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**SOME MYCOLOGICAL STUDIES ON SUPERFICIAL  
KERATITIS IN THE HORSES  
IN ASSIUT GOVERNORATE**  
(With One Table)

By

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بعض الدراسات الفطرية عن التهابات السطحية للقرنية فى الخيول  
فى محافظة أسيوط

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أجريت هذه الدراسة على ٣٢ حصان من مناطق مختلفة بمحافظة أسيوط يعانون من التهابات سطحية لقرنية العين وتعرض خمسة منهم لإصابات مباشرة فى العين. وحيث سبق علاج جميع هذه الحالات لفترة تتراوح من ٩-٢١ يوم وذلك باستعمال المضادات الحيوية الموضعية المختلفة بالإضافة إلى مشتقات الكورتيزون ولم تظهر أى تحسن ملحوظ. وقد أجرى الفحص الميكولوجى لمعرفة الأسباب الحقيقية لهذه الأصابات. وقد أوضحت النتائج أن ٩٣,٧٥% من الحالات إيجابية للفحص الميكولوجى. وقد لوحظ أن فطر الاسبرجلس وخصوصاً الاسبرجلس فيوميجاتس والاسبرجلس نيجر من أهم أسباب التهابات القرنية فى الخيول. كما تم عزل أنواع أخرى من الفطريات ولكن بمعدلات بسيطة.

**SUMMARY**

A total of 32 horses suffered from superficial keratitis form different localities in Assiut Governorate were used in this study. Five of them had exposed to traumatic injuries to the eyes. All cases were treated from 9-21 days with a variety of topical antibiotic or antibiotic/steriod ophthalmic preparations without any improvement. Mycological study was performed in a trial to detect the possible mycotic causative agents. 93.75% of the examined infected eyes (30/32) showed mycotic keratitis. The pathogens were isolated in pure and heavy growth from the swabs and also demonstrated directly in clinical material by the potassium

hydroxide technique. This high incidence was attributed to mis use of the above mentioned drugs. *Aspergillus* species (68.77%) especially *Aspergillus Fumigatus* (31.25%) and *Aspergillus niger* (15.62%) were the main predominant fungal causative agents. Another fungal species had been isolated but of low incidence.

**Key Words:** *Mycotic, Keratitis in the Horses.*

## INTRODUCTION

Most ocular pathogenic fungi in the horse are opportunist pathogens which form part of the population of commensal flora harboured in conjunctival fornices, lid margins and proximal nasolacrimal drainage system of the normal healthy eye. Mycotic keratitis with or without ulceration appears to be the most prevalent fungal eye affection in horses, especially after injury with objects of vegetable origin (Slatter, 1990 and Matthews, 1994). Cases of ulceration in the cornea of equines as a result of infection with *Cryptococcus neoformans* were recorded by Bennett (1944). Epiphora, blepharospasm, photophobia, hyperaemia and ulcerative keratitis were observed by Hodgson and Jacobs (1982) and Pal and Mehrotra (1986) in equines infected with *Fusarium* and *Aspergillus* species. Abd El-Galil *et al* (1993) recorded that, *Aspergillus spp.*, *Cladosporium spp.*, *Alternaria spp.*, *Rhizopus spp.* and *Candida albicans* were incriminated in ocular lesions in horses.

The main aim of the present study was planned to isolate and identify different fungi responsible for causing keratitis in horses in order to reach rapid treatment and control corneal infection.

## MATERIAL and METHODS

Thirty-two horses suffered from sporadic cases of superficial keratitis distributed in different localities in Assiut Governorate from October 1998 to March 1999 were examined in this study. Five of them (draft horses) had exposed to traumatic injuries. All cases were treated from 9-21 days with a variety of topical antibiotic or antibiotic/steroid ophthalmic preparations e.g oxytetracycline, neomycin sulphate, polymyxin B, chloramphenicol, tetrahydrozoline hydrochloride, and betamethason. No improvement occurred with this treatment regime. The initial clinical signs of keratitis were recorded.

### **Collection of Samples:**

Examined eyes were wiped with a piece of cotton soaked in warm water to remove any dirty particles and remnants of any medicament that would make mycological examination difficult, then eyes were dried. Two sterile swabs were aseptically taken from each case and quickly transferred using sterile technique for mycological examination.

### **Fungal isolation:**

Each specimen was immediately inoculated onto the surface of four Sabouraud's dextrose agar tubes (Emmons *et al.*, 1977) supplemented with chloramphenicol (0.05 mg/ml). Two tubes of which were incubated at 37°C, while the others were incubated at 25°C. Some of the material was mounted in a drop of 10% potassium hydroxide and examined microscopically for the presence of fungal hyphae (Pal and Mehrotra 1986). Incubated tubes were examined periodically at 5, 10, 15, and 21 days for the detection of any fungal growth. The fungal growths were picked up from the primary cultures and subcultured onto Sabouraud's dextrose agar plate with chloramphenicol for purification. Pure colonies were subjected to identification by gross appearance and microscopically, in case of moulds according to Raper and Fennell (1965) and Campbell and Stewart (1980), while yeasts were cultured onto rice agar for identification into genera then fermentation and assimilation tests were done according to Lodder (1970) for species identification.

## **RESULTS**

The most prominent clinical signs of the eye infected horses were long-standing resistant unilateral superficial corneal erosion accompanied by epiphora, photophobia, and blepharospasm. Focal, cloudy, yellow opacities at the edge of the lesions were observed in some cases.

Thirty of the 32 specimens cultured from diseased eyes showed mycotic keratitis, 22 (68.77%) of them yielded pure heavy growth of *Aspergillus* species at 37°C. In these cases septate, dichotomously branched hyphae 3 to 4µm thick compatible with *Aspergillus* species could be detected in the potassium hydroxide preparation during the microscopical examination of the corneal material. Types, incidence and percentage of different mycological isolates were demonstrated in Table 1.

**Table 1:** Types, incidence and percentage of different Fungal species isolated from horses suffered from superficial keratitis

Fungal isolates	Incidence	Percentage
<i>Aspergillus spp.</i>	22	68.75%
<i>A. fumigatus</i>	(10 31.25%)	
<i>A. niger</i>	(5 15.625 %)	
<i>A. flavus</i>	(2 6.250 %)	
<i>A. versicolor</i>	(2 6.250 %)	
<i>A. terreus</i>	(1 3.125 %)	
<i>A. japonicus</i>	(1 3.125 %)	
<i>A. parasiticus</i>	(1 3.125 %)	
<i>Alternaria alternaria</i>	1	3.125 %
"    "    + <i>Ulocladium</i>	1	3.125 %
<i>Neurospora crassa</i>	2	6.25 %
<i>Candida albicans</i>	2	6.25 %
<i>Rhizopus stolonifer</i>	1	3.125 %
<i>Penicillium</i> species	1	3.125 %
Negative samples	2	6.25 %
<b>Total</b>	<b>32</b>	<b>100 %</b>

## DISCUSSION

The swabs collected over a period of six months have shown, an association between fungus infection and superficial keratitis in horses. This association was established in 30 cases, giving a prevalence of 93.75% (Table1). The diagnosis in these cases depended upon the microscopical demonstration of hyphae in the eye material and the isolation of fungus in pure culture on Sabouraud's medium. The same criteria for establishing the diagnosis of keratomycosis have been described by Rebell and Forster (1974) Pal (1983) and Ball, *et al.*, 1997.

This high rate of eye mycotic infections may be due to the widespread distribution of the fungus in the atmosphere and vegetable matters which form the main source of the animal food especially during the winter season (Hodgson and Jacobs, 1982 and Abd El-Galil *et al.*, 1993).

Keratomycosis is an uncommon condition in domestic animals but it may be suspected in corneal ulceration or in cases of keratitis (Gelatt 1981). The normal fungal flora of the eyes are usually non-pathogenic and non-invasive but are disrupted after damage to the cornea by

infectious disease, trauma, or prolonged therapy with antibiotics and corticosteroids, which disrupts the balance of the microorganisms in the conjunctival sac and predispose the horse to mycotic infection (Frenkel 1962, Mitchell and Attleberger 1973, Beech et al., 1983, Pal and Mehrotra 1986). This was the case in the present investigation in which most of the horses had received topical application of these drugs and five of them had exposed to traumatic injuries.

The data present in table (1) shows that *Aspergillus spp.* (68.77%) especially *A. fumigatus* (31.25%) and *A. niger* (15.62%) were the predominant fungal isolates. While the other *Aspergillus spp.*, *Alternaria alternaria*, *Neurospora crass*, *Candida albicans*, *Rhizopus stolonifer* and *Pencillium spp.* were of lower incidences. These results of fungal prevalence were identical with those reported by Beech et al. (1983), Moore et al., (1983), Pal (1983) and Pal and Mehrotre (1986) they isolated 6 genera of fungi from horses with diseased eyes vis: *Aspergillus spp.*, *Alternaria spp.*, *Mucor spp.*, *Rhizopus spp.*, *Candida spp.* and *Penicillium spp.* *Aspergillus spp.* were the most prevalent ones in these studies. A similar study was conducted by Abd El-Galil et al. (1993) to identify the fungal infection in 45 donkeys suffered form different ocular affections. They isolated 10 species of fungi. *Aspergillus spp.* were recovered from most cases of the keratomycosis.

On the other hand, the dimorphic fungi such as *Histoplasma spp.*, *Blastomyces dermatitides* and *Coccidioides immitis* were not isolated in this study and these contradict to findings of Bennett (1944), Kapur (1952), and Camata (1956), who isolated the above mentioned dimorphic fungi. Failure to isolate such fungal organisms may be due to differences of geographic area, season, husbandry and past history.

From this study it was concluded that to facilitate a correct and early diagnosis before starting treatment, it is probably best that a mycological investigation of clinical material from cases of keratitis, keratoconjunctivitis and other ophthalmological disorders should be combined with bacteriological examination.

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