بعض الدراسات على الخضبات المعزولة من قطيع يد إسبوتو

ősبراب، محمد مصطفى، صلاح موسى

- جمعت مياه من 480 حالة تراوية 3-10 أسابيع ثم تفحصت ومحاولة لعزل الخضبات منها.
- وقد تم التسريع إلى 198 آسياب على 16 إسبراجس 176 كانت بدا 12 بنسيلوم.
- تم إجراء الحيوان الصناعية في كتاكيت عبر 5 أسابيع وقد ثبت أن أنواع الإسبراجس والكبد بينما كثيرة.
- أثبت اختبار الحساسة أن مركبات النستين وسلفات النحاس ذات فعالية أكبر من التيتريدين والجريزيو طلقين.

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رئيس القسم: أ.د. عماد كامل ناقع
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. 197 Aspergillus species were isolated from lungs in addition to 12 Penicillium species, 102 Candida albicans and 6 Aspergillus spp. from crops and 34 isolates of C. albicans from intestinal samples. Experimental infections using some isolates were carried out on 5-week-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 thalabendazole 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 airsacs were the most characteristic lesions found in 2078 cases examined for pneumonia. 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 airsacs were only seen in A. fumigatus infected chicks.

SAIF (1976) reported that thalabendazole and nystatin proved to have fungicide effect on A. flavus cultures, while copper-sulphate had no such effect. SAIF & REFAI (1971) recorded that thalabendazole 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.  (Memphis)
- Thiabendazole.  (M.S.D.)
- Copper-sulphate.  (El-Nasr)

Mycological Examinations:
Dead chicks were subjected to post-mortem examination, and macroscopic lesions were recorded. Direct smears 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 mg/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:
Molds 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 into 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,
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), SALEM (1976), and ABOU-CABAL 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-BATRAWY (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-CABAL, et al., (1977) who isolated low percentage of moulds.

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

The frequency of Penicillium species recorded in our study resembles to some extent that reported by ABOU-CABAL, 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-BATRAWY (1980). SINGH & MALHOTRA (1974), who found granulomatus 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 results accord with those of WYATT & HAMILTON (1974) and EL-BATRAWY (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 QUENNORST (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 (6, 12 mg/ml.) used by EL-BAYEY, 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.
ACKNOWLEDGEMENT

The authors wish to express their sincere thanks to Prof. Dr. I.M.H. Sokkar, Head of Animal Medicine & Poultry Diseases Dept. Fac. of Vet. Med., Assiut University for his continuous advice, and useful discussion.

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

<table>
<thead>
<tr>
<th>Examined organ</th>
<th>No. of specimens</th>
<th>C. albicans</th>
<th>A. fumigatus</th>
<th>A. flavus</th>
<th>A. niger</th>
<th>Penicillium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop</td>
<td>480</td>
<td>102 21.6</td>
<td>6 1.25</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Intestines</td>
<td>480</td>
<td>54 11.25</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lungs</td>
<td>480</td>
<td>--</td>
<td>96 20</td>
<td>66 13.75</td>
<td>30 6.25</td>
<td>12 2</td>
</tr>
</tbody>
</table>

Table (II)

Showed the results of sensitivity of isolates against some fungicides

<table>
<thead>
<tr>
<th>Tested fungi</th>
<th>Thiabendazole 2 mg./ml.</th>
<th>Griseofulvin 10 mg./ml.</th>
<th>Nystatin 1250 i.u./ml.</th>
<th>Copper-sulphate 20 mg./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


