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بعض الدراسات على الفطريات المعزولة من قطع بيدا رى أسيوط

عوض ابراهيم ، محمد عطية \* ، مصطفى شحاتة ، صلاح موسى

- جمعت عينات من ٤٨٠ حالة نافعة ٣ - ١٠ أسابيع وتم فحصها ومحاولة لعزل الفطريات منها .  
وقد تم التعرف على ١٩٨ اسيرجلس ١٥٦ كنديدا ١٢ بنسليوم .
- تم اجراء العدوى الصناعية فى كتاكيت عمر ٥ أسابيع وقد ثبت أن أنواع الاسيرجلس والكنديدا كانت معدية .
- أثبت اختبار الحساسية أن مركبات النستاتين وسلفات النحاس ذات فاعلية أكثر من الشيبندازول والجريزو ملفين .

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SOME STUDIES ON FUNGI ISOLATED FROM A BROILER FLOCK IN ASSIUT  
(With Two Tables)

By

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SUMMARY

1440 samples taken from crop, lungs, and intestines of 480 dead broiler chickens 3-10 weeks old were subjected to mycological examinations. 192 *Aspergillus* species were isolated from lungs in addition to 12 *Penicillium* species, 102 *Candida albicans* and 6 *Aspergillus* spp. from crops and 54 isolates of *C.albicans* from intestinal samples.

Experimental infections using some isolates were carried out on 5 weeks old chicks. Reisolations of the fungal spp. from airsacs, crops and intestines of infected chicks were carried out. The sensitivity of the isolated fungi to different drugs was studied and it was found that nystatin and copper-sulphate were more effective than thiabendazole and griseofulvin.

INTRODUCTION

It is well known that Mycotic diseases of poultry are of high economical losses particularly when associated with other conditions.

In Egypt several studies were carried out to investigate the different aspects of fungal infections among poultry.

EL-BATRAWI (1976) isolated 60 *Candida albicans* from crops of 159 chickens and found that experimental infection of 7 day-old chickens via oral route was milder than the natural one. SALEH (1976) found that bright greenish-yellow caseous nodules in the lungs and air-sacs were the most characteristic lesions found in 2078 cases examined for pneumo-mycosis. The result of isolation showed that 54.6% were yeast and yeast-like fungi. *Aspergillus fumigatus* was the most prevalent species of fungi followed by *Aspergillus flavus* and at least *Aspergillus niger*.

EL-BATRAWI (1980) described a respiratory and nervous signs in 2-week-old chicks experimentally infected with *A. fumigatus*, *A. flavus* or *A. niger* spores, the characteristic nodules in the lungs and air-sacs were only seen in *A. fumigatus* infected chicks.

SAIF (1976) reported that thiabendazole and nystatin proved to have fungicide effect on *A.flavus* cultures, while copper-sulphate had no such effect. SAIF & REFAI (1971) recorded that thiabendazole tablets eliminated completely *A. fumigatus* from incubators, hatcheries without any effect on egg hatchability when used as fumigation.

As this problem appeared severally in the above mentioned farm, it was then necessary to carry up the following studies:

- 1- To isolate and identify the fungi causing this recurrent problem in this farm.
- 2- To study the pathogenicity of the isolated fungi on susceptible birds.
- 3- To study the sensitivity of the isolated fungi to some fungicides.

MATERIAL and METHODS

Specimens:

480 dead broiler chickens 3-10 weeks old were used in this study.

Media:

- Sabouraud's dextrose agar " plates and tubes "
- Rice agar media.
- Sugar fermentation media.

Sugar assimilation media.

Reagents and Stains:

Physiological saline.  
Lactophenol cotton blue.

Antibiotic for isolating media:

Penicillin.  
Streptomycin.

Chicks for Experimental Infections:

50 chicks, 5-weeks-old were used in pathogenicity test, the chicks were obtained from Bani-Mur poultry farm, Assiut Governorate.

Fungicides for Sensitivity Test:

Nystatin and Griseofulvin.	(Memphes)
Thiabendazole.	( M.S.D.)
Copper-sulphate.	(El-Nasr)

Mycological Examinations:

Dead chicks were subjected to post-mortem examination, and macroscopic lesions were recorded. Direct swabs were taken from crops, lungs and intestines, streaked on slope Sabouraud's dextrose agar to which penicillin and streptomycin were added in the proportion of 20 i.u. & 40 ug/ml. of media respectively.

The cultured media were incubated at 37°C for 48 hours, then at room temperature for 2 weeks before recording the results.

Identification of the Isolates:

Moulds were identified according to their morphological appearance as the mycological literature. ATIA (1975). Yeasts were identified according to their fermentation and assimilation properties as described by LODDER, *et al.*, (1967), POSE & HARRISON (1971).

Pathogenicity Test:

Subcultures from the isolated *Aspergillus* species were made on Sabouraud's agar plates, incubated for 10 days at 37°C then fungal suspensions using fresh distilled water were prepared. *Candida albicans* suspensions of 48 hours cultures were also made.

The chicks were divided into five groups each of 10 birds.

- Birds of group "A" were inoculated with  $4 \times 10^6$  *A. fumigatus* spores / chick via the left abdominal air-sac.
- Birds of group "B" were inoculated by the same dose and route using *A. flavus* spores.
- Birds of group "C" were inoculated by the same dose and route using *A. niger* spores. SINGH & MALHOTRA, (1974).
- Birds of group "D" were inoculated with  $6 \times 10^6$  *C. albicans*/chick via the crop.
- Birds of group "E" were left as controls.

All chicks were kept under observation for 2 weeks.

Reisolation of the inoculated fungal species were carried out from crops, air-sacs and intestines of infected chicks.

Sensitivity of the isolates to some fungicides:

Known concentrations of drugs as shown in (Table II) were dissolved in 20 ml. distilled water and thoroughly mixed with 980 ml. of Sabouraud's agar medium after autoclaving and cooling to 60°C then the media were poured in plates. The isolated fungi were inoculated into this plates. The plates were incubated for 10 days at 37°C, at the same time control cultures of fungi without fungicides were also incubated. ATIA (1975).

## DISCUSSION

Because of the high mortality rate among the flock of broilers belonging to the Faculty of Agriculture,

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Assiut University which ranged between 20-25% and the presence of respiratory manifestations as well as lesions of pneumonia, airsacculitis together with ulcerative crop lesions in P.M. examinations of dead birds, these birds were subjected to mycological examination. Mycological identification of the isolated organisms revealed that *C. albicans* was the most common isolate from the examined crops and the only species detected from intestinal samples. "21.6 and 11.25% respectively". Similar results were recorded by WYATT & HAMILTON (1974), SALEH (1976), and ABOU-GABAL *et al.*, (1977), although high percentage of *C. albicans* "40.5, 75.9 and 37.5%" were isolated from crop swabs examined by ALLER (1967), MOS, *et al.*, (1973) and EL-BATRAWI (1976) respectively. In the present investigation 1.25% of the isolated fungi from the crop were identified as *A. fumigatus*, contrary to our results ALLER (1967) isolated *A. fumigatus* from the examined crop samples in high percentage (13.5%).

Concerning the species isolated from lungs, *A. fumigatus*, *A. flavus*, *A. niger* and *Penicillium* species could be detected from 20, 13.75, 6.25 and 2.50% of the examined samples respectively. Some of the examined cases showed pneumonia, airsacculitis, but typical granuloma was not observed in any. Our results are in agreement with those described by CUBILLOS & PRUSSING (1979) who isolated *Aspergillus* species from 26.05% of apparently healthy broilers and disagree with the results reported by ALLER (1967) and ABOU-GABAL, *et al.*, (1977) who isolated low percentage of moulds.

SINGH & RAOK (1976) found that *A. niger* was present in 6.25% out of 81 poultry carcasses showing granulomatous lesions. Similar percentage was reported by the authors without observing granulomatous lesions.

The frequency of *Penicillium* species recorded in our study resembles to some extent that reported by ABOU-GABAL, *et al.*, (1977).

The first three groups of chicks inoculated with *A. fumigatus*, *A. flavus* and *A. niger* showed general signs. Post-mortem lesions reported in sacrificed chicken groups two-weeks after infection revealed pin-headed grayish-white nodules on the airsacs, pneumonia, tracheitis, streaks of haemorrhages on liver and congestion of brain. Similar findings have been reported by EL-BATRAWI (1980), SINGH & HALHOTRA (1974), who found granulomatous lesions in 1-2 week-old chicks four days after infection with sublethal and lethal doses of *A. fumigatus* spores in lungs, airsacs, trachea, heart, liver and skeletal muscles. It was concluded that this reaction was dose-related and the lesions were more severe in chicks infected via intra-tracheal route than air-sacs inoculation.

No clinical signs could be observed in chicken group inoculated with *C. albicans* during the whole period of observation. Yellowish white nodules of different sizes in the crops were the most pathognomonic lesions. These result accord with those of WYATT & HAMILTON (1974) and EL-BATRAWI (1976) who reported that the crop lesions were the only characteristic feature in the chicks inoculated orally with *C. albicans*.

Results of sensitivity test clarified that nystatin in dose of 1250 i.u./ml. media inhibited all the tested isolates. Similar finding has been recorded by QUERNHORST (1963), ZIGER (1971), SAIF (1976) and HAMARA & PANKIEWICZ (1977). Griseofulvin (10 mg./ml.) was of moderate effect on the tested fungal species. EL-BADRY (1979) found that griseofulvin (16 mg./ml.) inhibited the growth of *A. flavus* and *A. niger*. On using copper-sulphate in a concentration of 20 mg/ml. it resulted in complete inhibition of *C. albicans*, *A. flavus*, *A. niger* and slight growth of *A. fumigatus*. Low concentrations of copper-sulphate (8, 12 mg/ml.) used by EL-BAHEY, *et al.*, (1968) and SAIF (1976) had no fungicide effect, while moderate inhibition on the growth of *Aspergillus* spp. was reported by EL-BADRY (1979) who used 20 mg./ml. Using of thiabendazole as a fungicide it has been found that 2 mg./ml. of the medium had no effect on *C. albicans*, moderate effect on *A. fumigatus*, slight effect on *A. niger* and complete inhibition of *A. flavus* growth.

Some-what similar results were reported by SAIF (1976), EL-BADRY (1979), while those reported by ZIGER (1971) were completely different.

From the present study it could be concluded that the high losses recorded in broiler flock might be attributed to infection with pathogenic fungi. Bad hygienic condition of the flock in addition to other stress factors as malnutrition played an important role in complication of this mycotic infections.

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The percentage of the isolated fungi after their identification were illustrated in Table (I)

Examined organ	No. of specimens	Isolated Fungal Species									
		C. albicans		A. fumigatus		A. flavus		A. niger		Penicillium	
		No.	%	No.	%	No.	%	No.	%	No.	%
Crop	480	102	21.6	6	1.25	--	--	--	--	--	--
Intestines	480	54	11.25	--	--	--	--	--	--	--	--
Lungs	480	--	--	96	20	66	13.75	30	6.25	12	2

Table (II)

Showed the results of sensitivity of isolates against some fungicides

Tested fungi	Thiabendazole	Griseofulvin	Nystatin	Copper-sulphate
	2 mg./ml.	10 mg./ml.	1250 i.u./ml.	20 mg./ml.
C. albicans	+++	++	---	---
A. fumigatus	++	++	---	---
A. flavus	---	++	---	---
A. niger	+	++	---	---

## REFERENCES

- Abou-Gabal, M.; Enab, S.M.; El-Agroudi, M.A. (1977): Studies on the incidence of pathogenic fungi in poultry. J. Egypt. Vet. Med. Ass. Vol. 36, (1), 90-102.
- Aller, B. (1967): Fungi in the upper digestive and respiratory tract of hens. Br. Vet. J. 123, 431.
- Atia, M.A. (1975): Ecological investigation on dermatophytes and its role in the epidemiology of fungus infections of man and animals. Thesis, Assiut Univ., Fac. of Vet. Med.
- Cubillos, A.; Prussing, H. (1979): Mycological studies on the respiratory organs of broilers. Zentrablatt für Veterinärmedizin 26 B (8), 605-613.
- El-Badry, A.A. (1979): A study of mycotic diseases in turkeys. Thesis, Assiut Univ., Fac. of Vet. Med.
- El-Bahey, G.M.; El-Mossalmi, E. and Refai, M. (1968): The use of some disinfectants as fungicides. Mykosen, 11, 807.
- El-Batrawi, A.M. (1976): Studies on Candida albicans infection in poultry. Thesis, Cairo Univ., Fac. of Vet. Med.
- El-Batrawi, A.M. (1980): Studies on infections in poultry with particular references to Aspergillus fumigatus infections in intensive poultry farms. Thesis, Cairo Univ., Fac. of Vet. Med.