Case Report: Angioinvasive Pulmonary Aspergillosis in an Adult Captive Green Java Peafowl (*Pavo muticus*)

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Abstract. *An adult Green Java peafowl, from a private aviary, was presented for treatment at the Riphah Pet Hospital, Lahore, Pakistan. Physical examination of the bird revealed lethargy, gasping and head jerking but no other clinical signs. The bird died before any intervention. At necropsy, multiple off-white hard nodules were observed in the lungs and two off-white flat growths in abdominal air sacs. Systemic spread of the fungus to the liver causing hepatitis was also noticed. Kidneys were pale, yellow and atrophied but fungus hyphae or conidia could not be detected in kidneys.*

Microscopically, the lung nodules were typical granulomas consisting of Aspergillus spp. hyphae and necrosis in the centre surrounded by inflammatory cells, mainly heterophils and macrophages and rare multinucleated giant cells. From the literature search, it appears to be the first report of angioinvasive mycotic pneumonia in Green Java peafowl.

Statement of novelty

Pulmonary aspergillosis is not uncommon in poultry and avian wildlife. This is the first report describing the condition in Green Java Peafowl kept in captivity.

History and clinical signs

An adult Green Java peafowl of age 2.5 years was presented for examination with severe illness. History included anorexia, depression, weight loss and severe respiratory distress. The bird received, poorly described, extensive medication by the owner using antibiotics, etc., but the sickness remained progressive. The live body weight of the bird was 2.84 kg.

Physical examination of the bird revealed depression and severe open-mouth difficult breathing. The bird died soon after arrival at the hospital before any intervention and no ante mortem tests could be performed.

Post-mortem lesions

Necropsy examination revealed pectoral muscle atrophy and fibrosis at the site of intramuscular injection(s).

Grossly, the lungs were congested haemorrhagic and consolidated with multifocal off-white nodules on the surface, as well as deep in the parenchyma, varying from 0.4 cm to 1.0 cm in diameter (Fig. 1A). Microscopically, the interalveolar septa were thickened with fibrin, heterophils, lymphocytes and macrophages. There was congestion, haemorrhages and extensive consolidation, and many areas contained abundant hemosiderin pigment (Fig. 2A) in the lungs. The nodules were typical fungal granulomas (Fig. 2B) comprised of septated branching hyphae of Aspergillus spp. (Fig. 3B) and conidia heads, which were stained with Haematoxylin and Eosin stain (Fig. 3A) with mainly heterophil infiltration followed by infiltration of macrophages and rare multinucleated giant cells. Fungal hyphae caused extensive necrosis and also invaded blood vessels (Fig. 4).

Two fungal growths were also present in the left abdominal air sac (9 mm diameter x 1mm thick and 4 mm diameter x 0.5 mm thick), off-white in colour, surrounded by general cloudiness of air sacs (Fig. 1B). The bigger colony was attached to the gizzard serosal surface but it had no physical contact with the lungs. Wet smears, stained with Wright's stain, revealed fungal hyphae and conidia in the thickened inflamed air sacs.

Grossly, the oral cavity showed stomatitis. Lesions consisted of yellowish-green, fur-like tongue and palate. Scrapings, stained with Wright's stain, showed conidial heads and fungal hyphae.

The liver was enlarged and inflamed with numerous tiny aspergillus colonies. Fungal hyphae were seen in impression smears from the cut surface of the liver stained with Wright's stain. Kidneys were pale and atrophied.

Discussion

Soil is the natural reservoir of Aspergillus from which aerosols of conidia are released that are inhaled and deposited deep in the respiratory tract. Aspergillosis, a respiratory disease, is common in young chickens (Dykstra et al., 2013), captive (and wild) birds (Khosravi et al., 2008; Samson et al., 2014; Hauck et al., 2020; Arne et al., 2021), aquatic birds (Melo et al., 2020). The published literature is limited to case reports in various birds, but information on

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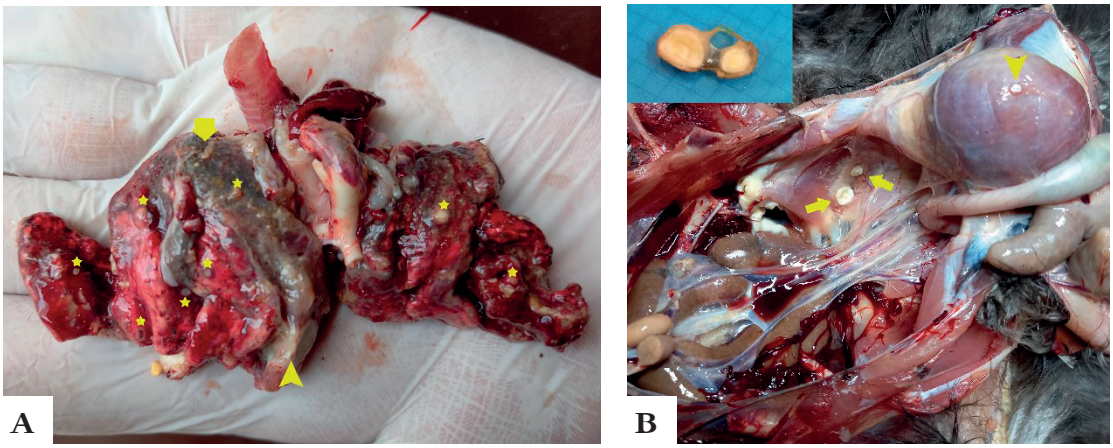

Fig. 1. The lungs of a Green Java Peafowl showing (A) congestion, consolidation and multifocal off-white nodules (*). The pleural covering of the lungs is thickened due to oedema and fibrinous inflammation (arrow). A large fungal colony involves pleura and lung parenchyma (arrowhead). (B) Grossly, two fungal growths were observed in the left abdominal air sac (arrows). The inset shows a closer view of the two colonies. The bigger colony was attached to the gizzard serosa (arrowhead).

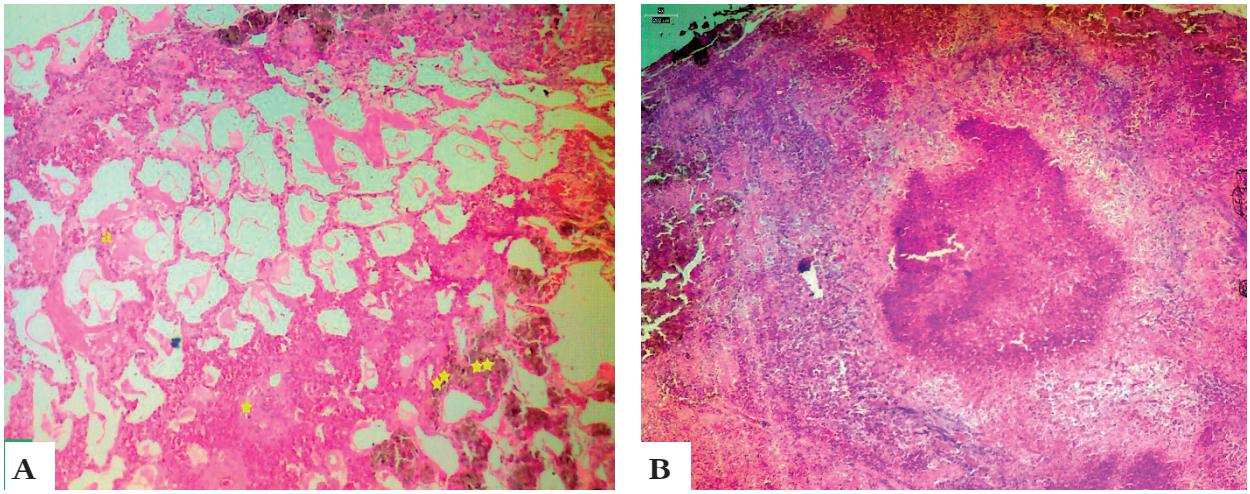

Fig. 2. A photomicrograph of the lung: (A) thickened inter-alveolar septa (a) with fibrin, heterophils, lymphocytes and macrophages; necrosis sounded by consolidation (*) and hemosiderin pigment (**); (B) a typical Aspergillus granuloma in the lungs (H&E stain).

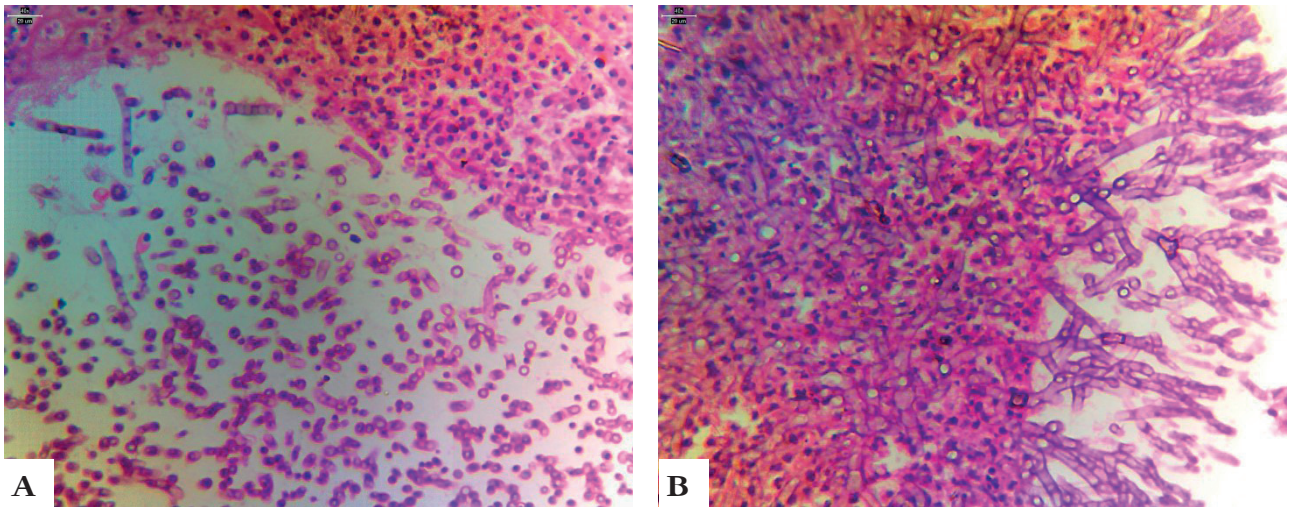

Fig. 3. (A, B) A photomicrograph of the lung of the Green Java peafowl showing extensive septated hyphae and conidia typical of Aspergillus spp., stained with Haematoxylin and Eosin stain.

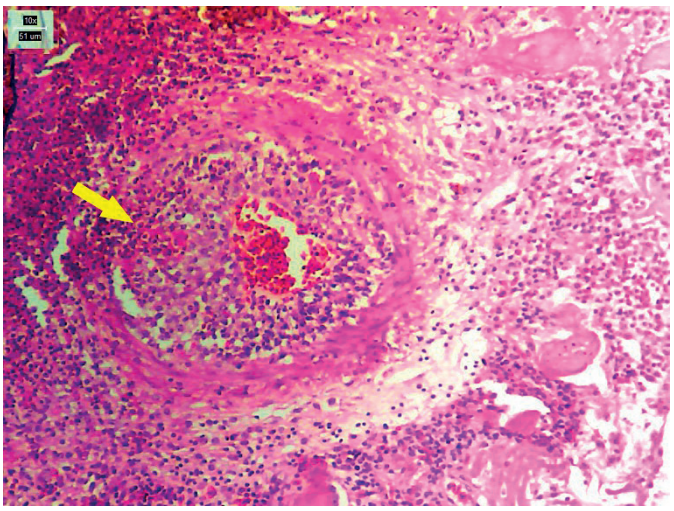

Fig. 4. The lung section of the Green Java peafowl shows an invasion of hyphae into an arteriole (arrow), and fungus growth has narrowed the arteriolar lumen to less than a half.

A photomicrograph of the lung with thickened interalveolar septa with fibrin, heterophils, lymphocytes and macrophages. HE × 320
A photomicrograph of the lung showing congestion and oedema. HE × 200

pulmonary mycosis in Green Java peafowl is scarce or not available.

Aspergillus flavus is a more prevalent species in Pakistan (Hedayati et al., 2007). However, in the Green Java peafowl, *Aspergillus fumigatus* was confirmed. Noteworthy, *A. fumigatus* conidia being smaller in size (2 to 3.5 µm) than *A. flavus* conidia (3 to 6 µm) allows *A. fumigatus* conidia to reach the pulmonary alveoli much easier than those of *A. flavus* (Hedayati et al., 2007).

Past case reports in various birds have emphasized a granulomatous type of lesion in the lungs. In the present case, an extensive and widespread inflammatory reaction was also seen in the lungs (Fig. 1). According to Ahamad et al. (2018), mycelial development causes tissue necrosis and incites a strong host reaction. Hence, a massive inflammatory response inducing severe necrosis and inflammation in the air sacs could be linked to extensive hyphae sprouting.

In humans, pulmonary nodules are a less common manifestation of aspergillosis in immunocompetent patients (Muldoon et al., 2016) but they have been associated with drastic immunosuppressive therapies (Hedayati et al., 2007). Also, Frank et al. (2005) induced severe mycotic air sacculitis and pneumonia with *A. fumigatus* by prolonged prednisolone therapy in parrots. There was no traceable obvious immunosuppressive factor in the present case. However, healthy birds exposed to large numbers of aerosol conidia may be infected without immunosuppression. Furthermore, *A. fumigatus* itself secretes molecules which are potentially immunosuppressive (Latgé, 1999).

In general, mononuclear cells predominate in chronic granulomatous lesions. However, in this case of aspergillus granuloma, heterophils predominated in the necrotic tissue. As reviewed by Latgé (1999), neutrophils remain responsible primarily for hyphal killing, and conidia are killed by macrophages. Therefore, extensive development of mycelium (see Fig. 3) justifies heterophil predominance in aspergillus lesions.

Aspergillosis can cause local lesions in the respiratory tract or systemic infections involving internal organs such as the liver, kidneys, or brain (Dykstra et al., 2013). The inflammation of the liver in this case appears to be systemic dissemination of the Aspergillus from the lungs (note angio invasion in Fig. 4).

There was no gross or histological evidence of fungal invasion in the kidneys. Pale, atrophied kidneys suggest chronic damage by mycotoxins; however, feed samples could not be obtained for spores or mycotoxins analysis due to the non-availability of feed consumed in the past. Kidney damage could also be related to injudicious and excessive medication or synthesis of mycotoxins by Aspergillus while growing in tissues.

The green Java was purchased around 6 months ago. Keeping in view the history and longevity of lung lesions in the case and hygienic management at the aviary, *a priori*, the bird was a latent carrier at the time of its arrival to this location. Five of the mates at this aviary are apparently healthy. Therefore, it appears that the *A. fumigatus* was not bird-to-bird transmitted.

Declaration of interest

The authors report no conflicts of interest. The research was conducted in the absence of any commercial or financial relationships. The authors are responsible for the content and writing of the paper.

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