Experimental aspergillosis in young chicks

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INTRODUCTION

Aspergillosis is a mycotic disease caused by various species of the genus Aspergillus. Fungi of this genus are ubiquitous saprophytes on dead material and exposure to the spores is very common. All domesticated, many wild mammals and birds were reported to be infected (5). Although the disease is uncommon in mammals, it is of great importance in birds, particularly young chicks. A. fumigatus is the most pathogenic species which cause avian aspergillosis. It causes severe losses in young chicks. Infection is generally by inhalation and the number of spores necessary to develop the disease is quite variable (1).

Chicken aspergillosis was first described in the Sudan in a domesticated fowl in 1955 (2). In 1975, A. fumigatus was isolated from nodular lesions in the lungs of a fowl (4).

Experimentally one-day-old chicks were susceptible to exposure to A. fumigatus airborne spores which infect them acutely causing high rates of morbidity and mortality (7). In older chicks, one or two weeks old, sublethal (L.D₅₀) and lethal doses inoculated in the air-sacs or trachea produced chronic form of the disease. There was loss in weight gain and granulomatous nodules were detected in various organs (6).

This experiment was designed to study the pathogenicity of A. fumigatus isolated from pigeons to young chicks. Lack of studies of the disease under the local conditions necessitates this investigation.
MATERIALS AND METHODS

Inoculum

The strain of *A. fumigatus* used in this experiment was isolated from natural pneumomycosis in pigeons. It was subcultured onto malt extract agar slants (Oxoid) containing chloramphenicol (0.5 mg/ml) and incubated at room temperature 25 °C-30 °C), sterile phosphate buffered saline (PBS) was poured into the tubes. They were allowed to stand for a while, then shaken gently. The spore-suspension thus obtained was washed three times in sterile PBS before transferring to sterile containers. Using a hemacytometer the suspension was adjusted to contain approximately $4 \times 10^5$ spores per ml.

Animals

Fifty one chicks one-day old were obtained from Kuku poultry farm. These were of Hisex white breed that was hatched locally. They were raised in one room for 5 days. Before inoculation, the chicks were randomly divided into 3 groups of 17 chicks each ; two inoculated and one control group. Each chick of group I was inoculated with 0.2 ml of the spore-suspension intraperitoneally. Group II chicks received 0.2 ml of the same suspension intra-tracheally each. The control (group III) were kept in a separate cage in a different room. All birds were reared on litter, fed commercial diets and were provided with water ad libitum. The chicks were observed for the development of clinical signs and mortality was recorded. Dead chicks were necropsied and examined for gross lesions. Slices from liver, lung, heart, kidney, proventriculus, bursa and brain were fixed in 10% 100 formalin, processed, sectioned and stained with hematoxylin and eosin, P.A.S. and Gridley's stains.

RESULTS

Signs

The birds in Group I and II looked dull, depressed, weak and showed respiratory distress. Chickens of the former group started to die on the third day post-inoculation and by the end of the 7th day all the 17 birds died, while in the latter group the death started on the 4th day and continued to the 9th day.

Gross pathology

The infected birds in group I and II showed miliary whitish nodules on the lungs and kidneys. Abdominal air-sacs were cloudy. The lesions in both groups were more or less similar but in addition peritonitis and pleuritis was evident in the first group.

Histopathological lesions

Liver : showed vaculation and degeneration of hepatocytes. Aggregates of mononuclear cells, mainly lymphocytes, heterophils and fibroblasts were evident, especially around the portal triads. Proliferation of the bile ducts was clear. Multinucleated giant cells of the foreign body type were scattered throughout the liver parenchyma. The blood vessels and sinusoids were dilated and congested with red blood cells (Photo 1).

Lungs: Granulomatous nodular lesions of variable sizes were scattered throughout the lung tissue. In some cases, the lesions coalesce leading to consolidation of the lungs. The nodular lesions consisted of central zone of homogenous eosinophilic material and cellular debris, surrounded by a wide zone of epitheloid cells namely macrophages, then multinucleated giant cells and fibroblasts (Photo 2). Blood vessels were congested. Young lesions showed in their centres multinucleated giant cells and/or epithelioid cells. Fungal hyphae were detected in affected tissues stained with hematoxylin and eosin. However, septated hyphae and fungal spores could be easily demonstrated with Periodic Acid Schiff and Gridley stains. They were very prominent in the zone of multinucleated giant cells and were detected in the cytoplasm of the giant cells. (Photo 3).

Heart: Myocardium revealed degenerative changes. Foci of mononuclear cells mainly lymphocytes and macrophages and giant cells were present (Photo 4), also edema and short branching filaments were seen.

Kidneys: Tubular nephrosis was evident in affected kidneys. Aggregates of lymphoid cells were scattered throughout kidney tissues. Small fungal particles were demonstrated by the special stains.
Photo 1. — Liver with scattered multinucleated giant cells, vacuolation of hepatocytes and congestion of sinusoids, of a chicken inoculated with *A. fumigatus* intraperitoneally at one week of age. H & E × 400.

Photo 2. — Inflammatory cells including multinucleated giant cells of a lung of a chicken inoculated intratracheally. H & E × 100.
Photo 3. — Fungal hyphae in cytoplasm of multinucleated giant cells present in nodular lesions in a lung of a chicken inoculated intraperitoneally. Gridley, × 400.

Photo 4. — Degeneration of muscle fibers, edema with few inflammatory cells and multinucleated giant cell formation in a heart of a chicken inoculated intratracheally. H & E, × 400.
DISCUSSION

This experiment shows that young chicks were readily susceptible to infection with A. fumigatus. This finding confirms the results of other workers (1, 6, 7). It is clear that this organism which was originally obtained from severely infected pigeons is virulent to young chicks as all the inoculated birds died in the acute stage of the disease.

It is interesting to note that both routes of inoculation produced minute miliary gross lesions in the lungs and kidneys. This suggests that these were the first organs to develop pathological changes in this infection, since microscopic lesions were detected in other organs as the heart and liver. The spread of infection seems to occur via blood stream from the inoculated site to other organs. However, the intraperitoneal route, in addition, causes lesions through the spread in internal cavities as evidenced by the presence of such lesions as pleuritis, peritonitis and air-sacculitis.

CHUTE and O’MEARA (3) suggested that the air-sac was the most effective route of inoculation. In our experiment, both the intratracheal and intraperitoneal routes were found very effective in producing the disease. The distribution of the lesions suggests that respiratory and circulatory insufficiency were mostly responsible for the death of chickens in the two inoculated groups.

The acute disease in young chicks follows the pattern of most respiratory diseases with high mortalities and this might pose a difficulty in diagnosis and control measures.

Differential diagnosis is essential as the condition could be confused with other diseases common to this age such as those due to Salmonella infection. Diagnosis can be achieved quickly based on post-mortem findings and demonstrating fungal particles with direct microscopy.

Work is needed to study this infection in our chicken’s flocks particularly at this stage where the industry is expanding fast. Assessment of the economic impact is not yet highlighted. Other aspects of the infection as toxigenicity and immunity need further studies.

RESUMEN


Se estudió la patogenicidad de A. fumigatus aislado a partir de palomos en pollos en las condiciones del Sudán. Inoculado por vías intraperitoneal e intratraqueal, provocó una enfermedad aguda con mortalidad de todos los pollos inoculados. Con las dos vías de introducción, se observaron lesiones miliare localizadas en los pulmones. Eran esencialmente granulomatosas con células epiteliales gigantes las lesiones microscópicas. Se encontraron también lesiones en el hígado, el corazón y los riñones.

Palabras claves: Aspergilosis - Aspergillus fumigatus - Sudán.

BIBLIOGRAPHIE