Systemic Aspergillosis in Emu Chicks in an organised farm in Kerala

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Abstract

Systematic post mortem examination was carried out on seven Emu chicks submitted for disease diagnosis to Clinical Laboratory, District Veterinary Centre, Palakkad. On examination, numerous small greyish white nodules were seen in the lungs, air sacs, kidney and serosal surface of proventriculus. Dark red liver with necrotic areas and dark coloured spleen were the other lesions. Microscopically the lungs revealed granulomas with central areas of caseation surrounded by mononuclear cells and fibroblasts. PAS positive fungal hyphae could be seen in the lesion. Aspergillus fumigatus could be isolated in Sabouraud Dextrose Agar from the lesions. This is the first report on the occurrence of systemic aspergillosis in Emus from Kerala.
Key words: systemic aspergillosis, Emu chicks, mycotic pneumonia

Introduction

The Emu (Dromaius novaehollandiae) is a large flightless bird native to Australia. It belongs to ratite group, which also includes ostriches, rheas and cassowaries. The emus are reared commercially for their meat, oil and skin. The skin is made into high quality leather, used in the manufacture of boots, handbags and other items.

The disease aspergillosis is caused by a fungus belonging to Aspergillus species. This fungus has the potential to infect a wide range of mammalian, avian and reptilian species including man and is probably the most common fungal infection found in birds. The most common species of Aspergillus causing disease in birds are *A. fumigatus, A. flavus* and *A. niger*. There are numerous other species of Aspergillus present in the environment but these rarely appear as a cause of disease.

The present paper documents a case of Aspergillus fumigatus infection in Emu chicks in an Emu farm in northern Kerala.

Material and methods

Seven Emu chicks in the age groups of one week to seven weeks submitted to the Clinical Lab, District Veterinary Centre, Palakkad District, from an Emu farm in Northern Kerala formed the materials for investigation. Detailed post mortem was conducted and gross lesions were recorded.

Heart blood smear and impression smears from lesions, liver and spleen were stained with Giemsa. Pieces of lung along with nodule, liver, spleen, kidney and sciatic nerve were collected and fixed in 10 per cent formalin for histopathology. They were then processed and paraffin embedded as described by Sheehan and Hrapchak (1980). Sections were cut at four micron thicknesses and stained with routine Haematoxylin & Eosin and special stain PAS (Bancroft & Cook, 1984). Heart blood, swabs from liver, spleen and kidney were aerobically cultured in Brain Heart Infusion Agar (BHIA) with 5 per cent sheep blood and in Mac Conkey's agar. In addition, portions of liver, spleen and intestine were cultured for Salmonella by incubating macerated tissues in Selenite F broth at 37 oC. This enrichment was followed by culture on Mac Conkey's agar, Brilliant Green Agar (BGA) and Xylose Lysine Agar (XLD) and incubated overnight at 37 °C.

Heart blood and pieces of liver tissue were cultured in Sabouraud's Dextrose Agar (SDA) aerobically at room temperature. Lungs along with nodule and liver were processed for lacto phenol cotton blue staining and with ten per cent potassium hydroxide solution for direct examination.

Results

The clinical signs shown by Emu chicks on the day prior to death were listlessness, unthriftiness and respiratory distress. The chicks showing symptoms of respiratory distress didn't show any response to the

antibiotic enrofloxacin suggested orally.

Gross lesions revealed upon necropsy were dark red liver with necrotic areas and dark coloured spleen. Numerous small nodules of approximately 0.5 cm thickness were noticed in the lungs and air sacs, spreading to rest of the viscera like kidney and serosal surface of proventriculus. The nodules were hard in consistency and were difficult to crush under ordinary glass slide. Proventriculus contained greenish ingesta. Mild enteritis and haemorrhages in the mucosa were noted throughout small and large intestine. Mesenteric and meningeal vessels were congested.

Histopathological examination of lungs revealed granulomatous inflammation with central area of caseation surrounded by mononuclear cells, predominantly lymphocytes and plasma cells, enclosed by strands of proliferating fibrous tissue. Per iodic acid Schiff's (PAS) staining revealed presence of branching fungal hyphae in the area of caseation.

Sections of liver showed subcapsular and extensive sinusoidal congestion. Vascular sclerosis, follicular hyperplasia and diffuse areas of necrosis were seen in the spleen. Intestine revealed goblet cell hyperplasia and mild enteritis. Congestion and haemorrhage in between muscle bundles could be observed in the heart.

Heart blood smear and liver impression smear upon Giemsa staining, revealed fungal hyphae. The heart blood smear also revealed RBC's and lymphocytes. Culture of heart blood and tissues in BHIA with 5 per cent sheep blood and in Mac Conkey's agar revealed no growth even after 48 hrs of incubation. Enrichment culture of liver, spleen and intestine also did not reveal any growth even after 48 hrs of incubation.

The lung nodules on lacto phenol cotton blue staining revealed fungal hyphae. Culture of heart blood, liver and spleen in SDA at room temperature revealed characteristic growth of fungus in five days. Colonies were white at first but later became bluish green and older colonies were slate-grey. Reverse of the colony remained cream colored. Lacto phenol cotton blue staining of colonies revealed septate hyphae- bearing conidiophore, conidiophore vesicle, sterigmata and chains of pigmented conidia. Conidiophore vesicle was partially covered with flask shaped sterigmata.

Discussion

Aspergillosis is an infection primarily associated with the respiratory system. Infection in other sites including visceral organs, liver, eye and brain, have been reported. Warm and humid environments tend to increase incidence of this disease. *Aspergillus fumigatus* is the most commonly isolated agent causing aspergillosis, although Aspergillus niger also has been isolated from poultry exhibiting respiratory distress (Jordan, 1990).

There is report of isolation of *Aspergillus flavus* as a cause of aspergillosis in Emus (http://www.dpi.qld.gov.au). Aspergillus species will proliferate in aerobic environments with a high humidity and relatively warm temperatures. Warm, moist areas such as in litter, around waterers, spoiled or damp feed, rotting vegetation etc provide suitable environment for the proliferation of the fungus.

Inhalation of large numbers of spores from this mould appears to be the primary route of transmission. Young chicks are most susceptible to infection. Infection results if the immune system is deficient as in very young chicks where the immune system is in the developing stage, or in birds that have been stressed through other diseases or problems such as overcrowding, insufficient food and water (Jordan, 1990). Infection can also result in normal birds where massive numbers of spores are inhaled and the immune system is overwhelmed. In the present report all the affected birds were young chicks within the age group of one week to seven weeks.

The chicks in the present case were infected from the hatchery itself. Initially no signs were seen but gradually affected chicks appeared unthrifty and less active and showed signs of gasping and respiratory distress. In the final stages the chicks were obviously depressed, did not move much and showed respiratory distress combined with open mouth breathing. This was the terminal stage and the chicks died soon after. One batch of chicks (3 days old) immediately after hatching showed nervous signs, probably due to fungus invading the brain.

Clinical signs were associated with spores germinating and growing inside the lungs. Firm, round and white nodules were found in lung tissue, air sacs and visceral organs like kidney and serosal surface of proventriculus. This disrupted normal functioning of lung and decreased the oxygen supply to the chicks and they could not survive. Chakravarty, (1976) reported a similar case of mycotic infection (aspergillosis) in an emu in Delhi zoo. The lesions reported were congestion of the lungs, numerous whitish nodules in the pleura and adjoining thoracic muscles and darkened and slightly enlarged liver. Aspergillosis was confirmed after histopathology. The histopathology of lung tissues also supported the present conclusion.

Elizabeth et al. (2002) reported similar case of mycotic pneumonia in great rheas due to Aspergillus fumigatus. Necropsy revealed several small yellow nodules in the lungs. Microscopically, the nodules consisted of granulomas containing numerous thin, 4-µm-diameter, septate, branching fungal hyphae.

Diagnosis of aspergillosis is dependent upon the microscopic detection of fungus in the tissue followed

by isolation and identification by appropriate microbiological method (Jordan, 1990). Granulomatous nodules and pneumonic lung can be used as specimens for isolation of fungus in Sabouraud's Dextrose Agar with or without chloramphenicol (Quinn et al., 2004). In the present case, fungus was isolated from heart blood and homogenized lung tissue in Sabouraud dextrose agar incubated aerobically at room temperature. The isolate obtained was confirmed as *Aspergillus fumigatus* based on morphological appearance of colony and conidial arrangement on sporing heads upon lacto phenol cotton blue staining of colonies. Homogenized pieces of lung tissues when examined directly using ten per cent potassium hydroxide also revealed hyphae of fungus.

Formation of septate hyphae in lung produces a local inflammatory reaction called nodular broncho-pneumonia and there is infiltration by polymorphs and macrophages. Macroscopically the lesion is nodular and microscopically, septate hyphae of fungus with the inflammatory cells may be found around a central caseous material (Sastry, 1983). Histopathology of lung tissues in the present case also supported this conclusion.

Deaths due to Aspergillus fumigatus infection in day old Emu chicks is discussed. Control measures should be directed at prevention of this disease as there is no effective treatment for this. In the present case, infection in the Emu farm could be controlled by fumigation of hatchers using potassium permanganate and formaldehyde and sanitization of eggs before setting in incubator, using egg sanitizers.

Acknowledgements

The present work was supported by R&D Project grant received from the Director, Department of Animal Husbandry, Kerala State and also the expert technical guidance received from Department of Veterinary Microbiology, and Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

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