Aspergillosis in Avian Species: A Review

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Abstract

Aspergillosis is an infectious, non-contagious fungal disease of wild and domestic birds caused by fungus Aspergillus species. It is characterized by primary involvement of respiratory tract, formation of yellow cheesy plaques and hard nodular masses in the lungs and air sacs, though other organs may also be generally involved. This fungal disease is of economic importance being the main cause of mortality in the captive birds. Major causative factors are inhalation of overwhelming amount of spores in immunocompromised birds. The review presents the current knowledge on avian aspergillosis, though in brief due to journal space limitations, from etiology, pathology, diagnosis to treatment and control.

Keywords: Aspergillosis, Aspergillus, Avian species, Pathology, Prevention and control.

1. Introduction

Avian Aspergillosis is an infectious fungal disease of wild and domestic birds caused by fungus Aspergillus species. It is characterized by primary involvement of respiratory tract, formation of yellow cheesy plaques and hard nodular masses in the lungs and air sacs, though other organs may also be generally involved. The disease is non-contagious and usually occur either in epizootic (acute) or sporadic (chronic) form.

Aspergillus species is a ubiquitous saprophytic mold with a worldwide distribution (Ben-Ami et al., 2010; McCormick et al., 2010). Aspergillosis is the most common opportunistic mycotic infection of respiratory tract in birds causing high morbidity and mortality (Tell, 2005) thus inducing a significant economic losses especially in poultry. Inhalation of A. fumigatus asexual spores (conidia) can cause a spectrum of clinical manifestations depending on the immunological status of the host (Ben-Ami et al., 2010; McCormick et al., 2010), besides anatomical and physiological factors also predisposed to such infection.

2. Etiology

Among the avian pathogenic species of Aspergillus, Aspergillus fumigatus is the main cause of aspergillosis. Other species like A. flavus, A. nidulans, A. niger and A. terreus can also cause the disease but less frequently than A. fumigatus. Aspergillus belongs to the Kingdom: Fungi, Division: Ascomycota, Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae. Genus Aspergillus was classified in 1729 by Micheli. The spore-bearing head is the first structure observed in a detailed study of the colony. The arrangement, shape, size and colour of such heads are characteristic of the species and to a lesser extent of the groups to which they belong (Table 1).

3. Transmission and Predisposing Factors

All birds are susceptible to aspergillosis. It is reported in domestic birds like poultry, duck, and quails as well as in wild birds as shown in Table 2. Inhalation of conidia or spores from contaminated feed, fecal material, soil and contamination of egg in ovo, infect the developing embryo. Higher susceptibility of birds to aspergillosis may be attributed to anatomic and physiologic characteristics of the avian respiratory system. The small non-expanding lungs and nine air sacs constitute a primary nidus for infection because the air (or conidia) reaches the caudal air sacs before it pass through those part of the lungs in which the gas exchange takes place (Nardoni et al., 2006). Higher body temperature also allows quick fungal growth. Other factors include chronic stress, unsanitary conditions, overcrowding, malnutrition, vitamin deficiencies especially vitamin A and overuse of certain medications (corticosteroids) as well as respiratory irritants (disinfectant fumes and aerosol sprays). Birds that are otherwise ill or are very young or old are also susceptible to aspergillosis.
fumigatus posterior thoracic and abdominal air sacs. Thus the mesobronchi which deliver the inhaled air to the respiratory system, trachea and to the primary bronchi through the nares (two holes in the beak leading to the nasal cavity). During inhalation, spores initially enter the bird by contacting epithelial surfaces in the lungs (Oglesbee, 1997). Microscopic examination revealed overwhelming numbers of small, hydrophobic fungal spores (conidia) into the lungs (Oglesbee, 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Brown et al., 1997).

Table 1: Summary of microscopic features and colour of colony of various Aspergillus species

<table>
<thead>
<tr>
<th>Species of Aspergillus</th>
<th>Characteristics of supporting asexual spores</th>
<th>Shape of vesicles</th>
<th>Colour of the colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. clavatus</td>
<td>Long, smooth</td>
<td>Clavate shape</td>
<td>Blue-green</td>
</tr>
<tr>
<td>A. flavus</td>
<td>Colourless, round</td>
<td>Round, radiate</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Short smooth, colourless, greenish</td>
<td>Round, columnar head</td>
<td>Blue-green to gray</td>
</tr>
<tr>
<td>A. glaucus group</td>
<td>Variable length, smooth, colourless</td>
<td>Round, radiate to very loosely columnar head</td>
<td>Green with yellow areas</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>Short, smooth, brown</td>
<td>Round, small columnar head</td>
<td>Green, buff to yellow</td>
</tr>
<tr>
<td>A. niger</td>
<td>Long, smooth, colourless or brown</td>
<td>Round, radiate, globose and large head</td>
<td>Black</td>
</tr>
<tr>
<td>A. terreus</td>
<td>Short, smooth, colourless</td>
<td>Round, compactly colourless head</td>
<td>Cinnamon to brown</td>
</tr>
<tr>
<td>A. versicolour</td>
<td>Long, smooth, colourless</td>
<td>Round, loosely, radiate head</td>
<td>White at the beginning turns to yellow, tan, pale green or pink</td>
</tr>
</tbody>
</table>

4. Pathogenesis

Aspergillosis is caused by inhalation of overwhelming numbers of small, hydrophobic fungal spores (conidia) into the lungs (Oglesbee, 1997). During inhalation, spores initially enter the bird through the nares (two holes in the beak leading to the respiratory system), trachea and to the primary bronchi (mesobronchi) which deliver the inhaled air to the posterior thoracic and abdominal air sacs. Thus the inhaled air reaches the posterior air sacs prior to contacting epithelial surfaces in the lungs (Nardoni et al., 2006). Air sacs are particularly prone to infection due to epithelial surface nearly devoid of a mucociliary transport mechanism and absence of macrophages (Brown et al., 1997). Conidia of small size (2-3 microns) enter and germinate in the lungs and air sacs (Fedde, 1998).

Aspergillus and Respiratory Epithelial Cells (REC) Interaction: After entry, the fungus comes in contact with the sticky mucus lining the respiratory tract and respiratory epithelial cells (REC). REC acts not only as a physical barrier but also an addition to the innate immune system. REC engulfed the conidia which are embedded in the atria and parts of the infundibula in the parabronchus (Maina, 2002). Féménia et al. (2009) evaluated that conidia of A. fumigatus and A. flavus inhibit human respiratory cell apoptosis. RECs internalized a portion of adherent conidia by endocytosis while some of the spores germinate externally forming hyphae which penetrates and damage the cells (Bellanger et al., 2009; Nardoni et al., 2006; Cacciuttolo et al., 2009). Invasion of hyphae, via spaces between and within the epithelium, cause cilia loss and cell detachment (Amitani and Kawanami, 2009) or the air sacs cause serosal inflammation and superficial necrosis in the adjacent organs (Tsai et al., 1992).

Aspergillus and Endothelial Cells Interaction: Fungal conidia and hyphae interact differently with endothelial cells. Conidia were endocytosed by the endothelial cells. Disseminated mycosis occurs by haematogenous spread. Hyphae are tissue and angio invasive and have a unique capacity to survive and proliferate within the host (Dahlhausen et al., 2004). Hyphus interacts with endothelial cells lining of the blood vessels by passing from the abluminal to the luminal surface and cause endothelial cell injury (Kamai et al., 2009). Some of these hyphal fragments can break off and circulate in the bloodstream resulting in disseminated lesions, involving pulmonary bone, peritoneum, internal organs or the CNS (Redig, 1993).

Aspergillus and Macrophages Interaction: The avian respiratory system responds efficiently to invasion by pathogens with a rapid influx of heterophils and macrophages from the subepithelial compartment and pulmonary blood vessels (Nganpiep and Maina, 2002). Macrophages are the main by the phagocytic cells of the respiratory tract and phagocytosed conidia in an actin-dependent manner through the recognition of pathogen-associated molecular patterns by host cell pattern recognition receptors, PRRs (Toll Like Receptors, TLR2 and TLR4 (Netea et al., 2003; 2006) and the C-type lectin receptor dectin-1 (Gersuk et al., 2006). A proinflammatory response is generated characterized production of cytokines (Phadke and Mehrad, 2005) and chemokines (Morrison et al., 2003). Dectin-1 specifically binds to fungal carbohydrates (1, 3)-glucan, which results in phagocytosis, activation of macrophages and generation of proinflammatory responses (Gersuk et al., 2006; Hohl et al., 2005;
Table 2: Aspergillosis in different avian species

<table>
<thead>
<tr>
<th>Avian species</th>
<th>Aspergillus sp</th>
<th>Lesions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emu (Dromaius novaehollandiae)</td>
<td>A. fumigatus</td>
<td>Pulmonary Aspergillosis</td>
<td>Shukla et al., 2013; Eswaran et al., 2011</td>
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<td>Emu Chicks</td>
<td></td>
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<td>Karunakaran et al., 2010</td>
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<td>Pigeon (Columbia livia)</td>
<td>A. fumigatus</td>
<td>Systemic aspergillosis</td>
<td>Elmubarak and Fadlelmula, 1991</td>
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<td></td>
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<td>Miliary aspergillosis</td>
<td>Gonzalez-Acuna et al., 2007; Tokarzewski, 2007; Beernaert et al., 2008</td>
</tr>
<tr>
<td>Duck (Anas platyrhynchos)</td>
<td>A. terreus</td>
<td>Disseminated aspergillosis</td>
<td>Pal, 1992</td>
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<td></td>
<td>A. fumigatus</td>
<td>Pulmonary aspergillosis</td>
<td>Savage and Isa, 1951; Parker, 2011; Rao and Choudary, 1980</td>
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<tr>
<td>Brahmini duck (Tadoroma ferruginea, Pallas)</td>
<td></td>
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<td>Sinha et al., 1978</td>
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<td>Khaki Campbell ducks</td>
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<td>Bhattacharya, 2003</td>
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<td>Mallard duck (Anas platyrhynchos)</td>
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<td>Adrian et al., 1978; Pearson, 1969; Hille and Lindner, 1968; Whirford and Robinson, 1980</td>
</tr>
<tr>
<td>Common peafowl (Pavo cristatus)</td>
<td>Aspergillus fumigatus</td>
<td>Respiratory aspergillosis</td>
<td>Ainsworth and Rewell, 1948</td>
</tr>
<tr>
<td>Japanese quail (Coturnix coturnix japonica)</td>
<td>Aspergillus flavus</td>
<td>Myotic salpingitis</td>
<td>Singh et al., 1994</td>
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<td></td>
<td>Aspergillus fumigatus</td>
<td>Pneumonic aspergillosis</td>
<td>Pandita et al., 1991</td>
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<td>Experimental aspergillosis</td>
<td>Chaudhari and Sadana, 1988; Gümüşsoy, 2004; Tell, 2010; Ghor i and Edgar, 1973; Borah et al., 2010</td>
</tr>
<tr>
<td>Turkey (Meleagris gallopavo)</td>
<td>A. flavus</td>
<td>Myotic pneumonia and pododermatitisArticular aspergillosis of hip joints</td>
<td>Stoute et al., 2010</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>Pulmonary Aspergillosis</td>
<td>Kunkle and Sacco, 1998; Ghor i and Edgar, 1973; Singh et al., 2009</td>
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<td>Omphalitis</td>
<td>Cortes et al., 2005</td>
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<td>Acute aspergillosis</td>
<td>Kunkle and Rimler, 1996</td>
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<td>Aspergillosis in the brains</td>
<td>Ozmen and Dorre stein (2004)</td>
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<td>Airsacculitis</td>
<td>Richard et al., 1996</td>
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<td>Davidson et al., 1985</td>
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<td>Goliath Heron (Ardea goliath)</td>
<td>Aspergillus sp</td>
<td>Pulmonary aspergillosis</td>
<td>Bonar et al., 2004</td>
</tr>
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<td>Vulture</td>
<td>A. fumigatus</td>
<td>Pulmonary aspergillosis</td>
<td>Mihaylov et al., 2008; Jung et al., 2009</td>
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<td>Bearded vulture (Gypaetus barbatus),</td>
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<tr>
<td>Eurasian black vultures (Aegypius monachus Linnaeus)</td>
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<tr>
<td>Himalayan Griffon Vulture (Gyps himalayensis)</td>
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<tr>
<td>Great horned owl (Bubo virginianus)</td>
<td>Aspergillus sp</td>
<td>Pulmonary carcinoma</td>
<td>Barathidasan et al., 2013</td>
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<td></td>
<td>A. niger</td>
<td>Pulmonary aspergillosis</td>
<td>Rettenmund et al., 2010</td>
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<tr>
<td>Himalayan Griffon Vulture (Gyps himalayensis)</td>
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<td>Herring Gulls (Larus a. argentatus L.), Seagulls (Larus cachinnans micaelis)</td>
<td>A. fumigatus</td>
<td>Pulmonary and Disseminated aspergillosis</td>
<td>Beer 1963; Nardoni et al., 2006</td>
</tr>
<tr>
<td>Love bird (Agapornis roseicollis)</td>
<td>A. fumigates, A. flavus</td>
<td>Pulmonary Aspergillosis</td>
<td>Corrasco et al., 1993</td>
</tr>
<tr>
<td>Red-faced love bird (Agapornis pullaria)</td>
<td></td>
<td></td>
<td>Ainsworth and Rewell, 1948</td>
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<tr>
<td>Species</td>
<td>Organisms</td>
<td>Disease Description</td>
<td>References</td>
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<tr>
<td>Stitchbird or hihi (Nofiomystis cincta)</td>
<td>A. fumigatus</td>
<td>Lung and air sacs affected</td>
<td>Alley et al., 1999; Cork et al., 1999</td>
</tr>
<tr>
<td>Geese (Anser species)</td>
<td>A. fumigatus</td>
<td>Pulmonary and systemic aspergillosis</td>
<td>Alley et al., 1999; Cork et al., 1999; Beytut et al., 2004</td>
</tr>
<tr>
<td>Wild geese (Chloëphaga poliocephala)</td>
<td>A. flavus</td>
<td>Syringeal aspergillosis</td>
<td>Stroud and Duncan, 1982</td>
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<td>Canada geese (Branta Canadensis)</td>
<td>A. fumigatus</td>
<td>Pulmonary aspergillosis</td>
<td>Beer 1963</td>
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<td>Pink-footed geese (Anser brachyrhynchus Bolliun)</td>
<td>Aspergillus sp.</td>
<td>Cerebral aspergillosis</td>
<td>Palya and Balogh, 1971, 1972</td>
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<td>Geese (Anser species)</td>
<td>A. flavus</td>
<td>Pulmonary aspergilloma</td>
<td>Hawkey et al., 1984; Stroud and Duncan, 1983</td>
</tr>
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<td>King shag (Phalacorax albivenier)</td>
<td>Aspergillus species</td>
<td>Spinal Aspergillosis</td>
<td>Bygrave, 1981; Barnett et al., 2011</td>
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<td>Pheasant</td>
<td>Aspergillus sp.</td>
<td>Pulmonary aspergillosis</td>
<td>Reissig et al., 2002; Copetti et al., 2004</td>
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<td>Rhea (Rhea Americana)</td>
<td>A. flavus and A. niger</td>
<td>Tracheal and systemic forms of aspergillosis</td>
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<tr>
<td>Penguin</td>
<td>Magellanic penguin (Spheniscus magellanicus), Gentoo penguin (Pygoscelis papua), Chinstrap penguin (Pygoscelis antarctica), King penguin (Aptenodytes patagonica), Little blue penguins (Eudyptula minor), Yellow-eyed penguins (Megadyptes antipodes), Rockhopper penguins (Eudyptes chrysocome), Adelie penguins (Pygoscelis adeliae), Peruvian penguin (Spheniscus humboldti), Black footed/Jackass Penguins (Spheniscus demersus)</td>
<td>A. fumigatus, A. niger and A. flavus</td>
<td>Pulmonary Aspergillosis</td>
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<td>Ostrich (Struthio camelus)</td>
<td>A. flavus and A. niger</td>
<td>Severe disseminated aspergillosis</td>
<td>Khosravi et al., 2008</td>
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<td>A. fumigatus</td>
<td>Mycotic rhinitis</td>
<td>Fitzgerald and Moisan, 1995</td>
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<td>Katz et al., 1996; Marks et al., 1994; Yokota et al., 2004; Perelman and Kuttin 1992</td>
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<td>A. fumigatus, A. niger and A. flavus</td>
<td>Multisystemic Aspergillosis with Granulomas</td>
<td>Kim et al., 2011</td>
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<td>A. flavus</td>
<td>Systemic mycosis</td>
<td>Sawale et al., 2012</td>
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<td>Sawale et al., 2011</td>
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<td>Pneumomycosis</td>
<td>Islam et al., 2009</td>
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<td>A. flavus</td>
<td>Mycotic tracheitis</td>
<td>Corkish, 1982; Pal et al., 1990; Singh et al., 1993</td>
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<td>Airsacculitis</td>
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<td>A. flavus</td>
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<td>A. flavus</td>
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<td>Steinlage et al., 2003</td>
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<td>A. flavus</td>
<td>Mycotic dermatitis</td>
<td>Grewal and Brar, 1987</td>
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<td>A. flavus</td>
<td>Concurrent aspergillosis and ascites</td>
<td>Zafra et al., 2008</td>
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Aspergillus Defences: Resting A. fumigatus conidia are resistant to macrophage killing by masking (1, 3)-glucan with hydrophobic rodlets protein and delaying macrophage activation. Melanin pigment in Aspergillus, serve a protective role against host defenses, specifically via scavenging ROS (Jacobson, 2000; Langfelder et al., 2003). Rodlets and superoxide dismutases (SODs) implicated in pathogenicity as scavengers of toxic ROS. Rodlets through the rod gene, rodA mutants, display increased susceptibility to alveolar macrophage killing (Paris et al., 2003). Rodletless conidia also induced a weak inflammatory response in a rat model of invasive aspergillosis (Shibuya et al., 1999). The eukaryotic SOD enzyme, Cu/Zn-SOD has been detected in the cell wall of conidia and hyphae (Hamilton et al., 1996).

5. Clinical Signs
Susceptible bird develops polymorphic clinical forms in relation to either localized or disseminated lesions. Aspergillosis occurs in acute and chronic form. Acute aspergillosis results from inhaling an overwhelming number of spores while chronic aspergillosis generally associates with immunosuppression (Vanderheyden, 1993).

5.1 Acute Form
The acute form usually develops less than a week. Young birds generally have a acute or peracute infection resulting in high morbidity and mortality. Clinical signs include difficult breathing, decreased or anorexia, polydypsia, cyanosis, foetid diarrhea and emaciation. Sometimes the birds may die suddenly without showing any clinical sign. Diagnosis is generally made through a post-mortem examination.

5.2 Chronic Form
The chronic form of aspergillosis may take weeks or months to develop. It is much more common in older birds. Clinical signs vary with the location of the infection. It includes inappetence, emaciation, dyspnea, gasping, increased thirst, fever, diarrhea and signs of nervous involvement (Jensen et al., 1997; Henrici, 1939). Green coloration in urates and hepatomegaly can be seen. Respiration may be noiseless and syrinx involvement leads to wheezing, rattling or clicking sound. Nares may become plugged or discharge with rhinitis (Tsai et al., 1992) along with malformation of the nostrils and beak (Bauck et al., 1992). Death occurs due to severe respiratory involvement. There may be ophthalmitis and keratitis (periorbital and eyelid swelling with cheesy yellow exudates in the conjunctival sac) (Beckman et al., 1994; Hoppes et al., 2000) as well as necrotic granulomatous dermatitis (Abrams et al., 2001). Wing droop can be observed when pneumatic bones such as the humerus gets involved (Forbes, 1991).

6. Lesions
The primary location of lesions is the lungs and air sacs although other organs may be involved. Extensive involvement of the respiratory tract can occur before development of clinical signs. Lesions vary in size from pinhead or miller seed (milliary <1mm in diameter) white to yellowish granulomas up to the size of a pea. Roughly spherical granulomatous nodules (>2 cm) may also be observed in serosa and parenchyma of the other organs involved.

6.1 Respiratory System
In acute aspergillosis, lungs showed the most striking lesions which are characterized by marked congestion and often studded with milliary yellow nodules. Each nodule is localized by a dark infiltrated zone while the other part of the lung appears normal. Air sacs are usually thickened with small whitish-yellow plaque-like lesions. In peracute pnemonic form, there is complete congestion of lung and no formation of nodules. Chronic aspergillosis is characterized by typical granulomatous lesions. It includes of variable sized nodules or multiple plaques that may be disseminated throughout the air sacs and
lungs (Fig 1). These lesions are especially observed in the periphery of the lungs and caudal thoracic and abdominal air sacs and may show sporulating fungal colonies. The serous membrane of the air-sacs is presented with yellowish-white plaque-like lesions or raised white nodules.

Trachea and bronchi may become blocked either by mucoid discharge or by the yellowish-white plaque-like lesions or raised white nodules. Nasal aspergillosis causes exudative rhinitis (Tsai et al., 1992). Malformation of the nostrils and beak were reported (Bauck et al., 1992). Mycotic tracheitis has been reported in chickens (Corkish, 1982) and domestic fowl (Singh et al., 1993). Fungal rhinosinusitis, with almost complete destruction of the premaxilla and deformation of the upper beak were reported by Mans et al. (2007).

6.2 Gastro-intestinal Tract

In turkeys, plaque-like lesions may occur in the mouth, gizzard and intestines (Lignires and Petit, 1898).

6.3 Liver, Kidney, Spleen, Heart, Brain, Ovary

Visceral organs were involved in aspergillosis with formation of nodular granulomatous lesions (Fig 2). Right ventricular dilatation or cor pulmonale due to pulmonary hypertension may occur with or without ascites in poultry (Julian and Goryo, 1990; Hofle et al., 2001). Abscesses in the cerebellum and cerebrum were reported (Raines et al., 1956; Jungherr and Gifford, 1944). It may occur with or without pulmonary and other lesions. In the cerebellum of broiler breeders and turkeys, circumscribed white to greyish areas were observed (Akan et al., 2002; Jensen et al., 1997). Granuloma formation was also seen in the brain and lungs of layer chicken (Kim et al., 2011). Nodular lesions have been reported in ovary (Emmel, 1929). Mycotic salpingitis associated with A. flavus was reported in adult female Japanese quail. White to grayish nodules 2-5 mm in diameter was present on the serosal surface of the oviduct (Singh et al., 1994).

6.4 Skin

Mycotic pododermatitis along with pulmonary aspergillosis was reported in turkeys. In footpads, keratinized epidermal disruption, encrustations and acute inflammation were noted (Stoute et al., 2009). Epidermal cysts associated with A. fumigatus have been described in the comb of a silky bantam chicken (Suedmeyer et al., 2002). Mycotic dermatitis was also reported in domestic fowl (Grewal and Brar, 1987).

6.5 Eyes

Lesions were observed in the eyes of baby chicks (Hudson, 1947) and in turkeys (Moore, 1953). Mycotic keratitis has been reported. It leads to periorbital swelling, swollen and adhered eyelids with turbid discharge, cloudy cornea and cheesy yellow exudates within the conjunctival sac (Beckman et al., 1994; Hoppes et al., 2000). In a peregrine falcon-gyrfalcon hybrid, blepharitis and dermatitis involving the eyelids and the head were recorded (Abrams et al., 2001). Dyar et al. (1984) reported non ulcerative or mildly ulcerative keratitis in a turkey flock. Dalton and Ainsworth (2011) reported mycotic keratoconjunctivitis in 12-day-old red-legged partridges (Alectoris rufa).

6.6 Bones and Joints

Involvement of ribs of broiler is observed as shown in Fig 3. Osteo-arthritis and granulomatous osteoarthritis of the hip joints with necrosis of the femur head was observed in turkey (Olias et al., 2010). Ribs of ostriches (Perelman and Kuttin, 1992) and sternum of broiler breeders with A. flavus (Martin et al., 2007) were reported.
7. Histopathology

Based on histopathological alterations, aspergillosis is distinguished into a deep nodular and a superficial diffuse form (Cacciuttolo et al., 2009). A well-organised granulomatous reaction develops in non-aerated as well as aerated parenchyma. An outer thick fibrous layer encapsulated these organised granulomas (Beytut et al., 2004; Copetti et al., 2004). In the adjoining tissues neither exudative inflammation nor vascular lesions were observed in this form (Nardoni et al., 2006, Cacciuttolo et al., 2009).

Fig 3: Aspergillosis, Lung, Poultry: Photomicrograph of Aspergillus granuloma structure showing necrotic area, inflammatory cells and fungal hyphae (Arrow). Grocott's methenamine silver, X10.

In superficial diffuse form, pyogranulomatous reaction containing fungal elements predominates in air sacs and lungs and was non-encapsulated (Jensen, 1997). Pyogranuloma is characterized by a centre with variable amounts of septate, dichotomously branching hyphae containing large numbers of conidiophores and conidia. These hyphae were surrounded by a cliff of radially arranged macrophages, heterophils, foreign body giant cells and lymphocytes (Nardoni et al., 2006; Cacciuttolo et al., 2009). Multinucleated cells phagocytized fungal elements. Lymphocytes infiltrated along the margins of the granuloma. In case of severe inflammation, parabronchioles obscured with eosinophilic necrotic material containing degenerated heterophils, erythrocytes and exfoliated epithelial cells (Martin et al., 2007; Beytut, 2004). A mixed type of both tissue reactions in the same tissue section were also reported (Tsai et al., 1992; Atasever and Guvenisoy, 2004).

In case of angioinvasive pulmonary aspergillosis by A. fumigatus (Barathidasan et al., 2013), there was vascular invasion by fungal hyphae involving numerous small to large veins of lungs and air sacs. Both alveolar epithelium and the blood vessel wall were severely damaged by penetrating fungal hyphae. Numerous vessels were thrombosed as a result of fungal hyphae invasion and intramural host reaction (Barathidasan et al., 2013).

8. Diagnosis

Antemortem diagnosis of aspergillosis can be very difficult since the signs of disease mimic those of many other illnesses, especially in the chronic form. Cases of aspergillosis in birds are often diagnosed based on postmortem findings of white caseous nodules in the lungs or air sacs of affected birds since clinical diagnosis is difficult (Beytut et al., 2004; Charlton et al., 2008). In case of exotic pet birds, cumulative diagnostic tests including biochemistry, haematology, radiography, laparoscopy or endoscopy (Jones and Orosz, 2000) may prove beneficial but these are not available in the poultry context.

8.1 Clinical History

A detailed history of the course of the illness and an accurate description of the diet and husbandry of the bird is required.

8.2 Necropsy Examination

Granulomatous nodules and/or cheesy plaques on the serosa and parenchyma of respiratory tracts as well as other organs are observed. But, definitive diagnosis is based on the isolation of Aspergillus species by culture or by the detection of the organism during histological examination (Kunkle, 2003).

8.3 Direct Microscopy (Wet Smear Examination)

Identification can also be made by preparing a wet smear. For this, a nodule can be dissected out and crushed on a slide beneath a cover slip in a drop of 20% potassium hydroxide and lactophenol cotton blue. The lactophenol cotton blue stains the fungal hyphae. Wet mounts can also be prepared from sputum or nasal swabs in either 10% KOH and Calcofluor or Parker ink and/or Gram stain.

8.4 Histopathological Examinations

The tissue samples (lungs, trachea, pharynx and thoracic air sacs as well as other organs) fixed in 10% neutral buffered formalin are processed and embedded in paraffin blocks and stain with haematoxylin and eosin (HE) method. Aspergillus hyphae stained poorly in H and E stained sections. Differential stains such as Periodic acid-Schiff (PAS), Bauer's and Gridley's stains differentiate and easily identify the hyphae and mycelia. Special stains for fungus Grocott’s and Gomori Methanamine Silver stain should be employed to detect the presence of fungal hyphae (Fig 3-Arrows).

8.5 Culture
For proper identification of the species, the pathogenic organism must be isolated by culturing it on differential media. Small pieces of lesions aseptically removed are placed onto plates or slants containing malt agar, Sabouraud's glucose agar or antibiotics and incubated at 37°C for 24 hours. Species of *Aspergillus* can be identified by observing the characteristic conidial head and colony as shown in Table 2.

### 8.6 Immunohistochemistry

Immunohistochemistry with monoclonal or polyclonal antibodies can be used to identify *A. fumigatus* in lesions.

### 8.7 Serology

A number of serological test have been applied in the diagnosis of aspergillosis. It includes counter immunoelectrophoresis (CIE), agar gel immunodiffusion (AGI) and enzyme-linked immunosorbent assays (ELISA). However, they should never be used alone, and must be correlated with other clinical and diagnostic data (Redig *et al*., 1997; Cray *et al*., 2006; 2009). In acute cases, antibody titre is low and thus detection of circulating *Aspergillus* antigen in the serum (Cray *et al*., 2006) while in chronic cases in which antigen levels may be low, detection of antibodies (Jones and Orosz, 2000) may be useful. It should be noted that serological tests have not been validated in poultry and are not currently used in farms to investigate Aspergillosis outbreaks. França *et al* (2012) reported serologic testing for aspergillosis in commercial broiler chickens and turkeys.

### 8.8 Differential Diagnosis

Avian aspergillosis signs are nonspecific and depend on the system involved. Pulmonary aspergillosis is usually differentiated from other avian respiratory diseases by the granulomatous lesions at necropsy, but needs to be differentiated from other mycoses and mycobacteriosis. Aspergillosis should be differentiated from chlamydiophylosis, tuberculosis, neoplasia, vitamin A deficiency, bacterial disease, candidiasis, ascitis, hepatomegaly and pneumonia.

### 9. Treatment

Treatment for aspergillosis is complicated and relies on the use of antifungal medication. The success of treatment depends upon the location and extent of the infection. However even the most potent drugs could not reach the fungal granulomas or the walled-off fungus by the inflammatory response. This disease has a poor prognosis in extensive infection in the tissues and using only systemic drugs. The best treatment results if the granulomatous lesions are debrided and a topical treatment in conjunction with a systemic therapy is given. The drugs used include itraconazole, fluconazole, clotrimazole, miconazole, ketoconazole and amphotericin B as shown in Table 3.

### 10. Prevention and Control

No vaccine against aspergillosis is available till date. Some autogenous vaccines have been applied but with little information about this vaccine. Although numerous antifungal protocols have been proposed to cure birds with aspergillosis, treatment of the disease in poultry farms is virtually impossible. Therefore, preventative measures should be practiced so that this disease does not become established in the flock. Since *Aspergillus* genus is an opportunistic pathogen, reduction of predisposing immunosuppressive factors such as malnutrition and stress should be encouraged. Standard of hygiene, nutrition and housing should be maintained. Mouldy litter or feed should be avoided. Feeders, waterers and incubators should be frequently cleaned and disinfected. Appropriate ventilation should be provided to maintain relative humidity so as to prevent wet litter. Environmental contamination should be control by sporadic or repeated antifungal treatment.

Spraying of fungistatic agents like nystatin, thiabendazole or copper sulphate (at 1 gram per 2 litre of water daily morning for 3 days) decreased fungal

### Table 3: Summary of the antifungal drugs used in Aspergillosis

<table>
<thead>
<tr>
<th>Anti-fungal drugs</th>
<th>Dosage</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>1 mg/kg for 20 minutes, 3 to 4 times a day for 10-14 days</td>
<td>Nebulization</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/Kg TID for 3-5 days</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>1.35 mg/kg</td>
<td>Topically</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>1% in 2 or 3 ml saline 1.5 hr/day for 4 to 6 weeks</td>
<td>Topical Nebulization</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>15mg/kg twice a day for 7 days</td>
<td>Orally</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>5mg-10 mg/kg twice a day for 7-21 days or 10mg/kg once a day for 7-21 days</td>
<td>Orally in feed</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>20 to 30mg/kg twice a day for 2 to 6 weeks</td>
<td>Oral</td>
</tr>
<tr>
<td>Miconazole</td>
<td>1% suspension in 2 or 3 ml saline for 1 week</td>
<td>Topical</td>
</tr>
</tbody>
</table>
contamination of litter. Itraconazole at 10mg/kg once a day for 10 days orally have been used as prophylactic measures in birds with high risk to develop aspergillosis. During outbreak, disease spread can be decreased by changing CuSO₄ solution at a dose of 1 gram per 2 litre of water daily orally for 5 days instead of drinking water. Poultry breeder should disinfect eggs soon after being laid and gathered in order not to have problems for future new born chickens.

11. Conclusions

References


Ainsworth GC and Rewell RE (1949). The incidence of aspergillosis. During outbreak, disease spread can be decreased by changing CuSO₄ solution at a dose of 1 gram per 2 litre of water daily orally for 5 days instead of drinking water. Poultry breeder should disinfect eggs soon after being laid and gathered in order not to have problems for future new born chickens.

In conclusion, aspergillosis is precipitated when the natural defenses and immunity of the bird is challenged by Aspergillus species. Since clinical diagnosis is difficult, avian aspergillosis is often diagnosed based on postmortem findings supported by microscopy, culture, immunohistochemical technique and molecular methods. Aspergillosis is preventable if proper intensive poultry farming with good husbandry practices like hygiene, food storage and preparation methods, cage location and avoiding stress factors and other conditions that could predispose a bird to the development of the disease.


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