CASE REPORT
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ASPERGILLOSIS IN BROILER CHICKEN- A CASE REPORT

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ABSTRACT

In a flock of 5000 broiler chicks, approximately 70 chicks were found dead within first 8 days of life showing respiratory signs. On necropsy, most of the birds showed multifocal to coalescing caseous nodules and in some, air sacs were covered with multifocal to coalescing cotton-like light green plaques. Microscopic examination of air sacs and lung lesions showed pyogranulomatous inflammation and presence of intralesional fungi. Fungi were observed on cytological examination and with Grocott's methenamine silver stain (GMS). On basis of clinical signs, gross and microscopic pathology, morphology in hematoxylin and eosin and GMS stains, fungi were diagnosed as *Aspergillus* spp.

Keywords: *Aspergillus* spp., Poultry, Gross pathology, Microscopic pathology, Morphology

INTRODUCTION

Among various poultry pathogens fungal organisms causing diseases show seasonal variation. In addition to season, presence of other factors like overcrowding in closed housing system particularly during summer season as well as contaminated poultry manure and litter material with fungal pathogens make eradication program inefficient (Soliman *et al.,* 2009). Death in poultry from fungal infections results mainly because pathogens target respiratory and nervous systems (Shivchandra *et al.,* 2004).

One of the oldest fungal pathogens known since long is Aspergillus (Ross, 1951) which causes significant economic loss in poultry by affecting the respiratory system of chicken as well as turkey and occasionally of other birds (Arne *et al.,* 2011). In 1863 a scientist named Fresenius reported Aspergillus fungi in the lung of great bustard (*Otis tarda*) and he is considered as the first person to use the term aspergillosis for the respiratory infection with this fungus (Samanta, 2015).

Aspergillosis is also known as brooder's pneumonia, which is primarily a disease of respiratory system commonly affecting chickens, turkey and characterised by granulomatous lesions in lung as well as air sacs. Clinically aspergillosis may appear in different forms, of which acute aspergillosis is common in young birds and chronic form is more prevalent in adult birds (Tell, 2005 ; Charlton *et al.,* 2008). The susceptibility of the infection may vary but newly hatched turkeys, chickens and ducks are at particular risk of being infected (Brown *et al.,* 2008).

CASE HISTORY AND OBSERVATION

The present investigation was carried out in a flock of 5000 broiler chicks, in which approximately 70 chicks were found dead during their initial 8 days of life. Before death, affected chicks appeared dull and depressed, and showed gasping. Dead birds were presented to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Sardar krushinagar, Gujarat, India for necropsy. The carcasses were subjected to detailed post mortem examination and most of the birds showed multifocal to coalescing caseous nodules that expanded the lung tissue and air sacs. In few necropsiedbirds, air sacs were covered with multifocal to coalescing cotton-like light green plaques (Fig. 1). Appropriate samples were taken in 10 percent neutral buffered formalin for detailed histopathological examination. The formalin-fixed tissues were trimmed, washed thoroughly under running tap water, dehydrated in different grades of alcohol, cleared, embedded in paraffin wax and blocks were prepared. Five micron thick sections were obtained and stained by routine Hematoxylin and Eosin (H&E) staining method. On microscopic examination, air sacs appeared markedly thickened due to infiltration of heterophiles, macrophages, multinucleate giant cells, lymphocytes, plasma cells, and intralesional fungi. Air exposed layer of air sacs showed multiple reproductive bodies of fungi characterised by conidiophore with globose vesicle, phialide, and radiate chains of conidia (Fig. 2). The deeper area contained septate, branched, parallel walled hyphae (up to 10 um in width) with a rare short globose terminal end. Lung nodules showed pyogranulomatous inflammation characterised by necrosis and infiltration of heterophiles, macrophages, multinucleate giant cells, lymphocytes, plasma cells, and intralesional fungi. Impression smear prepared from air sacs and stained with field stain (Eosine&Methylene blue) showed conidia mixed with ciliated epithelial cells and macrophages (Fig. 3). The sections prepared from formalin fixed tissue were subjected to Grocott's methenamine silver stain (GMS) in which black reproductive

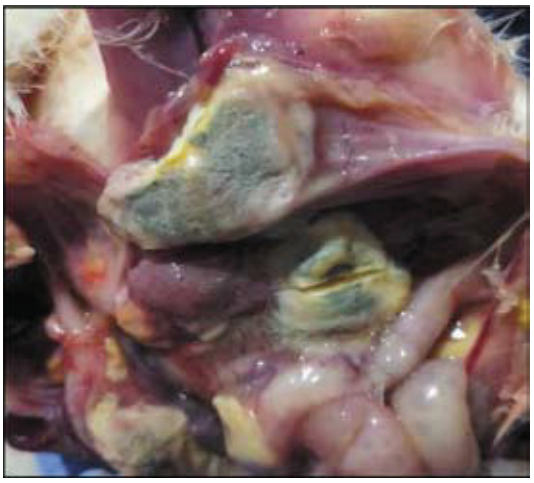

Fig. 1: Thickened air sacs with caseous exudate and covered with multifocal to coalescing cotton-like light green plaques

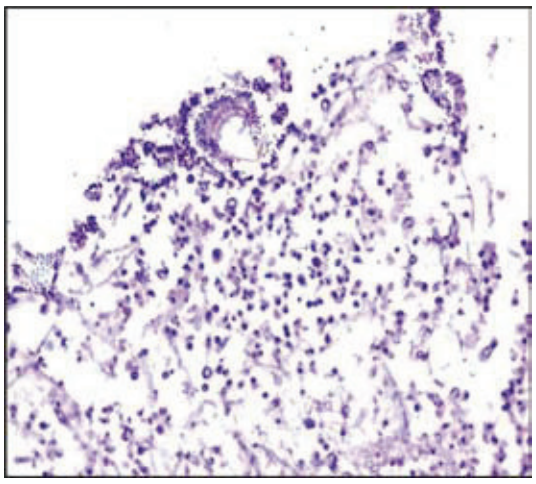

Fig. 2: Thickened air sac due to infiltration of heterophils, macrophages, lymphocytes, plasma cells, intralesional hyphae and reproductive body. H & E 200X

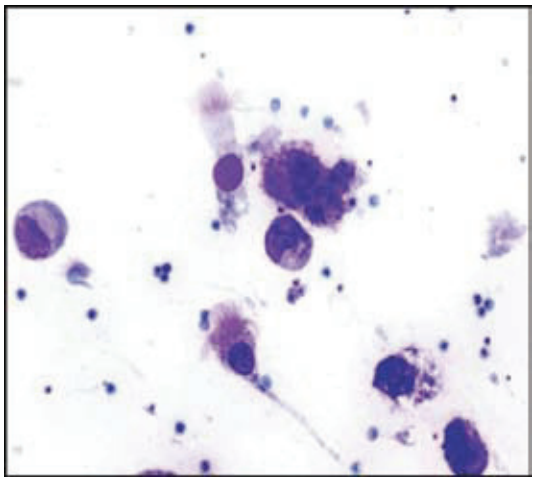

Fig. 3: Impression smear of air sac showing conidia mixed with ciliated epithelial cells and macrophages. Eosine and Methylene blue, 1000X

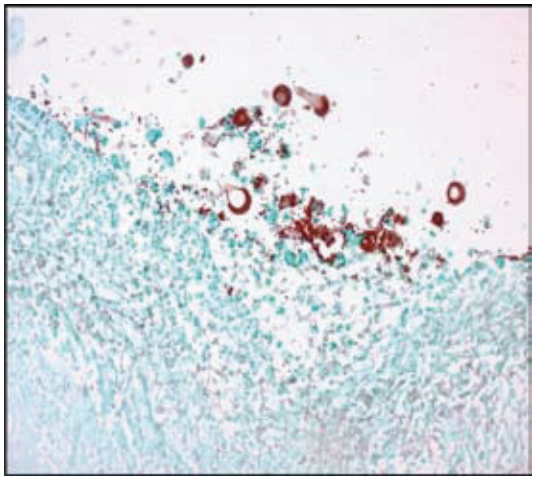

Fig. 4: Air sac showing multiple reproductive bodies of fungi characterised by conidiophore with globose vesicle, phialide, and radiate chains of conidia and deeper area showing septate, branching, parallel walled hyphae with rare short globose terminal ends Grocott's methenamine silver 100X

bodies and deeper fungi were noted **(Figure 4)**. On the basis of clinical signs, gross and microscopic pathology as well as morphology in hematoxylin and eosin and GMS stained sections, fungi were diagnosed as *Aspergillus* spp. and the case as aspergillosis.

DISCUSSION

Aspergillosis is a most common opportunistic mycotic infection of respiratory system of chicken, mammals as well as human and wild birds, caused by

fungi of the genus Aspergillus. The most common causative agent is *Aspergillus fumigatus*. The fungi Aspergillus is ubiquitously present in soil as saprophyte, also in indoor environment, vegetables and feed (Samanta, 2015).

Most probably infection is acquired through exposure of contaminated poultry house environment and some authors suggest that aspergillosis is not a transmissible disease (Charlton *et al*., 2008). *Aspergillus fumigatus* can also contaminate recently purchased bedding material leading to exacerbation of the condition. Some investigators reported that freshly hatched chicks up to two days of hatching in forced-draft incubator may easily acquire *A. fumigatus* infection in presence of contaminating spores (O'Meara and Chute, 1959). The conidia (asexual spore) are easily dispersed in air and inhaled conidia initially reach abdominal as well as posterior air sacs before coming in contact with epithelial surfaces of lung, hence air sacs are considered to be the first site of infection (Nardoni *et al.,* 2006).

Among different species of Aspergillus fungi, infection with *A. fumigatus* is more common owing to the fact that the spores of this organism are smaller (about 3 µm in diameter) than that of other fungi of Aspergillus genus (Richard and Thurston, 1983; Arne *et al.,* 2011) and as a result upon inhalation they get easily deposited deep in respiratory system. Approximately within 25 hours of formation of germ tube, conidia develop in to septate hyphae either at intra cellular or at extra cellular location. The death of infected cells may ensue concurrent inflammatory response with massive infiltration of inflammatory cells, predominantly heterophiles (Charlton *et al.,* 2008). The gross pathological findings may vary greatly in birds infected with *Aspergillus fumigatus*, of which granulomatous lesions in lung and air sacs are common.

As aspergillosis in many instances results from inefficient management practices, it is advised to take appropriate corrective measures to reduce the incidences of the disease. Improvement in ventilation of poultry houses, maintenance of proper disposal facility for unutilised feed as well as bedding material, appropriate management of diverse environmental conditions etc. are suggested to reduce the rate of generation of fungal spores and also individual susceptibility of birds to infection (Tell, 2005; Khosravi *et al.,* 2008).

SUMMARY

An investigation was carried out in a flock of 5000 broiler chicks, in which approximately 70 chicks were

found dead during their initial 8 days of life showing signs of dullness, depression and gasping. Dead birds were subjected to necropsy. On the basis of clinical signs, gross and microscopic pathology as well as morphology in hematoxylin and eosin and GMS stained sections, fungi were diagnosed as *Aspergillus* spp. and the case as aspergillosis.

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