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Fungal flora of the hair coat of domestic golden hamster (*Mesocricetus auratus*) with and without skin lesions in Mashhad, Iran

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Abstract:

The objective of this study was to investigate fungal flora of the hair coat of domestic golden hamsters with and without skin lesions. Sixty hamsters were examined in the Small Animal Teaching Hospital of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, and Mashhad, Iran. A sterile toothbrush was used for sample collection, from the entire body of the hamsters, all skin lesions were scraped as well with a scalpel. The mycological analysis was performed using direct microscopic examination and culture media. The direct microscopic examination and culture results were negative for dermatophytes. Of the 60 hamsters examined, 20 (33.3%) resulted in positive cultures for yeast species; of which 11 (18.3%) were *Malassezia* spp., 8 (13.3%) *Candida* spp. and 1 (1.7%) *Rhodotorula* rubra. *Rhizopus* spp. (50%), *Penicillium* spp. (38.3%), *Cladosporium* spp. (23.3%), *Aspergillus* (A.) fumigatus (23.3%), *A. flavus* (20%) and *Mucor* spp. (13.3%) were the most frequently isolated saprophytes. It is concluded that the skin of hamsters, as that of other animals, is contaminated by a variety of saprophytic fungi without concurrent skin lesions, some of which are opportunistic or allergens.

Keywords: dermatophyte, fungal flora, golden hamster, saprophyte, yeast.

Introduction

The coats of non-domestic as well as of domestic animals serve as reservoirs of different fungal organisms, some of which can cause opportunistic infections (Sparkes et al., 1993). Dermatophytes are cited among the most frequent causes of dermatological problems in pet animals. However, only a few species belonging to the genera *Microsporum* and *Trichophyton* are usually the cause of dermatophytosis in these animals (Ranganathan et al., 1997). Several studies have reported the occurrence of dermatophytes on the apparently healthy skin of pet animals (Cabanes et al., 1997; Khosravi and Mahmoudi, 2003; Romano et al., 1997). In addition to dermatophytes, many species of saprophytic fungi (predominantly *Rhizopus* spp., *Aspergillus* spp., *Alternaria* spp., *Mucor* spp., *Malassezia* spp. and *Candida* spp.) have been prominent in veterinary medicine. In this regard, when immune depressing conditions debilitates the host defense, saprophytic fungi can proliferate and elicit moderate to severe fungal infections (Khosravi, 1996). It is obvious that the prevalence of fungal flora of hair coats varies according to geographic and climatic regions; so different epidemiological studies on the isolation of fungal flora from the hair coats of different pet animals are performed (Cabanes et al., 1997; Khosravi, 1996; Moriello and Deboer, 1991; Ranganathan et al., 1997).

Golden hamster (*Mesocricetus auratus*) is a small rodent, which lives in the northern and western provinces of Iran. In recent years, although increasing attention has been paid to keep hamsters as pet animals in Iran and develop a close

relationship with humans in indoor areas, there is relatively little evidence about the zoonotic dermatoses of this animal (d'Ovidio and Santoro, 2014). To the best of our knowledge, this is the first study on fungal flora of the hair coat of domestic golden hamster carried out in Iran and our objectives were to investigate the prevalence of saprophytic fungi in the pet golden hamster as well as to determine the incidence of dermatophytes in this species.

Materials and Methods

Ethical approval: The animal maintenance and experimental protocols were approved by the Ferdowsi University of Mashhad Research Office.

Hamsters: Golden hamsters (n= 60) which had been referred to the Small Animal Teaching Hospital of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran were examined. Dermatologic examinations were performed on all hamsters and the results were recorded in the standard dermatology forms. Animals were from various parts of the metropolitan region of the city of Mashhad and were from both sexes and from 2 months to 2 years of age. They had been brought to the veterinary hospital for vaccination and routine checkup.

Sample collection: Three different sampling protocols were used. The first protocol involved using a sterile toothbrush to obtain samples from normal skin of the hamsters by hair brushing technique. The entire body of the hamsters was brushed from the head and followed by the neck, dorsum, trunk, ventrum, limbs and tail. The second protocol involved collecting small amounts of hairs from different parts of the body with a sterile pincette. In the third protocol, skin lesions were scraped using a sterile scalpel. All sample packages were submitted immediately to the mycology laboratory of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

Fungal identification: Direct microscopic examination was carried out on the samples mounted in 20% KOH/DMSO (Merck Co., Darmstadt, Germany). Samples were cultured onto Sabouraud glucose agar (Merck Co., Darmstadt, Germany) supplemented with chloramphenicol (0.005%), Mycosel agar (Merck Co., Darmstadt, Germany) and modified Dixon's agar for identification of saprophytes, dermatophytes and *Malassezia* species, respectively. Plates were then incubated aerobically at both 25ºC and 32ºC and examined daily for 2-4 weeks.

Homogenized mixtures were prepared from the hairs, which had been collected by pincette and inoculated into the media as well. Subsequently, the colonies were examined under a light microscope to determine the morphological structures of the fungi on slide mounted in lactophenol-cotton blue. *Aspergillus* species were identified following Raper and Fennell's keys (1965), while identification of other filamentous fungi was achieved to the genus level. Fungal genera were identified based on micro- and macro morphology, reverse and surface coloration and size of colonies grown on the above-mentioned media. Yeast colonies were identified for macro- and micro morphological characteristics, and based on physiological characteristics, such as germ tube test, CHROM agar, urease test, cornmeal agar containing Tween-80.

Independent variables: Relevant socio-demographic data such as age (adult/ immature), sex (male/female), bedding materials (sawdust/newspaper), the manner of keeping (solitarily/socially), the environment of protecting (house/shelter) and place of housing (cage/aquarium) were obtained by observation or from the owners through a structured questionnaire.

Data analysis: Chi-squared and Fischer exact tests were conducted to examine whether the independent variables were associated with frequency of any of the isolated fungi. A value of $P<0.05$ was considered significant for all analyses. All statistical analyses were performed using SPSS version 15.0.

Results

1. Demographic information: Sixty pet golden

Table 1. The total number and percentage of hamsters according to the animal's sex, age, bedding materials, manner of keeping, the habitat and place of housing.

Hamster Factor		n	%
Sex			
	Male	27	45
	Female	33	55
Age			
	Immature	12	20
	Adult	48	80
Bedding materials			
	Wood shavings	43	71.66
	Newspaper	17	28.33
Manner of keeping			
	Living alone	17	28.33
	Living with other hamsters	43	71.66
The habitat			
	House	28	46.66
	Shelter	32	53.33
Place of housing			
	Cage	18	30
	Aquarium	42	70

Table2. The frequency of different fungal isolates from the hair coat of 60 golden hamsters referred to the mycology laboratory of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. a Percentage of all hamsters from which organisms were isolated. b Percentage of total fungal isolates.

Fungus	Golden hamster		
	n	%a	%b
Aspergillus flavus	12	20	7.3
Aspergillus fumigatus	14	23.3	8.5
Aspergillus nidulans	3	5	1.8
Aspergillus niger	5	8.3	3
Acremonium spp.	1	1.7	0.6
Nocardia spp.	4	6.7	2.4
Alternaria alternata	4	6.7	2.4
Aureobasidium spp.	1	1.7	0.6
Chrysosporium spp.	4	6.7	2.4
Cladosporium spp.	14	23.3	8.5
Exophiala spp.	4	6.7	2.4
Malassezia spp.	9	15	5.4
Malassezia pachydermatis	2	3.3	1.2
Mucor spp.	8	13.3	4.8
Paecilomyces spp.	3	5	1.8
Penicillium spp.	23	38.3	13.9
Pseudoallescheria spp.	1	1.7	0.6
Rhizopus spp.	30	50	18.1
Rhodotorula rubra	1	1.7	0.6
Scopulariopsis brevicaulis	6	10	3.6
Scytalidium spp.	3	5	1.8
Trichothecium roseum	4	6.7	2.4
Ulocladium spp.	1	1.7	0.6
Candida albicans	5	8.3	3
Candida tropicalis	3	5	1.8
Total	165		

hamsters, 27 males and 33 females, were included in this study. Forty-eight hamsters were adult (80%) and 12 were immature (20%). Overall, 100% of the sampled hamsters were proven positive for fungi by either direct microscopic examination or culture. Age, sex, bedding materials, mode of keeping and the habitat of hamsters are summarized in Table 1. Seven (11.66%) out of 60 hamsters had skin lesions. The lesions were markedly alopecia, hyperpigmentation, skin erythema, crust and scales. Wood's lamp examination was negative in all cases.

2. Fungal isolates: The results of fungal cultures from the hair and skin samples are presented in Table 2. There were 60 positive specimens (100%), originating from 60 hamsters, 3 by microscopic examination only, 24 by culture only, and 33 on both microscopic examination and culture. Of the 60 hamsters, 36 (60%) cases were positive in direct microscopic examination. The number of fungal isolates per hamster varied from zero to six (mean = 2.45, standard deviation = 1.33). The Direct microscopic examination and culture results were negative for dermatophytes. Twenty (33.3%) resulted in positive cultures for yeast species; of which 11 (18.3%) were *Malassezia* spp., 8 (13.3%) *Candida* spp. and *Rhodotorula* rubra 1(1.7%). From 11 culture positive cases for *Malassezia* spp., two cases were identified as *Malassezia pachydermatis*. The culture positive results obtained for *Candida* spp. showed that *Candida albicans* and *Candida tropicalis* were isolated from five (8.3%) and three (5%) samples, respectively.

Rhizopus spp. (50%), *Penicillium* spp. (38.3%), *Cladosporium* spp., *A. fumigatus* (23.3%), *A. flavus* (20%) and *Mucor* spp. (13.3%) were the most frequently isolated saprophytes.

Despite not being the dominating species, *Scopulariopsis brevicaulis* (10%), *A. niger* (8.3%), *Actinomyces* spp., *Chrysosporium* spp., *Alternaria* spp., *Trichothecium* spp. and *Exophiala* spp. (6.7%), *Paecilomyces* spp., *Scytalidium* spp., *A.nidulans* and *C. tropicalis* (5%) were also recovered from the samples.

The frequency of *Rhizopus* spp. was significantly higher in the hamsters were kept on sawdust than those who were kept on newspaper ($P<0.05$). In addition, the statistical data showed that *Scopulariopsis brevicaulis* was significantly more frequent in animals that were dwelled in shelters than those were housed at home ($P<0.05$). In addition, the occurrence of *Rhizopus* spp. was significantly greater in hamsters, which were kept in social than those, which were kept solitarily ($P<0.05$). Likewise, the same fungus was more frequent in those hamsters who were housed in aquarium than those were kept in the cage ($P<0.05$). No significant differences in frequency of isolation of fungi were observed with respect to age and gender of hamsters. In addition, there was no significant difference in the number of fungal isolates between the hamsters with and without skin lesions ($P>0.05$).

Discussion

This study is the first investigation on cutaneous fungal flora in hamsters in Iran. Dermatophytosis is a common skin infection in different pet animals. In this study, dermatophytes agents were not isolated from hamsters. Similar to our findings, Bagy et al. (1997, 1998) in Egypt demonstrated that hamsters' hair had been free from true dermatophytes. In contrast, Stenwig (1985) isolated the *Microsporum canis* from hamsters; nevertheless, he reported that *M. canis* infection in the hamster could be expected. In a review by Dvorak and Otcenasek, *Trichophyton mentagrophytes* was listed as the only dermatophytes isolated from these species. In the same study, more than 30 animal hosts, including rodents, were considered susceptible to *M. canis* infection (Bagy et al., 1998; Dvorak and Otcenasek 1982). Dermatophytes are known to grow best in warm and humid environments and are therefore more common in tropical and subtropical regions. However, the geographic distribution varies with the organism (Nweze, 2011). Spontaneously occurring dermatophytosis is extremely rare in golden hamsters (*Mesocricetus auratus*) (Paterson 2006). Appraisal of similar studies by Aho (1980, 1983) on the hair coat of domestic and laboratory animals, and Khosravi (1996) on stray cats revealed the more prevalence of dermatophytes in animals with skin lesions than those without clinical signs, either. Therefore, the culture-negative results for dermatophytes in the present study may have been due to low number of hamsters with skin lesions.

Several studies have demonstrated that the frequency of positive findings is higher in pet animals with suspected dermatophytosis than in those without visible lesions (Aho, 1980; Khosravi and Mahmoudi, 2003). The data achieved by these studies and the current investigation support the opinion that dermatophytosis is seen less commonly in skin-lesion free hamsters, although the approval of a similar like statement is in the bail of direct evaluation of presence or absence of such a relation in further studies.

In the current investigation, 20 (33.3%) resulted in positive cultures for yeast species; of which11 (18.3%) were *Malassezia* spp., 8 (13.3%) *Candida* spp. and 1(1.7%) *Rhodotorula* rubra.

In the study undertaken by Bagy et al. (1997), the authors revealed that *Candida* spp. was isolated, the only isolated yeast species, from four (12.5%) hamsters. Rostami et al. (2010) reported that *Malassezia*, *Rhodotorula* and *Candida* species were isolated from nine (15%) squirrels.

Besides dermatophytes and yeasts, other mycoses have been prominent in veterinary medicine. Among these diseases are the mycoses resulting from saprobe fungi, which classically belong to skin microbiota. When the host is debilitated by a chronic disease, anticancer therapy, prolonged antibiotic treatment, steroids therapy, or immune suppressing conditions, saprobe fungi can proliferate and elicit an infection (Nichita and Marcu, 2010). Therefore, this situation associated with the improvement of diagnostic techniques, could explain, at least in part, the role of these fungi as primary pathogens. There have been fewer studies documenting saprophytic growth than studies involving dermatophytes.

In the present study, the mycological analysis of hamster hair coats demonstrated that moulds classified in 20 genera and 12 species were isolated. Bagy and Abled-Malek (1998) isolated 34 species and 2 varieties of 17 genera from the hair of Golden hamsters. They also found 23 genera and 53 species from the hair of small mammals (rabbits, guinea pigs, mice, cats and rats) in another study (Bagy, 1986). The six most prevalent species isolated from the hair coats in this study, in the order of frequency were, *Rhizopus* spp., *Penicillium* spp., *Cladosporium* spp., *Aspergillus fumigatus*, *Aspergillus* flavus, and *Mucor* spp., whereas Bagy et al. (1997) reported *Paecilomyces variotii* and *Aspergillus niger* as the most frequent fungal agents. *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., *Mucor* spp., Aurobasidium spp., *Alternaria* spp., *Scopulariopsis* spp. and *Trichothecium* spp. were recovered with high frequency. In a study by Aho (1983) on the hair of domestic and laboratory animals (cat, dog, horse, guinea pig, goat, lesser panda, mink, rat and parakeet) which basically was the same as those of Jaksch (1963) on clinically healthy and sick horses, dogs, cats, birds and rodents, although the media they had used differed with each other.

The following species of saprobe fungi, *Scopulariopsis brevicaulis*, *Aspergillus niger*, *Nocardia* spp., *Chrysosporium* spp., *Alternaria alternata*, *Exophiala* spp., *Paecilomyces* spp., *Scytalidium* spp. and *Aspergillus* nidulans were also isolated in our study.

When worldwide studies are included, the most common isolated saprophytes on the hair coats of different animals were *Penicillium*, *Aspergillus*, *Mucor* and *Cladosporium* species (Aho, 1980; Khosravi, 1996; Rostami et al., 2010; Moriello and Deboer, 1991). These fungal agents are ubiquitous in nature, are constantly in contact with animals, and considered to as secondary disease agents rather than primary factors. Interestingly, there appears to be much in common between the saprophytic organisms seen in these studies and the results obtained in our laboratory. However, these findings disagree with the view of Bagy and Abdel-mallek (1998) who reported *Paecilomyces variotii* and *Aspergillus niger* as the commonest fungal saprophytes of the hair coat of hamsters. These observations suggest that the geographical variations in the distribution of saprobe fungi may reflect climatic differences. In most reviews, *Alternaria* spp. is mentioned as one of the most frequent fungal agents recovered from the skin of animals. This mould has been described as opportunistic skin pathogen to humans and animals that is one of the most common fungal allergens and air-borne fungi in Europe (Aho, 1980). However, this mould is just isolated from four samples in this study. Bagy (1986) also had previously documented this agent as a rare frequent fungus. Oppositely, Moubasher et al. (1993) isolated *Alternaria* spp. as one of the most abundant agents from patients of skin diseases. The reason may have been either that the isolation media was used in the current study had not sufficient sensitivity, or due to the low number of symptomatic hamsters. However, the fungal flora of the skin of these animals seems not to be constant; it varies sporadically.

Although some fungal isolates were observed in a small number of hamsters and the comparison of their frequency at the various levels of independent variables due to in sufficient power. However, a significant relationship between the occurrence of some fungi and independent variables similar to bedding materials, the manner housing/keeping, the environment of protecting demonstrates that the characteristics of the environment and animal husbandry may increase the frequency of fungal flora on the coat hairs of the pet animal.

Conclusion

In conclusion, this study has clearly proven that the skin of the hamsters, as that of other animals, is contaminated by a variety of saprobe fungi without regarding the presence of skin lesions, some of which are opportunistic or allergens. Thereby producing an opportunity to become invasive on the skin or hair under particular circumstances, the presence of the opportunistic fungi on the coat hairs of these miniature pet animals and may cause

primary or secondary infections, which has serious implications on human health, considering the closeness of humans to these animals. It is also necessary to collect skin samples periodically to monitor the prevalence of these different fungal agents.

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