

STUDIES ON EXPERIMENTAL INFECTION WITH ASPERGILLUS FUMIGATUS IN OSTRICH CHICKS

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ABSTRACT

This work was aimed to study *Aspergillus fumigatus* infection (alone or preceded by immunosuppressive agents) in ostrich chicks. The birds (n=15) were randomly divided into three groups of 5 ostrich, each: The birds in the group (1) were inoculated intratracheally with 5 ml of *A. fumigatus inoculum*s (2.7x10⁶ spores/ml). The birds in the group (2) received three dexamethasone injections (2 mg/kg intramuscularly every 48h) preceding to the intratracheal inoculation with 5 ml of *A. fumigatus inoculum*s (2.7x10⁶ spores/ml). The birds in the group (3) were kept as control and were inoculated with only 5 ml of suspending media. After one week post infection, the birds in the group (II) showed cough, anorexia, dyspnea and weight loss. On radiograph the chest showed diffuse hazy infiltrates with a fine nodular appearance. At necropsy, multifocal white nodules were seen distributed on the surfaces of the air sacs and throughout the lung lobes. Histologically, multifocal necrotic, granuloma were seen with central radiating septated and branching hyphae of *Aspergillus fumigatus* in the lungs and liver. To our knowledge, it is a first time for experimental induction of *Aspergillus fumigatus* infection in ostrich. It is also concluded that the diagnosis of the disease in the ostrich is quite similar to other avian species including a thorough history, clinical signs, radiography and histologic demonstration of the organism

Key words: *A. fumigatus*, ostrich, histopathology.

INTRODUCTION

In recent years, interest has been focused on ostrich breeding in Saudi Arabia because of the value of ostrich products, such as meat, hide, eggs and feathers. Aspergillosis is a common term used to describe fungal infections caused by different species of *Aspergillus* (Verweij and Brandt, 2007). Most cases of aspergillosis are caused by *Aspergillus fumigatus* (*A. fumigatus*). *A. fumigatus* is the main type responsible for infections in different avian species (Beytut *et al.*, 2004; Copetti *et al.*, 2004; Martin *et al.*, 2007; Tokarzewski *et al.*, 2007; Olias *et al.*, 2010), wild birds (Mihaylov, 2008; Beernaert *et al.*, 2010; Rajamani *et al.*, 2013; Kheirandish *et al.*, 2013) and ostrich (Shathele *et al.*, 2009; Hasan *et al.*, 2011). The acute form of the disease is characterized by mortality rates of 70% to 90% in young ostriches (Hasan *et al.*, 2011). In the subacute and chronic forms of the disease, developing various clinical and histopathological findings (Ahmet *et al.*, 2009). Beside direct losses due to mortality, feed conversion and growth rate remain poor, airsacculitis is a major reason for carcass condemnation (Sancak and Paracikoglu, 2005). The diagnosis has been based on a thorough history, clinical and radiographic features, histopathology, along with serological or mycological evidence of *A. fumigatus* (Takashi *et al.*, 2004; Khosravi *et al.*, 2008 and Shathele *et al.*, 2009). Previously, experimental infections have been carried out in quails, starlings, pigeons, turkeys and chickens

(Gümü soy *et al.*, 2004; Atasever and Gümü soy, 2004; Van Waeyenberghe *et al.*, 2012; Femenia *et al.*, 2007). To our knowledge, it is the first time to induce experimental *A. fumigatus* infection in young ostrich. Therefore, The objective of this work is to study the susceptibility of ostrich to experimental *A. fumigatus* infection, as well as the immunosuppressive effect on this infection

MATERIALS AND METHODS

Experimental birds: A total of 15 three-weeks-old chick ostrich were used. The birds were randomly divided into three groups (5 ostrich for each): Group 1 (infected), group 2 (infected with preceding immunosuppression) and group 3 (control). The birds were placed in separate, cleaned and disinfected cages and fed with chick ostrich commercial pellet and received water supply *ad libitum*.

Preparation of inoculum: An isolate of *A. fumigatus* from a case of fatal aspergillosis in an ostrich in Saudi Arabia was used (Shathele *et al.*, 2009). Subculture was made into malt extract agar, incubate at 26^o C for 7 days. Harvest the growth in sterile Dist. Water, filter, collect the spore suspension. The concentration of 2.7x10⁶ spores/ml was obtained by using a counting chamber.

Experimental design: The birds in group (1) were inoculated intratracheally with 5 ml of *A. fumigatus inoculum*s (2.7x10⁶ spores/ml). The birds in group (2) received three dexamethasone injections (2 mg/kg

intramuscularly every 48h) to suppress the immune system followed by intratracheal inoculation with 5 ml of *A. Fumigatus inoculums*(2.7×10^6 spores/ml). The birds in group (3) were kept as control and were similarly inoculated with 5 ml of suspending media. Birds were observed closely, clinical signs were recorded throughout the experiment. After one week of last treatment, necropsy was performed on dead and euthanized birds (of various groups) after dislocation of the upper cervical vertebrae. The experiment follow the guide for the care and use of laboratory animal according to the Ethical Committee of King Faisal University.

Histopathological examination: Tissue specimens from the detected lesions were collected and then fixed in 10% neutral buffered formalin, processed, embedded and blocked in paraffin wax and then 4-5 μ thick sections were prepared, mounted and stained with haemotoxylin and eosin (H&E) according to Bancroft and Gamble (2007).The gross findings as well as the histopathological changes were recorded and photographed.

Mycological examination: Tracheal swap samples and entire internal organs of the infected birds with or without lesions at necropsy were cultured in Sabouraud-Dextrose Agar (SDA; oxoid, Hampshire, UK) at 25 °C for Mycological examination. Macroscopic and microscopic examination of colonies was carried out according to the method described by Raper and Fennel (1977).

A chest x-ray examination: The chest of all ostrich groups was examined radiographically in ventro-dorsal (VD) view in Sternalrecumbency using 40 KV and 10 meals and the radiograph was assessed for any lung lesions (Wagner and Kirberger, 2001).

Statistical analysis: Differences among treatment groups (1,2,3) for each of the recorded parameters were evaluated using Fisher's exact test.

RESULTS

Clinical signs: Recumbency is the first sign appeared after three days post-infection in the birds in the group (2). Three birds in this group were recumbent, cannot stand and unable to walk. Four days later, 4 birds showed cough, anorexia, dyspnea and weight loss, while 3 of them were dying (Figure 1a), however, the birds in the other two groups did not reveal any clinical signs (Table 1).

X- ray findings: A chest radiograph in three birds in the group (2) showed diffuse hazy infiltrates with a fine nodular appearance (Figure 1b).In control and another group, a chest x ray did not reveal any abnormalities (Table 1).

Mycological findings: *A. fumigatus* was isolated from trachea, lung, air sacs and liver in all birds in the group (2) and could not be isolated from other groups (Table 1).

Gross findings and microscopic examination: The birds in all groups were necropsied and the most common gross findings were noticed in group (2). There were whitish cheesy materials of fungal growth appeared dispersed in the air sacs and lung tissue (Figure 1c). Marked congestion of the lungs and thickening of the air sac membranes occur. Miliary necrotic foci develop in lungs around the sites of fungal growth and result in the formation of nodules. These nodules were discrete, circumscribed, white in color and randomly distributed throughout the lung tissue and air sacs(Figure 1d). Similar micronodules were observed in the liver associated with prominent meningeal and endocardial hemorrhage in most cases. Histopathologically, bronchi and bronchioles were frequently filled with large masses of necrotic debris, inflammatory cells admixed with fungal hyphae. Multifocal necrotic and granulomatous foci of variable sizes embedded in different parts of the lung tissue (Figure 2a). The peripheral part of these foci consisted of inflammatory cells, especially heterophils, macrophages and lymphocytes (Figure 2b). In the centers of these nodules, there were necrotic debris admixed with radiating septated and branching hyphae with conidia head (Figure 2c).Pulmonary blood vessels revealed vasculitis and angioinvasion of fungi associated with thrombus formation (Figure 2d). Liver also revealed granulomatous foci with radiating hyphae surrounding with macrophages, lymphocytes and heterophils.The birds in groups (1,3) did not show any gross or microscopic findings (Table 1).

Table 1.Numbers of ostrich with clinical signs, pathological lesions and isolation of *A. fumigatus*

Clinical signs, lesion and isolation	Groups			P-Value
	Group 1	Group 2	Group 3	
Recumbency	0	3/5	0	0.083
Clinical signs (cough and weight loss)	0	4/5	0	0.024
Mortality	0	3/5	0	0.083
X-ray finding	0	3/5	0	0.083
Lung lesion	0	4/5	0	0.024
Isolation of <i>A.fumigatus</i>	0	5/5	0	0.004

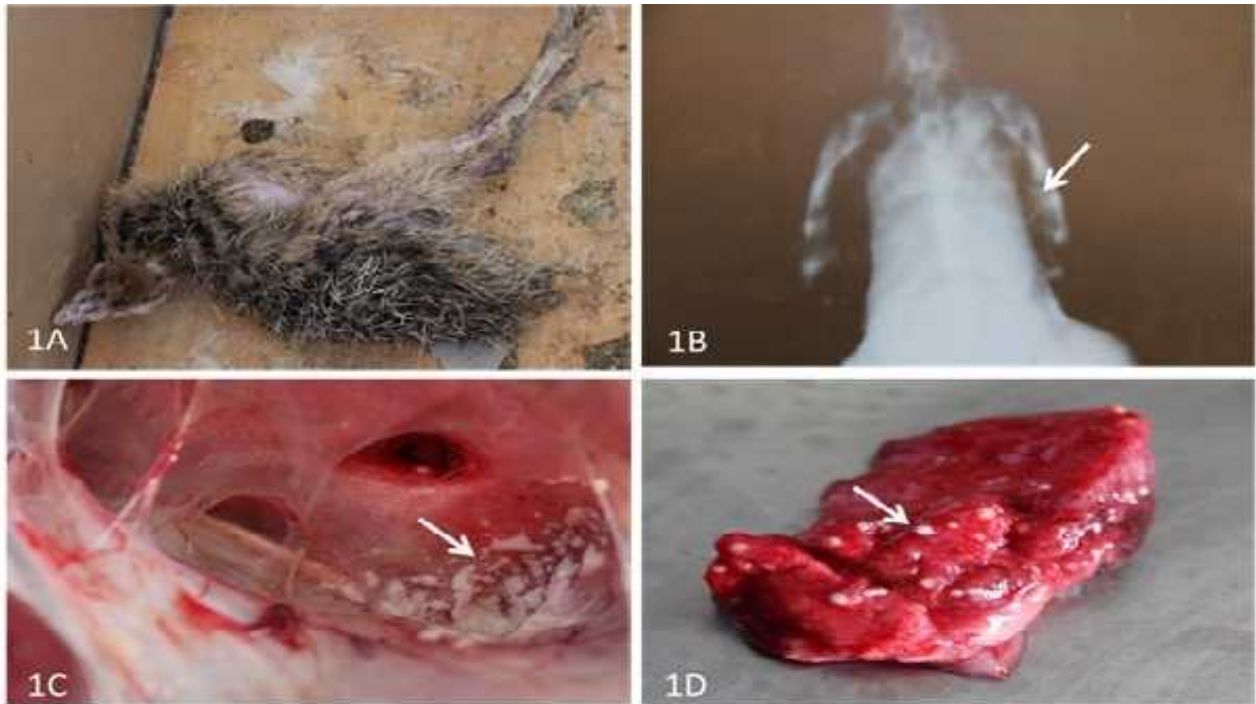


Figure 1: A- Dead ostrich chick 10 days post-infection. B- Chest x ray shows diffuse hazy infiltrates with a fine nodular appearance (arrow). C- Whitish cheesy material dispersed throughout air sacs and lung tissue (arrow). D- Whitish multifocal nodules of variable size distributed throughout lung tissue (arrow).

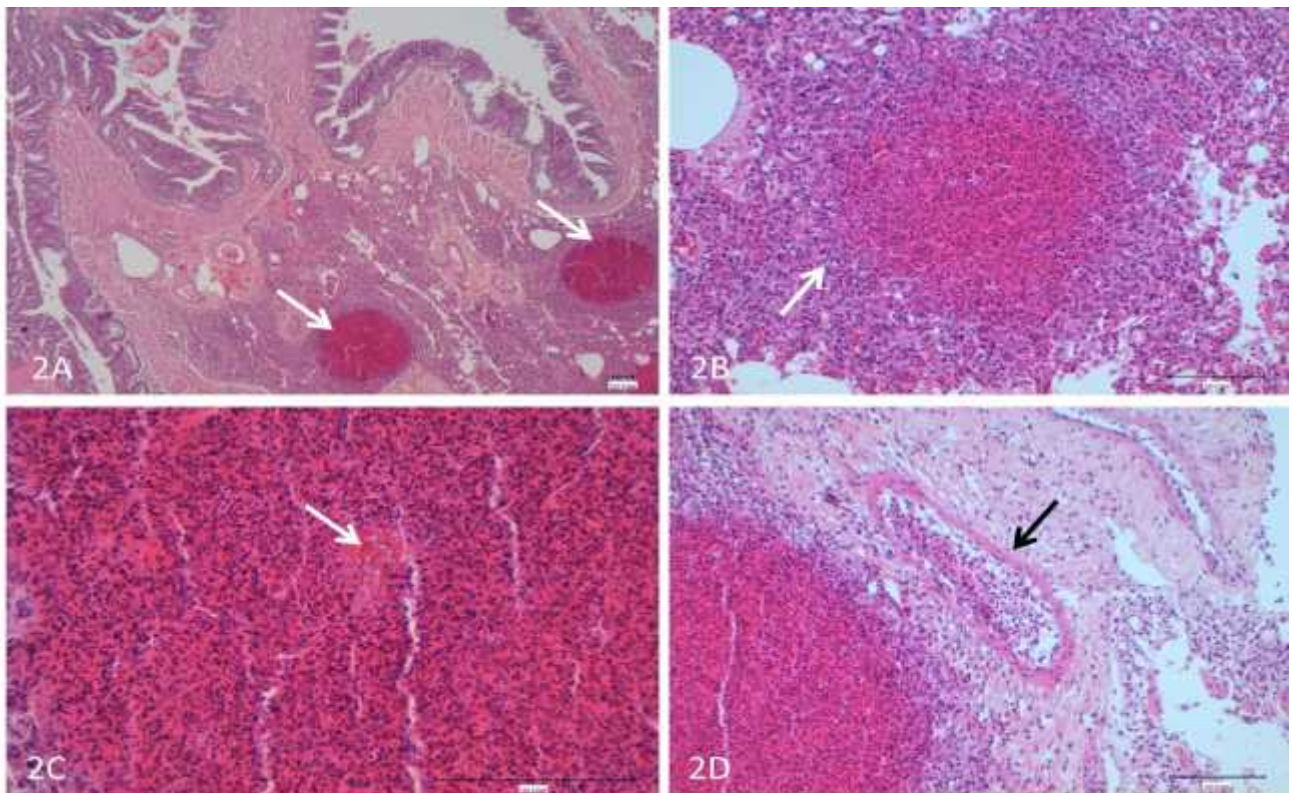


Figure 2: A- Multifocal granulomatous foci embedded in lung tissue (arrows). H&E, scale bar = 100 μ m B- granulomatous foci surrounding with inflammatory cells (arrow). H&E, scale bar = 100 μ m. C- Branched and septated hyphae with conidia head (arrow). H&E, scale bar = 100 μ m. D- Vasculitis and thrombosis of pulmonary blood vessels (arrow). H&E, scale bar = 100 μ m.

DISCUSSION

Ostrich chicks are particularly susceptible to fungal disease, especially aspergillosis, the most susceptible individuals being young birds kept in enclosed facilities and exposed to dust or hay (Yegani and Korver, 2008.). In the present study, the clinical signs, chest x ray examination, necropsy and histopathologic findings were seen in immunosuppressed group rather than control and immunocompetent groups. This finding suggests that clinically healthy ostriches are not prone to develop aspergillosis due to effective immune responses (Maina, 2002; Van Waeyenberghe *et al.*, 2012). Additionally, Copetti *et al.* (2004); Kunkle, 2003; Olias *et al.* (2010) reported that aspergillosis appears to be more significant where predisposing factors such as stress and immune suppression are usually involved. The clinical signs were coughing, anorexia, weight loss, dyspnea and these signs depend on which form of aspergillosis in the bird develops and which organs are involved (khosravi *et al.*, 2008). Although radiographs may not be helpful, radiograph revealed a fine nodular appearance. This was concomitant with Jones and Orosz (2000) who stated that lateral and ventrodorsal views can be taken in a bird suspected of having aspergillosis. At necropsy, there was numerous granulomatous foci in the air sacs, lungs and liver. This finding was in agreement with Jenkins (1991) in acute aspergillosis. The hepatic lesion indicates hematogenous spread of infection and hyphae were known to be tissue and angio-invasive (Beernaert *et al.*, 2010). However, a negative culture from the heart and brain, hemorrhage and congestion were observed and this could be attributed to a number of *A. fumigatus* toxins, including gliotoxin, helvolic acid and fumagillin (Takashi *et al.*, 2004). Histopathological findings in lung, air sacs and liver were quite similar to those observed in ostrich by Shathele *et al.* (2009); Hasan *et al.* (2011); Araghi *et al.*, (2014) and in other birds (Atasever and Gümü soy, 2004; Van Waeyenberghe *et al.*, 2012; Femenia *et al.*, 2007). Hence, aspergillosis should be included in the differential diagnosis of respiratory tract and systemic diseases in birds (Jones and Orosz, 2000). It can be concluded from the results of the present study that a young ostrich under stress factor is susceptible to experimental infection with *A. fumigatus*, and the signs and lesions were similar to those observed in other avian species. Furthermore, isolation of fungus alone does not confirm the infection and also, a negative culture does not rule out aspergillosis, eventually, the diagnostic tools of aspergillosis in ostrich are quite similar to other avian species and should include a thorough history, clinical signs, radiography and histopathologic demonstration of the organism.

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