp*, although a few itraconazole-resistant *Aspergillus fumigatus* isolates have been identified. Itraconazole is given orally, often using a dose of 5mg/kg twice a day, or 10mg/kg once or twice a day. Specific dose rates for each avian species are yet to be established as resultant plasma concentrations will vary depending on the type of bird.Some practitioners have used a higher dose of 15mg/kg twice a day but at these higher doses, depression, anorexia and vomiting may result. The adverse effects have been related to hepatic toxicity and can vary between different bird species, but are particularly common in African Grey Parrots, even at lower dose rates. Itraconazole has been used in waterfowl, shorebirds,

poultry, ratites and penguins without serious side effects. It is now one of the most commonly used systemic anti-fungal agents used in the treatment of avian aspergillosis often in conjunction with or immediately after initial short-term treatment with amphotericin B.

Amphotericin B is often used for the initial treatment of severe infections, either intravenously or via intratracheal injection. It is fungicidal and resistance problems are rarely encountered with *Aspergillus* species. This drug is not well absorbed when it is given orally and causes irritation with intramuscular or subcutaneous injections but can also cause granulomatous reactions when it is administered locally. Amphotericin B is almost completely insoluble in water and can only be made as a suspension. The intravenous form is a deoxycholate micellar suspension. It should not be mixed with sodium chloride or any other electrolyte solution because the drug readily precipitates. Diluents containing preservatives should also be avoided (Orosz 2000). Care should be taken to prevent exposure of veterinary staff to aerosolized amphotericin B due to the propensity of the drug to cause nephrotoxicity. Amphotericin B is also potentially nephrotoxic in birds, but the prolonged use in raptors and psittacine birds has not been associated with nephrotoxicity (Bauck 1994, Orosz 2000).

Terbinafine has, primarily, fungicidal activity against a number of fungi including *Aspergillus* species and is fungistatic against *Candida albicans*. Studies also have shown that terbinafine is similar to, or more effective than amphotericin B, ketoconazole and itraconazole against *Aspergillus* species. It can be used orally at a dose of 10-15 mg/kg every 12–24 hours and appears to be well tolerated by a number of avian species. It can also be administered by nebulisation as a 1mg/ml aqueous solution. The excellent antifungal activity of terbinafine, along with its good absorption after oral administration, wide tissue distribution and relative safety make this drug a safe and effective alternative to more conventional anti-fungal therapeutic agents (Dahlhausen et al 2000).

Enilconazole also has good efficacy against *Aspergillus* sp., although not as great as itraconazole. Enilconazole can be used via nebulisation and smoke inhalation therapy using enilconazole-impregnated smoke generators has been used to successfully treat ostriches with advanced aspergillosis (Black 1993).

Voriconazole, a second-generation triazole has some distinct benefits compared with other antifungal agents, such as reliable oral bioavailability and excellent activity against many Aspergillus species that are resistant to currently available therapy. It can be used orally at a dose of 12.5 mg/kg PO twice daily for 60–90 days or by nebulisation as a 1mg/ml solution for 60minutes once daily (Di Somma et al 2007).

Fluconazole, in contrast to ketoconazole and itraconazole, is highly water soluble and is readily absorbed from the gastrointestinal tract regardless of acidity or food intake and is normally used at a dose rate of 15mg/kg twice a day, orally. It penetrates the cerebrospinal fluid, brain tissue, ocular fluids and sputum and is therefore a useful anti-fungal agent in situations where penetration into the cerebrospinal fluid is desirable.

Birds with significant aspergillosis often are suffering severe immunosuppression and can be debilitated. Thus, support therapy including fluids, crop feeding and a warm environment is important to help improve the chances of survival.

Aspergillosis treatment is likely to be needed for some months so close monitoring of the response to treatment through haematology, indirect ELISA serology, serum protein electrophoresis, radiology and endoscopy are useful prognostic tools.

Prevention and Control

Given the difficulty in early diagnosis and the often poor response to treatment, prevention of aspergillosis is the highest priority. Aspergillosis in birds is generally considered to occur secondary to predisposing immunosuppresive factors such as stress, malnutrition, other underlying disease, concurrent drug therapy, immaturity, poor management practices such as overcrowding and genetic predisposition. In view of this, addressing and hopefully eliminating most of these factors is crucial to any preventative program.

When treating other illnesses, the benefits of long term or repeated antibiotic treatment must be weighed against the possibility of opportunistic mycotic infections. Fungal spore formation and consequent inhalation needs to be avoided through optimal ventilation, reduced humidity (if possible), adequate hygiene and, if necessary, disinfection of nests, incubators, hatchers, brooders, chick rearing nurseries and other chick or bird housing areas, avoidance of overcrowding and frequent changing of floor or ground substrate.

In-ovo, and often automated, vaccination of commercial poultry eggs has dramatically increased the speed and efficiency of vaccination in hatcheries. However, it may also be a potential source of *Aspergillus* infection, in a contaminated environment, as the opening created by vaccination may allow spores to enter and grow on the air cell membranes. In-ovo vaccination should therefore be carried out under strict hygienic conditions such as clean, uncontaminated air, aseptic vaccine mixing procedures and strict cleaning and antifungal disinfection procedures.

Prophylactic dosing with anti-fungal agents such as itraconazole, at an oral dose rate of 5-10 mg/kg once a day for at least 10 days has been used in birds deemed to be at high risk to the development of aspergillosis such as transport, capture or relocation of penguins, other marine birds, waterfowl and certain raptor species or during certain developmental stages (e.g. post-fledgling in goshawks and gyrfalcons) (Redig, 1993). The course of prophylactic treatment may be extended if clinical indications warrant it and treatment may also be commenced prior to the stress event such as transport or relocation if planned. Prophylactic treatment should also be considered in any susceptible bird (especially wild birds) during protracted antibiotic treatment or during long periods of capture, quarantine or hospitalisation.

Breeding programs to eliminate susceptible genetic strains of birds is also worthwhile. This has been done in ostriches (Black 1993) and in some raptors where the susceptibility of gyrfalcons to aspergillosis was reduced by crossing them with the relatively resistant saker falcon (Abundis-Santamaria 2003).

The use of vaccination may, in the future, offer an alternative to extensive prophylactic treatment programs. Some autogenous vaccines have been applied and seem to be effective in decreasing cases of Aspergillosis but further research and development is needed. Vaccination with a heat-killed culture filtrate preparation has been reported to reduce mortality in eiders and waterfowl (Donnelly et al cited by Redig 1993). A commercial biologic was used in the treatment and prophylaxis of aspergillosis in penguins and other avian species and was effective in reducing mortality in susceptible species (Stoddard 1990). An Aspergillus-specific humoral immunoresponse was demonstrated in pigeons after immunization was performed by administering weekly injections to *Aspergillus fumigatus* antigens (Martinez-Quesada et al 1993). Maternal anti-*Aspergillus* spp. antibodies have been shown to be transferred from wild African black-footed penguins to their embryonated eggs.

Conclusion

Aspergillosis continues to be a common disease in many species of birds. Early diagnosis is a key to successful treatment of the disease yet early diagnosis remains challenging. The best current treatment regimes involve physical removal of lesions (where feasible) in conjunction with a combination of systemic and topical (nebulisation, intratracheal, intranasal, intrasinus or surgical air sac flushing) anti-fungal therapeutic agents. The favoured anti-fungal therapeutic agents are terbinafine, itraconazole, amphotericin B, clotrimazole, voriconazole, fluconazole and enilconazole. The use of topical treatment with F10, a veterinary disinfectant is becoming increasingly popular with avian practitioners. Future research into the diagnosis, treatment and possible immunisation of the disease may improve the prognosis for cases of avian aspergillosis.

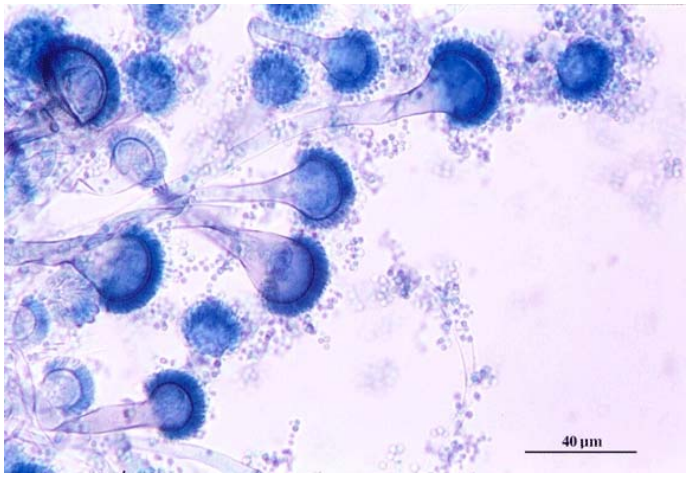

Figure 1
Aspergillus hyphae and conidiophore

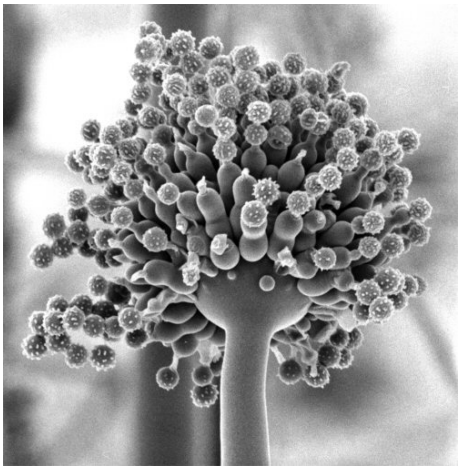

Figure 2.
Electron micrograph of *A niger* conidiophore showing hypha, vesicles, phialides and conidiospores

Figure 3 Acute aspergillosis in right anterior and posterior thoracic air sacs of a Southern Boobook owl

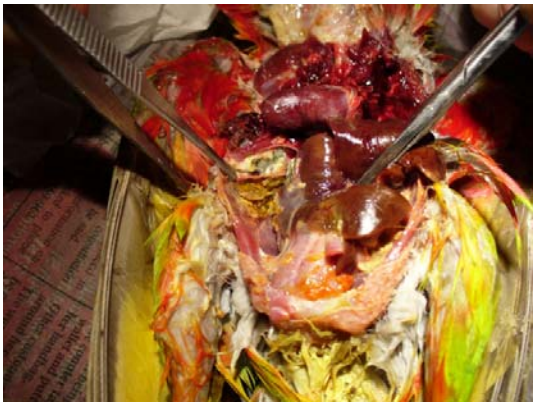

Figure 4 Chronic aspergillosis in right anterior and posterior thoracic air sacs of a mustard mutation rainbow lorikeet

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