

قسم : النبات - كلية العلوم - جامعة أسيوط .
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الفلورا الفطرية لحالات من الجاموس المصاب بالالتهاب الرئوي

بهي الدين نازن ، اسماعيل القاضي ، أحمد عبد الفتاح* ، عادل الياس**

في دراسة عن الفلورا لجميع اجزاء الجهاز التنفسي لافراد من الجاموس المصابة بالالتهابات الرئوية ، تم عزل وتعريف ٣٠٠ معزولة مختلفة تنتمي الى خمسة عشر نوعا واربعة اجناس من الفطريات . كان جنس الاسرجيلس هو اكثر الاجناس سيادة في الحالات المختبرة . تم عزل سبعة أنواع من هذا الجنس كان اكثرها شيوعا انواع الاسرجيلس فيوميجاتس ، اسرجيلس نيجر اسرجيلس فلافس . كان الجنس التالي في السيادة هو الميوكر وكان ممثلا بأربعة أنواع . أما جنس الرايزوبس والبنتسليم والمثلان بنوعين لكل منهما فقد احتلا المرتبة الثالثة والرابعة من ناحية الشيوع على التوالي . ثبت أن الحيوانات صغيرة السن هي اكثر الحيوانات قابلية لاصابة الجهاز التنفسي بالفطريات وتزداد المناعة ضد الاصابة بزيادة سن الحيوان .

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THE MYCOFLORA OF CLINICALLY POSITIVE PNEUMONIC CASES
OF BUFFALO CALVES
(With 5 Tables)

By
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SUMMARY

A total of 200 isolates of fungi belonging to fifteen species and four genera were isolated from one hundred and eighty five clinically positive pneumonic cases of buffalo calves. Aspergillus (7 species) was the most frequent genus. A. fumigatus, A. niger and A. flavus were the most common species. Mucor (4 species) was the second common genus. Rhizopus and Penicillium (2 species each) were the third and fourth common genera. Young animals are more susceptible to pulmonary fungal infection. About 38%, 24%, 21% and 16% of the positive cases were recorded in animals of different age ranging from 1-3, 3-6, 6-9 and more than 10 months, respectively.

INTRODUCTION

Outbreaks of respiratory infections either alone or complications other diseases have reached serious proportions in some areas of the world (MCKERCHER, 1968). The percentage of respiratory infections among diseased calves may reach up to 14% (OXENDER et al., 1973). Respiratory infections constitute the most costly and troublesome disease problem in cattle in Egypt.

On the bases of current etiologic knowledge MCKERCHER (1968) classified respiratory infections in cattle into three categories: those which are caused by virus only, those apparently initiated or triggered by virus followed by secondary bacterial infection and lastly those which are solely by bacteria. Role played by fungi as either singly or mixed with the above mentioned causes responsible for respiratory diseases in cattle was neglected by the author. This situation encourage the authors to investigate the mycoflora of positive pneumonic cases of buffalo calves which may reflect that fungi one of the causative agents of pneumonia in these animals.

MATERIAL and METHODS

185 buffalo calves of clinically positive pneumonia collected from different localities at Assiut were used in this study. The number age groups of the tested animals was as follows:

Age (Month)	No. of calves	Slaughter	Dead
1-3	39	21	18
3-6	45	40	5
6-9	56	52	4
10 or more	45	45	-

Isolation and identification of fungi:

The surface of affected organs were seared with hot spatula then opened with sterile scalpel. The pus material was taken with a sterile platinum loop and inoculated to culture media. Swabs from the nose, larynx and samples

from the affected lung tissues of each animal were collected in a clean plastic bags under complete aseptic conditions. Each sample of the collected materials was cultured on six sterile petri-dishes containing Sabouraud's dextrose-agar medium supplemented by Actidione (MESS and MAQUOWN, 1969). The dishes were incubated at 28°C for ten days during which they were examined daily. The colonies of growing fungi were transferred to slant and then to plates for identification. Identification was based on observation, classification, description of GILMAN (1957); RAPER and FENNELL (1965); BARON (1968) and ELLIS (1971) and (1976).

RESULTS

A total of 200 isolates of fungi which belong to fifteen species and four genera were isolated from one hundred and eighty clinically positive pneumonic cases (Table 1). *Aspergillus* (7 species) was the most frequent genus, from which 125 isolates were encountered. *Aspergillus fumigatus* (42 isolates), *A. niger* (25 isolates) and *A. flavus* (23 isolates) were the most common species. The genus *Aspergillus* was represented in all cases in which three fungi were recorded in the positive pneumonic cases (15 cases) (Table 2); in 33 out of 38 cases in which two fungi were recorded (Table 3) and in 58 out of 85 cases in which one fungus was recorded (Table 4).

Examination of the collected samples indicated that high number of cases of isolation of the different fungal species was recorded in nasal cavity rather than of trachea and lungs (Table 5).

With regards to the age of animals of the different pneumonic cases, it has been noted that about 38%, 24%, 21% and 16% of the positive cases were recorded in animals of different ages ranging from 1-3, 3-6, 6-9 and more than 10 months, respectively (Table 6).

DISCUSSION

The present results show that *Aspergillus* was the most frequent genus recorded from the different positive pneumonic cases examined. This is agreed with the finding of MULLINS (1974), who reported that the distribution of *Aspergillus*, on a world-wide basis, varies from 1.1 to 22% of the total airspora samples, being the most common organism in some countries. In Egypt, ABU EL-SOUOD (1974) and MOUBASHER and MOUSTAFA (1974) reported that *Aspergillus* spores predominated over those of any other fungal genera in the air at Assiut, constituting 10.2% of total air borne fungi. *Aspergillus*, was the most common fungus in Egyptian soils as reported by MOUBASHER and collaborators (1970 and 1978).

The genus *Aspergillus* is a group of free living saprophytic moulds. However, some species are capable of causing disease either by straight-forward tissue invasion, or colonization or by means of reactions involving the immune system. Since the spores of the organism (approx. 5 µm in diameter for *A. fumigatus*) can become air born, the most frequently affected organs is the lung and respiratory tract. This support the results obtained during this study, in which *Aspergillus* was the frequent genus, from which *A. fumigatus* was the most common species, constituting 34.4% of the total aspergilli isolated during this work.

Seven species belonging to the genus *Aspergillus* were isolated during this study, namely *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, *A. parasiticus*, *A. terreus* and *A. ustus*. In this respect, YOUNG *et al.* (1972) showed that pulmonary aspergillosis was not limited to *A. fumigatus*, mycological analysis of 39 cases with invasive aspergillosis showed that 59% were caused by *A. fumigatus*, 31% by *A. flavus* and 10% by *A. glaucus*.

As indicated by VOISIN *et al.* (1964), some *Aspergillus* species (usually *A. fumigatus* but occasionally *flavus*, *niger* or *nidulans*) can grow saprophytically. These aspergilli can colonize old tuberculous or abscess cavities and other pulmonary cavities e.g. caused by carcinoma, infarction, bronchiectasis (KRAKOWKA *et al.*, 1970), producing a fungal ball. The apparent saprophytic nature and delicate balance between the *Aspergillus* and the immune system may becomes an increasing hazard if resistance to infection is reduced by drug (EDWARD and AL-ZUBAIDY, 1977).

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Two genera namely Mucor and Rhizopus of the order Mucorales were the second and third dominant genera, they constituting 26% of the total isolated fungi. The phycomycetes (Mucor, Rhizopus and other genera) are widely disseminated in nature. Man and animals are more or less constantly exposed to them. They are found in soil, water, food and decomposing organic matter where they exist in free-living state (KAPLAN et al. 1960).

BENEKE (1962) reported that various species of Mucor and Rhizopus may be pathogenic, unfortunately, cultures have not been obtained from most of the mucormycosis cases reported in the literature. Mucormycosis is ordinarily a rapidly fatal disease usually affected the lung and rarely the central nervous system. It also has been reported in the nose, skin, nails and ear. The disease has been reported in dogs, birds, horses, cows and pigs (BAKER and SEVERANCE, 1948; BAKER, 1957 and DAVIS et al., 1955).

Mycotic lesions were described in a great variety of organs of cattle, most commonly in mycotic placentitis and/or acute mycotic pneumonia (CORDES and SHORTRIDE, 1968). Spontaneous pulmonary disease caused by Mucor pusillus has been reported in harp seal (KAPLAN et al., 1960).

BENEKE (1962), reported that in cases of mucormycosis and pulmonary aspergillosis, the increase in frequency of the disease is apparently due to the use of antibiotic, cortisone and antileukemic drugs. In attempts to produce experimental aspergillosis in mice, SIDRANSKY and FRIEDMAN (1959) observed that the number of animal infected by air borne A. flavus spores could be increased by giving them either antibacterial antibiotics or adrenal cortical steroids, and that the number increased markedly by giving them both. The administration of cortisone and antibiotics or cortisone alone rendered animal high susceptible to fatal pulmonary aspergillosis.

Penicillium species were isolated only from 23 positive cases out of 185 examined. This low occurrence is in harmony with the finding of MOUBASHER and MOUSTAFA (1974), who noted that Penicillium spores contributed only 3.2% of total air-borne fungi at Assiut. This result also in agreement with that reported by BENEKE (1962) who stated that penicillosis in rare cases has included pulmonary involvement. Penicillium strains alone or with other fungi have been reported as excitants in some allergic bronchial asthma cases (AIME et al., 1933 and GREGOIRE et al., 1953).

Investigation of the mycotic flora of the different sites of respiratory system indicted that high number of cases of isolation of fungi was recorded in nasal cavity, followed by trachea then the lungs. This agrees with the opinion of SINGH and MALIK (1968), who observed that many microorganisms present in the respiratory tract of apparently normal buffalos were found in the nasal cavity more than that in the trachea and lungs.

During this study it has been noted that higher incidence of fungal infection was recorded at the age between 1-3 months and decreased regularly with the increase of age. The higher incidence of fungal agents at the young animals than at higher ages may be explained by the fact that the resistance of the body tends to increase with age and thus rendering the animal more resist to infection.

In all the positive cases examined during this study, the absence of fungal viability or growth in vivo distinguishes these mycotic diseases. This class of pulmonary diseases known as (Toxomycosis) as reported by NORTHUP and KILBUPN (1979). Inhaled fungi, particularly spores may affected the tracheobronchial tree, the alveoli, or both. This attributed to fungal toxins acting as chemical agents or as allergens. The manifestations and duration of disease are highly variable, governed by the intensity of exposure, site of deposition, residence time in the pulmonary, zones and sensitivity of the individual host. Studies dealing with toxicity and mycotoxin-producing potentialities of the different fungi isolated during this study are in progress.

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Table (2): Pneumonic cases from which three fungal species were isolated

Isolated fungi	No. of cases
<u>A. fumigatus</u> + <u>A. niger</u> + <u>M. hiemalis</u>	1
<u>A. fumigatus</u> + <u>A. niger</u> + <u>P. notatum</u>	1
<u>A. fumigatus</u> + <u>A. flavus</u> + <u>Rh. oryzae</u>	1
<u>A. fumigatus</u> + <u>A. flavus</u> + <u>Rh. stolonifer</u>	1
<u>A. fumigatus</u> + <u>A. nidulans</u> + <u>M. racemosus</u>	1
<u>A. fumigatus</u> + <u>M. hiemalis</u> + <u>P. funiculosum</u>	1
<u>A. fumigatus</u> + <u>M. hiemalis</u> + <u>Rh. oryzae</u>	1
<u>A. niger</u> + <u>A. flavus</u> + <u>A. terreus</u>	1
<u>A. niger</u> + <u>A. flavus</u> + <u>P. notatum</u>	1
<u>A. niger</u> + <u>A. terreus</u> + <u>M. hiemalis</u>	1
<u>A. flavus</u> + <u>A. nidulans</u> + <u>P. notatum</u>	1
<u>A. flavus</u> + <u>A. parasiticus</u> + <u>P. funiculosum</u>	1
<u>A. flavus</u> + <u>A. nidulans</u> + <u>P. funiculosum</u>	1
<u>A. nidulans</u> + <u>A. parasiticus</u> + <u>Rh. oryzae</u>	1
<u>A. parasiticus</u> + <u>M. racemosus</u> + <u>Rh. stolonifer</u>	1

Table (3)

Pneumonic cases from which two fungal species were isolated

Isolated fungi	No. of cases
<u>A. fumigatus</u> + <u>A. nidulans</u>	3
<u>A. fumigatus</u> + <u>A. niger</u>	3
<u>A. fumigatus</u> + <u>M. hiemalis</u>	1
<u>A. fumigatus</u> + <u>M. fragilis</u>	1
<u>A. flavus</u> + <u>A. niger</u>	2
<u>A. flavus</u> + <u>Rh. stolonifer</u>	1
<u>A. flavus</u> + <u>P. funiculosum</u>	1
<u>A. flavus</u> + <u>P. notatum</u>	1
<u>A. niger</u> + <u>P. funiculosum</u>	1
<u>A. niger</u> + <u>P. notatum</u>	2
<u>A. niger</u> + <u>M. circinelloides</u>	1
<u>A. niger</u> + <u>M. fragilis</u>	1
<u>A. niger</u> + <u>M. hiemalis</u>	1
<u>A. nidulans</u> + <u>A. parasiticus</u>	2
<u>A. nidulans</u> + <u>M. racemosus</u>	1
<u>A. parasiticus</u> + <u>A. ustus</u>	1
<u>A. parasiticus</u> + <u>P. notatum</u>	2
<u>A. parasiticus</u> + <u>M. hiemalis</u>	1
<u>A. terreus</u> + <u>Rh. stolonifer</u>	1
<u>A. terreus</u> + <u>M. circinelloides</u>	1
<u>A. ustus</u> + <u>P. notatum</u>	1
<u>P. notatum</u> + <u>M. fragilis</u>	1
<u>P. notatum</u> + <u>Rh. oryzae</u>	2
<u>P. funiculosum</u> + <u>Rh. stolonifer</u>	1
<u>P. funiculosum</u> + <u>fragilis</u>	1
<u>M. fragilis</u> + <u>M. hiemalis</u>	1

Table (4)
Pneumonic cases from which single fungus was isolated

Isolated fungi	No. of cases
<u>A. fumigatus</u>	26
<u>A. flavus</u>	11
<u>A. nidulans</u>	2
<u>A. niger</u>	10
<u>A. parasiticus</u>	--
<u>A. terreus</u>	3
<u>A. ustus</u>	6
<u>M. circinelloides</u>	--
<u>M. fragilis</u>	--
<u>M. hiemalis</u>	4
<u>M. racemosus</u>	6
<u>Rh. oryzae</u>	9
<u>Rh. stolonifer</u>	4
<u>P. funiculosum</u>	1
<u>P. notatum</u>	1
Total	85

Table (5)
Distribution of fungal flora and the number of cases of isolation (out of 185 cases)
in the different sites of respiratory system

Isolated fungi	Nose	Pharynx	Lungs lobes			Pulmonary lymph nodes
			Right	Left	Right + left	
<u>A. fumigatus</u>	40	42	10	2	30	42
<u>A. flavus</u>	22	23	4	4	15	20
<u>A. nidulans</u>	10	12	-	3	9	12
<u>A. niger</u>	25	25	1	3	21	25
<u>A. parasiticus</u>	9	8	1	2	6	8
<u>A. terreus</u>	6	6	-	-	6	6
<u>A. ustus</u>	7	8	1	-	7	8
<u>M. hiemalis</u>	12	13	-	-	13	10
<u>M. racemosus</u>	9	9	-	-	9	7
<u>M. fragilis</u>	4	4	1	-	4	5
<u>M. circinelloides</u>	2	2	-	-	2	2
<u>Rh. oryzae</u>	13	14	2	2	10	14
<u>Rh. stolonifer</u>	9	6	-	-	6	9
<u>P. notatum</u>	15	10	5	5	5	15
<u>P. funiculosum</u>	8	8	-	-	8	8