Advances in Microbiology, Infectious Diseases and Public Health: Fungal Occurence in the Hair and Skin of Symptomatic Pets in Turin, Italy [*V.Allizond and V.Tullio contributed equally to this work;, ** A.M.Cuffini is the corresponding author]

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Abstract

Companion animals, often asymptomatic *reservoir* of fungi, can be important sources of infection in humans, due to the close contact with their owners. The present study was aimed to assess the occurrence of dermatophytes and other fungi isolated from pet dermatological lesions in Turin, Italy. Dermatological specimens were examined for fungal elements by direct microscopy and cultured to detect dermatophytes, other filamentous fungi and yeasts: 247 pets (118 cats, 111 dogs and 18 dwarf rabbits) were positive for fungal detection in culture. *Microsporum canis* was the most frequent dermatophyte in cats and dogs, whereas *Trichophyton mentagrophytes* was the most common in rabbits. Among the other fungi, for all examined pets, dematiaceous fungi were the most isolated, followed by *Mucorales*, penicilli, yeasts and yeast-like fungi, and aspergilli. No gender predisposition was detected for dermatophyte growth; on the contrary, for the other fungi male cats were more susceptible than female. The highest fungal occurrence was recorded in $<$1-year-old cats for dermatophytes, and in $<$5-year-old cats and dogs for the other fungi. Autumn was the period associated with a relevant incidence of fungal infection. Finally, fungi were more frequent in non pure-breed cats and in pure-breed dogs. These data underline the importance to timely inform pet owners about the potential health risk of infection caused not only by dermatophytes but also by non-dermatophyte fungi, routinely considered to be contaminants or harmless colonizers, since their role as source of zoonotic infections is not to be excluded.

Keywords (separated by ‘-’): Dermatophytes - Non-dermatophyte fungi - Pets - Hair and skin lesions

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Advances in Microbiology, Infectious Diseases and Public Health: Fungal Occurrence in the Hair and Skin of Symptomatic Pets in Turin, Italy

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Abstract

Companion animals, often asymptomatic *reservoir* of fungi, can be important sources of infection in humans, due to the close contact with their owners. The present study was aimed to assess the occurrence of dermatophytes and other fungi isolated from pet dermatological lesions in Turin, Italy. Dermatological specimens were examined for fungal elements by direct microscopy and cultured to detect dermatophytes, other filamentous fungi and yeasts: 247 pets (118 cats, 111 dogs and 18 dwarf rabbits) were positive for fungal detection in culture. *Microsporum canis* was the most frequent dermatophyte in cats and dogs, whereas *Trichophyton mentagrophytes* was the most common in rabbits. Among the other fungi, for all examined pets, dematiaceous fungi were the most isolated, followed by *Mucorales*, penicilli, yeasts and yeast-like fungi, and aspergilli. No gender predisposition was detected for dermatophyte growth; on the contrary, for the other fungi male cats were more susceptible than female. The highest fungal occurrence was recorded in <1-year-old cats for dermatophytes, and in <5-year-old cats and dogs for the other fungi. Autumn was the period associated with a relevant incidence of fungal infection. Finally, fungi were more frequent in non pure-breed cats and in pure-breed dogs. These data underline the importance to timely inform pet owners about the potential health risk of infection caused not only by dermatophytes but

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also by non-dermatophyte fungi, routinely considered to be contaminants or harmless colonizers, since their role as source of zoonotic infections is not to be excluded.

Keywords
Dermatophytes • Non-dermatophyte fungi • Pets • Hair and skin lesions

1 Introduction

Considering the close contact between pets and their owners, especially between children and cats and dogs, these animals, often asymptomatic carries of dermatophytes, can be important sources of infection and/or carriers of infection (Mattei et al. 2014). In addition, evidence exists that rodents, such as rabbits, may be a risk of infection for their owners and for those who work closely with them (Torres-Rodríguez et al. 1992; Hata et al. 2000; Spiewak and Szostak 2000). It is widely known that animals are the *reservoir* of many dermatophytes belonging to the genera *Microsporum* spp. and *Trichophyton* spp., and that dermatophytoses are usually disseminated among domestic animals. *M. canis*, *M. gypseum* and *T. mentagrophytes* are the main etiological agents of clinical dermatophytosis in pets (Bond 2010; Kraemer et al. 2012). The disease is characterized by alopecia, scaling and crusting; however, other filamentous fungi could mimic dermatophyte lesions rendering them indistinguishable from that of dermatophytes. These non-dermatophytic fungi isolated from animal lesions could have pathogenic potential and/or keratinolytic activity. In fact many of these species, such as *Alternaria* spp., *Scopulariopsis* spp., *Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp., are reported to be involved in fungal disease development and are increasingly recognized as agent of diseases both in animals and humans (Aho 1983; Bagy and Abdel-Mallek 1991; Seyedmousavi et al. 2015). Therefore, the aim of this report was to determine the occurrence, in Turin (Italy), of dermatophyte and non-dermatophyte fungi from living indoor cats, dogs and dwarf rabbits with lesions, referable to mycoses, for health monitoring since they are out by an appropriate health check.

2 Animals and Methods

2.1 Animals

In the period between March 2007 and November 2014, clinical dermatological specimens from 362 indoor domestic animals (195 cats, 149 dogs and 18 dwarf rabbits) were collected at Veterinary Clinics located in Turin. Pets, with suspected dermatophytosis, presented dermatological clinical signs such as scales, folliculitis, crusts and alopecic areas with variable degrees of inflammation and itch. Specimens (hair, scaling, crusts and/or skin scraping) were taken from head, abdomen, back and legs using a sterile lancet or pliers. The samples were submitted to the Bacteriology and Mycology Laboratory, Department of Public Health and Pediatrics, University of Torino, Turin, and processed.

2.2 Epidemiological Data Collection

The age, sex, breed, habitat in which animals lived and the presence of clinical signs were recorded for each animal. To assess the seasonal pattern of fungal infections, the sampling period was divided into four groups: spring (March–-May), summer (June–August), autumn (September–November) and winter (December–February).

2.3 Fungal Isolation and Identification

Specimens were examined for fungal elements by direct microscopy at 400× magnification after imbibitions in 20 % KOH. Multiple *inocula* (at least five) of the clinical specimens were cultured on Mycosel agar (MYC; Merck, Germany) to detect dermatophytes and Sabouraud dextrose agar (SAB; Sigma, St. Louis, Mo) for other filamentous fungi and yeasts. If the lesions were treated with antimycotics or covered in pus or other materials, they were first carefully washed with soap and water. The plates were incubated at 25 °C for at least 4 weeks and examined twice weekly. Cultures were held for at least 4 weeks before being considered negative. Each developing colony was isolated in pure culture on the following media: MYC (dermatophytes), Czapek's dox agar (Merck; aspergilli and penicillia), Potato dextrose agar (Merck; *Fusarium* spp.), modified Dixon agar (Merck; *Malassezia* spp.) and SAB (other filamentous fungi, yeasts and yeast-like fungi). The filamentous fungi, *Malassezia pachydermatis* and the yeast-like fungi were identified according to their colonial morphology and the microscopic appearance of the fungal elements (Raper and Fennell 1965; Rebell and Taplin 1979; Ellis 1993; Gueho et al. 1996; Guillot et al. 1996; de Hoog et al. 2000; Pitt 2000), whereas the yeasts were identified by API ID 32C (bioMérieux Italia S.p.A.; Italy).

2.4 Statistical Analysis

The chi-square test was performed for the analysis associations of the categorized variables: sex, age, season and breed. A p value of <0.05 was considered significant.

3 Results

This study included 362 symptomatic pets with marked skin lesions, characterized by alopecic areas, more or less itching, scabbed, disseminated in several body regions (head, abdomen, back, legs; data not shown), indistinguishable between dermatophytic and non-dermatophytic ones.

Out of 362 domestic animals, 282 were positive for fungal elements at direct examination and 247 were positive for fungal detection in culture (118 cats, 111 dogs and all 18 dwarf rabbits; Table 1). 54.25 % of cat samples, 38.75 % of dog samples and 27.78 % of rabbit samples were positive for dermatophytes: *M. canis* was the most frequent dermatophyte isolated from cats and dogs, whereas *M. gypseum* and *T. mentagrophytes* were isolated from 2 dogs and 5 rabbits, respectively.

The remaining fungal cultures (54.66 %; Table 1) were positive for other filamentous fungi and yeasts. In details: dematiaceous (*Alternaria alternata, Epicoccum nigrum*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *C. herbarum*, *Aureobasidium pullulans* and *Nigrospora* spp.) for 34.44 %; hyaline mycetes, represented by penicilli (*Penicillium brevi-compactum, P. griseofulvum*, *P. waksmanii*), aspergilli (*Aspergillus niger*, *A. versicolor* and *A. fumigatus*), *Trichoderma harzianum*, *T. viride* and *Fusarium* spp. for 10.11 %; *Mucorales*, represented by *Rhizopus oryzae* and *Mucor hiemalis,* for 6.07 %; yeasts and yeast-like fungi, represented by *Candida* spp., *M. pachydermatis* and *Geotrichum candidum*, for 4.04 %.

In all positive animals, males were more than females (Table 2); however no gender predisposition was detected for dermatophyte growth; on the contrary, male cats were significantly ($p = 0.0224$) more susceptible than female for other fungi. It can be highlighted the highest dermatophyte occurrence in <1-year-old cats ($p < 0.0001$) and the presence of other fungi in <5-year-old positive cats ($p < 0.0001$) and dogs ($p = 0.0276$; Table 2). All positive rabbits were less than 1-year-old. Positive samples for dermatophytes and other fungi were recorded in autumn (September–November) for all companion animals: a significant seasonal difference was detected for dogs ($p = 0.0168$; Table 2). Finally, fungi were more frequent in pure-breed dogs and in non pure-breed cats (Table 2), without statistical significant differences.

Table 1 Isolation and occurrence of fungal species (%)

	Cats		Dogs		Rabbits		Total	
	118/195[a]		111/149		18/18		247/362	
	(60.51 %)		(74.50 %)		(100 %)		(68.23 %)	
	Positive animals examined							
	n	%	n	%	n	%	n	%
Dermatophytes								
Microsporum canis	64	54.25	41	36.95	–	–	105	42.51
M. gypseum	–	[illegible]	2	1.80	–	–	2	0.81
Trichophyton mentagrophytes	–	[illegible]	–	–	5	27.78	5	2.02
Total	**64**	**54.25**	**43**	**38.75**	**5**	**27.78**	**112**	**45.34**
Dematiaceous mycetes								
Alternaria alternata	16	13.56	18	16.22	–	–	34	13.78
Epicoccum nigrum	11	9.32	14	12.61	–	–	25	10.12
Cladosporium cladosporioides	5	4.24	7	6.31	–	–	12	4.87
C. sphaerospermum	2	1.69	2	1.80	–	–	4	1.62
C. herbarum	–	–	2	1.80	–	–	2	0.81
Aureobasidium pullulans	–	–	2	1.80	4	22.22	6	2.43
Nigrospora spp.	2	1.69	–	–	–	–	2	0.81
Total	**36**	**30.50**	**45**	**40.54**	**4**	**22.22**	**85**	**34.44**
Hyaline mycetes								
Penicillium brevi-compactum	5	4.24	2	1.80	4	22.22	11	4.46
P. griseofulvum	1	0.85	–	–	–	–	1	0.40
P. waksmanii	–	–	2	1.80	–	–	2	0.81
Aspergillus niger	2	1.69	–	–	–	–	2	0.81
A. versicolor	–	[illegible]	1	0.90	–	–	1	0.40
A. fumigatus	–	[illegible]	4	3.61	–	–	4	1.62
Trichoderma harzianum	1	0.85	–	–	–	–	1	0.40
T. viride	1	0.85	–	–	–	–	1	0.40
Fusarium spp.	–	–	2	1.80	–	–	2	0.81
Total	**10**	**8.48**	**11**	**9.91**	**4**	**22.22**	**25**	**10.11**
Zygomycetes								
Rhizopus oryzae	3	2.54	5	4.50	5	27.78	13	5.26
Mucor hiemalis	2	1.69	–	–	–	–	2	0.81
Total	**5**	**4.23**	**5**	**4.50**	**5**	**27.78**	**15**	**6.07**
Yeasts and yeast-like fungi								
Candida tropicalis	1	0.85	–	–	–	–	1	0.40
C. albicans	–	–	2	1.80	–	–	2	0.81
Malassezia pachydermatis	2	1.69	3	2.70	–	–	5	2.02
Geotrichum candidum	–	–	2	1.80	–	–	2	0.81
Total	**3**	**2.54**	**7**	**6.30**	**–**	**–**	**10**	**4.04**

[a]Positive/total; n = number of cases of isolation; % = percentage frequency of occurrence (calculated per number of positive animals sampled)

4 Discussion

Over the past two decades, studies of dermatophytoses from domestic or wild animals have been described worldwide (Brilhante et al. 2003; Khosravi and Mahmoudi 2003; Cafarchia et al. 2004; Bond 2010; Kraemer et al. 2012). In some countries, such as Italy and France, *M. canis* is the most common etiological agent, whereas in Spain it varies in relation to the geographical area (Torres-Rodríguez

Table 2 Prevalence of dermatophytes and other fungi in cats, dogs and rabbits in relation to epidemiological variables[a]

		Cats				Dogs				Rabbits			
		Dermatophytes		Other fungi		Dermatophytes		Other fungi		Dermatophytes		Other fungi	
		Positivity/n	%	Positivity/n	%	Positivity/n	%	Positivity/n	%	Positivity/n	%	Positivity/n	%
Sex	Male	34/121	28.10	39/121	32.23	24/85	28.23	39/85	45.88	–	–	13/13	100
	Female	30/74	40.54	15/74	20.27	19/64	29.69	29/64	45.31	5/5	100	–	–
		$\boldsymbol{p = 0.0224}$				$p = 0.7867$				$\boldsymbol{p < 0.0001}$			
Age	<1 year	41/96	42.71	17/96	17.71	22/62	35.48	24/62	38.71	5/18	27.78	13/18	72.22
	1–5 years	16/81	19.75	33/81	40.74	9/45	20.0	25/45	55.55	–	–	–	–
	>5 years	7/18	38.89	4/18	22.22	12/42	28.57	19/42	45.24	–	–	–	–
		$\boldsymbol{p < 0.0001}$				$\boldsymbol{p = 0.0276}$				N.A.			
Seasons	Spring	14/38	36.84	9/38	23.68	4/21	19.04	12/21	57.14	–	–	4/4	100
	Summer	4/15	26.67	5/15	33.33	7/23	30.43	10/23	43.48	–	–	–	–
	Autumn	32/101	31.68	29/101	28.71	22/78	28.21	36/78	46.15	5/5	100	–	–
	Winter	14/41	34.15	11/41	26.83	10/27	37.04	10/27	37.04	–	–	9/9	100
		$p = 0.3695$				$\boldsymbol{p = 0.0168}$				N.A.			
Breed	Cross-breed	–	–	–	–	15/39	38.46	14/39	35.90	–	–	–	–
	Pure-breed	23/59	38.98	13/59	22.03	28/110	25.45	54/110	49.09	5/18	27.78	13/18	72.22
	Other breed	41/136	30.15	41/136	30.15	–	–	–	–	–	–	–	–
		$p = 0.1216$				$p = 0.1216$				N.A.			

[a]The chi-square test was used for the analysis associations of the categorized variables: sex, age, season and breed
A p value of <0.05 was considered significant

et al. 1992). In our study (Table 1) *M. canis* was the most frequent dermatophyte isolated in cats and dogs, confirming previous reports in Turin and in other sites in Italy, indicating that this fungus did not vary over the years (Marchisio et al. 1995; Mantovani 1978; Chermette et al. 2008; Bond 2010); *M. gypseum* and *T. mentagrophytes* were isolated from dogs and rabbits, respectively, underlying that these dermatophytes affect other pets (Chermette et al. 2008; Bond 2010). Additionally, our data report 5 *M. canis* isolated from asymptomatic cats (data not shown) whose owners manifested skin mycoses, indicating that cats are at present recognized as major sources of infection for their owners, confirming literature data (Cafarchia et al. 2006). As reported by Bond (2010), asymptomatic carriers cats are especially risky for humans, because no precautions are taken to prevent potential transfer; however, such cats may progress to develop overt infection and more abundant arthroconidia shedding. Infected cats have been shown to cause substantial environmental contamination and a significant airborne load of viable fungal elements, whereas dogs are of lesser importance in this regard.

Other filamentous fungi are common in the environment and their conidia are transported by air currents and settled on pet fur. Among these moulds, dematiaceous fungi and *Fusarium* spp., isolated in this study (Table 1), are nowadays well recognized as etiological agents of mycosis in animals and humans too (Bagy and Abdel-Mallek 1991; Noble et al. 1997; Huttova et al. 1998; Kluger et al. 2004; Walsh et al. 2004; Sanchez and Larsen 2007; Fan et al. 2009; Ryoo et al. 2009). For example, a case of *Alternaria* peritonitis after contact with a cat and the involvement in pet skin infections of *Fusarium* spp., a well-recognized cause of human diseases, were reported (Kluger et al. 2004; Ryoo et al. 2009). In this study *Alternaria*, *Epicoccum*, *Cladosporium* and *Fusarium* isolates probably played a role in the pathogenicity: they were no sporadic and many colonies were seen on the plates in each case.

Furthermore, we isolated some saprophytic fungi, commonly found in air and soil, such as *Mucorales* besides penicillin and aspergilli (Table 1). Albeit the recovery of these fungi was consistent with the findings of other authors (Bagy and Abdel-Mallek 1991; Keller et al. 2000; Efuntoye and Fashanu 2002; Ledbetter et al. 2007), further studies are required to verify and confirm their pathogenesis in companion animals.

Trichoderma spp., a saprophytic fungus commonly found in soil, isolated only from a cat in our study, has been reported among emerging fungal pathogens for both animals and humans (Table 1) (Kluger et al. 2004; Kantarcioğlu et al. 2009).

From a veterinary point of view, our findings related to the yeast *M. pachydermatis* from cat and dog skin lesions may have a great significance (Table 1). It can be found in very large proportion on the skin of healthy animals and it is the only lipid-independent species in the genus *Malassezia*; however since the early 1990s *M. pachydermatis* was isolated from lesions of atopic dermatitis, flea allergic dermatitis, otitis externa, pyoderma and seborrheic dermatitidis in dogs and cats (Aizawa et al. 2001; Dorogi 2002; Khosravi et al. 2010). Although *M. pachydermatis* is not normally isolated from human skin, there have been several reports of *M. pachydermatis*-associated fungaemia in infants in neonatal intensive care unit and in adults with serious internal diseases (Bond et al. 2010; ESCCAP Guideline 2011).

Literature data on sex, age, seasonality and breed are still controversial (Khosravi and Mahmoudi 2003; Cafarchia et al. 2004; Cabanes et al. 1997). With regard to the sex, from our results, in both cats and dogs no significant difference between the sexes for dermatophyte growth has been detected. Among cats, males were significantly more susceptible than females to other fungi occurrence (Table 2): this may be accounted for a different composition of sebum between males and females, as suggested by Cafarchia et al. (2004). For age, our data show that young animals are more susceptible to fungal infections (Table 2). Adult animals tend to be more resistant to infections than young animals in relation to their changes in the skin and secretions (quantity and nature of sebaceous lipids in the epidermis), hair replacement cycle, and development of an immune response to keratinophylic moulds (Bond 2010; Cafarchia et al. 2004; Rotstein et al. 1999; Khosravi and Mahmoudi 2003). Although the risk of dermatophyte infection is greater for puppies, kittens and aged or debilitated animals, the infection is not strictly age or health status-related, and so the risk continues throughout life. Consideration should be given to provide all dogs and cats with appropriate dermatophyte control throughout their lives (ESCCAP Guideline 2011). From our study autumn was the period with the highest risk for fungal infection (Table 2), according to Mancianti et al. (2002) and Iorio et al. (2007). The prevalence of non-dermatophyte and dermatophyte filamentous fungi varies according to the climate, temperature, relative humidity and rainfall of different geographical regions or natural *reservoir* (Brilhante et al. 2003; Cabanes et al. 1997; Mancianti et al. 2002; Iorio et al. 2007). Moreover, the life style such as the tendency to live in the outdoor environment in contact with soil, in groups, in isolation or in proximity to humans; the hygiene; the differences in non-specific cutaneous defenses are the general conditions related to the higher prevalence of fungal infections (de Hoog et al. 2000; Brilhante et al. 2003; Cafarchia et al. 2006). In our study in both cats and dogs there was difference in fungal isolation related to breed since fungi were more frequent in non pure-breed cats and in pure-breed dogs ($p < 0.05$; Table 2). Actually, breed is not proved to be a predisposing factor for infection (Cafarchia et al. 2006; Mancianti et al. 2002).

"The disease is not clear, unless we seek it": contact with animals or contaminated environments represents the major risk of infection for humans and people in contact with infected animals should be advised of the risk. In fact, nowadays, lack of connection between the monitoring of diseases in animals and humans is still great. The best way to bypass infection is to prevent the contact: this prophylactic strategy is very simple but not always feasible because infected animals do not show obvious clinical signs. When lesions are evident, the dermatophyte clinical lesion appearance is often indistinguishable from that caused by other fungi, suggesting the need for greater and accurate control, monitoring and identification of these last species to avoid the overestimated clinical diagnosis of dermatophytoses and to address the appropriate therapy. The role of animals as source of zoonoses in dermatophyte is widely accepted; on the contrary further investigations to evaluate the considerable zoonotic and zoopathogenic potential of other fungi, routinely considered to be contaminants or harmless colonizers, are necessary. A better understanding of diseases in pets could have direct relevance for the prevention and the fight against infectious diseases of humans.

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