ASPERGILLUS INFECTION IN LAYER HENS

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ABSTRACT

Two hundreds of layer chickens were sampled from 100.000 layer hens of different ages that showing clinical signs and gross lesions from different farms in Sharkia Governorate from January to April-2008. Aspergillus spp. were isolated and identified morphologically on cultural basis. Morbidity rate was reached 40% and mortality rate more 7%. Clinical signs were mainly respiratory characterized by gasping, dyspnea and accelerated breath. The most common gross lesions were caseated nodules distributed in lung, air sacs, intestine, heart and liver. Microscopic picture revealed granulomatous lesions with or without hyphae. Those lesions represented by caseated nodules,numerous inflammatory cells mainly heterophils, lymphocytes, macrophages and giant cells.The nodules were encapsulated with thin connective tissue capsule.The fungal hyphae appared as branching thread-like either at the periphary or inside the organs parenchyma. Control of aspergillosis in our present study illustrated that there was no curative treatment for aspergillosis.

INTRODUCTION

Mycotic disease in chicken had been reported early by **Rowell (1949)**, it cause high economic losses particularly when associated with other stress conditions **(Ononiwu and Momoh 1983)**.

Many authors isolated Aspergillus spp. as an etiological agent of mycotic infection causing death, **(Pandita et al., 1991; Droual et al., 1994; Ali 1996 and Sami & Sabry 2004)**.

Fungal infection consider to be a stress factor affecting hatchability, growth and development of birds **(Cutsen and Fram 1987 and El-Badry and Sokkar 1988)**.

Avian aspergillosis caused by Aspergillus fumigatus, Aspergillus flavus or Aspergillus nidulans and characterized by granulomatous lesion in respiratory tract **(Crokish 1982; Ibrahim et al., 1983; Mieoyie et al., 1986; El-Bardy and Sokkar, 1988 and Pal et al., 1990)**.

Hygienic conditions of chicken flocks such as warm, humidity, floor brooders and houses play an important role in the pathogenesis of this fungal infection **(Beckman et al., 1994)**.

The aim of the present work was to study the incidence of Aspergillosis (clinical signs, isolation of causative agents, the gross and microscopic lesions) among sick and or healthy layer flocks.

MATERIAL AND METHODS

A total number of 200 chickens from 100.000 layer hens of different ages that showing clinical signs and gross lesions were collected from different farms at Sharkia Governorate during the period from January to April-2008. Investigated chickens were either clinically sick or recently dead. Layers were fed on well balanced ration that contain recommended requirements and vaccinated with the recommended vaccines beside having prophylactic antibacterial and anticoeldial drugs.

Clinical signs and gross pictures were recorded. Specimens from lesions in different organs were collected for isolation and identification Asperigilus according to **Al-Doory (1980)**.

Swabs from air sacs, lung, heart, liver, spleen and kidneys from diseased chickens were cultured on Sabouraud's agar **(Al-Doory, 1980)** containing 100ug/ml gentamycin sulfate and incubated at 24°C to isolate Aspergillus spp.

Pathogenesity of Aspergillus fumigatus: 90 layer chickens of 4 weeks old were distributed into 3 groups (A, B and C). Group (A) was kept as a control (non infected and non treated). Groups (B & C) were inoculated I/M with 1ml containing 10.5 x 10^4C.F.U. of aspergillus fumigatus **(Beckman et al., 1994)**.

Group (C) was treated 24 h. post infection with griseoflvin 20 mg/kg body weight for 5 successive days in drinking water. All birds were kept under observation for 14 days.

Histopathological examination:

Fresh specimens from lung, air sac, liver and heart were fixed in 10% neutral buffered formalin. Paraffin sections of 5 microns thick were prepared and stained with hematoxylin and eosin according to **Lillie, 1984 and Bancroft et al., 1990**.

Pathogenicty of Aspergillus fumigatus to 4 weeks old layer chicks:

Inoculcated birds of group B and C showed high morbidity compared with birds in control group (A). birds in group C (treated) showed low mortality. We can re-isolating Aspergillus fumigatus from both groups B and C. (Table 2).

DISCUSSION

This work declared that the pereentage of mortality was 7% among flocks of layer naturally infeeted with Aspergillosis. This was in accordance with **Beckman et al., (1994)** who mentioned that the suseeptibility of the young chicks to Aspergillosis increased due to immaturity of phagocytes or due to environmental factors.

Aspergillus infection in the present study was characterized by high morbidity and low mortality.

The clinical signs in the present study were mainly respiratory manifestations characterized by gasping and dyspnea. These signs were also reported by **Edris et al., (1995); Calneck et al., (1991) and Sami and Sabry (2004)**. These signs indicated that aspergillosis was considered an air born disease and the severity of outbreak was related to the spore coneentration in the air.

Calneck et al., (1991) mentioned that the outbreak oeeurred when the organism was present in sufficient quantities to establish the disease or when birds resistance was impaired by immuno suppressive compounds.

The gross lesions of aspergillosis in the present investigation were detected in the lung, air sac, heart, liver and intestine. The widely disseminated infection to these organs may be due to hematogenous or lymphatic dissemination of the organism from respiratory tract to these organs, these observations agreed with **Beckman et al., (1994)**. The gross lesions were small whit-yellowish and fragile. Caseatcd nodules of 1-2mm thickness distributed in the infected organs. **Gab-Allah, (1994); Richard et al., (1994) and Ali (1996)** described white nodular lesions of variable thickness in lungs, air sacs, liver and intestine.

The microscopic picture was characterized by granulomata with or without hyphae, the granuloma represented by caseated nodules with numerous inflammatory cells mainly heterophils, lymphocytes, maerophages and giant cells. Thin fibrosis capsule was encapsulated the nodules.

These lesions showed partial agreement with **Mahmoud (1988)** and **Edris et al., (1995)** who found passive congestion and wide infiltration of inflammatory eells in pulmonary tissue of pigeon and Guinea fowl, respectively. They also detected granulomatous lesions of different sizes containing fungi hyphae in air sacs, lungs, liver and intestine. Hemosiderosis seen in liver was due to destruetion of erytherocyte by fungi and their toxins, this result agreed with **Edris et al., (1995)** who dctected hepatic hemosiderosis following natural Aspergillosis in Guinea fowl. Heavy lymphoeytic infiltration was observed in the eardiac muscles and that in agreement with **Mitroiu et. al. (1962)** who observed histocytle and lymphocytic infiltration in the cardiac muscles while **Mahmoud (1988)** noticed neither gross nor histopathological changes in the heart.

Pathogenisty of Aspergillus fumigatus to layer chicks revealed that clinieal symptoms and postmortem lesions were as that of the natural infeetion. Control of aspergillosis at the present study illustratcd that there was no feasible effective treatment for aspcrgillosis.

It could be concludcd that the mycotic diseases of chicken particularly aspergillosis induced some losses among infeeted farms and usually has systemic infection. Control of aspergillosis at the present study indicated that there was no feasible treatment for aspergillosis, so affectcd birds should be destroyed and the poultry houses should be vigorously cleaned and disinfected.

Table (1): Species numbers, locality of the farm and ages of the affected flocks.

No	*Layer species*	*Locality of the farm*	*Number of layer per flock*	*Age of chicks*
1	Shever	Belbies	20000	4 weeks
2		Mashtol	15000	8 weeks
3	Native layer	Kafr Sager	15000	2 weeks
4		Dearp Negem	5000	24 weeks
5		El-Salhia	20000	6 weeks
6		El-Salhia	25000	18 weeks

Table (2):

Group	*Infection*	*Treated*	*Morbidity*	*Mortality*	*Reisolation*
A	-	-	-	-	-
B	+	-	24/30	7/30	5/5
C	+	+	23/30	4/30	4/5

Fig. (1 and 2) : Caseous nodules on lung (L), air sacs (A), liver (V) and heart (H).

Fig. (3): Aspergillus culture on Sabouraud's agar.

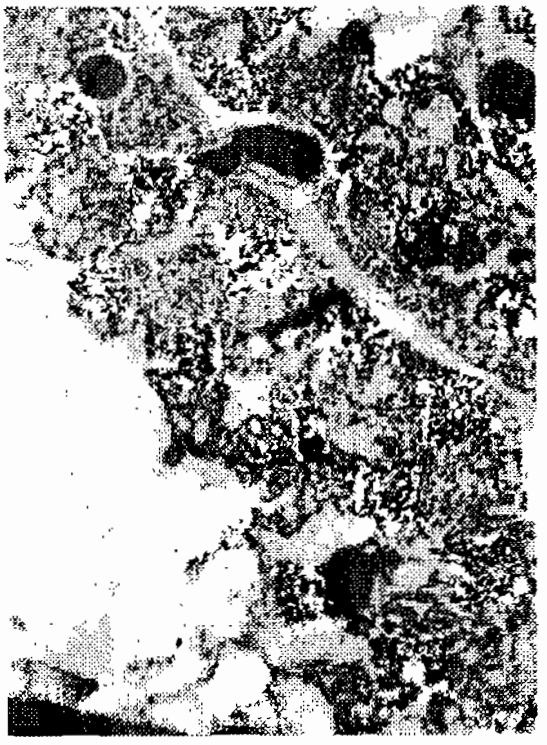

Fig. (4): Lung showing heavy lymphocytic infiltration and nodular formation (H & E x 150).

Fig. (5): Lung showing areas of granulomatous necrosis, inflammatory cell infiltration, fibrosis and fungal hyphae (H & E x 150).

Fig. (7): Wall of air sac showing thickness with nodular formation (H & E x 150).

Fig. (6): High power of fig. (5) showing the hyphae, giant cells, granulomatous nodules (H & E x 300).

Fig. (8): Wall of air sac showing hyphae and granulomatous nodules (H & E x 150).

Fig. (9) : High power of fig. (8) showing the nodules (H & E x 300).

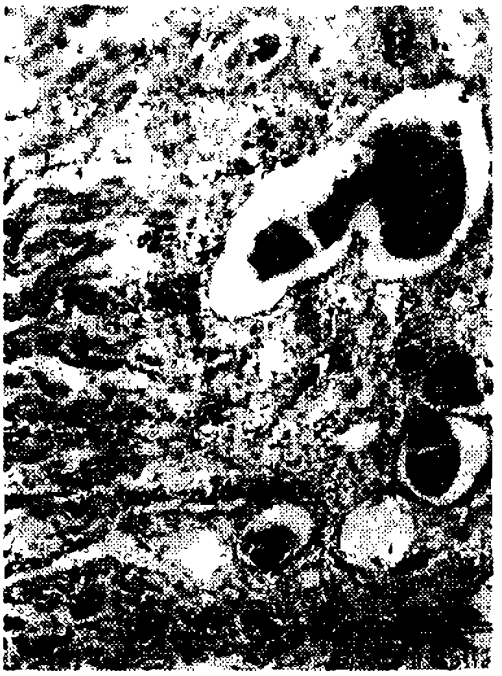

Fig. (11): Heart showing thickening of the epicardium, necrotic nodules and lymphocytic infiltration (H & E x 150).

Fig. (10): Wall of air sac showing hyphae, spores and granulomatous nodules (H & E x 300).

Fig. (12): Heart showing myocardium with lymphocytic infiltration (H & E x 150).

Fig. (13): Heart showing fungal hyphae (H & E x 600).

Fig. (15): Liver showing necrotic area and heavy reactive inflammatory cells, notice newly formed bile ductules (H & E x 300).

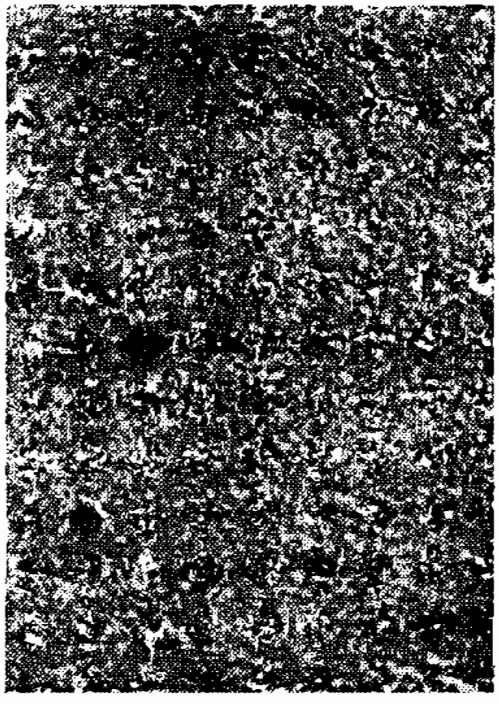

Fig. (14): Liver showing multiple easeous nodules (H & E x 150).

Fig. (16): Liver showing hyphae and hydropic degeneration of hepatic cells, notice the hemosidrosis (H & E x 600).

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الملخص العربى

عـــدوى الإســـبرحلوزس فى الدجـــاج البيـــاض

إبراهيـم فكـرى علـون ، منـال السيد محمد المسلمى ، حسـام حسـن عـلام

معهـد بحـوث صـحة الحيـوان - فـرع الزقـازيـق

أجريت الدراسة على ١٠٠٠٠٠٠ دجاجة بياض تم تجميع عدد ٢٠٠ دجاجة بياض منها تعانى من الإصابة بفطر الإسبرجلس بمحافظة الشرقية، وكانت الأعراض الظاهرة على الدجاج المصاب بهذا الفطر أعراض تنفسية، ونسبة الدجاج المصاب ٤٠٪ والنافق يزيد عن ٧٪، وتمثلت الصفة التشريحية بوجود عقد تنكرزية متجبنة لونها أبيض مصفر مع بعض الإحتقانات فى الأحشاء الداخلية مثل الرئتين، الأكياس الهوائية، القلب، الكبد.

وبالفحص المجهرى للأحشاء الداخلية وجد أن الصورة المجهرية للعدوى الطبيعية والصناعية متشابهة، وتتميز بإلتهاب تنكرزى حبيبى وإنتشار للخلايا الليمفاوية فى الأعضاء المختلفة - وتوجد خيوط الفطر متفرعة سطحياً على الأعضاء أو داخلها.

ولاتوجد طريقة للسيطرة على هذا المرض إلا بالوقاية فى المزارع ومعامل التفريخ ولم ينتج طريقة خاصة أو دواء فعال للعلاج، ويجب التخلص من القطيع المصاب وتنظيف حظائر التربية جيداً وتطهيرها.