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FUNGAL FLORA OF THE EYE AND NOSE OF HEALTHY DROMEDARY CAMELS (*Camelus dromedarius*) IN IRAN

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ABSTRACT

This study was carried out to isolate and identify the eye and nose fungal flora from healthy dromedary camels living in Iran during January and May 2009. The samples were taken using premoistened swabs from the right and/or left eye (n=63) and nose (n=69) of camels, seeded onto Sabouraud glucose agar and incubated at 30°C over a period of 7-10 days. Moulds identification was achieved to the genus level and yeast colonies were identified for macro and micro-morphologic and physiological characteristics. A total of 162 and 646 fungal isolates were obtained from eye and nose samples, respectively. The most predominant fungal isolates were *Cladosporium* (38.2%) and *Candida krusei* (34.9%) in the eye and *Cladosporium* (32.1%), *C. tropicalis* (30.9%) and *C. glabrata* (29.2%) in the nose of animals ($P<0.05$). The yeasts were associated with moulds. Also, 22 certain pathogens identified as *Nocardia asteroides* and *Actinomyces bovis*, which was not commonly related to fungal flora of animals' nose were also found. Our results showed that *Cladosporium* and *Candida* species were the most frequent fungal isolates obtained from eye and nose of camel.

Key words: *Candida*, *Cladosporium*, dromedary camel, mycoflora

Over 300 species of fungi have been reported to be animal pathogens (Scott *et al*, 2001). The microbial flora of the normal eye has been found in many animal species such as cows (Samuelson *et al*, 1984), pigs (Davidson *et al*, 1994), birds (Miller *et al*, 1995), rabbits (Cooper *et al*, 2001), horses (Moore *et al*, 1988), bison (Davidson *et al*, 1999), dogs and cats (Samuelson *et al*, 1984). Several factors including age, geography, habitat, husbandry and climate are described to influence its composition (Andrew *et al*, 2003). Corneal or conjunctival tissue can become infected by saprophytic fungi following a trauma, underlying ocular pathology or improper use of topical antibiotics and/or corticosteroids (Brooks, 1999). Many reports showed that healthy eye often yield bacteria or fungi, which some of them were potential pathogens (Whitley and Moore, 1984; Gerding *et al*, 1993a; Gerding *et al*, 1993b).

On other hand, primary mycotic infections of the animal nasal cavities are uncommon but nasal mycosis often occurs secondary to other disorders, such as sinusitis, nasal neoplasia or traumatic damage to the nasal mucosa. These infections can be very destructive, resulting in erosion of the nasal conchae (Barakzai, 2007). They may occur in immuno-competent or immuno-compromised animals, may have an acute or chronic course and may spread to the orbit, structures of the eye and the brain (Sanabria *et al*, 1992).

Since mycologic diagnostic tests are not routinely performed on initial presentation for eye and nasal infections, a wide spectrum antibiotic is usually used. In order to increase the accuracy of treatments, knowledge of fungal flora found in healthy eye and nose of camels would be most valuable. Therefore, the study of fungal flora of this animal is vital to understanding eye and nasal diseases and critical to guide the selection of suitable anti-infective agents. In our knowledge, we did not find the data of camel eye and nose mycoflora and details of various fungal species in the literature. The purpose of the present paper is to culture, describe and quantify moulds and yeasts from eye and nose of healthy camels living in Iran, and to identify the most common fungal species.

Materials and Methods

Right and/or left eye (n=63) and nose (n=69) of camel of different sexes and aged from 1.5 to 12 years old were examined. The subjects were from different farms in Iran with different management systems. All the animals were considered clinically healthy on ophthalmic and nasal examinations.

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Samples were taken during January and May 2009. Eye specimens were obtained by retropulsing each eye through the closed upper eyelid and running a sterile swab along the surface of the ventral conjunctival fornix. Special care was taken to ensure that the swab did not come into contact with the vibrissae, eyelids or eyelashes. Camels with active inflammation or a red eye were excluded. In addition, dry and sterile cotton swabs in sterile glass tubes were used to culture the nose samples of the isolated camels.

Each sample was maintained in 2 ml of sterile water with 50 µl/ml gentamicin added. Samples were seeded onto Sabouraud glucose agar (*Merck Co, Darmstadt, Germany*) supplemented with antibiotic (chloramphenicol 0.005%) and kept at 30°C for up to 7-10 days. Moulds were identified to the genus and species levels; *Aspergillus* species were identified following the keys of Raper and Fennel (1965). Yeast colonies were identified for macro and micro-morphological characteristics and on the basis of physiological characteristics such as, presence of capsule by India ink testing, urease production at 25°C, germ tube and sugar fermentation and assimilation tests by RAPID yeast plus system (*Remel Inc, USA*). Tests were performed on all isolates. Each test was carried out in duplicate to confirm the results.

Statistics

The chi-square (χ^2) test was used to assess statistical differences between the groups. Probabilities of 5% were taken to be statistically significant. Results were analysed and described by way of frequency and percentual distribution expressed in tables and graph.

Results

Thirty-eight of the 63 camels (39.7%) had a positive culture for fungi from one eye. Seventy-six moulds and 86 yeasts isolates (totally 162) were screened and identified from the samples as indicated in tables 1 and 2. The yeasts were always associated with moulds. Three different fungal species were found in 7 camels, 2 in 18 and 1 in 13 camels. The most frequent fungal genera and species were as follows: *Cladosporium* (38.2%), *Penicillium* (17.1%), *A. fumigatus* (11.8%) and *A. alternata* (10.5%) among moulds and *C. krusei* (34.9%), *C. tropicalis* (14%) and *G. candidum* (11.6%) among yeasts. As shown in Figure 1, there was no significant difference between moulds (46.9%) and yeasts (53.1%) isolated. The values found on *Cladosporium* species and *C. krusei* were significantly higher ($P<0.05$) in comparison with the results obtained on the other moulds and yeasts, respectively. Significance of age of camel and isolated of fungi has not been presented in table but is mentioned in the text.

Concerning the nose mycoflora, fungal agents 82.6% were isolated from (57) nose samples of healthy camels. A total of 646 fungal isolates were recovered

Table 1. Frequency of the isolation and percentage of moulds from 38 healthy eyes and 57 healthy noses of dromedary camels.

	Eye No. (%)	Nose No. (%)
A. niger	6 (7.9)	30 (6.7)
A. fumigatus	9 (11.8)	16 (3.6)
A. flavus	1(1.3)	27 (6)
A. clavatus	0	4 (0.9)
Mucor spp	4 (5.3)	57 (12.7)
Rhizopus oryzae	0	15 (3.3)
Penicillium spp	13 (17.1)	61(13.6)
Chrysosporium spp	0	6 (1.3)
A. alternata	8 (10.5)	17 (3.8)
F. solani	0	37 (8.2)
F. oxysporum	0	7 (1.6)
Acremonium spp	0	8 (1.8)
Absidia corymbifera	2 (2.6)	2 (0.4)
Paecilomyces variotii	4 (5.3)	2 (0.4)
Cladosporium spp	29 (38.2)	144 (32.1)
Ulocladium spp	0	6 (1.3)
Syncephalastrum spp	0	2 (0.4)
Cunninghamella spp	0	8 (1.8)
Total	76 (100)	449 (100)

Table 2. Frequency of the isolation and percentage of yeasts and actinomycetal agents from 38 healthy eyes and 57 healthy noses of dromedary camels.

	Eye No. (%)	Nose No. (%)
Yeast		
C. albicans	5 (5.8)	19 (10.9)
C. tropicalis	12 (14)	54 (30.9)
C. glabrata	5 (5.8)	51(29.1)
C. krusei	30 (34.9)	3 (1.7)
C. guillermondii	5 (5.8)	0
G. candidum	10 (11.6)	5 (2.9)
R. rubra	9 (10.5)	10 (5.7)
Cr. Albidus	3 (3.5)	8 (4.6)
T. Beigelii	7 (8.1)	25 (14.3)
Total	86 (100)	175 (100)
Actinomycetes		
N. asteroides	0	10 (45.5)
A. bovis	0	12 (54.5)
Total	0	22 (100)

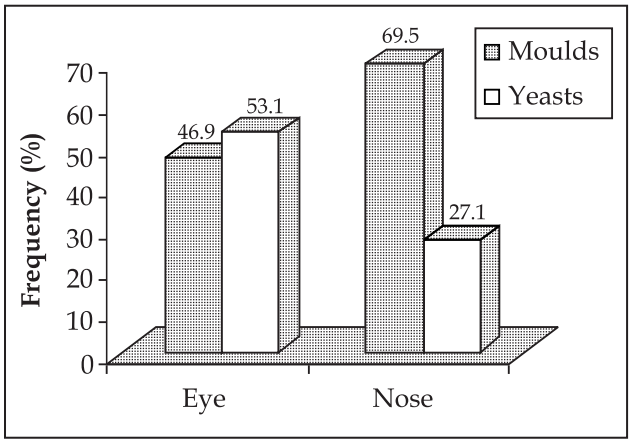

Fig 1. Comparison of percentage of moulds and yeasts isolated from eyes and nasal cavity of dromedary camels.

and identified from the samples. After investigation, mixed yeasts and moulds were determined, in which 6 different fungal isolates were found in 1 camel, 5 in 5, 4 in 3, 3 in 25, 2 in 16 and 1 in 7 camels. It was found that all these fungal isolates belonged to 21 genera; *Cladosporium* (32.1%), *Penicillium* (13.6%), *Mucor* (12.7%) and *C. tropicalis* (30.9%), *C. glabrata* (29.2%) and *T. Beigelii* (14.3%) predominantly among moulds and yeasts, respectively. The isolates were identified as moulds in 69.5% of cases ($P<0.05$), yeasts in 27.1% and actinomycetes in 3.4% (Fig 1). As shown in Tables 1 and 2, *Cladosporium* was the highest frequent species among moulds and *C. tropicalis* and *C. glabrata* were the highest frequent species among yeasts, indicating significant differences in comparison with other fungal isolates ($P<0.05$). Two actinomycetal isolates were also identified as *N. asteroides* and *A. bovis* in camels' nose whereas, they did not found in healthy eye.

Discussion

The resident eye and nose fungal flora are still unknown in dromedary camels and this knowledge would be very useful in assessing the accuracy of treatments. The micro-organisms isolated in this study and their relative frequencies were quite similar to the previous investigations from eye of mammals and exotic birds (Samuelson *et al*, 1984; Dupont *et al*, 1994). A large range of fungal flora were isolated from eyes of our sample population, indicating the presence of fungi in healthy camels living in Iran. Most frequently recovered fungal genera were *Cladosporium*, followed by *Penicillium* and *Aspergillus* spp. Dematiaceous fungi like *Cladosporium* species are frequently encountered as fungal contaminants and they are considered one of the most common causes of keratomycosis in animals (Andrew *et al*, 2003). *Alternaria alternata* was isolated occasionally from both eyes of the same animals. This fungus is a major cause of fungal keratitis and is considered as the most capable of producing disease owing to their ability to invade and destroy the cornea (Tu, 2009). Fungi are usually isolated more frequently in large animals such as equine and bovine species, since their environment contains more organic material. Our results agree with those of Barsotti *et al* (2006) and Nardoni *et al* (2007), who found a large number of *Cladosporium*, *Aspergillus* and *Penicillium* species from horse and donkey eyes. In an another study conducted by Samuelson *et al* (1984), conjunctival swab specimens were obtained from both eyes of 43 horses, 25 cows, 50 dogs and 25 cats without keratitis or other ophthalmological problems. Fungi were isolated from 95% of the horses, 100% of the cows, 22% of the dogs and 40% of the cats. *Aspergillus* species were isolated from 56% of the horses, 12% of the cows, 8% of the cats, and none of the dogs. *Penicillium* and *Cladosporium* species were isolated ubiquitously. In birds, Dupont *et al* (1994) showed only 2 fungal species, *Aspergillus* and *Cladosporium* in healthy eyes of birds of prey. The overall results of this study are similar to previous studies carried out in humans and other animals.

Predominant isolation of yeasts such as *Candida* species also occurred. Among them, *Candida krusei* was the predominant fungal isolate in healthy eyes. *Candida krusei*, which was found as the most general isolated yeast, is considered nonpathogenic; however, it can become pathogenic as a result of underlying ocular pathology. To our knowledge, there are no reports in the literature concerning camel keratomycosis in Iran.

In present study, no difference was observed based on animals' age and this finding was in agreement with Barsotti *et al* (2006) report on horse's eyes. In contrast, Andrew *et al* (2003), reported that the likelihood of detecting fungi depended upon horses' age, younger animals being more frequently colonised. Stabled animals were more frequently positive than those living outside, as observed in previous reports (Whitley *et al*, 1983; Moore *et al*, 1988; Rosa *et al*, 2003), confirming that stabling or blocked places may be considered a risk factor. The age variation among the different studies may be due to varying methods of handling, hygiene and the geographical regions where the research was made.

Studies that have attempted to quantitate and identify as to species, the fungi in the nasal flora of animals are few in number. In this study, 57 out of 69 (82.6%) were positive for fungi. Most frequently

recovered fungal genera were *Cladosporium*, *Penicillium*, *Mucor* among moulds and *C. tropicalis*, *C. glabrata* and *T. Beigelii* among yeasts. Previous studies on the nasal mycoflora of different animals revealed a diverse grouping of moulds and yeasts. It was reported *Rhodotorula*, *C. krusei*, *Geotrichum* and *T. beigelii* in the nares of dog (Balish *et al*, 1977), *Aspergillus* species and *Conidiobolus coronatus* in horse (Korenek *et al*, 2008), dematiaceous fungi including *Bipolaris* and *Drechslera* species in cow (Penrith *et al*, 1994), *Cryptococcus* species in cat (Malik *et al*, 2008) and *Aspergillus* species in ostrich (Fitzgerald and Moisan, 1995). This report and other data on nasal mycoflora of animals demonstrated that their mycoflora is diverse and made up of a variety of fungi. Our finding was in close consistent with some studies. The reasons for these differences are attributed to geographical conditions, such as plant flora, humidity and temperature as important factors to spread the fungi.

During this study, some filamentous bacteria, *N. asteroides* and *A. bovis*, were observed in camels' nose. The isolation of these organisms from nose appears not to have been recorded earlier in animals, although, their involvements in the paranasal sinuses and lungs mycoses are well documented (Roberts *et al*, 1995; Menendez *et al*, 1997; Ozcan *et al*, 2004; Ono *et al*, 2006). Actinomyces existing in the nose, paranasal sinuses, oral cavity, pharynx, salivary gland and intestine as indigenous bacteria can induce actinomycosis following dental manipulation, trauma or aspiration (Ono *et al*, 2006). In contrast, the presence of *N. asteroides* in nasal cavities must be interpreted with caution and the fact, that the manner in which these saprophytes become established in the normal flora of mammals requires further study. Interestingly, in 2 previous studies performed by Khosravi *et al* (2008), *N. asteroids* was isolated from vagina, uterine body and external ear canals of several dromedary camels examined as well. Although this micro-organism is one of saprophytic and thermophilic agents found in soil and infection usually takes place through wound contamination (Harvey and Lloyd, 1995), it seems that the presence of this organism into the camels' nasal cavity occur through close contact with soil and desert plants (*Astragallus* spp); and are treated not as the component of physiological flora, but as the contamination.

This study demonstrated the similarity between the mycoflora of healthy eye and nose of camels and those of other animal species. Also, a survey of the most common organisms recovered from diseased eye and nose of camels would be most valuable. As our knowledge of the camels' eye and nose grows, so will our capacity to give them the best possible care.

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References

Andrew SE, Nguyen A, Jones GL and Brooks DE (2003). Seasonal effects on the aerobic bacterial and fungal conjunctival flora of normal thoroughbred brood mares in Florida. Veterinary Ophthalmology 6:45-50.

Balish E, Cleven D, Brown J and Yale CE (1977). Nose, throat and faecal flora of beagle dogs housed in "Locked" or "Open" environments. Applied and Environmental Microbiology 34:207-221.

Barakzai S (2007). Handbook of Equine Respiratory Endoscopy. 1st Edn. W.B. Saunders. pp 20.

Barsotti G, Sgorbini M, Nardoni S, Corazza1 M and Mancianti (2006). Occurrence of fungi from conjunctiva of healthy horses in Tuscany, Italy. Veterinary Research Communications 30:903-906.

Brooks DE (1999). Equine ophthalmology. In: Veterinary Ophthalmology, K.N. Gelatt (Ed.) 3rd Edn, Lippincott Williams and Wilkins, Philadelphia. pp 1053-1116.

Cooper SC, Mc Lellan GJ and Rycroft AN (2001). Conjunctival flora observed in 70 healthy domestic rabbits. Veterinary Record 149:232-235.

Davidson HJ, Rogers DP, Yeary TJ, Stone GG, Schoneweis DA and Chengappa MM (1994). Conjunctival microbial flora of clinically normal pigs. American Journal of Veterinary Research 55:949-951.

Davidson HJ, Vestweber JG, Brightman AH, Van Slyke TH, Cox LK and Chengappa MM (1999). Ophthalmic examination and conjunctival bacteriologic culture results from a herd of North American bison. Journal of the American Veterinary Medical Association 215:1142-1144.

Dupont C, Carrier M and Higgins R (1994). Bacterial and fungal flora in healthy eyes of birds of prey. Canadian Veterinary Journal 34:699-701.

Fitzgerald SD and Moisan PG (1995). Mycotic rhinitis in an ostrich. Avian Diseases 39:194-196.

Gerding PA, Cormany K and Weisiger R (1993a). Survey and topographic distribution of bacterial and fungal micro-organisms in eyes of clinically normal dogs. Canine Practice 18:34-38.

Gerding PA, Cormany K and Weisiger R (1993b). Survey and topographic distribution of bacterial and fungal micro-organisms in eyes of clinically normal cats. Feline Practice 21:20-23.

Harvey RG and Lloyd DH (1995). The distribution of bacteria (other than *Staphylococci* and *Propionibacterium acnes*) on the hair, at the skin surface and within the hair follicles of dog. Veterinary Dermatology 6:79-84.

Korenek NL, Legendre AM, Andrews FM, Blackford JT, Wan PY, Breider MA and Rinaldi MG (2008). Treatment of mycotic rhinitis with itraconazole in 3 horses. Journal of Veterinary Internal Medicine 8:224-227.

Khosravi AR, Shokri H, Ziglari T and Niasari-Naslaji A (2008). A study of mycoflora of the external ear canals in dromedary camels in Iran. Journal of Camel Practice and Research 15:155-159.

Malik R, Martin P, Wigney DI, Church DB, Bradley W, Bellenger CR, Lamb WA, Baras VR, Foster S, Hemsley S and Canfield PG (2008). Nasopharyngeal cryptococcosis. Australian Veterinary Journal 75:483-488.

Menendez R, Cordero PJ and Santos M (1997). Pulmonary infection with *Nocardia* species: a report of 10 cases and review. European Respiratory Journal 10:1542-1546.

Miller PE, Langenberg JA and Hartmann FA (1995). The normal conjunctival aerobic bacteria flora of 3 species of captive crane. Journal of Zoo and Wildlife Medicine 26:545-549.

Moore CP, Heller N, Majors LJ, Whitley RD, Burgess ES and Weber J (1988). Prevalence of ocular microorganisms in hospitalised and stabled horses. American Journal of Veterinary Research l:773-777.

Nardoni S, Sgorbini M, Barsotti G, Corazza M and Mancianti F (2007). Conjunctival fungal flora in healthy donkeys. Veterinary Ophthalmology 10:207-210.

Ono T, Yoshida Y, Izumaru S and Nakashima T (2006). A case of nasopharyngeal actinomycosis leading to otitis media with effusion. Auris Nasus Larynx 33:451-454.

Ozcan C, Talas D, Görür K, Aydin O and Yildiz A (2004). Actinomycosis of the middle turbinate: an unusual cause of nasal obstruction. European Archives of Oto-Rhino-Laryngology 262:412-415.

Penrith ML, Van der Lugt JJ, Henton MM, Botha JA and Stroebel JC (1994). A review of mycotic nasal granuloma in cattle, with a report on three cases. Journal of South Africa Veterinary Association 65:179-83.

Raper KB and Fennell DR (1965). The Genus *Aspergillus*. The Williams and Wilkins Co. Baltimore.

Roberts SA, Bartley J, Braatvedt G and Ellis-Pegler RB (1995). *Nocardia asteroides* as a cause of sphenoidal sinusitis: Case report. Clinical Infectious Diseases 21:1041-1042.

Rosa M, Cardozo LM, da Silva Pereira J, Brooks DE, Martins ALB, Florido PSS and Pedroso Stussi JS (2003). Fungal flora of normal eyes of healthy horses from the State of Rio de Janeiro, Brazil. Veterinary Ophthalmology 6:51-55.

Samuelson DA, Andersen TL and Gwin RM (1984). Conjunctival fungal flora in horses, cattle, dogs and cats. Journal of the Veterinary Medical Association 10:1240-1242.

Sanabria Gomez F, Cenjor Espanol C, Marquez Dorsch FJ and Sarasa Corral JLA (1992). Severe rhinological mycosis: mucormycosis. A report of 3 cases. Acta Otorhinolaryngology Espanola 43:273-278.

Scott DW, Miller WH and Griffen CE (2001). Muller and Kirk's Small Animal Dermatology, 6th Edn, Philadelphia PA, W.B. Saunders Co.

Tu Ey (2009). *Alternaria* keratitis: clinical presentation and resolution with topical fluconazole or intrastromal voriconazole and topical caspofungin. Cornea 28:116-119.

Whitley RD, Burgess EC and Moore CP (1983). Microbial isolates of the normal equine eye. Equine Veterinary Journal 2:138-140.

Whitley DR and Moore CP (1984). Microbiology of the equine eye in health and disease. Veterinary Clinics of North America: Equine Practice 6:451-465.