

Pathomorphological studies on respiratory Aspergillosis in a Japanese quail farm

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ABSTRACT

Three ailing and eight breeder quail carcasses of both sexes at 10 weeks old were brought to Department of Veterinary Pathology for clinical diagnosis and postmortem examination respectively with the history of regular mortality for the past five days. Clinically, ailing birds showed dullness, depression with respiratory distress and nasal discharges. Gross examination revealed yellow to whitish caseous nodules on the lung parenchyma. Air sacs were thickened with multiple tiny whitish nodules. Histopathological examination of air sacs revealed granulomatous inflammation characterised by the presence of tubuliform structures which were surrounded by caseous exudate and infiltration of macrophages and lymphocytes. Lungs revealed caseous central core mass contained numerous septate fungi and was surrounded by heavy infiltration of heterophils, macrophages, lymphocytes and giant cells. This was confirmed by special stains namely Periodic acid-Schiff (PAS) and Grocott methenamine silver nitrate (GMS) which showed pink and black coloured septate hyphae respectively. Based on the cultural characteristics, gross, cytology, histopathological and histochemical findings, it was concluded as aspergillosis infection in the breeder quail farm.

Keywords: Air sacs, Aspergillosis, caseous exudate, giant cells, lungs, quail

Aspergillosis is a non-contagious fungal disease that can affect humans, animals and birds due to inhalation of fungal spores found in feed, bedding and litter materials. It is a ubiquitous conidial saprophytic fungus that affects almost all variety of birds like chicken, ducks, quails and wild birds and often leading to high morbidity and mortality rates^{1,2}. Avian aspergillosis is caused by different species: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Aspergillus glaucus*. Among these, *Aspergillus fumigatus* is the most common pathogen involved in the avian aspergillosis³.

The primary route of transmission for aspergillosis is through inhalation and it affects lower respiratory disease mainly air sacs and lungs^{2,4}. The fungal hyphae on air sacs and lungs either produce localized acute infection like air sacculitis and bronchopneumonia or chronic granulomatous inflammation. In some cases, hematogenous spread causes chronic granulomatous lesions in all visceral organs⁵. However ocular and neural form of aspergillosis also reported in birds². In some cases, it may cross Blood Brain Barrier (BBB) and localise in brain tissue causing encephalitis marked by neurological symptoms⁶. The present report describes the pathomorphological studies of respiratory aspergillosis in breeder Japanese quails.

A breeder Japanese quail flock with the capacity of 350 birds had daily mortality of seven to eight birds regularly. Three ailing (One male and two female) birds and eight (three female and five male) dead Japanese breeder quail carcasses of both sexes were presented to the Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal for disease investigation and postmortem examination respectively. Detailed clinical examination was done and clinical signs were noted. Systematic necropsy was carried out and gross lesions were recorded.

Impression smears taken from the cut section of nodules from lungs and air sacs were subjected to Gram staining, acid fast staining and lactophenol

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cotton blue staining to rule out the etiological agent. Lung and air sac tissue suspensions were inoculated into Sabouraud dextrose agar (SDA) and incubated at 37°C for one week⁷. Smears were prepared from the cultured fungus and subjected to lactophenol cotton blue staining technique for identification of fungal organisms.

Organs showing lesions were collected in 10 percent neutral buffered formalin. Paraffin embedded tissue sections were cut at four-micron thickness and stained with Haematoxylin and Eosin (H&E). Additionally, histochemistry techniques like PAS and GMS were performed for further confirmation⁸.

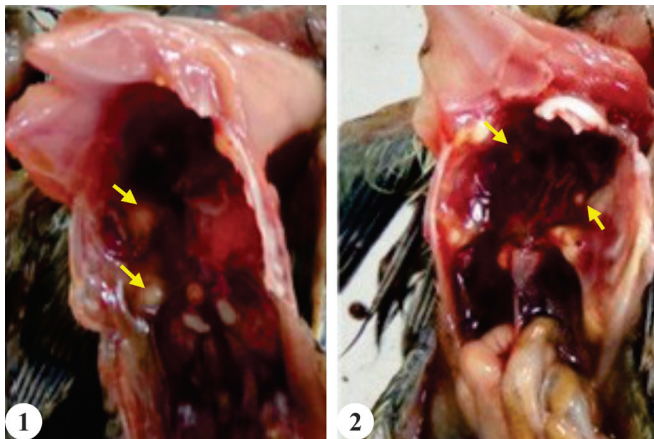


Fig. 1. Air sacs showing whitish nodules; **Fig. 2.** Lungs revealing yellowish white nodules studded in the parenchyma.

Mortality was noticed within 24-48 hrs of onset of symptoms about 37 birds out of 350 breeder quails over a period of five days. Clinically, ailing birds were emaciated, anorectic, dull, depressed and showed respiratory distress, nasal discharge and incoordination. On necropsy, external examination revealed emaciated and dehydrated carcasses with ruffled feathers. The keel bone was prominent with atrophy of breast muscles. Internal examination revealed thickening of both thoracic and abdominal air sacs with scattered whitish nodules (Fig. 1). The cut section of nodules exhibited cheesy mass of varying diameter (2-5 mm). There were yellowish

white caseous nodules studded on the lung parenchyma (Fig. 2). However, the other viscera were normal devoid of any specific lesions.

In cytology, impression smears from crushed nodules of lung and air sacs showed no observable bacteria or acid-fast organisms in Gram staining and Acid-fast staining respectively. Whereas, numerous branched septate fungal hyphae along with characteristic conidial structures were noticed in Lactophenol Cotton Blue staining under light microscopy. *Aspergillus fumigatus* was recovered from SDA culture of lung and air sacs. Dark green velvety colonies appeared within 48 hrs with white reverse pigmentation. The colony showed spiny green conidiophores with the short and smooth spores on the upper half of the vesicle.

Histologically, air sacs showed nodule with caseous necrotic area in the centre surrounded by fibrous capsule (Fig. 3). It showed tubuliform fungal hyphae in the centre surrounded by heavy infiltration of heterophils, lymphocytes and macrophages (Fig. 4). Lungs showed multifocal caseous necrotic areas surrounded by heterophils, lymphocytes, macrophages and numerous giant cells (Fig. 5). There were pink coloured septate and branching hyphae observed in PAS-stained lung sections (Fig. 6). In GMS staining, black coloured fungal hyphae were dispersed in the caseous necrotic mass as well as in the surrounding pulmonary parenchyma (Fig. 7).

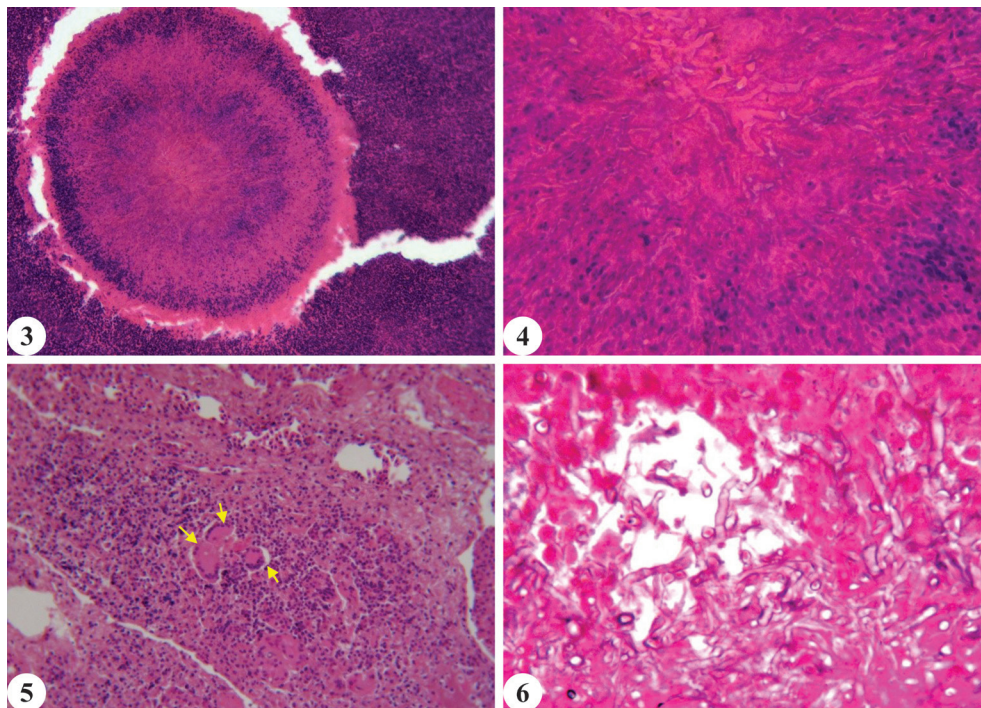


Fig. 3. Air sac showing caseous mass containing fungal hyphae surrounded by inflammatory cells and fibrous capsule (H&E x100); **Fig. 4.** Air sac showing caseous necrosis with radiating fungal hyphae and infiltration of inflammatory cells (H&E x400); **Fig. 5.** Lung showing granulomatous reaction with heterophils, lymphocytes and giant cells (H&E x100); **Fig. 6.** Lung showing caseous mass containing numerous pink coloured fungal hyphae (PAS x400).



Fig. 7. Lung showing blackish fungal material in caseous mass and surrounding parenchyma (GMS x400).

Based on the colonial morphology, reverse pigmentation and cytology by using LCB technique, the isolated fungus was identified as *A. fumigatus*. In addition, histomorphology and histochemistry further confirmed the fungal pneumonia by *A. fumigatus*.

The affected birds exhibited the symptoms of acute aspergillosis viz. anorexia, respiratory distress, depression and death and these clinical signs were supported by the earlier report⁹. In the present study, lesions were confined to the respiratory system rather than a systemic manifestation¹⁰. The extremely small size of *A. fumigatus* conidia (2-3 µm) enables them to evade the mucociliary clearance mechanism and allow the conidia to reach the lower respiratory tract, facilitating pulmonary deposition and potential colonization¹⁰.

The air sacs appeared abnormally thickened and opaque due to caseous exudates which was deviated from their thin and transparent appearance. Similar air sac lesions were also recorded by previous author¹¹. Granulomatous lesions in lungs and air sacs are the hallmark of pulmonary aspergillosis. Microscopic lesions showed typical granulomatous lesion in lungs and air sacs with central caseous necrotic mass with fungal hyphae surrounded by fibrous capsule and infiltration of lymphocytes and giant cells. These histopathologic observations were very well correlated with gross pathology and were in accordance with recent reports on pulmonary aspergillosis^{1,12}.

Although Japanese quails are generally hardy, fungal pneumonia caused by aspergillosis may occur due to predisposing immunosuppressive conditions such as infectious bursal disease and chicken infectious anemia.

These diseases are more commonly found in nearby commercial layer farms, can increase the susceptibility of quails to opportunistic fungal infections.

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