Indian J Anim Health (2024), 63(2)- Special Issue: 248-252
DOI: https://doi.org/10.36062/ijah.2024.spl.11324

Short Communication

Neuro-ocular aspergillosis: A rare systemic form of aspergillosis in Japanese quails (*Coturinx Coturnix Japonica*)

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Abstract

The paper describes the neuronal and ocular form of aspergillosis in Japanese quail with rare cerebral vascular lesions. Two-week-old Japanese quail flock holding 300 birds reported gradual mortality and had clinical signs of weakness, respiratory distress, incoordination, tilting of the head and blindness. Systematic postmortem was carried out in three randomly selected dead birds. Samples from the brain and lungs were collected for histopathological and microbiological studies. Samples were processed as per standard protocols. Necropsy showed variable-sized yellowish-white caseous nodules in air sacs, lungs, kidney, liver, spleen, adrenal and pericardium. Cloudiness of the lens and caseous exudate in the vitreous chamber was obvious. The cerebrum showed yellowish-white firm areas with indistinct borders. Microscopically, tissues revealed mycotic granulomatous lesions characterized by a caseous necrotic centre with radiating fungal hyphae surrounded by inflammatory cells. The eye showed pyogranulomatous uveitis and cataracts with septate fungal hyphae. Cerebrum revealed necrosis with septate fungal hyphae, glial cell proliferation, congestion and chondroid metaplasia of blood vessels. *Aspergillus fumigatus* was isolated from lung and brain lesions by cultural examination. GMS stain in tissues showed radiating fungal hyphae from the centre of lung nodules and in the brain. Proteases of fungus and intracellular germinating fungal spores within macrophages resulted in invasive aspergillosis and haematogenous spread causing systemic chronic aspergillosis. Encephalitis with chondroid metaplasia of the brain and uveitis were found to be rare lesions which were responsible for neuronal signs and visual disorders. Overexpression of TGFβ1 from lesions could cause the above changes, which was plausible in the present study with earlier reports.

Keywords: Aspergillosis, Chondroid metaplasia, Encephalitis, Japanese quail, Uveitis

Highlights

- Systemic aspergillosis appears to be uncommon in Japanese quails.
- Documenting the rare neuronal and ocular form of aspergillosis in Japanese quails.
- Mycotic uveitis and encephalitis with choroid metaplasia of cerebral vessels are unique findings.

Aspergillosis is a non-contagious fungal disease of human beings, animals and birds caused by the saprophytic fungus *Aspergillus* spp. *Aspergillus* spp. are ubiquitous conidial fungus found commonly in bedding, litter material and feed. Birds are comparably susceptible among vertebrates. All species of aviary birds are equally susceptible, including domestic/captive birds, pet birds, and penguins (Arne *et al.*, 2011; Sanduri *et al.*, 2023). *Aspergillus fumigatus* and *A. flavus* are found to cause aspergillosis in birds, the former being the most common cause in poultry (Cheng *et al.*, 2020). Inhalation of conidia forms the primary route of transmission and causes lower respiratory disease, primarily affecting the air sacs and lungs. Fungal hyphae and mycelium in tissues produce either acute localized lesions characterized by airsacculitis and bronchopneumonia or may spread haematogenously producing chronic granulomatous lesions. Vascular spread causes multisystemic lesions in all visceral organs (Beernaert *et al.*, 2010; Girma *et al.*, 2016). Seldom, it perches into the eye, causing mycotic endophthalmitis, uveitis and cataract (Khambati *et al.*, 2022). On a rare account, it may cross the blood-brain barrier and seeds on brain tissue, causing encephalitis characterized by neurological signs (Arulmozhi *et al.*, 2016; Hauck *et al.*, 2020). This paper describes the rare neuro-ocular form of aspergillosis in Japanese quail with uncommon chondroid metaplasia of cerebral blood vessels.

A private poultry flock holding 300 two-week-old Japanese quails reported the gradual mortality of two quails every day for four weeks. Clinically, quails were dull and depressed and showed gasping, dyspnoea, blindness, incoordination and tilting of the head. Two dead birds randomly selected were brought and

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submitted for postmortem examination to the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Thanjavur. Systematic necropsy was carried out. Crushed impression smears were collected from caseous nodules for cytology. Enucleated nodules from the lungs and a piece of brain tissue lesion were collected in a sterile container for microbial culture. Tissue samples from lungs, air sacs, kidney, liver, heart, eye and brain were collected in 10% formalin for histopathology. Crushed impression smears were subjected to wet film examination with lactophenol cotton blue stain. Nodules were triturated and processed as per the standard fungal culture method using Sabouraud Dextrose Agar (SDA). Formalin fixed tissue samples were processed as per standard paraffin embedding technique. Tissue sections of 4-5 µm thickness were cut and stained with Haematoxylin and Eosin as per routine standard technique (Bancroft and Layton, 2019) and duplicate tissue sections were stained with Grocott methenamine silver (GMS) impregnation technique (Morris *et al.*, 2019).

Externally, carcasses were thin with dry and ruffled feathers. The right eye of a bird revealed cloudiness of the lens and exudate in the posterior chamber (Fig. 1a). On internal examination, cervical, clavicular, thoracic and abdominal air sacs showed multiple scattered yellowish-white firm raised nodules of varying diameter (2-5 mm) and contained thick, yellowish-white caseous content. Lungs showed 1-3 mm multiple scattered yellowish-white firm raised caseated nodules embedded over and within the lung parenchyma. Cerebral hemispheres showed yellowish-white foci of varying diameter (1-2 mm) deeper in the white matter. Caseous nodules were also noticed over the surface of the liver, pericardium, heart, spleen, adrenal and kidneys (Fig. 1b).

Cytological examination of crushed contents revealed branching septate hyphae. Cultural examination on SDA plate revealed dark green velvety colonies with white reverse pigmentation (Fig. 1c). On microscopic examination under lactophenol cotton blue stain, the hyphae showed conidiophore bearing vesicle with chains of conidia arranged over uniseriate row of phialides (Fig. 1d). Based on cultural and morphological features, the fungus was identified as *Aspergillus fumigatus*.

Histologically, air sacs showed thickening of the air sac wall, which was infiltrated by heterophils, lymphocytes, macrophages and fungal hyphae (Fig. 2a). Lungs showed multifocal caseous necrosis with basophilic fungal hyphae at the centre surrounded by granulomatous inflammation characterized by heterophils, lymphocytes, macrophages and few giant cells with the fibroblastic capsule. Few of these granulomatous lesions were noticed in parabronchioles (Fig. 2b & c). The cerebrum revealed moderate to severe congestion of white matter, multifocal necrosis (Fig. 3a) and vacuolation of neuropil of white matter. Necrotic tissue was intervened by basophilic radiating branched septate fungal hyphae surrounded by glial cell proliferation (Fig. 3b). Blood capillaries of nearby necrotic area revealed chondroid metaplasia. Metaplastic cells showed single isogenic matured chondron depicting central chondrocyte surrounded by peripheral matrix connected by thin intercellular matrix (Fig. 3c). Few of the blood capillaries revealed complete distortion of the vessel wall by cartilaginous tissue depicting two to three isogenic chondron with thick basophilic intercellular matrix (Fig. 3d). Eye showed pyogranulomatous uveitis. Pyogranulomatous lesion was obvious within the anterior uvea of the vitreous chamber (Fig. 4a). The choroid layer was thickened and disorganized with necrotic tissue, inflammatory cells (macrophages) and melanocytes. The entire choroid lesion was commingled with diffuse radiating septate fungal hyphae. Necrotic tissue showed scattered bacterial colonies, vascular congestion and calcification. Fungal hyphae were found spread to the sclera (Fig. 4b), ciliary body and lens. The ciliary body was disorganized and distended with necrotic tissue and fungal hyphae. The lens was condensed and found to be adhered to the ciliary body and choroid (Fig. 4c). The lens showed a degenerative change of fibres with obvious Morgagnian globules. The lens was rimmed and infiltrated with radiating septate fungal hyphae (Fig. 4d).

Histochemistry by GMS impregnation technique revealed black-coloured fungal hyphae radiating from the centre of the caseous mass of granulomatous nodules from the lung (Fig. 5a) and air sacs. Fungal hyphae were found beyond the fibrous capsule. Similar hyphae were also evidenced in cerebral necrotic tissue (Fig. 5b) and in the eye (Fig. 5c).

Based on the cytological features, cultural characteristics, histopathological changes and histochemistry, the lesions were inferred as mycotic granulomatous bronchopneumonia, mycotic airsacculitis, pericarditis, choroiditis, cataract, uveitis and leucoencephalitis caused by *Aspergillus fumigatus* with rare cerebral lesions.

Aspergillus spp. are common soil saprophytic fungus found in contaminated feed and bedding material. *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. glaucus* and *A. terreus* are known to cause aspergillosis in birds. *Aspergillus fumigatus* was first identified as a pathogenic

Neuro-ocular aspergillosis in Japanese quails

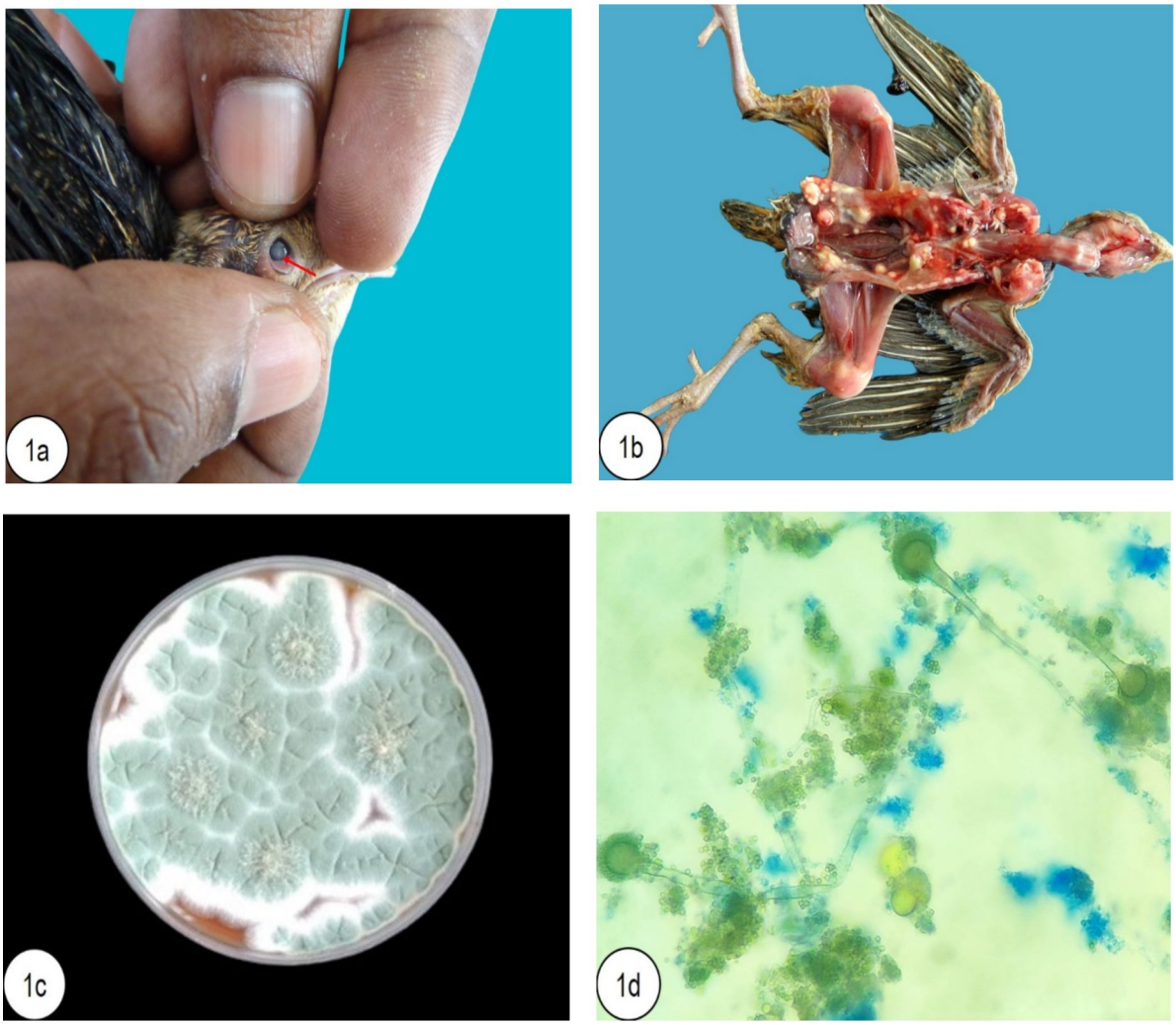
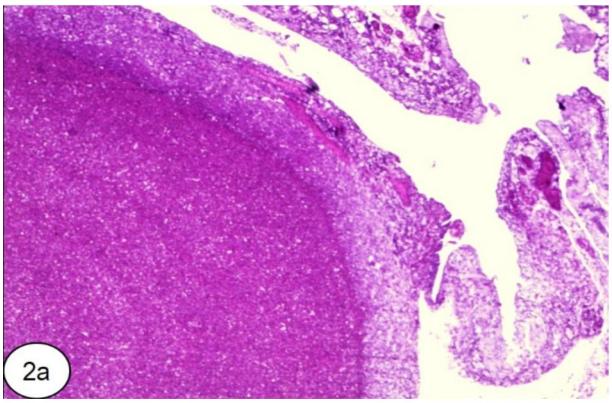
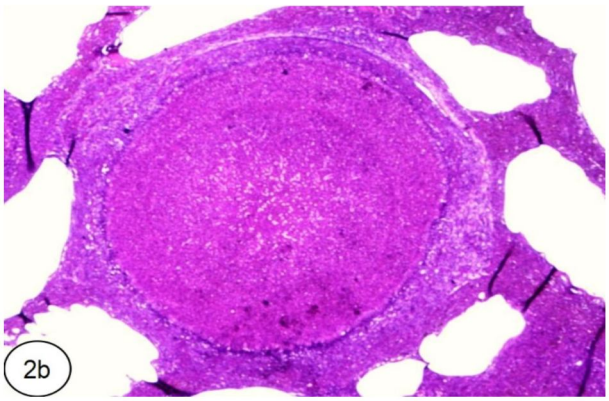


Fig. 1. Gross pathology neuro-ocular aspergillosis in Japanese quails. a. Cloudiness of lens; b. Emaciated carcass with multiple yellowish-white nodules in air sacs and lungs Japanese quail; c. *Aspergillus fumigatus* - SDA culture - green velvety colonies; d. *Aspergillus fumigatus* - SDA culture - Wet film examination - hyphae showed conidiophore





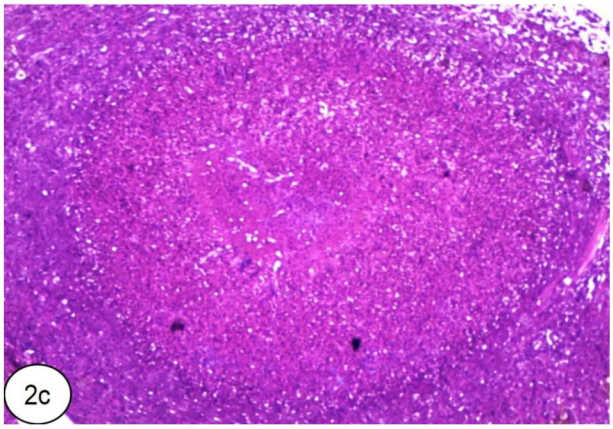
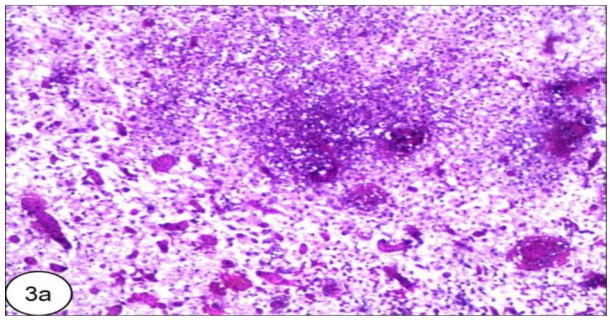
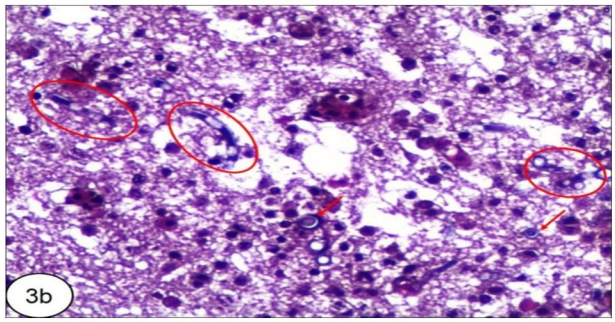
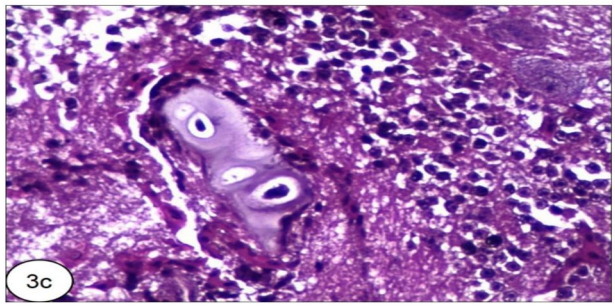


Fig. 2. Histopathology of neuro-ocular aspergillosis in Japanese quails (H&E stain). a. Granulomatous airsaculitis with thickening of epithelium, x100; b. Granulomatous obstructive bronchopneumonia, x40; c. Granulomatous obstructive bronchopneumonia intervened by fungal hyphae, x100







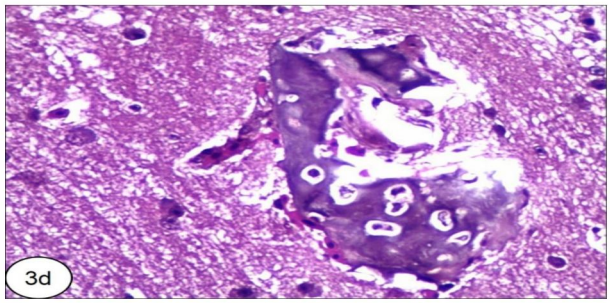


Fig. 3. Histopathology of neuro-ocular aspergillosis in Japanese quails (H&E stain). a. Necrosis with glial cell proliferation, congestion - Infiltrative mycotic leucoencephalitis, x40; b. Vacuolation of white matter with radiating septate fungal hyphae (circle & arrow) - Infiltrative mycotic leucoencephalitis, x400; c. Chondroid metaplasia of cerebral blood vessel - Infiltrative mycotic leucoencephalitis, x400; d. Chondroid metaplasia with distorted cerebral blood vessel - Infiltrative mycotic leucoencephalitis, x400

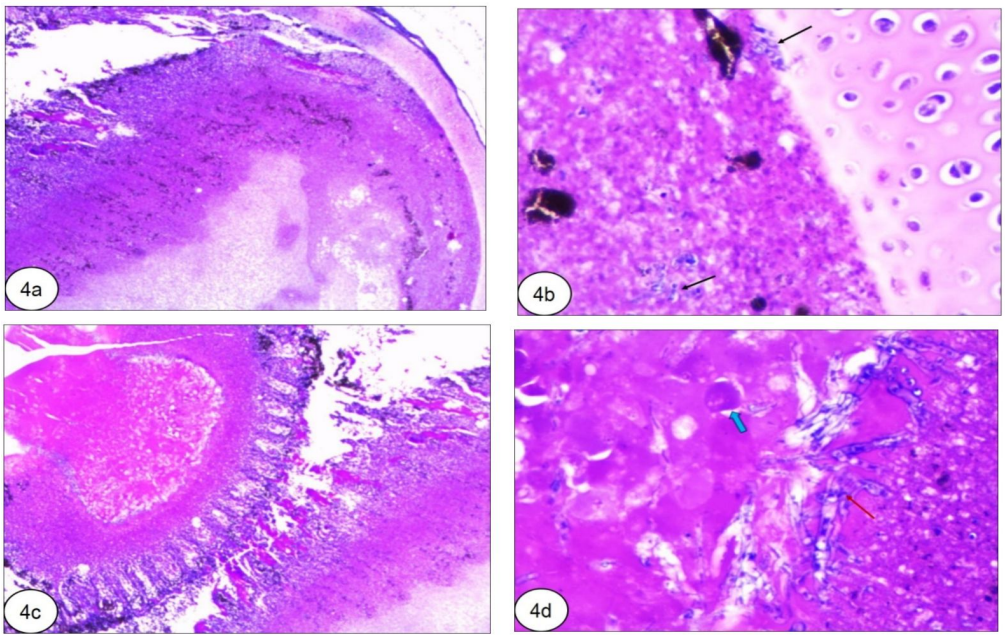


Fig. 4. Histopathology of neuro-ocular aspergillosis in Japanese quails (H&E stain). a. Pyogranulomatous choroditis, x40; b. Choroditis with fungal hyphae spreading into sclera, x40; c. Mycotic uveitis with degeneration of lens, x40; d. Cataract with obvious Morgagnian globules (broad arrow) and radiating septate fungal hyphae (narrow arrow), x400

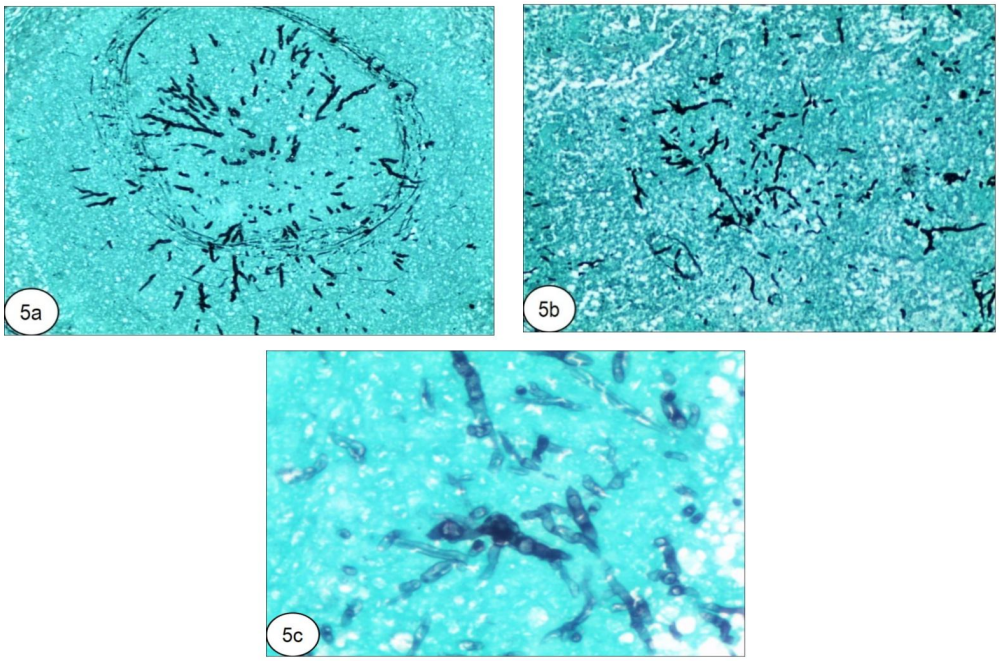


Fig. 5. Histochemistry of neuro-ocular aspergillosis in Japanese quail (Grocott methanamine silver stain). a. Radiating fungal hyphae beyond the connective tissue fiber - Obstructive bronchopneumonia, x100; b. Diffuse scattered fungal hyphae Mycotic leucoencephalitis, x100; c. Diffuse scattered fungal hyphae - Mycotic uveitis and cataract, x400

filamentous fungus in the lungs of bustard (*Otis tarda*) by Johann Baptist Georg Wolfgang in 1863 (Schmidt and Schmidt, 1999). Among domestic birds, Japanese quails were found to be highly susceptible, followed by turkey and chicken (Ghori and Edgar, 1973). Ghori and Edgar (1973) proved that Japanese quails showed high mortality during the first week after experimental exposure to spores of *Aspergillus fumigatus* when compared to chicken and turkey. Further, infected birds showed acute inflammatory lesions, such as congestion of the lungs during the first week and progressed as nodular lesions with decreased mortality after the first week of infection. This was in line with the present study, where the affected Japanese quail flock showed 19% mortality with nodular lesions recorded at the fourth week of age.

Clinical signs observed in the present study were in accordance with previous reports on quails (Ambily and Mini, 2017) and chickens (Sawale *et al.*, 2011; Nururrozi *et al.*, 2020; Zamboni *et al.*, 2020). Respiratory signs such as dypnoea and gasping were the reflection of respiratory lesions primarily due to the caseous necrotic tissue or the granulomatous nodules causing parabronchiolar obliteration (Cheng *et al.*, 2020; Nururrozi *et al.*, 2020). This was justifiable in the present study, which observed multiple parabronchiolar granulomatous nodules in the lungs and granulomatous airsacculitis. Incoordination and tilting of the head reported in the present malady might be due to mycotic leucoencephalitis. Brain lesions were in parallel with earlier reports on broiler breeders (Akan *et al.*, 2002; Sawale *et al.*, 2011), and their observed neurological signs in the affected flock had encephalitis. Similar observations were reported in aspergillosis induced experimental model with Lohmann broilers (Nururrozi *et al.*, 2020).

Aspergillosis occurs as an acute epizootic disease in young chicks under thirteen days old and infected *in ovo* or the hatchery (Nururrozi *et al.*, 2020). Acute aspergillosis was found to be characterized by localized lesions in lungs and air sacs with high mortality. Rather, the chronic sporadic form is characterized by high morbidity and low mortality and is usually found in adults or in breeders (Cheng *et al.*, 2020). Chronic aspergillosis was mostly found to be a disseminated disease with multiple granulomatous nodules (deep nodular form) in different organs (Throne Steinlage *et al.*, 2003). Infective spores carried intracellularly by respiratory macrophage (Beernaert *et al.*, 2010) were found to be the cause of haematogenous spread (Girma *et al.*, 2016; Ambily and Mini, 2017; Anjum and Khan, 2022). Proteases released by *Aspergillus* spp. (Arne *et al.*, 2011; Girma *et al.*, 2016) could cause tissue or angio-invasiveness (Anjum and Khan, 2022; Sree Lakshmi *et al.*, 2022). Altogether, the above properties of *Aspergillus* spp. enhance the pathogenesis of invasive aspergillosis (Savelieff *et al.*, 2018; Cheng *et al.*, 2020). This was evident in GMS impregnation stain of lung tissue where fungal hyphae radiated beyond the fibrous capsule in granulomatous nodules. The above possible explanation justifies that hematogenous spread mediated by infective macrophages could cause cerebral lesions, neurological signs and ocular lesions observed in the present study.

Gross and microscopic lesions in the present findings indicated that the disease was a chronic malady with nodular, caseous lesions in air sacs, lungs, kidneys, adrenal, pericardium, spleen, and liver were in parallel with earlier observations in chicken (Arne *et al.*, 2011; Girma *et al.*, 2016; Shoukat *et al.*, 2018). Lesions of aspergillosis in the brain were reported in turkeys (Arulmozhi *et al.*, 2016) and in broiler breeder flocks (Sawale *et al.*, 2011) and were observed from the cerebrum and cerebellum, respectively. These lesions were characterized by congestion with mild necrosis of the neuronal tissue. This was similar to the present case where the lesion was observed in the cerebrum as infiltrative diffuse form, characterized by necrosis of the white matter immingled with fungal hyphae, glial cell proliferation and surrounded by areas of severe vascular congestion, chondroid metaplasia of vascular channel around the necrotic tissue was unique. Cloudiness of the ocular lens due to aspergillosis was reported in turkeys (Dyar *et al.*, 1984) and in broiler breeders (Akan *et al.*, 2002) was analogous in the present cases. Although histopathology of ocular lesions were inaccessible in birds, thickening of choroid, pyogranulomatous exudates observed in the present study was parallel with *Aspergillus* endopthalmitis in immunocompromised human patient reviewed by Khambati *et al.* (2022). Exuberant growth of the fungus in the choroid and lens of the present observation unbox that it was due to immunosuppression due to primary fungal lesions. Endogenous spread from the lung *via* blood was the prime role for the fungal burden, tissue damage, and inflammation in the choroid, uvea, and lens proposed earlier (Spadea and Giannico, 2019; Khambati *et al.*, 2022). It was proved that retinal innate secretion of Toll-Like receptor (TLR) and upregulation of pro-inflammatory interleukins were responsible for *Aspergillus*-induced endophthalmitis (Khambati *et al.*, 2022).

It was hypothesized that transforming growth factor β1 (TGFβ1) was crucial in regulating vascular

architecture. Over-expression of TGFβ1 from vascular endothelium modulates the vascular phenotype with a pronounced effect on cellularity and matrix content and differentiates the state of cells of tunica media (Schulick *et al.*, 1998). It was postulated that vascular disease or tissue with inflammation initiates overexpression of TGFβ1, which mediates hyperplasia, apoptosis and cartilaginous metaplasia of vascular endothelium or tunica muscularis (Schulick *et al.*, 1998). In addition, bone morphogenetic proteins (BMP) were found to be involved in osseous metaplasia (Jacono *et al.*, 2001; Wood *et al.*, 2020). Chondroid or osseous metaplasia of neuronal vasculature was reported to be rare and observed in neuronal injury, neoplasms and its related inflammations (Cambruzzi and Pegas, 2014; Alkhaibary *et al.*, 2020). Acute to chronic inflammation and active fibroblast proliferation were postulated to mediate osseous metaplasia in rectal inflammatory polyp in human (Wood *et al.*, 2020). Intramuscular vascular osseous metaplasia of skeletal muscle was thought to be due to increased endothelial turnover by signal transducer molecules from intramuscular haemangioma (Panda *et al.*, 2018). Further, glial fibrillary acidic protein (GFAP) was found to increase the incidence of chondro-osseous metaplasia in ependymoma which lacked fibrous capsule in human brain (Alkhaibary *et al.*, 2020). The above theories suggested that chondroid metaplasia of neuronal tissue with leucoencephalitis induced by *Aspergillus fumigatus* might be of either GFAP-induced or TGFβ1-induced phenotypic change in the present case.

This report concludes that the property of angio-invasiveness of the fungal hyphae and intracellular germination of spores in macrophages might cause hematogenous spread from the lung-air sac system to other organs, including the brain and eye. Lesions observed in the cerebrum were of focally extensive infiltrative form. Further, overexpression of TGFβ1 and or elevated GFAP in the brain in acute to chronic lesions and in neoplasm, respectively, played a vital role in chondro-osseous metaplasia. Hence, the present malady was termed as neuroaspergillosis due to rare neuronal changes by *Aspergillus fumigatus*.

Although no quintessential antifungal is available for curative to neuro-ocular aspergillosis in birds, effective control measures can be achieved with good bedding/litter management, appropriate ventilation with controlled humidity in the shed, avoiding in-all-possible ways for fungal contamination in feeds and finally, creating stressless rearing with immunocompetency of the birds be the best way to avoid mortalities and loss of production.

Conflict of interest: The authors have disclosed no conflicts of interest in the study.

Author's contribution: NB: Conducted postmortem examination of dead birds, collected details regarding the outbreak and prepared the manuscript; JS: Assisted in interpretation of histopathological lesions and did proof reading of the manuscript; PS: Done the microbial study and confirmed the etiology agent.

Data availability statement: All the data supporting the findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

ACKNOWLEDGMENT

We acknowledge the Dean, Veterinary College and Research Institute, Orathanadu and Tamil Nadu Veterinary and Animal Sciences University for the facilities provided.

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Received- 24.08.2024, Accepted- 21.11.2024, Published- 01.12.2024 (Print)
Section Editor: Dr. C. K. Jana, Editorial Board Member