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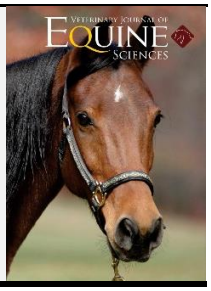

Original Article

Ocular fungal flora in healthy donkeys in Iran

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ABSTRACT

Fungi are normal inhabitants of the equine ocular surfaces. However, they can cause keratomycosis under certain conditions. This work was aimed at identifying the fungal flora of the normal conjunctiva of donkeys in Iran and to determine the effect of gender and age variations on the flora. Samples were collected from both eyes of 120 healthy donkeys, aged 1-12 years, and subsequently cultured on Sabouraud's dextrose agar. Totally, 92.08% of specimens were culture-positive for one to six different species of fungi (overall 10 genera and 13 species were isolated). The most common fungal genera were Candida (33.01%), Alternaria (25.91%), Penicillium (16.89%) and Aspergillus (11.70%). Yeasts (Candida, Rhodotorula and Geotrichum spp) represented 38% of the isolates. Age and sex of donkeys appeared to influence the isolation rate of some fungal species. A positive correlation was found between the age and number of fungal species isolated per eye (r=0.187; P=0.008). Isolated fungi were similar to those reported previously for equine species, although the prevalence of yeasts was higher which may be due to geographical differences.

فلور قارچی چشم الاغ های سالم درایران

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چکیده

قارچ ها ساکنین طبیعی سطح چشم در اسب سانان می باشند، لیکن در شرایط خاص باعث ایجاد کراتومایکوز می گردند. هدف از این مطالعه شناسایی فلور قارچی ملتحمه سالم الاغ ها در ایران وتعیین اثر جنس و سن میزبان بر روی این فلور می باشد. نمونه ها از هر دو چشم ۱۲۰ راس الاغ سالم با گستره سنی ۱ تا۱۲سال اخذ و در محیط سابرودکستروز آگار کشت گردید. در کل۹۲/۰۸٪ از نمونه ها برای یک تا ۶ گونه قارچی مختلف مثبت بودند . در مجموع ۱۰ جنس و ۱۳ گونه قارچی جدا گردید. فراوانترین قارچ های جدا شده شامل جنس های کاندیدا(۳۳/۰۱٪)، آلترناریا(۲۵/۹۱٪)، پنی سیلیوم(۱۶/۸۹٪) و آسپرژیلوس(۱۱/۷۰٪) بودند. مخمرها (جنس های کاندیدا، رودوتورلا و جئوتریکوم)۳۸٪ از جدایه ها را بخود اختصاص دادند. جنس و سن میزبان میزان جداسازی تعدادی از قارچ ها را تحت تاثیر قرار داد. یک همبستگی مثبت ما بین سن و تعداد گونه های قارچی جدا شده به ازای هر چشم دیده شد(P =۰/۰۰۸، r=۰/۱۸۷). قارچ های جدا شده مشابه موارد قبلی گزارش شده برای دیگر اسب سانان بود، اگر چه فراوانی جدا سازی مخمرها بیشتر بود که می تواند ناشی از تفاوت های جغرافیایی باشد.

واژه های کلیدی: کاندیدا، الاغ، چشم، فلور قارچی، ایران

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INTRODUCTION

Keratomycosis is a painful and sight-threatening disease, most commonly diagnosed in horses than in other domestic species [1] comprise it 4.8 to 39% of total ulcerative keratitis [2, 3], and accounts for 13% of total corneal problems reported in horses [4]. Reportedly, 2 to 56% of these eyes were to be enucleated due to lack of response to treatment [5, 6].

Keratomycosis usually follows corneal trauma. Abrasion or ulceration of the protective layers and particularly corneal epithelium, as the most effective defensive mechanisms of ocular surface, can lead to the invasion of corneal stroma by fungi and consequently typical melting corneal lesions [7].

Factors believed to predispose horses to keratomycosis include innate species-specific susceptibility and debility of the defensive mechanisms of outer eye, large eyes that protrude from the orbital fossa and eyes with large exposed corneas that are prone to trauma. A relatively slow rate of corneal fibrovascular infiltration and healing can provide a niche for opportunistic infective agents following a mechanical, chemical or enzymatic insult [5].

In equine species, fungi involved in corneal infection are generally considered to be those of the fungal flora of normal conjunctiva [8]. Therefore, knowledge of fungal species most likely to be encountered on ocular surface is important to select an antifungal drug as empirical treatment of corneal mycoses. Aspergillus, Cladosporium, Alternaria, Penicillium and Mucor are the predominant species in most mycological studies of the equine normal conjunctiva [9, 10, 11]. The ocular fungal flora is thought to be transitory and related to seeding from surrounding environment, therefore, may vary with the season, geography and habitat [8]. The aim of this work was to describe the conjunctival fungal flora in healthy donkeys living in the northwest of Iran. Differences between prevalence of isolated fungi in relation to age and sex of donkeys were also investigated.

MATERIALS AND METHODS

Study Area

This study was carried out from October 2011 to April 2012 in rural areas around the lake Urmia, northwest of Iran (37.7000°N, 45.3167°E), with altitude of 2,120m above sea level, 342 mm annual precipitation and an average minimum and maximum temperatures of 5 °C and 19.6 °C, respectively.

Animals and Sampling Procedures

A total of 120 donkeys (52 females, 43.33%; 68 males, 56.67%) with an age range of 1 to 12 years old (median 5.0 years) were selected and divided into two age groups (group A: below 5 years of age, 57 heads; group B: above 5 years of age, 63 heads). The eyes were carefully examined and proved to be clinically healthy. The specimens were obtained from the inferior conjunctival sac of both eyes by sterile dry swab without touching the eyelids, eyelashes and vibrissae. The swabs were placed in tubes containing sterile normal saline and submitted immediately to microbiology laboratory in a cool box.

Mycology

In the laboratory swabs were cultured on Sabouraud dextrose agar with chloramphenicol (HiMedia, India) and incubated at 30°C. The plates were evaluated daily at least for 3 weeks. Growing colonies were investigated under microscope using lactophenol cotton blue stain. In some cases colonies were transferred on potato dextrose agar to induce fungal sporulation for identification purposes [12]. Except Aspergillus that was identified to

species level, other fungi identified to genus level.

Statistical analysis

Statistical analysis was performed with the use of SPSS (ver. 21). Mann-Whitney test was used to compare the rate of fungal isolation between sex and age groups. Kendall's tau correlation test was used to assess the relation between age of donkey and number of fungal species isolated per eye. Significance was set at $P < 0.05$.

RESULTS

Examination of the conjunctival swabs from 120 donkeys without any ophthalmic disease showed the presence of fungi in 100% of cases and 92.08% of eyes. A total number of 521fungal isolates belonging to 10 genera and 13 different species were recovered (Table 1). Single species were isolated in 47 eyes (19.58%). Two up to 6 different species were isolated in 174 eyes (72.5%). Candida spp, Alternaria spp, Penicillium spp and Aspergillus spp were the most prevalent isolates. The filamentous fungi composed the majority (62% of all isolates) vs. yeasts (Candida, Rhodotorula and Geotrichum spp). Aspergillus species included A. flavus (34.43%), A. niger (29.51%), A. terreus (24.59%) and A. fumigatus (11.47%).

According to statistical analysis (Table 1), isolation rate of Mucor spp ($P<0.01$) and A. flavus ($P<0.05$) in males and isolation rate of A. flavus ($P<0.05$) in B age-group was significantly higher. The prevalence of the fungal species isolated per eye was significantly correlated to age of donkey ($r = 0.187$; $P = 0.008$).

Table 1. Fungal genera, number of species isolated and frequency analysis of isolates in relation to sex and age of donkeys

Fungi	No. of isolates (%)						
	Total	Sex		P value	Age group		P value
		Male	Female		A	B	
Candida spp	172 (33.01)	97 (56.40)	75 (43.60)	0.904	83 (48.26)	89 (51.74)	0.939
Alternaria spp	135 (25.91)	76 (14.31)	59 (11.45)	0.901	66 (48.89)	69 (51.11)	0.749
Penicillium spp	88 (16.89)	48 (54.55)	40 (45.45)	0.450	37 (42.05)	51 (57.95)	0.254
Mucor spp	29 (5.57)	22 (75.86)	7 (24.14)	0.017**	11 (37.93)	18 (62.07)	0.238
Aspergillus flavus	21 (4.03)	16 (2.86)	5 (1.14)	0.048*	6 (28.57)	15 (71.43)	0.049*
Rhodotorula spp	19 (3.65)	11 (57.89)	8 (42.11)	0.793	7 (36.84)	12 (63.16)	0.534
Aspergillus niger	18 (3.45)	9 (50.00)	9 (50.00)	0.302	6 (33.33)	12 (66.67)	0.363
Aspergillus terreus	15 (2.88)	11 (73.33)	4 (26.67)	0.166	7 (1.33)	8 (1.52)	0.630
Aspergillus fumigatus	7 (1.34)	5 (71.43)	2 (28.57)	0.419	3 (42.86)	4 (57.14)	0.801
Geotrichum spp	7 (1.34)	5 (71.43)	2 (28.57)	0.419	1 (14.29)	6 (85.71)	0.071
Gliocladium spp	5 (0.97)	3 (60.00)	2 (40.00)	0.878	3 (60.00)	2 (40.00)	0.569
Trichoderma spp	3 (0.58)	2 (66.67)	1 (33.33)	0.724	1 (33.33)	2 (66.67)	0.620
Cladosporium spp	2 (0.38)	2 (100)	-	0.382	2 (100)	-	0.293
Total	521 (100)	307 (58.93)	214 (41.07)	0.131	231 (44.33)	290 (55.67)	0.084

*: $P < 0.05$; **: $P < 0.01$; A: below 5 years of age B: above 5 years of age

DISCUSSION

To our knowledge, this is the first published report of the ocular fungal flora from healthy donkeys in Iran and second in world. In the current study, positive fungal cultures were obtained from 100% of donkeys. The rate of fungal growth of samples from normal equine eyes differs between studies, from 13% [13] to almost 100% [14] in horses and 79.4% in Italian donkeys [10]. It has been suggested that fungal organisms are typically transitory inhabitants of the ocular microbiota, and their presence or absence can thus be affected by geographic, environmental and husbandry conditions [8, 15, 16]. Further, it is likely that sampling technique also be involved in these differences [13].

In various studies on the normal equine eyes molds predominated over the yeast organisms [9, 10, 11, 13] consistent with our data in which 62% of the isolates were filamentous fungi. In most of these studies, the major isolated fungi were Aspergillus spp, which were reported up to 71% of isolates [3, 10, 11, 17]. However, in contrast to these reports, Candida spp was found to be the most prevalent fungal species in our study. Similar result has been echoed in one study in Brazil on the horses [18]. It has been suggested that the ocular surface could be contaminated with Candida spp by direct contact, as the organism can be frequently isolated from the hands of people. In Amiata donkeys the yeasts with 11% prevalence [10] (vs. 38% in the current study) were sparse on the ocular surface. In our unpublished data, Candida spp were also the most prevalent isolates of normal eyes from horses of the same geographical distribution. It seems that geography plays an important role in composition and frequency of the outer eye flora. Genera Aspergillus, Fusarium and Candida have been reported to be most often fungi isolated in equine keratomycosis [5, 19] however, unlike Aspergillus spp and Fusarium spp that are reported frequently in most works, Candida spp are isolated more often in temperate regions than in tropical regions [5, 19, 20, 21].

In this study, Alternaria spp, Peni [19] cillium spp and Aspergillus spp were other prevalent isolated fungi. In study conducted in Italy on donkeys, Aspergillus spp, Penicillium spp, Cladosporium spp and Acremonium spp were the most common fungi found in normal eyes [10]. However, Alternaria spp have been reported frequently in horses [8]. In a recent study in Switzerland, this genus was reported to be most prevalent isolate of normal eyes in sixty-four horses [22]. Alternaria spp are dematiaceous fungi usually present on the ground or plants in decomposition and have been described as a cause of equine keratomycosis [8, 23]. Aternaria spp, Cladosporum spp, Penicillium spp and Aspergillus spp spores are the most frequently recovered fungal spores from the air [24]. Culturing common aerial fungal spores from the ocular surface of donkeys is no surprise, consistent with great exposure of their eyes due to the anatomical characteristics.

In our study, different Aspergillus species were identified; of which A. flavus, A. fumigatus and A. niger are known toxigenic and pathogenic fungi, frequently isolated from mycotic keratitis [2, 6, 9, 20]. Since these fungi can readily contaminate the feed and their spores are easily airborne, the defects in the eye defense mechanisms, corneal trauma or use of immunosuppressive drugs can lead to colonization/penetration and then keratomycosis.

All of the fungal genera isolated in this report are considered to be saprophytic; however, most of them have been reported from the equine keratomycosis [25]. Unlike horses,

there is no report in the literature on the ocular mycosis in donkeys. More closely observation of eyes in horses and extensive topical use of antibiotic and corticosteroid medications, that may enhance indigenous fungal replication, could be considered as reasons for high rate of keratomycosis in horses vs. other equine species

It was proven that age and sex have various effects on the normal conjunctival flora in humans and animals. Liu et al [26] gave evidence that age has a significant positive correlation with the number of microbial species isolated per eye in human [26]. Similar conclusion can be derived from our study, where number of fungal species isolated per eye was significantly higher in the older donkeys. This may be due to decreasing immune functions, reduction of lacrimal secretions, and partially compromised or weakened local immune system in the older donkeys. Additionally, we found that age and sex affect the isolation rate of some fungi. Similar findings have been observed in various studies performed on the conjunctival microflora of different animals, as in one report from camelids, Bacillus spp was isolated more frequently in females [27]. Sex differences have also been seen in male horses [8], male goats [28] and female pigs [29], which had higher prevalence of the conjunctival bacterial and fungal isolates. A potential age difference was noted in study of the equine conjunctival flora where the authors found that the highest number of Gram-negative and fungal isolates in younger horses [16]. To understand how sex and age of host affect the conjunctival microflora, and its clinical relevance more studies are required.

CONCLUSION

In this work multiple saprophytic fungi with potential opportunistic pathogenicity were isolated from the majority of the eyes. The isolated fungal species are comparable to the isolates of previous studies on equine species. However, the frequency of yeast isolation was higher which may reflect a geographic difference.

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ETHICS

All ethical standards have been respected in this study.

CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Voelter-Ratson K, Pot S, Florin M, Spiess B. Equine keratomycosis in Switzerland: a retrospective evaluation of 35 horses (January 2000–August 2011). Equine veterinary journal. 2013;45(5):608-12.

[2] Brooks D, Andrew S, Dillavou C, Ellis G, Kubilis P. Antimicrobial susceptibility patterns of fungi isolated from horses with ulcerative keratomycosis. American journal of veterinary research. 1998;59(2):138-42.

[3] Gemensky-Metzler AJ, Wilkie DA, Kowalski JJ, Schmall LM, Willis AM, Yamagata M. Changes in bacterial and fungal ocular flora of clinically normal horses following experimental application of topical antimicrobial or antimicrobial-corticosteroid ophthalmic preparations. American journal of veterinary research. 2005;66(5):800-11.

[4] Pearce JW, Giuliano EA, Moore CP. In vitro susceptibility patterns of Aspergillus and Fusarium species isolated from equine ulcerative keratomycosis cases in the midwestern and southern United States with inclusion of the new antifungal agent voriconazole. Veterinary ophthalmology. 2009;12(5):318-24.

[5] Gaarder J, Rebhun W, Ball M, Patten V, Shin S, Erb H. Clinical appearances, healing patterns, risk factors, and outcomes of horses with fungal keratitis: 53 cases (1978-1996). Journal of the

American Veterinary Medical Association. 1998;213(1):105-12.

[6] Reed Z, Thomasy S, Good K, Maggs D, Magdesian K, Pusterla N, et al. Equine keratomycoses in California from 1987 to 2010 (47 cases). Equine veterinary journal. 2013;45(3):361-6.

[7] Matthews A. The aetiopathogenesis of infectious keratitis in the horse. Equine veterinary journal. 1994;26(6):432-3.

[8] Moore C, Heller N, Majors L, Whitley R, Burgess E, Weber J. Prevalence of ocular microorganisms in hospitalized and stabled horses. American journal of veterinary research. 1988;49(6):773-7.

[9] McLaughlin S, Brightman A, Helper L, Manning J, Tomes J. Pathogenic bacteria and fungi associated with extraocular disease in the horse. Journal of the American Veterinary Medical Association. 1983;182(3):241-2.

[10] Nardoni S, Sgorbini M, Barsotti G, Corazza M, Mancianti F. Conjunctival fungal flora in healthy donkeys. Veterinary ophthalmology. 2007;10(4):207-10.

[11] Sgorbini M, Barsotti G, Nardoni S, Mancianti F, Rossi S, Corazza M. Fungal flora of normal eyes in healthy newborn foals living in the same stud farm in Italy. Journal of equine veterinary science. 2008;28(9):540-3.

[12] Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology E-Book: Elsevier Health Sciences; 2013.

[13] Johns IC, Baxter K, Booler H, Hicks C, Menzies-Gow N. Conjunctival bacterial and fungal flora in healthy horses in the UK. Veterinary ophthalmology. 2011;14(3):195-9.

[14] Rosa M, Cardozo LM, da Silva Pereira J, Brooks DE, Martins ALB, Florido PSS, et al. Fungal flora of normal eyes of healthy horses from the State of Rio de Janeiro, Brazil. Veterinary ophthalmology. 2003;6(1):51-5.

[15] Whitley R, BURGESS EC, Moore C. Microbial isolates of the normal equine eye. Equine Veterinary Journal. 1983;15(S2):138-40.

[16] Andrew SE, Nguyen A, Jones GL, Brooks DE. Seasonal effects on the aerobic bacterial and fungal conjunctival flora of normal thoroughbred brood mares in Florida. Veterinary ophthalmology. 2003;6(1):45-50.

[17] Araghi-Sooreh A, Navidi M, Razi M. Conjunctival bacterial and fungal isolates in clinically healthy working horses in Iran. Kafkas Univ Vet Fak Derg. 2014;20:625-7.

[18] Pisani EHR, de Moraes Barros PS, de Avila FA. Microbiota conjuntival normal de eqüinos Departamento de. Brazilian Journal of Veterinary Research and Animal Science. 1997;34(5):261-5.

[19] Andrew S, Brooks D, Smith P, Gelatt K, Chmielewski N, Whittaker C. Equine ulcerative keratomycosis: visual outcome and ocular survival in 39 cases (1987–1996). Equine Veterinary Journal. 1998;30(2):109-16.

[20] Ledbetter EC, Patten VH, Scarlett JM, Vermeylen FM. In vitro susceptibility patterns of fungi associated with keratomycosis in horses of the northeastern United States: 68 cases (1987–2006). Journal of the American Veterinary Medical Association. 2007;231(7):1086-91.

[21] Galán A, Martín-Suárez E, Gallardo J, Molleda J. Clinical findings and progression of 10 cases of equine ulcerative keratomycosis (2004–2007). Equine Veterinary Education. 2009;21(5):236-42.

[22] Voelter-Ratson K, Monod M, Unger L, Spiess BM, Pot SA. Evaluation of the conjunctival fungal flora and its susceptibility to antifungal agents in healthy horses in Switzerland. Veterinary ophthalmology. 2014;17(s1):31-6.

[23] Coad C, Robinson N, Wilhelmus K. Antifungal sensitivity testing for equine keratomycosis. American journal of veterinary research. 1985;46(3):676-8.

[24] Verneuil M, Durand B, Marcon C, Guillot J. Conjunctival and cutaneous fungal flora in clinically normal dogs in southern France. Journal de Mycologie Médicale/Journal of Medical Mycology. 2014;24(1):25-8.

[25] Brooks D. Equine keratomycosis: an international problem. Equine Veterinary Education. 2009;21(5):243-6.

[26] Liu J, Li J, Huo J, Xie H. Identification and quantitation of conjunctival aerobic bacterial flora from healthy residents at different ages in Southwest China. African Journal of Microbiology Research. 2011;5(3):192-7.

[27] Gionfriddo J, Rosenbusch R, Kinyon J, Betts D, Smith T. Bacterial and mycoplasmal flora of the healthy camelid conjunctival sac. American journal of veterinary research. 1991;52(7):1061-4.

[28] Araghi-Sooreh A, Mokhber-Dezfuli M, Mohammadi-Chorsi M. Identification of fungal isolates from conjunctival sac in healthy goats. Journal of Veterinary Research. 2013;68(4):327-32.

[29] Davidson H, Rogers D, Yeary T, Stone G, Schoneweis D, Chengappa M. Conjunctival microbial flora of clinically normal pigs. American journal of veterinary research. 1994;55(7):949-51.