Pathology of Systemic Aspergillosis in a Desi Chicken

D.Basheer Ahamad, V.Ranganathan, N.Punniyamurthy, S.Sivaseelan & B.Puvarajan

Veterinary University Training and Research Centre, Tamil Nadu Veterinary and Animal Sciences University, Thanjavur, Tamil Nadu

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Abstract

Systemic aspergillosis in desi chicken was recorded. Clinically, 25% of birds showed anorexia, whitish droppings, dullness, depression and difficulty in respiration with 6% morality . Grossly, nodular swellings on subcutis near the right eye, head, neck and wings were seen. Cut section of those nodular growths revealed whitish grey cheesy contents. On internal examination, air sacs, lungs, proventriculus, kidney and abdominal wall revealed diffuse mild congestion, multi focal grayish foci of varying sizes. Histopathologically, mycotic granulomatous dermatitis, mycotic granulomatous pneumonia, mycotic air sacculitis, toxic hepatitis, lymphocytolysis in spleen, bursal atrophy, sub acute pericarditis, epicarditis and acute to subacute myocarditis were recorded. On histochemical examination, nodules in skin and lungs showed positive for fungus by Grocott - Gomori's hexamine (Methenamine) silver staining. On fungal cultural examination, Aspergillus species was isolated from lungs and skin nodules. Based on pathomorphological, histochemical, fungal culture examination, the disease outbreak was diagnosed as aspergillosis in desi chicken farm.

Keywords: *Pathomorphology, Aspergillosis , Desi chicken*

Introduction

Aspergillosis is a non-contagious disease of avian species. It is caused by a fungal species under the genus Aspergillus (Saif *et al*., 1999). The genus Aspergillus organisms cultured from affected organ in poultry in decreasing frequency are: *Aspergillus fumigatus, A flavus, A niger, A glaucus* and *A terreus* (Girma *et al*., 2016). Aspergillosis can be acute or chronic. Acute aspergillosis generally occurs in young birds and results in high morbidity and mortality. Acute aspergillosis is thought to be the result of inhaling an overwhelming number of spores, while chronic aspergillosis is generally associated with immune suppression (Vanderheyden, 1993). Chronic form is sporadic which causes lesser mortality and generally affects older birds, especially with a compromised immune system due to poor husbandry condition (Jordan *et al*., 2002). There are three common presentation of the disease such as diffuse lower respiratory tract disease, syringeal granuloma and focal central nervous system (CNS) granuloma particularly in the brain (Pattisson et al., 2008;). Aspergillosis may involve many body systems. Fungal spores most commonly invade trachea, air sacs and lungs. Signs depend on the number of spore that enter the body and organ system affected but can be generally reflected as disease of respiratory tract (Atlaman , 1997; Ahamad *et al*., 2017). Visceral lesions typically have concurrent clinical signs of

respiratory disease in turkey poults (Ghazikhanian, 1989), chicks (Pal *et al*., 1989) and broiler breeder pullets[9] (Martin et al., 2007). The following disease outbreak describes aspergillosis in a desi chicken farm.

Materials and Methods

Two desi chicks of two months age maintained under deep litter system from a commercial farm with a capacity of 200 chicks were brought for necropsy with the history of clinical signs such as whitish droppings, dullness and depression. All the birds were vaccinated with Ranikhet disease (RD) vaccine F1 on 7th day, infectious bursal disease vaccine on 14th day and RD lasota on 45th day. The birds were fed with commercial chicken feed. A thorough necropsy was conducted and lesions were recorded. The impression smears from cutaneous nodules were examined. Nodules from the skin and lung were subjected to fungal culture. Tissue samples were collected from skin, lungs, air sacs, liver, heart, kidneys, proventriculus, intestine, pancreas spleen and bursa of Fabricius in 10% formalin, processed routinely, sectioned at 4-5 µm and stained with haematoxylin and eosin for histopathological examination. Selected sections were also stained with Grocott- Gomori's hexamine (Methenamine) silver staining procedure for fungi (Bancroft et al., 1996).

Results and Discussion

Clinically, 20% affected birds showed anorexia, whitish droppings, dullness, depression and difficulty in respiration. Grossly, the dead chicken showed swelling in the subcutis of near right eye, head, neck and left and right wings (5 to 8 mm in diameter). On cut section grayish cheesy material was observed. On internal examination, air sac and abdominal wall revealed multi focal grayish foci of varying size from 2 to 6 mm in diameter. Lungs revealed diffuse mild congestion and multi focal grayish foci of 1.5 to 3.0 mm in diameter (Fig. 1.). Anterior cervical, clavicular and caudal air sacs showed cloudiness, thick and greyish white foci of 1.5 to 3 mm in diameter. Kidneys revealed patchy areas of congestion and grayish white foci of 2-3 mm in various lobes (Fig.2.). Pericardium revealed cloudy appearance with oft whitish deposition. Peritoneum was thick, opaque and adhered with the intestine. Yellowish caseous flakes adhered with serosal surface of small and large intestine. Liver showed diffuse mild mottling, enlargement and multifocal pin point whitish foc of 2-3 mm in diameteri. The lumen of duodenum and jejunum revealed yellowish ingesta mixed with mucus and their mucosa showed multifocal patchy congestion.

On histopathological examination, nodular swelling on subcutis, air sac. lungs, proventriculus, gizzard, liver and proventriculus- esophagus junction revealed mycotic granulomatous lesions and it was characterized by eosinophilic central necrotic area containing large number of interlacing fungal hyphae surrounded by lymphocytes, macrophages, giant cells and fibrous tissues (Fig. 3.) Fungal hyphae were septate and branching type. Tracheal mucosa showed diffuse moderate subacute tracheitis. Air sacs showed severe congestion, hyperplasia of epithelium and chronic mycotic

granulomatous air sacculitis charecterised by infiltration of lymphocyte and a few heterophils, fungal hyphae with fibrin deposition. Lung parenchyma revealed diffuse moderate congestion, subacute bronchitis and mycotic granuloma .

Liver revealed toxic hepatitis characterized by diffuse sinusoidal congestion, diffuse micro to macrovacuolar changes in hepatocytes, diffuse acinar transformation, diffuse mild bile duct hyperplasia, multifocal mild subacute hepatitis and chronic fibrinous perihepatitis. Serosa of esophagus and proventricular junction revealed mycotic granuloma. Proventriculus revealed hyperplasia of proventricular gland epithelium and diffuse mild subacute granulomatous proventriculitis. Gizzard showed diffuse mild subacte granulomatous ventriculitis and erosion of kaoline layer. Intestine showed diffuse moderate subacute enteritis. Serosa of duodenum revealed focal chronic mild steatitis with grnulomatous duodenitis. Kidneys showed diffuse moderate congestion and solitary mycotic granuloma. Heart showed diffuse subacute pericarditis, epicarditis and diffuse mild acute to subacute myocarditis. Spleen revealed multifocal lymphocytolysis. Bursa of Fabricius revealed atrophy of lymphoid follicles.

On histochemical examination of granulomatous nodules in subcutis, fungal hyphae showed black colour with pale green back ground in Grocott- Gomori's hexamine (Methenamine) silver staining (Fig.4). On fungal cultural examination, *Aspergillus species* was isolated from lungs and skin nodules.

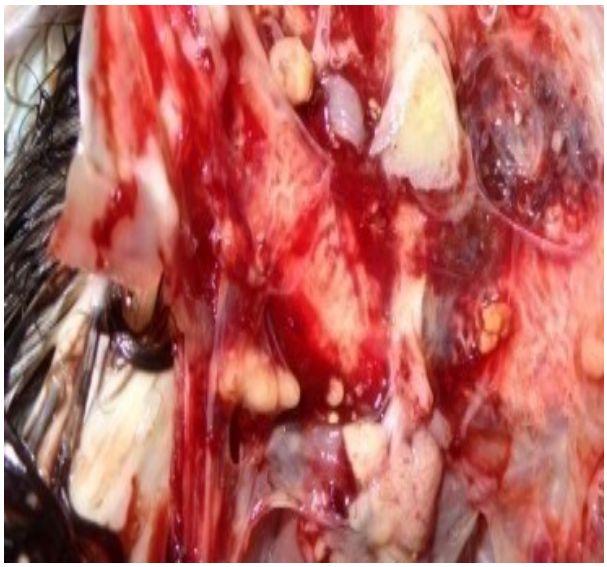

Figure 1 Lungs, air sac and abdominal wall revealed multi focal grayish foci of varying size

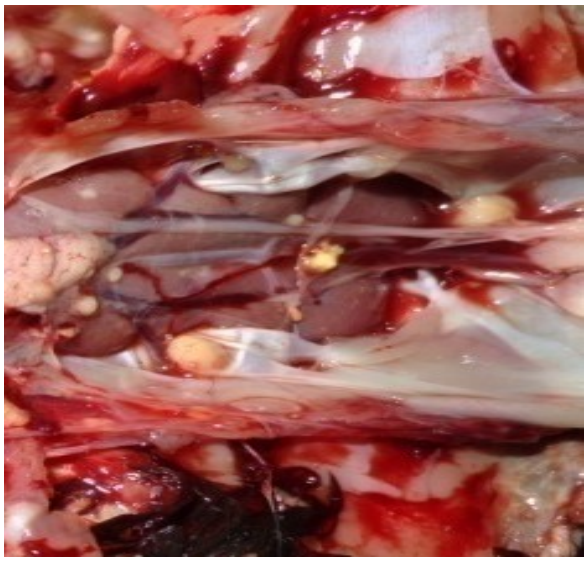

Figure 2 Kidney revealed multi focal grayish foci

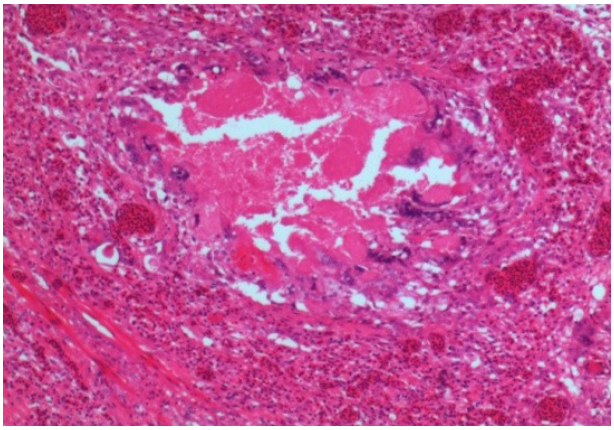

Figure 3 Lungs showing chronic granulomatous lesion H&E 200x

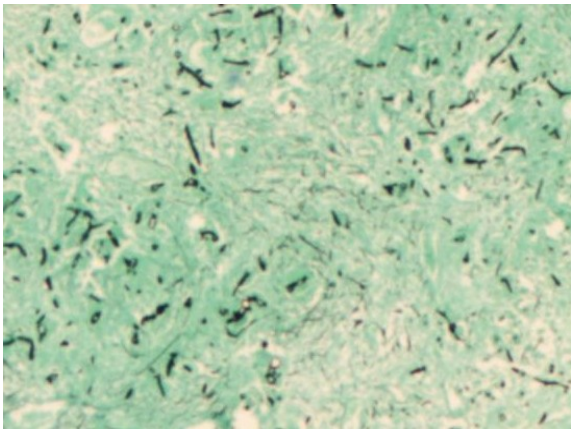

Figure 4 Lungs showing septal hyphae in mycotic granuloma G&G stain 40x

Discussion

Aspergillosis may involve many body systems. Fungal spores most commonly invade trachea, air sacs and lung. Signs depend on the number of spore that enters the body and organ system affected but can be generally reflected as disease of respiratory tract, (Atlaman, 1997). Aspergillosis may include a variety of non specific clinical signs such as anorexia, lethargy, ruffled feathers, respiratory signs, polydipsia, polyuria, stunting or sudden death (Arn´ e *et al*., 2011;). Lesions in the respiratory tract were greatest in the anterior cervical and clavicular air sacs followed by multifocal areas of mycotic granuloma in the lungs and were least in the caudal air sacs. Similar condition was recorded by Martin *et al*. (2007).

In the present study, the primary location of fungal granulomatous lesion was recorded in air sacs and lungs. However, one bird showed disseminated form of mycotic granuloma in subcutis of head, neck, wing, lungs, air sacs, and esophagus-proventriculus junction. Microscopic lesions revealed classical granuloma in various visceral organs which were in accordance with the observation of Sawale *et al*. (2012). Aspergillosis is caused by inhalation of overwhelming numbers of small, hydrophobic fungal spores into the respiratory tract. After infective spores invade tracheal, nasal, bronchial and air sac epithelium, they penetrate the respiratory tissue, reproduce by single division of tubular hyphae to form mycelia and they initiate granulomas lungs and air sacs and produce mycotic granulomatous pneumonia and air sacculitis (Ahamad *et al*., 2017). Then they are disseminated hematogeneously to the other tissue like brain, pericardium, bone marrow, kidney and other soft tissue. Tissue invasion creates a chronic inflammatory condition with heterophils, lymphocytes, macrophages and some giant cells (Kunkle and Rimiler, 1996). Perhaps the distribution of lesions made it possible for the birds to maintain normal respiration by compensating for loss of gas exchange and likely aerodynamic changes suggested by the histologic tissue damage (Martin *et al*., 2007). *Aspergillus fumigatus* could colonize skin and surgical wounds as observed in caponized cockerels and

induce necrotic mycotic granulomatous dermatitis or even systemic aspergillosis (Arn´ e *et al*., 2011). In the present study, mycotic dermatitis was recorded based on gross and histological study. The spores of *Aspergillus sp* get deposited within the airways or caudal air sacs before reaching the anterior air sacs (Richard *et al*., 1981; Martin *et al*., 2007). Spread to the lungs occurred secondarily to anterior air sac involvement because these lesions were smaller, more discrete and localized. Similarly, nodular growth adjacent to kidney lesions probably occurred secondarily to infection of the caudal air sac (Martin *et al*., 2007). White to yellowish granulomatous nodules involving serosa and parenchyma of multiple organs were also recorded by earlier workers (Martin *et al*., 2007; Arn´ e *et al*., 2011).

In the present study, multifocal lymphocytolysis in spleen and bursal atrophy could lead to immunosuppression in affected birds. An impaired immune system is also an important risk factor in development of aspergillosis in both human and animals (Kunkle., 2003). However, fungemia through spores has been demonstrated from the blood and visceral tissues of turkeys and presence of viable spores within macrophages that entered the bloodstream was considered to be the source of the fungus and spread of the fungus within infected birds in this flock was most likely via contiguous dissemination (Richard and Thurston, 1983). Nodular lesions located at the thoracic inlet , the anterior cervical and interclavicular air sacs were the sites for initial infection (Martin *et al*., 2007). Frequently, cases of aspergillosis are linked to fungal contamination of either litter or feed. Avoidance of moldy feeds by proper storage and litter management in conjunction with good cleaning and disinfection protocols are needed to minimize the risk of aspergillosis (Debey *et al*., 1995). The birds were potentially immunosuppressed based on histological finding of bursal atrophy and splenic lymphocytolysis. Toxic hepatitis could have been due to aflatoxin B_1 contaminated feed by *Aspergillus species*. Generally, non infectious factor, such as stress and subclinical infectious bursal disease could have aggravated the disease caused by exposure to Aspergillus sp (Okoye *et al*., 1991).

In conclusion, the present study showed that desi chicken were highly susceptible to the infection with *Aspergillus sp.* in the respiratory tract and this may be disseminated to other organs. Based on pathomorphological, histochemical studies and fungal isolation, the present outbreak was diagnosed as systemic form of aspergillosis in chicken.

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