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DERMATOPHYTES AND OTHER FUNGI ISOLATED FROM SHEEP IN ASSIUT PROVINCE

(With 2 Tables & 3 Fig.)

By

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فطريات الأمراض الجلديه للأغنام في محافظة أسيوط

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تم اجراء دراسة عن مرض القراع فى الأغنام فى محافظة أسيوط ، وبالفحص الاكلنيكى لعدد ١٩٠٠ رأس من الغنم فى ثلاث مزارع وجد أن ٤٨ حاله مصابه بمرض القراع ، وبالفحص الميكروسكوبى لعينات الشعر والقشور وجد ان ٢٣ عينه ايجابيه وبالزرع على مستنبت السابارود الدكستروز آجار تم عزل فطر الترايكوفيتون فيروكوزيم فى ٢٦ حاله ووجد أنه المسبب الرئيسى لمرض القراع فى الأغنام وكذلك تم عزل فطر الترايكوفيتون منتاجروفيتس فى ٥ حالات وفطر الترايكوفيتون سودانيز فى حالة واحده وكذلك عترتين من الكانديدا البيكانز .

تمت دراسة كلوريد الصوديوم على نمو فطر الترايكوفيتون منتاجروفتس والترايكوفيتون فيروكوزيم ووجد ان تركيز ٥٪ يثبط النمو بنسبة ٣ ر ٩٣٪ ، ١٠٠٪ على الترتيب وذلك بأضافته لمستنبت السابارود دكستروز آجار .

تمت دراسة استخدام كلوريد الصوديوم ٥٪ فى علاج العدوى المحدثه بفطرى الترايكوفيتون منتاجروفيتس وفيروكوزيم فى خنازير غينيا وتم الشفاء بعد عشرة أيام من بداية العلاج .

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SUMMARY

The clinical examination for sheep ringworm was done in three sheep farms in Assiut provine (El-Awamer, Bani Sanad and Assiut University sheep farm). Out of 1900 animals 48 animals were affected. Positive results of the microscopic examination of the hairs and scales were 23 (47.90%). Culturing of the specimens on Sabouraud's dextroses media revealed that *Tricophyton verrucosum* (26 isolates) was the main causitive agent of sheep ringwaorm. *T. mentagrophytes* (5 isolates), *T. soudanese* (1 isolate), *Chrysosporium Keratinophilum* (4 isolates) and *Candida albicans* (2 isolates) were recognized. Also, Saprophytic fungi, Aspergillus, Penicillum, Scopulariopsis and Alternaria were isolated. The effect of sodium chloride on dermatophytes was investigated. It was found that the addition of sodium chlorids 5% to Sabouraud dextrose agar would inhibit the growth of *T. verrucosum* and *T. mentagrophytes* by 100% and 93.3% raspectively. Also the use of sodium chloride in the treatment of induced ringworm infection in Guinea-Pigs gave good results after 10 days.

INTRODUCTION

Dermatophytes play an important role in mycotic skin diseases in animals and man which are generally known as ringworm. The detection of such infection among demosticated animals is of agreat importance due to the great damage that affect the skin of the diseased animals. The effect of ringworm on domestic animals was reported by *OTCENASEK* et al., *(1988)*; *EL-ALLAWAY* et al., *(1989)*; *ABDEL HALIM* et al., *(1988)*; *GOLOVINA, 1988*; *MARCO* et al., *1989*; *ABDEL HAFEZ* et al., *(1990)* and *BAGY and ABDEL-MALLEK, (1991)*.

The present work aimed to study the dermatophytic and saprophytic fungi isolated from characteristic skin lesions of clinically infected sheep. Also, to study the effect of sodium chloride on the isolated dermatophytes *In vitro* and *In vivo*.

MATERIAL AND METHODS

Collection, examination and culturing of specimens:

Aseptic techniques were used in collecting the specimens from animals showing clinical picture of ringworm. The surfac

of the affected area was firstly scrubbed with 70% ethyl alcohol prior to sampling. Then after skin crust scales and hair samples were collected by using sterile scalpel and forceps, and put into sterile plastic bags.

Direct microscopic examination of the obtained samples was performed using a solution of 5% potassium hydroxide and 25% glycerol *(SOLYTIS and SMITH, 1969).*

Part of the collected meterial was culured on Sabouraud's dextrose agar *(MOSS and MCQUOWN, 1979)* containing chloromphenicol and cycloheximide (0.5 g/L for each) to inhibit the bacterial growth and to minimize the development of some saprophytic fungi. Cultured plates were incubated at 25^{0}C for 4 weeks. They were examined daily until growth became evident.

Identification of fungal colonies was made depending on macroscopic appearance of fungal colonies (growthh rate, colour, pigmentation in reverse) and microscopic characters (shape and dimensions of conidia and chlamydospores, mode of hyphal branching etc.). Several references were used for identification: *CARMICHEAL, 1962*; *RAPER* and *FENNEL, 1965* and *FREY* et al., *1979*).

Effect Of NaCl On dermatophytes in vitro and in vivo:

In Vitro:

Two sets, each of 3 plates of Sabouraud's dextrose agar containing 5% NaCl were prepared; One set was inoculated with *T.verrucosum* and the other with *T.mentaorophytes.* Two control sets of the medium containing no salt were similarly inoculated. The plates were incubated at 25^{0}C for 17 days, then the diameters of colonial growth were measured.

In vivo:

Four Guinea-pigs free from any skin lesion were used, two of them were used to receive infection of dermatophytes (first group) and the other two were kept as control (second group). Four quarters of each animal of the first group were shaved, scarified by sand paper, cleaned and disinfected with 70% ethyl alcohol. A suspen-sion of one of 2 dermatophytes (*T.verrucosum* and *T.mentagrophytes)* was rubbed on the cleaned 4 sites of each animal of the first group. The animals were kept in isolated boxes for daily examination to observe developed skin lesions.

To investigate the effect of NaCl on dermatophytic lesions, a preparation of NaCl 5% oientment was used. The treatment started after the characteristic lesions of ringworm were well developed and 5 applications of NaCl 5% oientment were tried daily.

RESULTS

The obtained results are recorded in tables 1, 2 and Figures 1, 2 and 3.

DISCUSSION

48 sheep out of 1900 showed clinical picture of ringworm; the lesions were located in different areas of the head, mostly on the nose, ears and around the eyes. The clinical symptoms of the disease were loss of hair and formation of sharply, greyish and circumscibed areas of scales. In advanced cases large crusted lesions were formed.

The direct microscopic examination of 48 specimens was positive in 23 (47.5%). Fungal propagules were detected in crust and hair specimens but the morphological features were not clear enough to allow good identification of these fungi.

The mycoloaical analysis of 48 specimens led to the isolaton of 23 strains of *T.verrucosum* from 23 specimens, 1 strain of *T.verrucosum* and one strain of *T.soudanese* from one specimen, 4 strains of *T.mentaarophytes* from 4 specimens, 2 isolates of *T.verrucosum* and other saprophytic fungi from 2 specimens, 1 strain of *T.mentagrophytes* and other saprophytic fungi from 1 specimen and different species of saprophytic fungi were isolated from 17 specimens (Table 1). The main causative dermatophytes of ringworm in sheep were *Trichophyton verrucosum* and *Trichophyton mentagrophytes* 26 (54.20%) and 5 (10.40%) respectively. wherease *Trichophyton soudanese* was recorded in one case. The results in this investigation were higher than that recorded by *EL-ALLAWAY* et al. *(1980)* who observed that the incidence of *T.verrucosum, T.mentagrophytes* and *T.terrestre* among infected Egyptian infected sheep by ringworm were 44.40%, 20.00% and 11.10%, respectively. The high incidence in our results may be due to bad hygenic measures of the farms, nutritional disorders and overcrowdness of the animals in the farms. However, the results agree with that obtained by *ABDEL HALIM* et al. *(1988)*; *MARCO MELERO* et al. *(1989)* who observed that the *T.verrucosum* and *T.mentagrophytes* were the main causative agents of sheep ringworm.

In the present investigation 2 isolates of *Candida albicans* were isolated. This finding agrees with that reported by *ALI SHTAYEH* et al. *(1988a)*; *MAHMOUD (1991)* who mentioned that 2 isolates of *Candida albicans* were isolated from 45 cases of sheep associated ringworm. In addition to the above mentioned dermatophytes, several saprophytic fungi were isolated from other cases as shown in Table (1). Some of the

mentioned species were encountered but with various frequencies from sheep and domestic animals (*AHO, 1983*; *ABDEL-HAFEZ, 1987*; *ALI SHTAYEH* et al., *1988* a&b; *ABDEL-HAFEZ* et al., *1990* and *BAGY* and *ABDEL-MALLEK, 1991*).

In this investigation it was found that NaCl 5% had a great effect on inhibition of dermatophytes both *In vitro* and *In vivo*. The addition of 5% sodium chloride to the media would inhibit the growth rate of both *T.verrucosum* and *T.mentagrophytes* 100% and 93.7%, respectively (Table 2 and Fig. 1) and the result was recorded after 17 days of culturing.

Our result is supported by *LOBITZ and DOBSON (1961)* who mentioned that the sweet of both normal and diseased persons containing sodium ions, might play a role to some extent with regard to the skin fungal infections. Besides, *BAGY* and *ABDEL-HAFEZ (1984)* recorded that no keratinolytic fungi could be isolated from soil samples-baited with different animal hairs treated by 10,20,30 & 40% NaCl whereas *M.canis, M.gypseum, T.mentagrophytes* and *Chrysosporium tropicum* were isolated from untreated soils. Also some reports showed that soils of salt markets in Egypt were completely free from any of keratinophilic fungi, when the total soluble salts fluctuated between 5.1-46.7% (*ABDEL-FATTAH* et al., *1982; ABDEL-HAFEZ* et al., *1989 and ABDEL-SATER, 1990*). Another record by *EL-SHANAWANY* (*1993*) who mentioned that *M.canis* and *T.violaceum* were significantly inhibited by three concentration levels (1. 2 and 2.5%) of NaCl, while the growth of *T.rubrum* and *E.floccosum* was suppressed by 2 and 2.5% of NaCl whereas *T.mentaqrophytes* and *C.tropicum* were not significantly affected by any of the concentrations used.

Induced infection by *T.verrucosum* and *T.mentagrophytes* to Guinea-pigs produced redness, mild itching and skin lesions after the first week. Severe itching and crust formation were observed after the second week (Fig. 2). The treatment of dermatophytic lesions by using NaCl 5% ointement showed firstly disappearance of scales and crusts. after that, hyperaemia of the skin was developed, begining of new hairs formation and finally complete recovery after 10 days from the start of treatment (Fig.3).

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Table (1):Incidence and percentage of incidence of the dermatophytes (A), and other than true dermatophytes (B) isolated from 48 cases of sheep ringworm.

Fungal species		Incidence	% of incidence
(A) Tricophyton:		32	66.70
T.mentagrophytes	(Robin) Blanchard	5	10.40
T.soudanese	Joyeux	1	2.10
T.verrucosum	Rodin	26	54.20
(B) Candida:		2	4.20
C.albicans	(Robin) Berkhout	2	4.20
Aspergillus:		25	52.10
A.flavus	Link	4	8.30
A.niduluns	(Fdiam) Winter	2	4.20
A.nigar	Van Tieghem	4	8.30
A.ochraceus	Wilhelm	1	2.10
A.oryzae	(Ahlb) Cohn	1	2.10
A.sulphureus	(Fres.) Thom&Church	1	2.10
A.sydowii	(Bain&Sart.) Thom&Church	5	10.40
A.versicolor	(Vuill) Tiraboschi	7	14.60
Alternari:		4	8.30
A.alternata	(Fr.) Keissler	4	8.30
Chrysosporium:		4	8.30
C.keratinophilum		4	8.30
Cladosporium:		2	4.20
C.cladosporioides	(Fres.) Derries	2	4.20
Cunninghamella:		1	2.10
C.echinulate	(Thaxter)	1	2.10
Penicillium:		9	18.90
P.chrysogenum	(Thom)	2	4.20
P.citrinum	(Thom)	3	6.30
P.corylophilum	(Dirckx)	3	6.30
P.funicubsum	(Thom)	1	2.10
Scopulariopsis:		14	29.20
S.brevicaulis	(Sacc.) Bainier	11	22.90
S.candida	(Gueguen) Vuillemin	3	6.30

* This identification according to Carmichael (1962), Raper & Fennel (1965) and Frey et al. (1979).

Table (2):Effect of Nacl 5% on growth of some dermatophytes.

Dermatophyte	Growth on S.D.A		Growth on S.D.A with 5% Nacl		Percentage of inhibition
	Diameter of colony (cm)	mean	Diameter of colony (cm)	mean	
T.mentagrophytes	4.2x4.5 4.4x4.5 4.6x4.6	4.7	0.4x0.4 0.4x0.4 0.2x0.2	0.3	93.30%
T.verrucosum	0.6x0.4 1.0x1.2 0.6x0.3	0.66	0 0 0	0.0	100.00%

S.D.A = Sabouraud's Dextrose Agar.

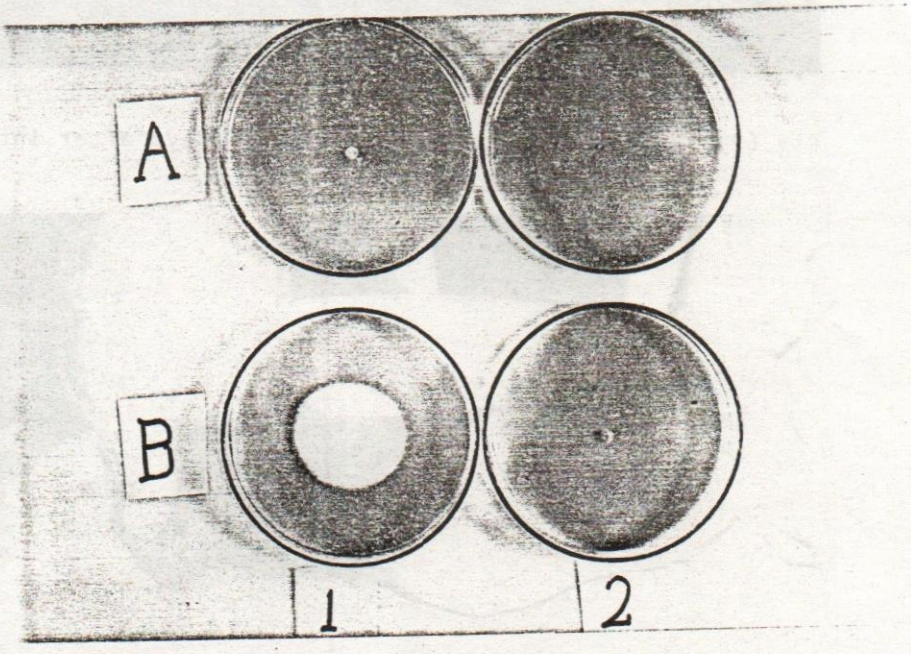

Fig.(1):A_1 & A_2 growth of T.verrucosum on S.D.A and S.D.A with 5% Nacl.
B_1 & B_2 growth of T.mentagrophytes on S.D.A and S.D.A with 5% Nacl.

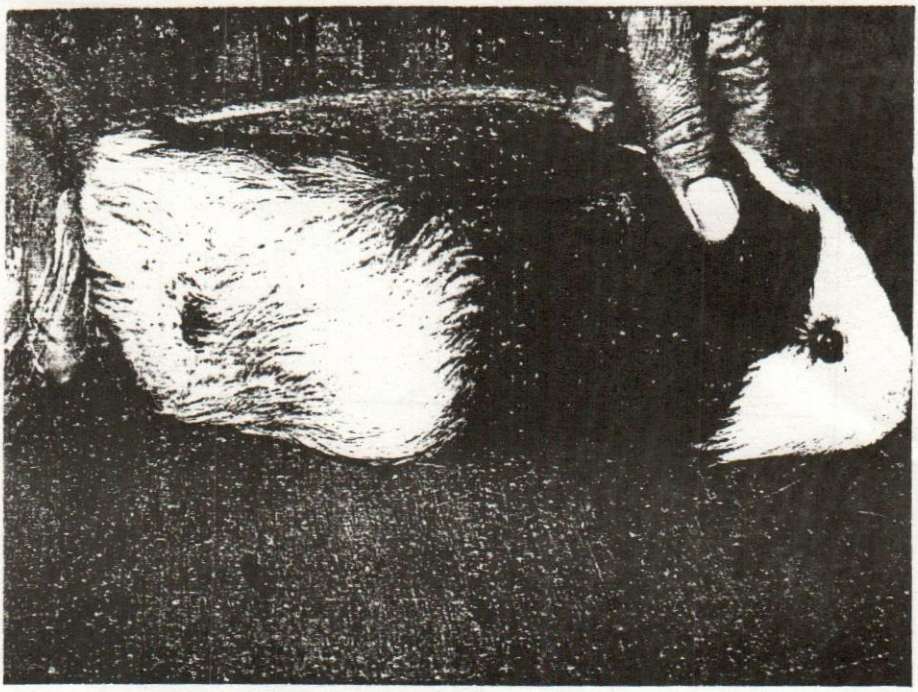

Fig.(2):Showing dermatophytic lesion 15 days after infection.

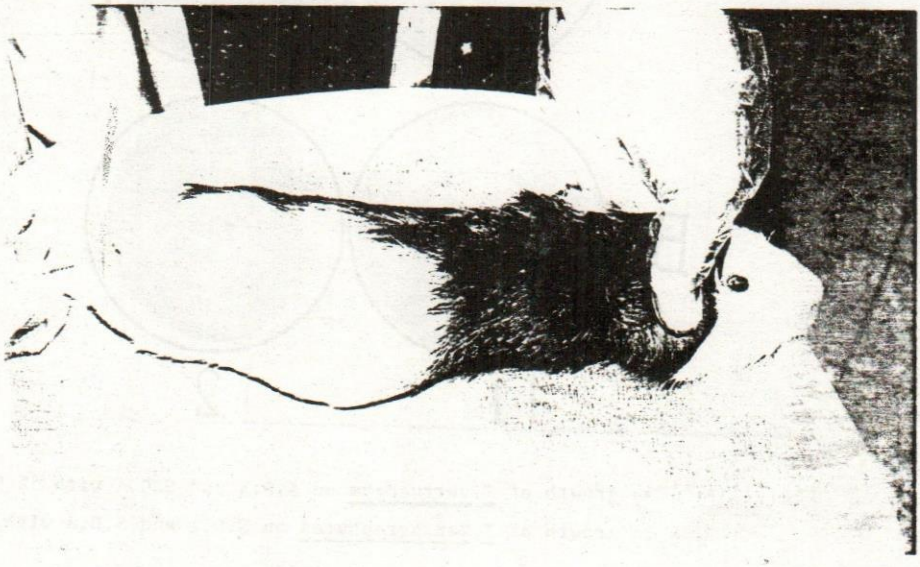

Fig.(3):Showing good recovery after 10 days of treatment.