



## SURVEY ON COMMON CAUSATIVE AGENTS OF DERMATOPHYTOSIS IN DIFFERENT ANIMALS IN EL-BIEDA- LIBYA

N. B. Eissa<sup>a</sup>, M. Ezzat<sup>a</sup>, A. El-Sawah<sup>a</sup> and M. EL-Hamoly<sup>b</sup>

<sup>a</sup> Faculty of Veterinary medicine, Omar Al Mukhtar University. <sup>b</sup> Faculty of Pharmacy, Omar Al Mukhtar University

### ABSTRACT

This work was planned to investigate the causative fungal agents of dermatophytosis and the rate of distribution of disease among animals in El-Bieda-Libya. 185 samples of skin infection were collected from different animals (75 camels, 62 cattle, 35 sheep and goats, 10 dogs and 3 cats). These animals were clinically diagnosed as dermatophytosis. Results of the microscopical examination of 185 hair and scabs samples revealed the presence of characteristics arthrospores in 165 samples with incidence of (89.2%). On the other hand, results of cultivated samples on specific media showed that 85 were positive culture with incidence of (45.9%). Wide varieties of dermatophytes were isolated from animals, but few zoophilic species were responsible for the majority of the cases. Our finding revealed the identification of two genus: Genus *Microsporum*, where 7 (8.2%) strains of *M. canis* were isolated and Genus *Trichophyton* 78 (91.8%) strains, which include 57 (67.1%) strains of *T. verrucosum* and 21 (24.7%) strains of *T. mentagrophytes*. In addition to many fungal contaminant were isolated, the most prevalent were *Aspergillus*, *Penicillium* and *Alternaria* species. Pathogenicity of isolated dermatophytes were tested in rabbits, with application of treatment using methanol extracts of *Thymus capitatus*. *Thymus capitatus* was effective in treatment of the infected rabbits.

**KEY WORDS:** *T. verrucosum*, Dermatophytosis, *M. canis*, *T. mentagrophytes*.

(BVMJ-24(2): 108-115, 2013)

### 1. INTRODUCTION

**D**ermatophytes are fungi that have the ability to invade the keratinized structures such as the superficial cornified skin layers, hair, and nails causing a superficial cutaneous infection called dermatophytosis, which commonly referred to as ringworm [32]. The lesions of the disease characterized by circular area of alopecia, scaling, crusts, erythema and pruritus present to varying degrees, affect the fur and cause deterioration of the quality of hide. Dermatophytosis are particularly common in cold climates, where animals stabled for long periods. Ringworm in cattle

often seen in winter [20]. Young animals are affected most often and asymptomatic infections are common particularly in adult animals. The reaction to dermatophyt infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection and local environment factors [32]. The etiologic agents of the dermatophytosis are classified in three anamorphic (asexual or imperfecti) genera, *Epidermophyton*, *Microsporum* and *Trichophyton*. Animals serve as reservoirs of the zoophilic dermatophytes, and their

infections have zoonotic importance [12]. *T. verrucosum* has been cited as a major agent encountered in cases of bovine, ovine and caprine ringworm. Other species such as *M. canis*, *M. gypseum*, *T. mentagrophytes* and *T. equinum* have been isolated from some ruminants [22, 28]. All domestic animals are susceptible to dermatophytes. The most common fungi vary with host, in cattle; *T. verrucosum* is the most important species. *T. mentagrophytes*, *M. gypseum* and *M. canis* in sheep and goats, *T. mentagrophytes* and *M. canis* in dogs and cats. *M. canis* is the most common species particularly in cats, *M. gypseum* and *T. mentagrophytes* are occasionally found in camels. While *T. mentagrophytes*, *T. verrucosum*, *M. nanum* and *T. equinum* is most common species in cattle. The incidence of the disease vary according to country [20]. The incidence of dermatophytosis in India were (0.59%) in buffaloes, (1- 0.56%) in cattle and (3.98%) in farm workers. In dogs and cats dermatophytosis occurred with low prevalence ranged from 4% to 18% [10]. Many literatures mentioned the uses of medicinal plant extracts for treatment of many infectious diseases. Reports had shown that *T. capitatus* possesses biological properties such as antimicrobial activity [4, 9], antifungal activity [13, 16, 25], and antioxidant activity [4, 3]. Our research through light on the causative agents of dermatophytosis in animals at Al-Gabal Al-Akhdar region; studies the virulence of isolated strains in rabbits and trails for treatment using methanol extracts of *Thymus capitatus*.

## 2. MATERIAL AND METHODS

### 2.1. Collection of samples

185 hairs and scabs samples were collected from clinically diseased animals (75 camels, 62 calves, 35 sheep and goats, 10 dogs and 3 cats) from the

active edges of lesions as described previously [17].

### 2.2. Microscopical examination of samples

According to the methods method adopted before [11], specimen were clarified with 15 – 20% KoH on clean slide and covered with cover slide and left for 15 – 30 min and examined using low and high power objective lenses for demonstration of arthrospores and their arrangement (ecto or endothrix) on hairs.

### 2.3. Isolation of causative fungi

Collected samples (hairs and scabs) were inoculated in Sabouraud's dextrose agar with chloramphenicol and cyclohexamide and incubated at 25 °C for 2 – 4 weeks [7].

### 2.4. Identification of isolated Fungi

According to the standard procedures [29] the following was performed: *a. Study of the culture characteristics:* which include size and rate of growth, texture and color of colonies in case of pigment production. *b. Microscopic examination of the culture:* Small part of culture were transferred to clean slide and stained with lactophenol cotton blue stain and examined microscopically for the presence of characteristics fungal elements (Micro and Macroconidia, Chlamyospores, septated mycelia with different shape) [31].

### 2.5. Experimental infection

Three rabbits (one month old) were used. The flank area was plucked and scarified. A three weeks subculture of *T. verrucosum*, *T. mentagrophytes* and *M. canis* were macerated with normal saline to form suspension of each strain. Inoculation was carried out with swab dipped into the inoculums of the macerated subculture and placed on the scarified area of the first, second and third rabbits respectively [26, 18].

### 2.6. Treatment

Infected rabbits were treated using ointment prepared from methanol extract of *Thymus capitatus* as follow: *a- Plant material:* Aerial parts from wild *Thymus capitatus* was collected in July 2009 from Al-jabal Al-Akhdar, El-Bieda area. The taxonomic identification was determined kindly by staff members of Botany, Plant Biology Department, Faculty of Science, Omar Al-Mukhtar University, El-Bieda, Libya. A herbarium specimen was kept as a reference in the Department of Pharmacognosy, Faculty of pharmacy, Omar Al- Mukhtar University, El-Bieda, Libya. Collected plant materials were dried in shade, and the plant leaves were separated from the stem, and grounded in a grinder to small particles. *b- Methanol extraction:* A total of 13 g of dry powdered *T. capitatus* (leaves and stems) were infused in absolute methanol and the mixture was heated for 15 min in water bath. The extract was filtered through a piece of cotton and dried to a residue (1.762 g residue represent 13.55%). This residue was dissolved in melted Vaseline (53.3 g), triturated till congealing smoothly to be ready for clinical topical application.

### 3. RESULTS AND DISCUSSION

*Clinical examination:* Ringworm is a common skin disease of calves. The disease occurred throughout the year with higher prevalence during winter season due to high humidity, which facilitate the growth of spores, and increases the susceptibility of animals to infection [20]. Lesions in diseased animals appeared circular circumscribed, grayish-white crusty raised lesions, alopecia and the lesions were most commonly found on the head, neck, dewlap and chest area. These signs were in agreement with previous studies [30]. Young animals after weaning

time are highly susceptible to ringworm infection. This may be in part due to their weak immunity and the high pH of their skin. [23].

*Direct microscopical examination of samples (hair and scabs):* it revealed the presence of arthrospores either outside the hair (Ectothrix) or inside the hair shaft (Endothrix) (Fig 1a & b). Our results revealed the presence of arthrospores in 165 hair and scabs samples with incidence of (89.2%). These finding were higher than those reported by Al-Ani, et al., [2] who found that (71%) of examined samples were positive for fungal spores. Other researchers reported that direct microscopical examination could provide a positive diagnosis in (40 to 60%) of samples [6, 27].

*Culture examination:* Culture characteristics of pathogenic dermatophytes were determined according to the growth rate of fungi, texture and color of colonies from up and down sides. Our results showed that 85 samples were positive by culture methods on specific media for dermatophytes with incidence of (45.9%). These finding were nearly similar to the previous studies [27].

*Identification of isolated Fungi:* as shown in table (1) our results revealed the isolation of two Genus: *Trichophyton*, which was the most frequent genus isolated with incidence of 91.8% and genus: *Microsporum* where 7 strains of *M. canis* with incidence of (8.2%) were isolated. Among *trichophyton* species: *T. verrucosum* was the most commonly found 57 strains with incidence of (67.1%) of the total isolated fungi. It characterized by slow growth on Sabouraud's, glucose agar and forming slightly folded, curled, heaped, glabrous, gray white colonies (Fig 2). Prepared culture slides stained with lactophenol cotton blue,

Table 1. Incidence of isolated dermatophytes from farm animals

Animal	Isolated Fungi	No. of isolates	Percentage
Camels	<i>T. verrucosum</i>	19	22.4
	<i>T. mentagrophytes</i>	10	11.8
Calves	<i>T. verrucosum</i>	22	25.9
	<i>T. mentagrophytes</i>	8	9.4
Sheep and goats	<i>T. verrucosum</i>	16	18.8
	<i>T. mentagrophytes</i>	2	2.4
Dogs and cats	<i>M. canis</i>	7	8.2
	<i>T. mentagrophytes</i>	1	1.2
Total	-	85	100

chlamydospores in chains, with microconidia and rare macroconidia may be found (Fig 3). The second frequent isolated fungi was *T. mentagrophytes* were 21 strains were identified with incidence of (24.7%) of total isolate of fungi. Their colonies appear after 2-4 weeks at 25<sup>0</sup>C characterized by buff to tan colour and exhibited radial folds (Fig 4). Microscopical examination revealed the presence of pyriform in shape microconidia and cegar shaped macroconidia with thin walls having 3 – 5 cells and spiral shape mycelia (Fig 5). In addition 7 strains of *M. canis* was isolated from dogs and cats with incidence of (8.2%). Their cultures characterized by rapid growth, which appear white cottony (Fig 6). Microscopical examination of *M. canis* revealed the presence of numerous spindle shaped with rough and thick wall macroconidia (Fig 7). From table (1), it was clear that the higher incidence of *T. verrucosum* was obtained from calves with incidence of (25.9%) followed by camels (22.4%) while sheep and goats (18.8%). While *T. mentagrophytes* were recovered with higher incidence from camels (11.8%), followed by calves (9.4%), sheep and goats (2.4%) and from dogs (1.2%). From the above mentioned results, it was appear that *T. verrucosum*, *T. mentagrophytes* and *M. canis* are the main causes of ringworm in farm animals in El Bieda- Libya with percentage of (67.1%,

24.7% and 8.2%) respectively and this was higher than those mentioned by Al-Ani, et al., [2] they isolated *T. verrucosum* and *T. mentagrophytes* from calves with incidence of (47.88% and 12.68%) respectively, also Abou-Gabal et al., [1]; Pal [1] and Renner [24], while Nooruddin and Singh [20] isolated *T. verrucosum*, *T. mentagrophytes* and *M. gypseum* from cattle and reported that the incidence of dermatophytosis in India were very low in buffaloes (0.59%), in cattle (1.56%) and in farm workers (3.98%). Concerning the incidence of dermatophytosis in dogs and cats, [12] reported low prevalence of infection ranged between (4%) to (10%), this agree with our finding (8.2%) but few studies show higher prevalence [5, 10], and *M. canis* was the most common species isolated from dogs.

*Experimental infection:* Rabbits experimentally infected with *T. verrucosum*, *T. mentagrophytes* and *M. canis* showed cutaneous lesions in the site of infection within 3 weeks following infection, the lesions appeared as inflammatory and scaly areas in the site of inoculation. Samples collected and cultivated on Sabouroud's, glucose agar with cyclohexamide and chloramphenicol revealed the re-isolation of the inoculated fungal strains. These finding were similar to the results of Narai et al., [19] and Al-Ani et al., [2], they reported

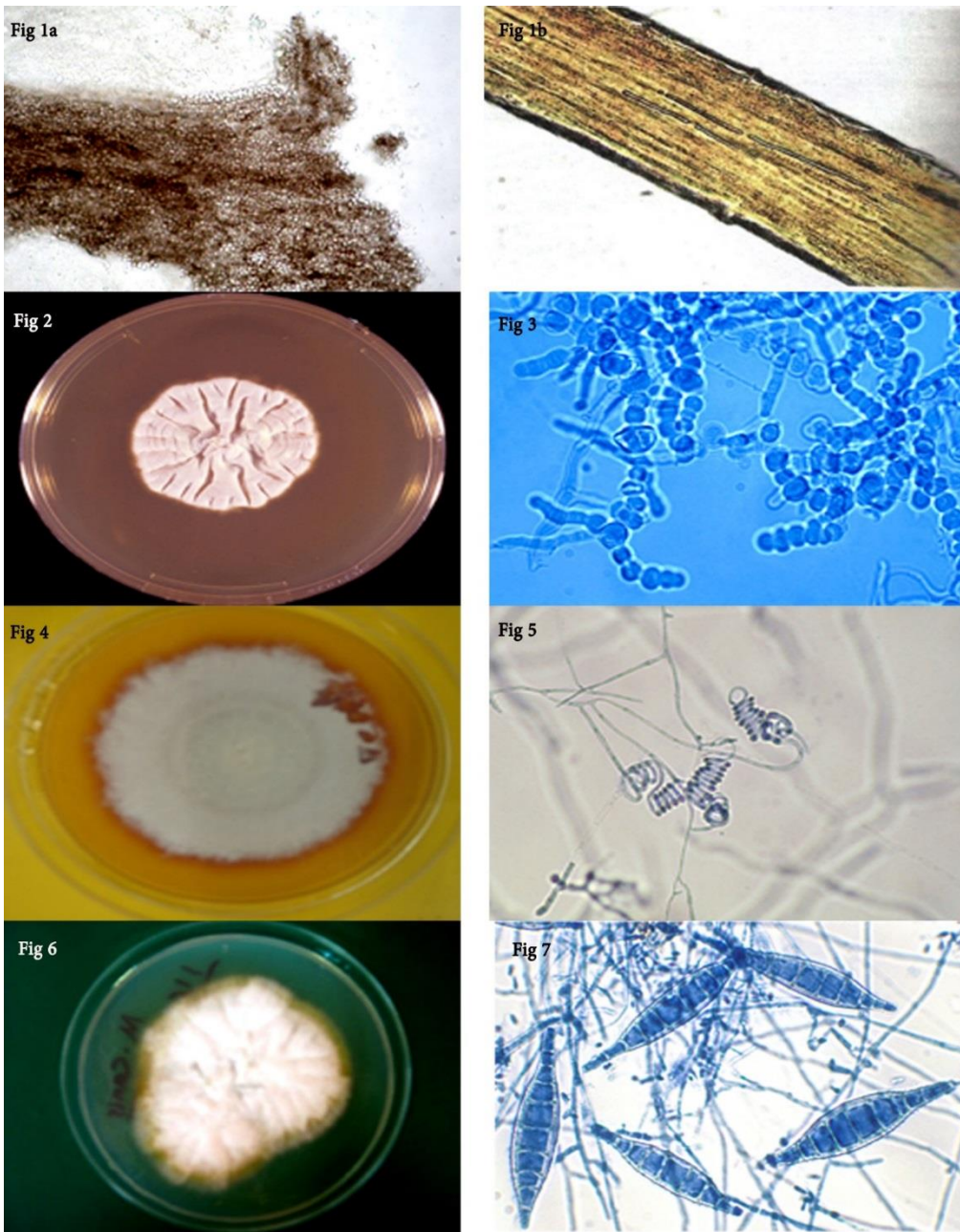


Fig 1a. arthrospores outside the hair (Ectothrix). Fig 1b. arthrospores inside the hair shaft (Endothrix). Fig 2. *T. verrucosum* (growth on Sabouraud's, glucose agar, forming slightly folded, curled, heaped, glabrous, gray white colonies). Fig 3. *T. verrucosum* prepared culture slides stained with lactophenol cotton blue, showed characteristic, septate hyphae with chlamydoconidia. Fig 4. Cultures of *T. mentagrophytes* characterized by buff to tan color and radial folds. Fig 5. Microscopic examination of *T. mentagrophytes* showed presence of spiral shape mycelia. Fig 6. Cultures of *M. canis* characterized by rapid growth and appear white cottony. Fig 7. Microscopic examination of *M. canis* showed the presence of numerous spindle shaped with rough and thick wall macroconidia.

inflammatory sparse scaly lesions developed at the flank area within 3 weeks after inoculation of rabbits with suspension of *T. verrucosum* and *T. mentagrophytes*.

*Treatment:* Results of application of local ointment prepared from methanol extracts of *T. capitatus* (13.55%) for 14 days showed effective cure of the affected rabbits skin. The lesions started to subside gradually and after 2 weeks, the hairs started to grow again, within one month there was a complete recovery. These finding were in agreement with those of Hamed et al., [15], who reported *In Vitro* antimicrobial activity of some Libyan medicinal Plant extracts that have anticandidal activity such as *P. harmala*, *P. crispa* and *T. capitatus* with MICs ranging from 0.25 to 1mg/ml. Chami et al., [8] concluded that eugenol and carvacrol (one of main constituents of thymol) are promising drugs for the treatment of oral candidiasis. Therefore, further studies on their pharmacokinetic and toxicological behaviors are warranted. Previous studies [2, 14] reported that sulfur with concentration of 1 – 10% was fungicidal. In contrast to the finding of Wabacha, et al., [30] who mentioned that calves did not respond to topical treatment with various antifungal drugs within periods of 9 weeks.

From the above mentioned data we could concluded that *T. verrucosum*, *T. mentagrophytes* and *M. canis* were the most prevalent dermatophytes among farm animals in El-Bieda – Libya and the use of methanol extracts of *T. capitatus* was effective in treatment of the infected rabbits.

#### 4. REFERENCES

1. Abo-Gabal, M., El-Galil, G.A., El-Nore, A. and El-Rehim, A. 1976. Animal ringworm in Upper Egypt. *Sabouraudia* 14: 33-36.
2. Al-Ani, F.K., Younes, F.A. and Rawashdeh, O.F. 2002. Ringworm infection in Cattle and Horses in Jordan. *Actavet. BRNO* 71: 55-60.
3. Al-Mustafa, A.H., and Al-thaunibat, O.Y. 2008. Antioxidant activity of some Jordanian medicinal plant used traditionally for treatment of diabetes. *Pak. J. of Bio. Sci.* 11(3): 351-358.
4. Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G. 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. Et. Link. *Food Chem.* 105: 146-155.
5. Cabanes, F.J., Abarca, M.L. and Bragulat, M.R. 1997. Dermatophytes isolated from domestic animals in Barcelona, Spain. *Mycopathologia* 137: 107-113.
6. Carman, M.G., Rush-Munor, F.M. and Carter, M.E. 1979. Dermatophytes isolated from domestic and feral animals. *N. Z. Vet. J.* 27: 136.
7. Champion, R.N., Burton, J.L., Burns, D.A. and Breathnach, S.M. 1998. *Text Book of Dermatology*. Sixth Edition. pp. 1277-1335.
8. Chami, N., Chami, F., Bennis, S., Trouillas, J. and Remmal, A. 2004. Antifungal treatment with carvacrol and eugenol of oral candidiasis in immunosuppressed rats. *Braz. J. Infect. Dis.* 8(3): 50-59.
9. Ebrahimi, S.N., Hadian, J., Mirjalili, M.H., Sonboli, A. and Yousefzadi, M. 2008. Essential oil composition and antibacterial activity of *Thymus capitatus* at different phenological stages. *Food Chem.* 110: 927-931.
10. Faggi, E., Saponetto, N. and Sagone, M. 1987. Dermatophytes Isoles des Carnivores domestiques a Florence (Itaile) euquete epidemiologique. *Bull Soc. Fr Mycol. Med.* 16: 297-301.

11. Falco, C.B.G., Plewing, H.H.W. and Winlelman, R.K. 1991. Dermatology "chapter 7" Spring Verlag. Co. U.S.A. pp. 220-222.
12. Francisce, T.C. 2000. Dermatophytes in domestic animals. Revisia, Iberoamericana, de Micologia. Apdo. 699: E-48080.
13. Grayer, R.J and Harborne, J.B. 1994. A survey of antifungal compounds from higher plants (1982-93). *Phytochem.* 37: 19-42.
14. Jungerman, P.F and Schwartzman, R.M. 1972. *Veterinary Medical Mycology*. Lea and Febiger, Philadelphia, pp. 3-28.
15. Hamed, A.A.B., Ladislav, K., Jaroslav, H., Pavel, K., Vojtech, R. and Karel, V. 2007. In Vitro antimicrobial activity of some Libyan medicinal plant extracts. *Pharmaceutical Biology* 45(5): 386-391.
16. Kalemba, D. and Kunicka, A. 2003. Antibacterial and antifungal properties of essential oils. *Current. Medicinal. Chemistry.* 10: 813-829.
17. Martin, A.G. and Kobayashi, G.S. 1993. Fungal Disease with cutaneous Involvement. Int. T. B. Fitzpatrick (ed) *Dermatology in general* Mc-Graw-Hill New York. pp. 2421-2451.
18. McGinnis, M.R. 1988. Current topics in Medical Mycology. Springer-Verlag. New York, pp: 261-265.
19. Narai, K., Nuner, E., Rodriguez, H., Gonzalez, M., Aibari, V. and Sarkisov, K.A. 1988. Use of ringworm vaccine. LTF-130 in the Republic of Cuba. *Veternariya* 6: 61-62.
20. Nooruddin, M. and Singh, B. 2009. Dermatophytosis in Buffaloes, Cattle and their Attendants. *Mycoses* 30: 594-600.
21. Pal, M. 1987. Dermatophytosis in Cattle, clinical and mycological studies. *Indian. J. Anim. Sci.* 57: 856-857.
22. Pier, A.C., Smith, J.M.B., Alexiou, H., Ellis, D.H., A.L. and Pritchard, R.C. 1994. Animal ringworm. Its etiology, public health significance and control. *J. Med. Vet. Mycol.* 32: 133-150.
23. Radostits, O.M., Blood, D.C. and Gay, C.C. 1997. *Veterinary Medicine* 8<sup>th</sup>ed Baillier, Tindall. London. pp. 381-390.
24. Renner, J.E 1992. A new treatment for ringworm in cattle. *Dtsche, Tierarztliche. Wchschr.* 99: 208-210.
25. Ricci, D., Fraternali, D., Giamperi, L., Bucchini, A., Epifano, F., Burini, G. and Curini, M. 2005. Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (Lamiaceae). *J. Ethnopharmacology* 98: 195-200.
26. Schmitt, J.A and Miller, R.G. 1967. Variation in susceptibility to experimental dermatophytosis in genetic strains of mice. *Mycopathol.* 32: 306-312.
27. Sparkes, A.H., Grwffydd, T.J., Shaw, S.W., Wright, A.I. and Stokes, C.R. 1993. Epidemiological and diagnosis features of Canine and Feline dermatophytosis in the United Kingdom from 1956 to 1991. *Vet. Rec.* 133: 57-61.
28. Stenwig, H. 1985. Isolation of dermatophytes from domestic animals in Norway. *Nord. Vet. Med.* 37: 161-169.
29. Suhonen, R.E., Dawber, R.P.R. and Ellis, D.H. 1999. Fungal infections of the skin, Hair and nail By Martun Dunitz Ltd. The Livery House. London, NWI-OAE.
30. Wabacha, J.K., Gitau, K.J., Bebor, L.C., Bwanga, C.O., Wamuri, Z.M. and Mbithi, P.M. 1998. Occurrence of dermatomycosis (ringworm) due to *Trichophyton verrucosum* in dairy calves and its spread to animal attendants. *J. S. Afr. Vet. Assoc.* 69: 172-173.
31. Weitzman, I. and Padhye, A.A. 1996. Dermatophytes, Gross and Microscopic. *Dermatol. clin.* 4: 9-22.
32. Weitzman, I. and Summerbell, R.C. 1995. The dermatophytes. *Clinic. Microbiol. Rev.* 8: 240-259.



## مسح على مسببات داء الجلد الفطري في مختلف الحيوانات بالبيضاء-ليبيا

نواره بالقاسم عيسى<sup>1</sup>، محمود عزت<sup>1</sup>، عزة السواح<sup>1</sup> و محمد الحامولي<sup>2</sup>

<sup>1</sup> كلية الطب البيطري جامعة عمر المختار، <sup>2</sup> كلية الصيدلة جامعة عمر المختار

### الملخص العربي

استهدفت هذه الدراسة التعرف على المسببات الفطرية لداء الجلد الفطري ومعدل انتشار هذا المرض بين الحيوانات في البيضاء ليبيا. تم جمع عدد 185 عينة من حالات إصابات جلدية من الحيوانات المختلفة (75 من الإبل، 62 من الأبقار، 35 من الأغنام والماعز، 10 من الكلاب و 3 من القطط) والتي شخّصت إكلينيكيًا على أنها إصابات فطرية للجلد. أظهرت نتائج الفحص المجهرى للشعر والقشور من الجلد المصاب عن وجود الأبواغ المميزة للفطريات في عدد 165 عينة بنسبة (89.2%). بينما أظهرت نتائج الزرع على الأوساط النوعية الحصول على عدد 85 مزرعة ايجابية للفطريات بنسبة (45.9%). وأشارت نتائج تصنيف الفطريات الى عزل 7 عترات من فطر تنتمي الى *M. canis* بنسبة (8.2%) وعدد 78 عترة تنتمي الى جنس *Trichophyton* بنسبة (91.8%)، والتي تشمل عدد 57 عترة من *T. verrucosum* بنسبة (67.1%) وعدد 21 عترة من *T. mentagrophytes* بنسبة (24.7%). بالإضافة الى عزل العديد من الفطور الملوثة ومن أكثرها شيوعا أنواع من فطر الأسبرجليس وأنواع من البنسلليوم وأنواع من اللتراناريا. تم اختبار ضراوة الفطريات الجلدية المعزولة في الأرانب واستعمال مرهم محضر من مستخلص الميثانول لنبات *Thymus capitatus* (الزعرتر) بتركيز (9% 13.55%) للعلاج وقد أثبتت فاعليته في الشفاء خلال 14 يوم بالنسبة للأرانب المصابة

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (2)، يونيو 2013: 108-115)