Detection of dermatophytes in healthy companion dogs and cats in eastern India

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Summary

In recent times increasing occurrence of dermatophytosis, especially among the school children in eastern India was evidenced along with increased tendency of keeping companion animals such as dogs and cats. This study was undertaken to detect the occurrence of dermatophytes with antifungal susceptibility among the companion animals. A total of 1501 healthy companion animals comprising 1209 dogs and 292 cats belonged to individual owners in and around Kolkata (West Bengal, India) were examined for the evidence of dermatophytosis during 2011-2013. The collected samples were subjected to direct examination by standard KOH mount technique. The samples were inoculated into both Sabouraud dextrose agar (SDA) with 0.05% chloramphenicol and 0.5% cycloheximide and dermatophyte test medium (DTM). Each of the fungal isolate was identified based upon its colony characteristics and hyphal and conidial cells it produced. Antifungal susceptibility of the isolates was tested by broth micro dilution assay using fluconazole, ketoconazole, itraconazole, miconazole, griseofulvin and amphotericin-B antifungals. Among the 1209 samples from dogs and 292 samples from cats, 253 (20.93%) and 109 (37.33%) samples were positive for dermatophytes by direct examination. Three identified species of dermatophytes with predominant occurrence were *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. Ketoconazole (0.06-0.5 µgm/ml), itraconazole (0.03-0.5 µgm/ml) and amphotericin-B (0.03-0.5 µgm/ml) showed lowest MIC values against *M. canis*, *T. mentagrophytes* and *M. gypseum*, respectively. This is the first systemic report of dermatophytes in healthy companion animals with large numbers of samples in India.

Key words: Cat, Dermatophytes, Dog, *Microsporum*, *Trichophyton*

Introduction

Dermatophytosis is a superficial fungal infection of hair and keratinized layers of the epidermis and is caused by keratinophilic and keratinolytic genera such as *Microsporum*, *Trichophyton* and *Epidermophyton*. It is an endemic infection in many countries throughout the world affecting companion animals (dogs, cats), domestic animals (calves), and laboratory animals (rabbits) as well as humans. High animal density in a farm and close contact between the companion animals and human facilitates transmission (Lund *et al*., 2014; Samanta, 2015).

The companion animals (dogs and cats) can act as carriers of dermatophyte (*Microsporum*) spores which cannot invade the healthy skin of the animals. This carrier stage may progress to infection based on certain predisposing factors such as young age, immuno-suppression, nutritional deficiency, high environmental temperature with high humidity and skin trauma. After penetration through the damaged skin the spores germinate in the stratum corneum and the fungal metabolites induce inflammatory reaction at the site of infection (Weitzman and Summerbell, 1995).

The 'gold standard' diagnostic techniques for identification of dermatophytosis involve direct microscopic examination of clinical specimens followed by *in vitro* isolation and identification (Nardoni *et al*., 2010). The antifungals commonly used in systemic treatment of dermatophytosis in dogs and cats include itraconazole, terbinafine and griseofulvin (Gupta and Del Rosso, 2000). Currently emergence of antifungal resistant clinical isolates leads to failure in the treatment of mycosis (Alcazar-Fuoli and Mellado, 2014). Therefore, *in vitro* antifungal susceptibility test could help to optimize the therapy and select an effective antifungal agent against the clinical isolates (Araújo *et al*., 2009).

Indian human population mostly suffers from *tinea corporis* type of dermatophytosis which is associated with age, occupation, prior exposure and personal hygiene (Mahapatra, 1989; Das *et al*., 2009). However, in recent times increasing occurrence of *tinea capitis* (scalp infection) especially among the school children in the study area (West Bengal, India) was evidenced which was not associated with personal hygiene (Kundu *et al*., 2012). An increased tendency of keeping companion animals such as dogs and cats was observed in the study area and the pets are very closely associated with the daily life of their owners, especially the children.

Historically very few reports regarding the occurrence of dermatophytes in pet animals in the study area were noted (Chakrabarty *et al*., 1954; Chatterjee *et al*., 1980). Furthermore, the studies were performed with limited number of samples and it could not provide the current trend of infection and their antifungal susceptibility.

Thus the present study reported the occurrence of dermatophytes carrier state and their antifungal susceptibility in healthy companion animals (dogs and cats) which are considered as the most potent carriers in India.

Materials and Methods

Study population

A total 1501 companion animals comprising of 1209 dogs and 292 cats belonging to individual owners in and around Kolkata (West Bengal, India) were examined for the evidence of dermatophytosis during 2011-2013. The skin of the animals was examined by veterinarian for any lesion and only the healthy animals without lesions were selected. The animals were both male and female. The dogs belonged to different breeds (German Shepherd, Spitz, Labrador, Golden Retriever) whereas the cats were indigenous. The age of the animals was divided into two groups i.e., group 1 (0-6 months) and group 2 (>6 months). The samples were collected during four seasons i.e., spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). The dogs were kept indoors and they often shared the common floor, bed, sofa with their owners specifically, the children. However the cats preferred to roam outside the house during the daytime and were dirty in appearance.

Sampling

The sampling was done either at pet clinics of veterinarians or at owner's house. The hairs and unused toothbrush or hair brush after brushing the animal skin over the back, shoulders, sides, hindquarters and legs for 5-7 min were collected. Both the hair and the brushes were wrapped in a coloured paper and were kept in a tight container preferably without moisture for transport to the laboratory.

Direct examination

The collected samples were subjected to direct examination by standard KOH mount technique (Rippons, 1983).

Isolation and identification

The clinical samples were inoculated into both Sabouraud dextrose agar (SDA) with 0.05% chloramphenicol and 0.5% cycloheximide and dermatophyte test medium (DTM). The SDA plates were incubated at 28°C for four weeks and were observed periodically for appearance of fungal growth. The DTM tubes were incubated at 28°C for three weeks to detect any change in colour (Robert and Pihet, 2008).

Each of the fungal isolate was identified based upon its colony characteristics and hyphal and conidial cells it produced. The conidia were identified after lactophenol cotton blue staining on the basis of their size, shape, presence of septa, thickness of conidial wall and arrangement of conidial cells around the hyphae (Pang *et al*., 2008).

Antifungal susceptibility test

Antifungal susceptibility of the isolates was tested by broth micro dilution assay using fluconazole, ketoconazole, itraconazole, miconazole, griseofulvin and amphotericin-B antifungals (CLSI, 2002).

Statistical analysis

Differences in occurrence rates of dermatophytosis were compared according to age, sex and season using Chi-square test (SPSS Inc., Chicago, IL).

Results

Occurrence of dermatophytes in cats and dogs

Out of 1209 samples from dogs, 253 (20.93%) samples were positive for dermatophyte spores by direct examination. Out of 253 samples, deramtophytes were isolated from 248 (248/253, 98.02%) samples. Three identified species of dermatophytes were *M. canis* (43.55%), *M. gypseum* (36.69%) and *T. mentagrophytes* (19.79%) (Figs. 1-3; Table 1). Younger pups (<6 months) showed statistically highly significant (P<0.0001) levels of occurrence of the disease than the adults (>6 months). Higher rate of carriage in dogs was observed during summer and autumn than the winter and spring (P=0.0091; Table 2). Male animals showed a relatively higher occurrence of dermatophytes than the females, however, this was not statistically significant (P=0.571; Table 2).

Among the 292 samples from cats, 109 (37.33%) samples were positive for dermatophyte spores by direct examination. Further, out of 109 samples, deramtophytes were isolated from 103 (103/109, 94.49%) samples (Table 1). Three identified species of dermatophytes were *M. canis* (55.34%), *M. gypseum* (31.07%) and *T.*

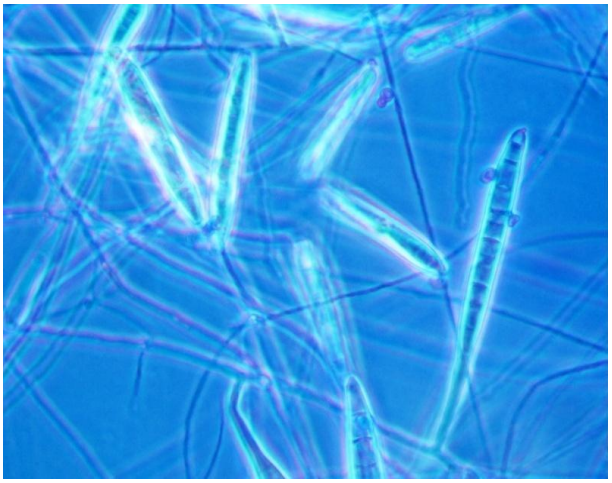

Fig. 1: Macroconidia of *M. canis* (lactophenol cotton blue) detected from healthy dogs in India

Fig. 2: Macroconidia of *M. gypseum* (lactophenol cotton blue) detected from healthy dogs in India

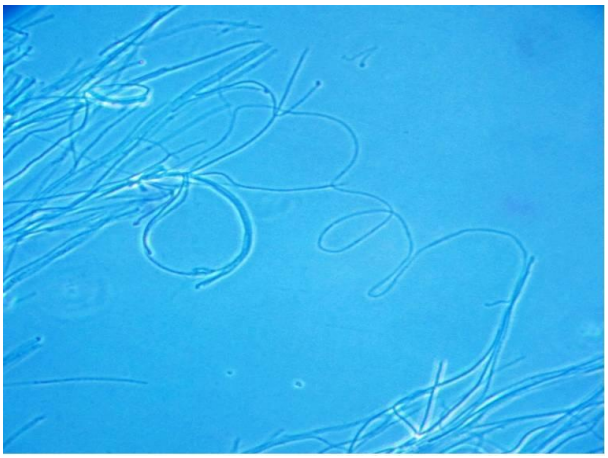

Fig. 3: Microconidia of *T. mentagrophytes* (lactophenol cotton blue) detected from healthy dogs in India

mentagrophytes (13.59%) (Table 2). The occurrence of dermatophytes in cats was significantly higher in summer and autumn (P=0.0039) than in spring and winter (Table 2). Younger (<6 months) and male cats showed a relatively higher occurrence of dermatophytes than the adult (>6 months) and female cats, however, this was not significantly different (P=0.2106 and P=0.5864; Table 2).

Table 1: Occurrence of dermatophyte species in dogs and cats in West Bengal, India

Dermatophytes	Dogs		Cats	
	n	Percentage	n	Percentage
M. canis	108	43.55	57	55.34
M. gypseum	91	36.69	32	31.07
T. mentagrophytes	49	19.76	14	13.59
Total	248	100.00	103	100.00

Table 2: Variables of age, sex and season on dog and cat dermatophytosis in West Bengal, India

Variables	No. of positive/No. of animals examined (percentage)	
	Dog	Cat
Age		
0-6	172/484 (35.54)	68/178 (38.20)
>6	76/725 (10.48)	35/114 (30.70)
	P<0.0001	P=0.2106
Sex		
Male	133/629 (21.14)	47/127 (37.00)
Female	115/580 (19.83)	56/165 (33.93)
	P=0.571	P=0.5864
Season		
Spring	14/119 (11.76)	09/53 (16.98)
Summer	103/454 (22.69)	43/94 45.74)
Autumn	117/528 (22.16)	38/87 (43.68)
Winter	14/108 (12.96)	13/58 (22.41)
	P=0.0091	P=0.0039

Table 3: *In vitro* antifungal susceptibility of dermatophyte isolates from cats and dogs in West Bengal, India

Species (No. of isolates)	Antifungal agents	MIC (µg/ml)		
		Range	MIC50	MIC90
T. mentagrophytes (63)	Fluconazole	4-64	16	64
	Ketoconazole	0.06-2	0.125	0.25
	Itraconazole	0.03-0.5	0.125	0.25
	Miconazole	0.03-1	0.06	0.125
	Griseofulvin	0.6-1	0.125	0.25
	Amphotericin-B	0.03-1	0.03	0.125
M. canis (165)	Fluconazole	4-64	16	32
	Ketoconazole	0.06-0.5	0.06	0.125
	Itraconazole	0.03-1	0.06	0.125
	Miconazole	0.03-0.5	0.06	0.25
	Griseofulvin	0.06-4	0.125	0.25
	Amphotericin-B	0.03-1	0.06	0.125
M. gypseum (123)	Fluconazole	8-64	16	32
	Ketoconazole	0.03-1	0.06	0.125
	Itraconazole	0.03-2	0.25	0.5
	Miconazole	0.03-0.25	0.06	0.25
	Griseofulvin	0.06-2	0.125	0.5
	Amphotericin-B	0.03-0.5	0.06	0.125

Antifungal susceptibility test

Antifungal susceptibility pattern of isolated dermatophytes by broth micro dilution method revealed minimal inhibitory concentration (MIC) of 6 antifungal agents for 351 (cat-103, dog-248) dermatophyte isolates. Ketoconazole (0.06-0.5 µgm/ml), itraconazole (0.03-0.5 µgm/ml) and amphotericin-B (0.03-0.5 µgm/ml) showed lowest MIC values against *M. canis*, *T. mentagrophytes* and *M. gypseum*, respectively. Further, 90% of *M. canis*, *T. mentagrophytes* and *M. gypseum* isolates were inhibited by 0.125 µgm/ml of ketoconazole (MIC_{90}), 0.25 µgm/ml of itraconazole (MIC_{90}) and 0.125 µgm/ml of amphotericin-B (MIC_{90}), respectively. Fluconazole and miconazole showed highest MIC value against the isolates indicating antifungal resistance (Table 3).

Discussion

The present study detected moderate occurrence (21%) of dermatophytes in the companion dog population in eastern India which is consistent with earlier findings throughout the world (Faggi *et al*., 1987; Seker *et al*., 2011). In corroboration with our findings, earlier reports are also available from India and abroad regarding isolation of *M. canis* as the predominant species of dermatophyte in dogs (Seker *et al*., 2011; da Costa *et al*., 2013; Beigh *et al*., 2014).

The present study detected moderately higher occurrence of dermatophytes (37.33%) in healthy cats without any skin lesions in eastern India. Similar prevalence of dermatophytosis (25-35%) in stray or companion cats was detected earlier in other countries such as New Zealand, Iran and Portugal (Carman *et al*., 1979; Khosravi, 1996; Duarte *et al*., 2010). Although lower prevalence (5%) of dermatophytosis in cats was observed in the UK and Italy (Patel *et al*., 2005; Proverbio *et al*., 2014). The unhygienic condition in which the companion cats were kept as detected in the present study is the probable reason of higher occurrence of dermatophytes. *Microsporum canis* was the most frequently isolated dermatophyte from the companion cats which is in agreement with earlier findings in India and throughout the world (Chatterjee *et al*., 1980; Duarte *et al*., 2010; Lopez *et al*., 2012).

Further, comparative occurrence rate of dermatophytes between the studied cats and dogs revealed higher occurrence in cats than the dogs which is also consistent with earlier findings (Guzman-Chavez *et al*., 2000). Probably the unhygienic conditions in which the cats were kept is responsible for more dermatophyte carriage than the dogs.

Group-1 pups (<6 months of age) showed a significantly higher occurrence ($P<0.05$) of dermatophytes than the group-2 (>6 months age). While, relatively higher occurrence of dermatophytes in group-1 cats (<6 month age) was detected than the group-2 cats (>6 months age), which was not significantly different. Higher susceptibility of young companion animals to dermatophytes was also reported earlier (Marchisio and Gallo, 1995; Seker *et al*., 2011). It might be due to their poorly developed immune system and deficiency of fungistatic linoleic acid (Al-Ali *et al*., 1997).

Further, male cats showed significantly higher occurrence of dermatophytes than the female cats. The samples were not collected equally from male and female cats which could explain the higher occurrence in male cats. Whereas, there was no significant difference of occurrence between male and female dogs. Similarly previous studies did not find the sex as a contributing factor in occurrence of dermatophytosis in cats and dogs (Guzman-Chavez *et al*., 2000; Seker *et al*., 2011; Lopez *et al*., 2012). The occurrence of dermatophytes in cats and dogs was significantly higher in summer and autumn than in spring and winter season. During summer the owners preferred to keep their dogs indoor only due to harsh external weather which could explain the higher occurrence as observed in temperate countries during winter (English, 1972).

Ketoconazole, itraconazole and amphotericin-B showed lowest MIC values against *M. canis*, *T. mentagrophytes* and *M. gypseum*, respectively. Whereas fluconazole and miconazole showed highest MIC value against the isolates, indicating the antifungal resistance. Similarly, itraconazole with lowest MIC activity (0.03-0.5 µgm/ml) and fluconazole with highest MIC activity (0-24 µgm/ml) was detected against human dermatophyte isolates (Santos *et al*., 2006; Araújo *et al*., 2009; Aktas *et al*., 2014).

Thus the present study identified companion animals like dogs and cats of West Bengal (India) as potential carriers of dermatophytes in their hair or skin without showing any clinical symptom. The infection may be transmitted to the owners, especially among the children who came in close contact and mingled with them.

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