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Occurrence of aspergillosis in Giriraja chicken from Aizawl, Mizoram

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ABSTRACT

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An outbreak of respiratory tract affection with failure of antibiotic treatment was investigated in a backyard poultry farm of 100 Giriraja birds in the age group of 6-8 weeks from Aizawl (Mizoram). Twenty five birds showed the clinical signs of respiratory tract affection with increasing severity over a week. *Aspergillus fumigatus* was isolated and found to be the main etiological agent detected in 18 dead birds. On necropsy, white to yellow caseous nodules were observed in the lungs, air sacs, liver and kidneys. Histological sections of lungs revealed focal granulomatous reaction and thickening of air sac membranes due to heterophil and mononuclear infiltrations. *Aspergillus* hyphae invading the lung parenchyma were observed in lacto phenol cotton blue stained sections. *Aspergillus fumigatus* was isolated from the inoculations prepared from the suspensions of organs showing lesions. Therapeutic treatment with copper sulphate orally to the birds and replacement of litter material treated with copper sulphate responded rapidly with decrease in severity in clinical signs and suspended mortality.

Keywords: Aspergillosis, giriraja chicken, Mizoram

Aspergillosis is a respiratory tract infection caused by fungi of the genus *Aspergillus*, of which *A. fumigatus* is the primary species responsible for infections in chicken, canary, pigeon, turkey, ostrich and penguin. Although aspergillosis is predominantly a disease of the respiratory tract, all organs can be involved, leading to a variety of manifestations ranging from an acute rapidly fatal disease to chronic infections[1,2,3,4]. Aspergillosis generally occurs secondary to stress, immunosuppression, prolonged antibacterial therapy or overwhelming exposure to the organism. This paper reports clinical, histopathological and mycological examinations of an outbreak of severe disseminated aspergillosis in a backyard flock of Giriraja birds.

A backyard poultry farm of hundred Giriraja grower birds of 6-8 weeks age at Aizawl, Mizoram witnessed a respiratory distress and associated symptoms during May, 2011. The birds were reared in a poorly ventilated house with damp deep litter materials. Exaggerated respiratory signs appeared 2-3 days later resulting in death of 18 birds in a period of 7 days. Detailed post mortem examination of the dead birds was carried out in the Department of Veterinary Pathology, College of Veterinary Sciences, CAU, Aizawl for diagnostic evaluation. Tissue samples of the lungs, air sacs, liver, spleen and gastrointestinal tract were aseptically collected and fixed in 10% buffered formalin for 24 hours and then embedded in paraffin. Sections of tissues were cut (5µm) and stained with haematoxylin and eosin (H&E) for histopathological examinations[5]. Direct microscopic examination was also performed by lacto phenol cotton blue staining of impression smear for detection of fungal elements. A portion of the samples was cultured on Sabouraud glucose agar (Merck Co.) and incubated in duplicate at 25°C and 42°C for 3-7 days. For further confirmation of fungal isolates, dox agar (Merck Co.) was also used[6].

On clinical examination, 25 birds (25%) showed anorexia, dyspnoea, depression, weight loss, emaciation, stunning, polydipsia and cyanosis followed by failure of several antibiotic regimens. Affected birds walked with their heads close to the ground and moved lethargically. The clinically affected birds showed overall morbidity of 25.00% with 18.00% mortality. Post-mortem examination of the affected birds showed severe emaciation and soft bones with distortion of the thoracic cage. Multiple cream colour nodules, 1 to 10 mm in diameter, were distributed in the lungs, air sacs, liver and kidneys in all the dead birds (Fig.1). Histopathologically, focal granulomatous reaction was observed in lungs (Fig. 2). The granulomas with a central core of necrotic cellular debris and heterophils with a peripheral palisade of macrophages, epithelioid cells and aggregates of lymphocytes were observed in the thickened parabronchi, which lead to compression of air passages. Hyphal invasion and a few *Aspergillus* fruiting heads were also observed in the parabronchial parenchyma (Fig. 3).

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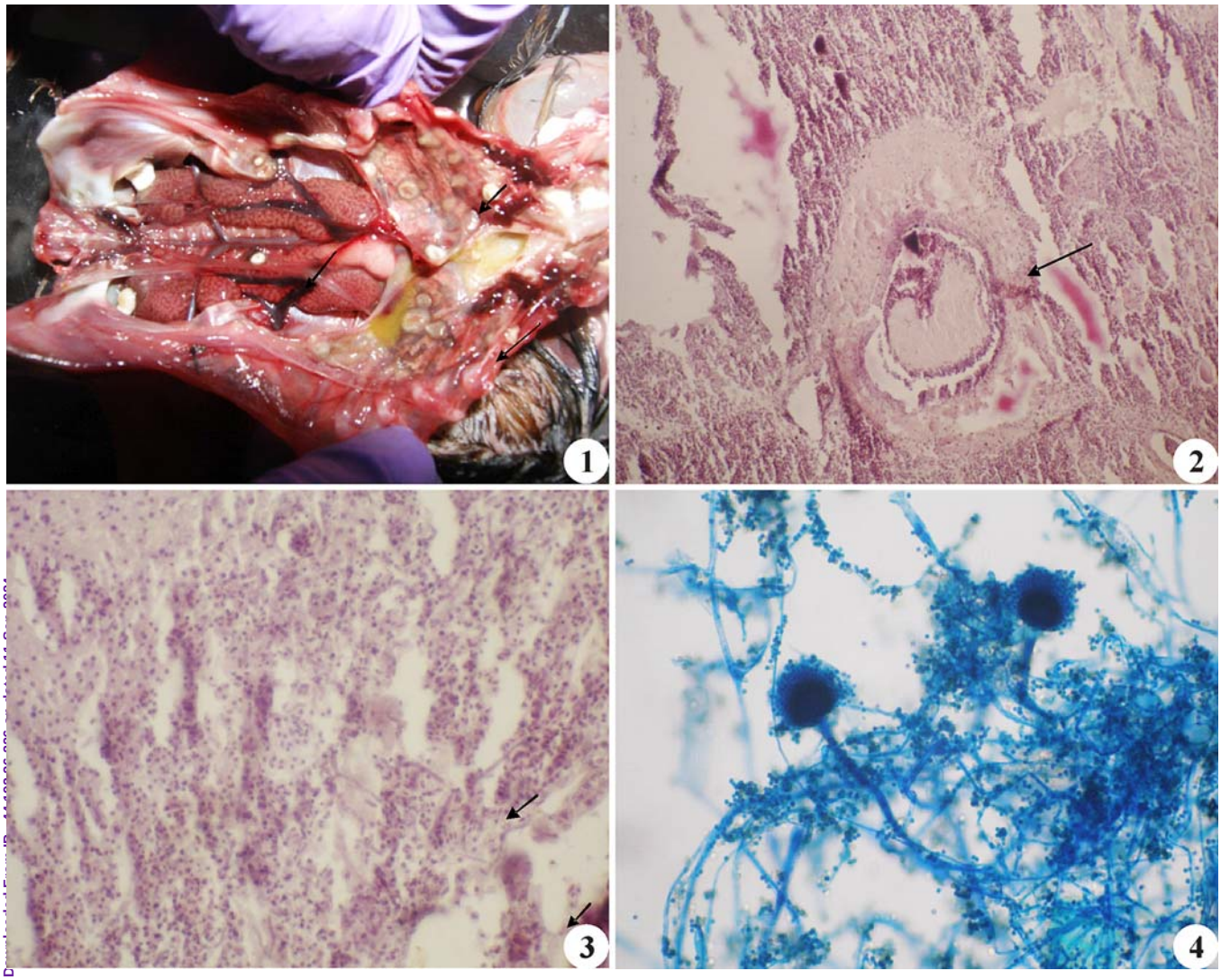

Fig. 1. Multiple cream colored nodules (arrows) distributed throughout the lungs, air sacs, liver and kidneys; **Fig. 2.** Lung: mycotic granuloma (arrow) with a central core of necrotic cellular debris and heterophils surrounded by periphery of macrophages, epithelioid cells and aggregates of lymphocytes in the thickened parabronchi of lung parenchyma. H& E x100; **Fig. 3**. Lung: Fungal hyphal invasion in the parabronchi of lung parenchyma. H& E x200; **Fig. 4.** Fungal mycelium with presence of conidiophores, sterigmata and conidia of *A. Fumigates* in the impression smear. Lacto Phenol Cotton Blue x100.

Air sac membranes are thickened due to massive infiltration of heterophils, multinucleated giant cells and other leukocytes. Granulomatous inflammatory lesions were also observed at many places in air sac membranes. Rib sections showed osteomyelitis with necrosis, inflammatory cells and septated hyphae. Direct microscopic examination of impression smears with lacto phenol cotton blue staining showed the presence of conidiophores, sterigmata and conidia indicative of *A. fumigatus* (Fig. 4). On isolation by culturing, the fungal colonies were observed in 5-7 days. Multiple colonies of *A. fumigatus* isolates were recovered and identified in all tissue specimens based macroscopic and microscopic characteristics of the colonies. The fungal colonies initially appeared as flat white and the colour changed to bluish green at the centre of the colonies by the time conidial mass matured with whitish colour in the surrounding edges. On microscopic examination, the suspected colonies showed the mycelia with septate hyphae. The septate hyphae revealed conidiophores with sterigmata. The birds in the affected flock were treated with copper sulphate at the dose rate of 1gm/litre of drinking water for the first two days and then the dose was reduced to 0.5 gm/ litre of water for the subsequent 5 days. At the same time the litter material was replaced with fresh copper sulphate treated litter @1gm/ litre of water.

The signs of aspergillosis are non-specific, making diagnosis difficult[1,7]. Diagnosis needs an accumulation of evidence from the history, clinic-pathological presen-

tation and culture of the fungus[8]. The disease condition in the growing Giriraja chicks in the outbreak was diagnosed as aspergillosis based on the history, clinical signs and failure to antibiotic treatment, post-mortem findings, microscopic detection, isolation and identification of the fungus. Aspergillosis in the grower chicks with high mortality rate might be occurred due to the improper hygiene and sanitation in poorly housed birds with improper ventilation and high humidity which led to mouldy litter at the onset of rainy season, in the month of May[9, 10]. All the dead birds showed invasive forms probably spreading hematogenously from air sacs[11]. In birds, inhaled air can reach the posterior thoracic and abdominal air sacs without first contacting epithelial surfaces in the lungs, thus, these membranes are usually primary sites of infection[3,12,13]. The infection to kidney, liver and rib cage might have reached through seeding from the granulomas with the fungal growth in these air sacs. Softening of ribs observed in the affected birds might be due to the chronic osteomyelitis with necrosis. Similar findings of nodular lesions in lungs, eyes, air sacs and cerebellum were also reported[13, 14]. The findings supported the isolation of *A. fumigatus* from tracheal samples of 8 weeks old white leghorn chicken[11]. The immaturity in phagocytes and environmental factors had been responsible for predisposing the outbreak of aspergillosis as the local climate was found favourable for the growth and multiplication of *A. fumigatus* spores and aspergillosis was typically acquired as a result of inhalation of fungal spores[10]. The treatment of the flock and litter with copper sulphate was found to be effective as the severity of clinical signs in the affected birds reduced from the third day and recovered completely within 5-7 days from commencement of treatment[15, 16].

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