



Outbreak of *Aspergillus fumigatus* in an Organized Emu Farm in Indore, India

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Abstract

Detailed postmortem examination was conducted on eleven emu chicks presented to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Mhow, MP, India from local emu farm in Indore. Birds had died with history of respiratory signs and mild nervous signs. On gross examination, numerous small grayish white nodules were seen in many organs particularly in the lungs. Histopathologically nodules on the lungs were characterized by granulomatous necrotic areas infiltrated with monocytes, lymphocytes and plasma cells. Numerous thin, tubular septate branching fungal hyphae with parallel-sided walls were seen in the parenchymatous tissue along with mononuclear cell infiltration. Liver of the affected birds revealed severe subcapsular and sinusoidal congestion. Diffuse areas of necrosis and severe congestion were noticed in the spleen. The disease was diagnosed as aspergillosis by correlating clinical signs with postmortem findings (Gross lesions and histopathology) and microscopic detection of fungus in the lung tissue followed by isolation and identification of the fungus. As the disease may take a very fatal course and no effective treatment has been established yet, so effective preventive measures should be taken to reduce the ensuing economic losses.

Keywords: *Aspergillus fumigatus*; Emu; farm; India

Introduction

The Emu (*Dromaius novaehollandiae*) is a large flightless bird native to Australia. It belongs to ratite group, which also includes ostriches, rheas and cassowaries. The emu is principally farmed for low-fat meat, high quality leather and oil and are generally processed at around 12-18 months age. As entrepreneurs raise birds to meet the potential demand, a worldwide interest in ratites has developed (Tully and Shane, 1996). Since the mid-1980s, as ratite production has increased, there have been advances in knowledge of the diseases of these species. *Aspergillus spp.* has been identified as a primary cause of death in juvenile emus (Marks *et al.*, 1994). The most susceptible individuals are young birds in enclosed facilities with exposure to dust or to hay, which is alternately wet and dry. *Aspergillus spp.* exposure can occur through egg contamination but, under current con-

ditions in the industry, poorly-managed intensive chick rearing seems to be the most likely place for an outbreak to occur (Tully and Shane, 1996). The most common species of *Aspergillus* causing disease in birds are *A. fumigatus*, *A. flavus* and *A. niger*, but *Aspergillus fumigates* accounts for 95% of the cases and *A. flavus* is the second most common organism associated with avian infections (Tell, 2005). To the authors' knowledge only a few reports of *Aspergillus* infection in juvenile emus have been reported in India (Eswaran *et al.*, 2011; Sunitha *et al.*, 2010). In this paper, authors' reported the occurrence of severe aspergillosis due to *Aspergillus fumigatus* in emus in an organized farm in Indore, India.

Materials and methods

The study material included the eleven emu chicks in the age group of two to five weeks from an organized local emu farm of 146 birds, presented to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow, M.P, for postmortem examination. The his-

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tory revealed that the birds were fed with a mixture of alfalfa hay, oats and rice bran. The affected chicks gradually appeared unthrifty and less active than other chicks in the group. The signs of gasping and respiratory distress were evident during a forced exercise. Later on birds showed labored respiration along with dyspnea, cough, anorexia, dullness, ataxia and incoordination. Some of the affected birds also showed nervous signs prior to death. No response was found to oral antibiotic therapy with enrofloxacin at 15mg/Kg body weight, twice daily.

Detailed necropsy was conducted and grossly visible lesions in different organs were noted. Impression smears were taken from the affected organs and stained with Wrights-Giemsa stain. Tissue specimens from various organs were collected in 10% formalin and processed by routine paraffin embedding technique (Sheehan and Hrapchak, 1980). Briefly, the fixed tissue samples were cut into pieces of 2-3mm thickness and washed thoroughly with water for several hours before putting in ascending grades of alcohol for dehydration, followed by clearance in benzene and embedded in paraffin. Sections of 4-5 micron thickness were cut and stained with Haematoxylin and Eosin stain (Luna, 1968).

For bacteriological examination the swabs from lungs, liver and spleen were taken and aerobically cultured on Brain Heart Infusion Agar (BHIA) with 5 per cent sheep blood and on MacConkey's agar. Also macerated tissues from liver and intestine were incubated in Selenite F broth at 37°C for culture of probable Salmonella infection. This enrichment was followed by culture on MacConkey's agar, Brilliant Green Agar (BGA) and Xylose Lysine Agar (XLD) and incubated overnight at 37 °C.

For isolation and identification of the fungus, lung and liver tissues were cultured in sabouraud dextrose agar (SDA, Hi media) supplemented with chloramphenicol (0.05mg/mL) and incubated under aerobic condition at 25°C for 3-5 days (Jung *et al.*, 2009). The colonies were transferred using Roth flag technique (Quinn *et al.*, 1994) to a clean microscopic slide containing few drops of Lactophenol cotton blue stain.

Results

Postmortem examination revealed congested liver with focal necrotic areas. Small greyish-yellow

nodules of varying sizes were present on the lungs (Fig. 1) and at times lungs were adhered to thoracic cavity. Trachea of some birds revealed severe congestion and formation of a caseous plug partially blocking the lumen (Fig. 2). Numerous small developing nodules were evident on the serosal surface of proventriculus and other visceral organs. Catarrhal enteritis and petechial haemorrhages were present on the mucosa of intestinal tract. In addition petechial haemorrhages were noted on the pericardium and in some birds meninges of the brain were severely congested. Kidneys were swollen and congested (Fig. 3).



Fig. 1. Lungs revealing small greyish-yellow nodules scattered along the parenchyma.

Histopathologically nodules on the lungs were characterized by granulomatous necrotic areas infiltrated with monocytes, lymphocytes and plasma cells. Numerous thin, tubular septate branching fungal hyphae with parallel-sided walls were seen in the parenchymatous tissue along with mononuclear cell infiltration (Fig. 4) and fibrous tissue proliferation at the periphery. Section of lungs from some birds revealed numerous hyphae arranged in radial pattern in the lumen of parabronchioles. Liver of the affected birds revealed severe subcapsular and sinusoidal congestion. Diffuse areas of necrosis and severe congestion were noticed in the spleen.



Fig. 2. Trachea of the affected bird revealing a caseous plug partially blocking the lumen along with severe congestion.

Impression smears stained with wrights Giemsa from lungs revealed fungal hyphae. No growth was observed on the BHIA, MacConkey's agar and on Selenite F broth after 48 hours incubation at 37°C, or even after extended period of incubation on any media, which were used in the study.



Fig. 3. Kidneys of an affected juvenile emu revealing oedema and severe congestion.

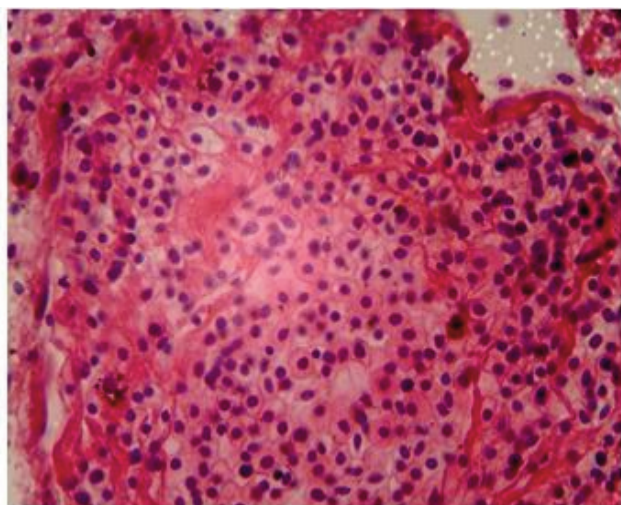


Fig. 4. Section of lung parenchyma revealing fungal hyphae along with mononuclear cell infiltration (H & E) ×400.

Culture of lung and liver tissues in Sabouraud dextrose at 25 °C revealed characteristic fungal growth in 3-5 days. The visible colonies were flat, white at first, and then bluish green as conidia began to mature, especially near to the center of the colony. On maturation conidial masses became gray-green, while the colony edge remained white. Lactophenol cotton blue staining of the suspected colonies revealed mycelia composed of septate hyphae bearing smooth conidiophores, conidiophores vesicle, sterigmata and chains of pigmented conidia. Conidia were echinucleated, spherical to semispherical and 2-3µm in diameter. Based on the colonial mor-

phology and microscopic characteristics, the isolated fungus was identified as *Aspergillus fumigatus*.

Discussion

Aspergillosis has been identified as a primary cause of death in juvenile emus and ostriches (Marks *et al.*, 1994) and most common identified species is *Aspergillus fumigatus* (Chute *et al.*, 1997; Jordan, 1990). Fungal infections generally occur via inhalation of air borne spores from moldy feed, dust, dirty pens, and dirty and improperly sanitized hatchery equipment (Oglesbee, 1997). The origin of the infection could not be traced in this study, but it is likely that overcrowding or dust could have predisposed the birds to infection. Eleven chicks had died in the concerned farm belonging to age group of two to five weeks, showing lesions typical of aspergillosis. Immune system in young chicks being in developing stage buttressed by stress such as overcrowding and malnutrition further make them more susceptible to infection with a very severe manifestation of disease (Jordan, 1990; Sunitha *et al.*, 2010). The postmortem findings in the present study were similar to those previously described by Sunitha *et al.* (2010) in emu chicks and for other avian species with pulmonary aspergillosis (Oglesbee, 1997; Tell, 2005). Grayish yellow nodules in the lungs and in other visceral organs were a predominant postmortem finding in all the affected birds. These pulmonary nodules could have disrupted the normal lung functioning and probably caused death due to respiratory failure (Chakravarty, 1976; Eswaran *et al.*, 2011). Histopathological features observed in present study were in concurrence with that of Eswaran *et al.* (2011). Based on the colonial morphology and microscopic morphology the isolated fungus was identified as *Aspergillus fumigatus*. Elizabeth *et al.* (2002) and Sunitha *et al.* (2010) reported similar findings from aspergillosis in great rheas and emus, respectively.

Based on the clinical signs, postmortem findings, microscopic detection, isolation and identification of the fungus, the disease was diagnosed as Aspergillosis. Efforts to control this problem should be directed at prevention, as there is no effective cure yet. Therefore every attempt should be made to reduce predisposing immunosuppressive factors such as stress and malnutrition. To avoid in-

halation of large number of spores, birds should be housed in ventilated area without overcrowding and prevented access to moldy feed.

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