Journal of Animal and Veterinary Advances 5(5) : 350-355, 2006

Cutaneous Fungal Flora in Asymptomatic Stray Cats in the North of Iran

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Abstract: In Sari (north of Iran), in spite of abundant presence of stray cats, no human infection caused by *M. canis* have been reported yet. The aim of this study was to evaluate the frequency of fungal flora health carriers among the stray cats in Sari and to determine the prevalence of *M canis* carrier status in population sampled. One hundred stray cats (n=100) were captured in different parts of Sari. Age, gender and Cutaneous lesions were recorded. All cats were free of skin diseases. The specimens were obtained from coat using a sterile toothbrush. Sabouraud Dextrose Agar (SDA), Mycosel agar (SCC) and Dermatophytes Test Medium (DTM) were used for initial fungal isolations. Plucked hairs and scraped scales were examined in 10% Potassium Hydroxide (KOH) with or without 0.1% calcofluor white (KOH+CFW) for fungal elements. Seventy seven cats (77%) were adult and 23 (23%) were kittens. Fifty-six cats (56%) were male and 44 cats (44%) were female. All cats were clinically normal. Eleven identifiable genera of fungi were isolated from these cats. Nine of the genera are commonly considered as saprophytes. M.gypseum was isolated in 3 cats and Trichophyton mentagrophytes Var. mentagrophytes in one. *M. canis* was not isolated from the samples under study. The most frequent saprophytic fungal isolates were *Aspergillus*, *Cladosporium*, *Alternaria*, *Penicillium*, *Mucor*, *Rhizopus* and *Scopulariopsis* sp. It was found that, 10% KOH-0.1% CFW preparation has higher sensitivity than 10% KOH preparation test. Normal fungal flora of the coat of stray cats is principally composed of saprophytic fungi, most likely seeded from the environment and that Stray cats are not a significant reservoir of M. canis in Sari.

Key words: Microsporum canis, dermatophytes, stray cats

INTRODUCTION

Cats are considered by some authors to be the reservoir of *Microsporum canis*, which is potentially pathogenic for people[1]. Several studies[2-4] indicate that *M.canis* can be isolated from the coat in up to 88% of the feline population sampled. Those studies were conducted at facilities where studying cats had contact with large numbers of other cats. Cats and to a less extent, dogs currently appear to cause substantial environmental contamination and provoke a substantial presence of viable airborne fungal elements[5-9]. A contaminated environment acts as a source of infection and reinfection for both animals and humans[10-11] and up to 1000 arthrospores per cubic meter of air space were recovered in a house with an *M canis* infected cat[12]. Previous studies have been conducted in Iran; *M canis* was isolated from all stray kittens with clinical signs of dermatophytosis[13].

Mapping, in each city, of the natural foci of zoophilic dermatophytes may thus be important for understanding the epidemiology of human dermatophytoses and for planning preventive measures. We have surveyed fungal flora on the coat of stray cats in Sari and to determine the prevalence of *M canis* carrier status in population sampled.

MATERIALS AND METHODS

Cats: Stray cats (n=100) were captured in different parts of Sari. Age, gender and Cutaneous lesions were recorded. All cats were free of skin diseases. Average number of 12 cats was examined each month from October 2003 to May 2004. Estimation of age was done by dental formula, maturity of external sex organ and body build. Cats are classified as kittens if dental formula is Di 3/3 Dc 1/1 Dp 3/3 =28, immature external sex organ and small body, as adult if dental formula is I 3/3 C 1/1 P 4/4, M 2/3 =42, mature external sex organ and big body.

Specimen collection: Specimens were obtained from coat of each cat as described by Mackenzie[14] using a sterile toothbrush. Prior to sampling in this study, all anaesthetized cats were examined by a trained person for clinical signs of dermatophytosis. Each cat was brushed for at least 3 min or until the bristles of the toothbrush

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contained hairs. The entire cat was combed by the neck, dorsum, trunk, ventrum, limbs and tail.

Fungal culture: Sabouraud Dextrose Agar (SDA), Mycosel agar (SCC) and Dermatophytes Test Medium (DTM) were used for initial fungal isolations. Plates were inoculated by repeatedly stabbing the bristles of toothbrushes onto surface of the medium, sealed with a parafilm to prevent dehydration of the medium, incubated at 25-28°C, then examined daily for 30 days. Plucked hairs and scraped scales were examined in 10% potassium hydroxide (10% KOH) and 0.1% Calco Fluor White (CFW) (M_2 R powder from polysciences) for fungal elements. Saprophytic fungi were identified to genous level and the dermatophytes were identified to species level, if possible. Identification was performed by macroscopic and microscopic features of isolated colonies using teased mount and slide culture, special nutritional requirements (Trichophyton agar) and *in vitro* hair perforation and urease tests. Fungi not identified initially were subcultured onto rice grain medium and observed for additional 21 days. Yeasts were identified to genus level by microscopic examination and on the basis of urease test and pigmented colony on Niger seed agar.

Statistical analysis: The fisher exact probability test was done to find out whether there is significant difference in gender and age between healthy cats and carriers of dermatophytes. A p-value <0.05 was considered significant.

RESULTS

Description of cats: One hundred stray cats (Persian cats with medium and long hair) were included in this study. Seventy seven cats (77%) were adult and 23 (23%) were kittens. Fifty-six cats (56%) were male and 44 cats (44%) were female (Table 1). All cats were clinically normal; the hair coat and skin did not have dermatologic signs compatible with dermatophytosis. No Statistically significant differences were found in terms of sex and age between carrier and non-carrier of dermatophyte cats (fisher exact probability test: p=0.2237, p= 0.2258), although dermatophytes were more frequently isolated in females stray cats

Fungal isolates: eleven identifiable genera of fungi were isolated from these cats. Nine of the genera are commonly considered as saprophytes. Two fungal genera are commonly regarded to be pathogenic (*Microsporum* and *Trichophyton* sp). *M. canis* was not isolated from any cats under this study. Only 4 cultures were positive for dermatophytes of which 3 for *Microsporum gypsum* (3%) and one for *Trichophyton mentagrophytes Var. mentagrophytes* (1%). The most frequent saprophytic fungal isolation were *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus* and

Table 1: Comparison of dermatophytosis positive and negative cats

					Gender			
	Adult		Kitten		Female		Male	
	N	(%)	N	(%)	N	(%)	N	(%)
Dermatophytosis+cats N=4	2	50	2	50	3	75	1	25
Dermatophytosis-cats N=96	75	78	21	22	41	43	55	57
Total cats N=100	77	77	23	23	44	44	56	56

Table 2: Fungal flora of the hair coat of asymptomatic stray cats in north of Iran

organism	No	(%)
Aspergillus sp.	49	17.1
Cladosporium sp.	46	16.1
Alternaria sp.	44	15.4
Penicillium sp.	39	13.6
Mucor sp.	24	8.4
Rhizopus sp.	23	8.1
Scopolariopsis sp.	19	6.7
Trichoderma sp.	18	6.3
Acromonium sp.	7	2.5
Paecilomyces sp.	1	0.3
T.menta Var menta	1	0.3
M. gypseum	3	1
Unsporulated growth	12	4.2
Total isolates	286	100

Table 3: Analysis of KOH and culture of asymptomatic stray cats with dermatophytosis Sari, Iran (2003-4)

	Culture	
KOH+CFW	Positive	Negative
Positive	3 Sens=75% PPV=100%	0
Negative	1	96 Spec=100% NPV=98.8%

Sens indicate sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value

Table 4: Analysis of Culture and KOH+CFW culture of asymptomatic stray cats with dermatophytosis Sari, Iran (2003-4)

	Culture	
KOH+CFW	Positive	Negative
Positive	4 Sens=100% PPV=100%	0
Negative	o	96 Spec=100% NPV=100%

Sens indicate sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value

Scopulariopsis sp. The potentially zoonotic yeast *Cryptococcus neoformans* was not isolated. The fungal flora of the hair coat are shown in Table 2.

The results for sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of 10% KOH preparation and 10% KOH-0.1% CFW for identification of fungi are shown in Tables 3 and 4. From samples under study 4 cases were positive for arthroconidia in 10% KOH-0.1% CFW method culture wise too. Whereas 3 cases with 10% KOH preparation were positive. In addition, their cultures were positive (sensitivity = 75%, NPV = 98.8%).Thus it was found that, 10% KOH-0.1% CFW preparation has higher sensitivity than 10% KOH preparation test.

DISCUSSION

Pathogenic dermatophytes species (*M. gypseum* and *Trichophyton mentagrophytes Var. mentagrophytes*) were cultured from coat of 4 cats and none of them have clinical sign of dermatophytosis. *M. gypseum* is considered geophilic dermatophytes that occasionally infect cats[1]. The question must be addressed as to whether these cats were merely carriers of the fungi on their coat or were actually infected. It is impossible to know whether these organisms were established and actively growing on the hair coat. *Trichophyton mentagrophytes* is a cosmopolitan sp and is most commonly isolated dermatophytes from man and animal. *Trichophyton mentagrophytes Var. mentagrophytes* is zoophilic species capable of infecting humans. The major animal hosts for *Trichophyton mentagrophytes Var. mentagrophytes* are rodents, monkeys, dogs, cattle, pigs and cats[15]. Favic scutula are sometimes found when the disease is caused by *Trichophyton mentagrophytes* in cats and dogs[1]. In one study[16], the most frequent dermatophytes isolated from clinically suspect to dermatophytosis were *M. canis* (95%), *Trichophyton mentagrophytes* (2.5%) and *M. gypseum* (2.5%). Mancianti *et al.*[17] reported that 97, 2.6 and 0.2% of the isolated dermatophytes from symptomatic cats were *M.canis, M. gypseum* and *Trichophyton mentagrophytes*, respectively. In another study[6], *M. canis* (*82* of 173), *Trichophyton mentagrophytes* (3 of 173) and *M. gypseum* (1 of 173) were isolated from asymptomatic stray cats. The isolation of *Trichophyton mentagrophytes* and *M. gypseum* in our study agreement with the two above mentioned studies[6,16].

Microsporum canis is the dermatophytes most frequently recovered from canine and feline ringworm cases. Several studies[2-4,11,16] indicate that *M. canis* can be isolated from the coat in up to 100% of feline population sampled. A few studies on the hair coat of stray cats in Iran have been published as regards different geographical part of Iran. In one study[13] in the city of Isfahan (center of Iran) *M. canis* was isolated from 22 of the 96 stray cats which all of them had clinical signs of dermatophytosis. In another study[18] in the city of Tehran *M. canis* was isolated from 9% of stray cats that all of them except one were clinically healthy. These two studies had sampling and culturing techniques similar to ours. In one study[6] in Italy *M. canis* was isolated from 82 (47.4%) of 173 stray cats without clinical sign of dermatophytosis. In another study[3] in Brazil *M. canis* was isolated from up to 88% of clinical normal stray cats. In contrast to the above mentioned findings, our study indicated that *M. canis* is uncommon inhabitant of the coat of healthy stray cats. Our failure to isolate *M. canis* from the coat cat was identical to the findings of another group of investigator. In those studies[19,20] *M. canis* was not isolated from any cats. These two studies were conducted on healthy pet cats while our study carried out on stray cats. Our failure to isolate *M. canis* is completely in agreement with the previous studies that have been carried out in the north of Iran. In these studies,[21-23] there were no *M. canis* isolated from patients clinically suspected to dermatophytosis.

Previous studies in Iran, Tehran[18] and Isfahan (the center of Iran)[13] M.canis was isolated in 87.2 and 22.9% stray cats with clinical sign of dermatophytosis, respectively. The frequencies of the isolation of *M. canis* from patients clinically suspected to dermatophytosis in these regions are in accordance with the it's isolation in cats. Dermatophytes isolated from the hair coat of cats in six studies in Iran and the world is shown in Table 5. Several possible explanations exist for these discrepancies. All those studies were conducted in different regions of Iran and the world. Climate and environmental differences can not be ignored. In addition, population we surveyed in our studies comprised stray asymptomatic cats while other studies were conducted on pet cats, cats from animal's shelters, cats from catteries and cats participating in shows with and or without clinical signs of dermatophytosis. The environment and living conditions of those cats is greatly different from our cats.

In this study, the most commonly isolated saprophytes on the hair coat belonged to the genera *Aspergillus*, *Cladosporium*, *Alternaria* and *Penicillium*. However, are markable commonality exists among fungal saprophytes isolated from the hair coat of cats all over the world and Iran (Table 6). These molds have

Table 5: Dermatophytes isolated from cats hair coat in eight studies

	Geographic location							
Organism	France [11]	USA [20]	Italy Siena [6]	Mexico [29]	Iran (Tehran) [18]	Iran (Isfehan) [13]	Brazil [16]	Italy (Tuscany) [17]
M.canis	×	×	×	×	×	×	×	×
T.mentagrophytes	…	…	×	×	…	…	×	×
M. gypseum	×	…	×	×	…	…	×	×
T.terrestre	×	…	×	×	…	…	…	…
M.persicolor	×	…	…	…	…	…	…	…
T. rubrum	…	×	…	…	…	…	…	…
M.vanbreusghemii	…	×	…	…	…	…	…	…

Table 6: Saprophytic fungi isolated from cats hair coat in three studies

	Geographic location		
Organism	France [11]	USA [20]	Iran(Isfehan) [13]
Aspergillus sp.	×	×	×
Cladosporium sp.	×	×	×
Alternaria sp.	×	×	×
Penicillium sp.	×	×	×
Mucor sp.	…	…	×
Rhizopus sp.	…	×	…
Scopolariopsis sp.	×	×	…
Trichoderma sp.	…	×	…
Acromonium sp.	…	×	…
Paecilomyces sp.	…	…	…
Chrysosporium sp.	×	…	…

a worldwide distribution and are typically found in association with soil and decaying plant debris. Our finding were similar to those already reported[11,13,20,24]. Saprophytic fungi isolated are not known skin pathogens; however, *Aspergillus* and *Cladosporium* sp. have been occasionally isolated from chronic non healing wound[1,25]. Most infections are suspected to be the result of traumatic inoculation of the organism into the skin. Moriello and Deboer[20] who isolated similar saprophytes to our findings suggest that since none of the cats have developed any clinical sign of infection, it seems reasonable to assume that the cats were the carriers of the saprophytic fungi. On the basis of the findings in this survey we concluded that normal fungal flora of the coat of cats is principally composed of saprophytic fungi, most likely seeded from the environment.

In this study dermatophytes often were associated with isolation of saprophytic fungi from the coat.

In our study, we did not use a specific lipid supplemented medium for the isolation of Malassezia from coat specimens.

There was no significant difference in terms of age (p=0.2258), also no significant differences were found for sex (p=0.2237) between carriers of dermatophytes and non-carriers of dermatophytes cats, although dermatophytes were more frequently isolated in females.

One study[13] which found no significant differences in sex between *M. canis* infected and *M. canis* free cats is consistent with our findings. In this study the age of the stray cats were not investigated. In other studies[5-7,16] were found no significant differences in terms of sex between dermatophytes carriers and dermatophytes non-carriers; however, all of these studies reported dermatophytes carriers were significantly more frequent in the 1-year age group. The reason for this difference in terms of age between our findings and the above mentioned studies might be due to the fact that the dermatophyte carrier cats are lower than them.

Ringworm infections can be diagnosed by direct microscopy of hair and scrapings, wood lamp examination, skin biopsy and culture. Of these, the latter is the most reliable. Direct microscopy although false negative up to 50% of cases, is a highly efficient screening technique. Adding CFW stain to 10% KOH often reduce false negative results. Culture is a valuable adjunct to direct microscopy and is essential to identify more dermatophytes.

Our study showed that 10% KOH-0.1% CFW Preparation was more sensitive than 10% KOH preparation. This is compatible with the results of other investigators[26-28]. They showed that since CFW stain bind to cellulose and chitin and fluoresces when exposed to UV radiation, is a highly sensitive and specific technique for the detection of dermatophytes. Therefore, it is suggested that, (10% KOH-0.1% CFW) preparation can be in common use instead of 10% KOH in direct microscopy of suspected specimen to having dermatophytes.

CONCLUSION

Normal fungal flora of the coat of stray cats is principally composed of saprophytic fungi, most likely related to seeding from the environment. Our study was concerned exclusively with stray cats without clinical

evidence of lesions and showed that, stray cats are not a significant reservoir of *M. canis* and source of infections for man. Finally, dermatophytes, such as *Trichophyton mentagrophytes Var. mentagrophytes* and *M.gypseum* can be isolated from the coat. This may represent a healthy risk for humans in contact with dermatophytes carrier cats. KOH-CFW preparation is a highly sensitive and specific technique for the diagnosis of dermatophytosis in cats.

ACKNOWLEDGEMENT

We are grateful to Vice-chancellor of research for Mazandaran University of Medical Sciences for financial support. We also thank Dr. AR. Khalilian for statistical analysis.

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