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Diagnosis of aspergillosis in avian species from Punjab

Jagmeet Kaur, Rajinder Singh Brar, Harmanjit Singh Banga and Mudit Chandra

Abstract
The aim of this study was to diagnose Aspergillus infection in avian species (Chicken and Emu) in Punjab. Twenty samples suspected for Aspergillosis were studied grossly, histopathologically, through special stains, by direct microscopy and isolation. Out of 20 samples, Aspergillosis was detected in 6 chicken and in an Emu on the basis of gross, histopathology, special stains and culture of the fungus. Histopathogical studies showed edema, congestion, hemorrhage and sloughing of alveolar cells within the lumen of the lungs. Caseous necrotic areas with giant cells surrounded by fibrous tissue were also observed. Within the caseous necrotic areas numerous sprouting fungal hyphae were observed. The fungus was characterized as *Aspergillus fumigatus* and *Aspergillus flavus*. The present study detected *Aspergillus fumigatus* and *Aspergillus flavus* in the birds of Punjab.

Keywords: Aspergillosis, histopathology, pathology and poultry

Introduction
Poultry industry is one of the fastest growing segments of the agriculture segment in India today, but the major threat to the poultry industry is from infectious diseases. Among these infections, fungal infections cause huge economic loss. Losses can either be due to their direct effect or due to the production of mycotoxins. Aspergillus infection is the commonest invasive fungal infection which affects respiratory system.

Aspergillus species is a ubiquitous saprophytic mold with a worldwide distribution (Tell, 2005) [1]. It is reported in domestic birds like poultry, emu (Parker, 2011 and Shukla *et al*., 2013) [2-3], as well as wild birds (Jung *et al*., 2009) [4]. *Aspergillus fumigatus* accounts for 95% of the cases of fungal infections and *Aspergillus flavus* is the second most common organism associated with avian species (Tell, 2005) [1]. *Aspergillus fumigatus* was first found in the lungs of a bustard (Otis tarda) in 1863 by Fresenius (Arne *et al*., 2011) [5]. It is present mostly in the lungs but recent reports suggest their presence in the cecum also (Byrd *et al*., 2017) [6].

Disease prevails throughout the year and incidence of disease increases in the hot and humid months of the year and disease is significantly high in younger birds (Sajid *et al*., 2006) [7]. Disease is called brooder pneumonia when source of infection is hatchery and in older birds, disease is called Aspergillosis (Shankar, 2008) [8]. Lesions in birds are commonly confined to lungs and air sacs, although oral mucosa, trachea, eyes may be affected. Typical lesions are fungal nodules or plaques within the lungs and on the air sacs. Occasionally, the syrinx may be also affected.

In the present study, natural infection of aspergillosis in poultry was investigated using gross, histopathological, special stains, direct microscopy and isolation.

Material and Method
In the present study, twenty poultry birds and an emu bird formed the material for investigation which has been submitted for postmortem examination in the Department of Veterinary Pathology, College of Veterinary Science, GADVASU, and Ludhiana and also from the local Veterinarian. Detailed post mortem was conducted and gross lesions were recorded. For isolation, lung samples were streaked on Sabouraud's dextrose agar (SDA) and were incubated for 7 days at 37^0C. Direct microscopy with lacto phenol cotton blue stain was also done i.e., colony is mounted in a drop of lacto phenol cotton blue stain on a glass slide and examined microscopically. For histopathology, representative organs were collected in 10% neutral buffered formalin. Tissue samples were processed for routine hematoxylin and eosin staining as per the standard protocol**.** Special stain Periodic acid Schiff (PAS) and Grocott were also performed.

Result and Discussion

Aspergillus species are ubiquitous filamentous fungi in which Aspergillus fumigatus is considered as a major respiratory pathogen for birds. In the present study, out of 20 birds, 6 poultry birds (Chicken) and an Emu bird was diagnosed for aspergillosis on the basis of gross, histopathology, special stains and culture of the fungus. Grossly, there were thickened air sacs and granulomatous nodules on the lungs (Fig.1 & 2) as report earlier by (Akan *et al*., 2002 and Mitra *et al*., 2005) [9, 10] detected aspergillosis in broiler chicks in three poultry farms on the basis of clinical signs, gross and histopathological findings. Lesions were most frequently found in lungs and air-sacs that includes miliary pinhead to large millet size nodules depending on progress of the disease, occasionally caseous foci in lungs and caseous coating on the pleura and peritonium were also observed. Histopathogical studies showed edema, congestion, hemorrhage, and sloughing of alveolar cells within the lumen of the lungs. Caseaous necrotic areas with giant cells surrounded by fibrous tissue were also observed. Within the caseaous necrotic areas numerous sprouting fungal hyphae were observed. Further there was also presence of giant cells with or without fungal hyphae. These observations corroborated well with the (Sajid *et al*., 2006) [7] and (Karunakaran *et al*., 2010) [11] who have also reported granulomatous inflammation with necrotic areas infiltrated predominantly with lymphocytes and plasma cells. The presence of fungal hyphae and giant cells surrounded by fibrous tissue goes in exact accordance with the findings of (Eswaran *et al*., 2011) [12] who earlier reported that presence of granulomatous necrotic areas in the lungs containing inflammatory cellular infiltrations, numerous thin, tubular septate branching fungal hyphae with parallel-sided walls, spherical spores and fibrous tissue proliferation at the periphery. Focal necrosis of tracheal mucosa with mononuclear cellular infiltration was also observed. (Brar *et al*., 2014) [13] Has also reported the presence of fungal hyphae penetrating the mucous membrane of trachea along with caseative plug with central necrotic mass.

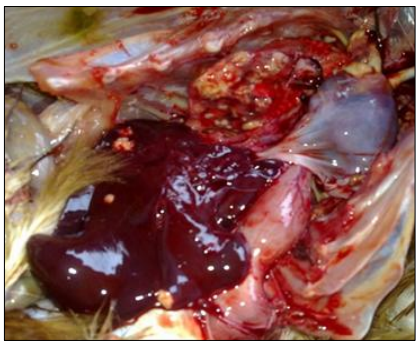

Fig 1: Thickened air sacs and granulomatous nodules on the lungs and liver

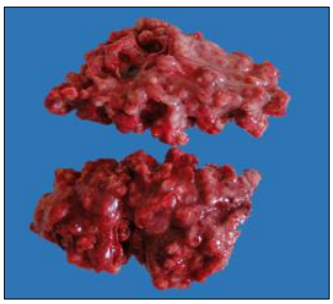

Fig 2: Granulomatous nodules on the lungs

Periodic acid Schiff's (PAS) staining and Grocott staining also revealed the presence of branching fungal hyphae in the area of caseation (Fig.3) which is similar to that of (Sajid *et al*., 2006) [7]. Pink coloured hyphae in PAS staining and black coloured hyphae in Grocott staining were observed as reported earlier by (Brar *et al*., 2014) [13].

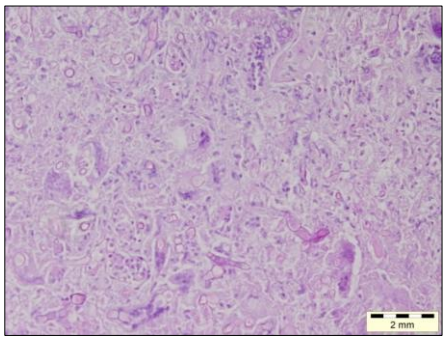

Fig 3: Branched fungal hyphae detected in the granulomatous areas. PAS X10

Isolation of Aspergillus species was also carried from these morbid samples on Sabouraud Dextrose agar. Six samples revealed positive for Aspergillus species (Aspergillus fumigatus and Aspergillus flavus) on culture, on the basis of colony characteristics as observed earlier by (Zafra *et al*., 2008) [14]. In 2 samples one chicken and one Emu, white colonies were observed which turns to yellow green in the centre with time (Fig.4) indicating Aspergillus flavus and dichotomously branched fungal hyphae were observed under microscope staining with lacto phenol blue stain as reported earlier by (Sajid *et al*., 2006) [7]. Further, in 5 cases of chicken, the white colonies turn blackish-green in the centre indicating Aspergillus fumigatus. The findings of the present study were in line with the findings of (Yokota *et al*., 2004) [15] who reported that white to green mold growth on the walls of caseous thickened air sacs when cultured yielded pure growth of Aspergillus fumigatus. (Jung *et al*., 2009) [4] isolated fungus from lung tissue, air sacs in Sabouraud dextrose agar (SDA). The colonies have a diameter of approximately 3 to 4 cm in 7 days. The flat colonies were white at first, and then bluish green as conidia began to mature, especially near to the center of the colony. As the colony matured, conidial masses became gray- green, while the colony edge remained white.

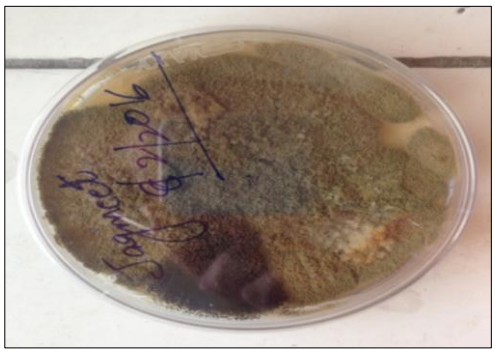

Fig 4: Isolation of fungus

Conclusion

Thus the present study detected Aspergillus fumigatus and Aspergillus flavus in the birds of Punjab. This study provides an additional knowledge in the field of avian aspergillosis.

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