FREQUENCY OF DERMATOPHYTES AND YEASTS ON TEGUMENT OF HEALTHY DOGS AND CATS

FREQUÊNCIA DE DERMATÓFITOS E LEVEDURAS EM TEGUMENTO DE CÃES E GATOS HÍGIDOS

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ABSTRACT

Skin is an organ that covers the body and acts as a protective layer against external aggressions. Changes in this organ, such as hair loss, evident blemishes, changes in skin pigmentation, and the presence of scaling or crusts, are the main signs of dermatological disease. The study aimed to verify the frequency of dermatophytes and yeasts in healthy cats and dogs. Samples of hair and shedding from 30 cats and 30 dogs were cultured on dermatophyte test medium and Sabouraud Dextrose agar enriched with yeast extract, thiamine, antibiotics (streptomycin and chloramphenicol) supplemented with cycloheximide and incubated at 25°C and 35°C for 10 days. The positive cultures were assessed macro and microscopically, and the fungi were identified by biochemical methods. It was found that 100% of the cats had a positive mycological culture for *Microsporum canis,* 33.33% for *Microsporum gypseum*, and 50% for *Trichophyton mentagrophytes*, with a prevalence of *Microsporum canis* (P>0.001). In dogs, 86.66% were positive for *Microsporum canis*, showing a predominance over the other fungal species (P<0.001). *Malassezia pachydermatis* was isolated in 50% of the dogs evaluated, although it was not found in cats, while positive cultures for *Malassezia spp* were found in 6.6% of cats and 26.66% of dogs. *Candida albicans* was isolated in samples of dogs and cats (26.66% and 33.33%, respectively). It was concluded that asymptomatic dogs and cats are carriers of agents of dermatophytosis and dermatomycoses and may be important sources of environmental spread and intra and interspecific infection.

Keywords: *Microsporum*; *Trichophyton*; *Candida, Malassezia*; Dermatophytosis.

RESUMO

A pele é um órgão que reveste o corpo e atua como uma camada protetora contra as agressões externas. Alterações neste órgão, como queda de cabelo, manchas evidentes, alteração na pigmentação na pele, presença de descamação ou crostas, são os principais sinais de doença dermatológica. O objetivo do estudo foi verificar a frequência de dermatófitos e leveduras em cães e gatos hígidos. As amostras de pelos e descamações de 30 gatos e 30 cães, foram cultivadas em meio de teste de dermatofito e ágar Sabouraud Dextrose enriquecido com extrato de levedura, tiamina, antibióticos (estreptomincina e

cloranfenicol) suplementado de cicloheximida e incubadas a 25ºC e 35°C por 10 dias. As culturas positivas foram avaliadas macro e microscopicamente, e os fungos identificados por métodos bioquímicos. Constatou-se que 100% dos gatos apresentaram cultura micológica positiva para *Microsporum canis,* 33,33% para *Microsporum gypseum* e 50% para *Trichophyton mentagrophytes*, verificando-se prevalência de *Microsporum canis* (p>0,001). Nos cães, 86,66% foram positivas para *Microsporum canis*, apresentando prevalência sobre as demais espécies fúngicas (p<0,001). *Malassezia pachydermatis* foi isolada em 50% dos cães avaliados, porém não foi encontrada em gatos, já culturas positivas para *Malassezia spp* foram constatadas 6,6% dos gatos e em 26,66% dos cães. *Candida albicans* isolada em amostras em cães e gatos (26,66% e 33,33%, respectivamente). Conclui-se que os cães e gatos assintomáticos são portadores de agentes de dermatofitoses e de dermatomicoses, podendo ser importantes fontes de contágio ambiental e de infecção intra e interespecífica.

Palavras-chave: *Microsporum*, *Trichophyton*, *Candida*, *Malassezia*, Dermatofitose.

1. Introduction

Dermatophytes are a group of imperfect keratinophilic filamentous fungi, taxonomically related, which have the capability of invading keratinized tissues of humans and animals, causing dermatophytosis, a contagious disease of high prevalence worldwide that affects these living beings, most common zoonoses worldwide [1]. These fungi have an essential enzyme system to metabolize keratin, used as a substrate to obtain nutrients and energy. They are classified into three genera: *Microsporum, Trichophyton* and *Epidermophyton* [2-4].

Dermatophytes are classified into groups, according to their natural habitat in geophilic, zoophilic, and anthropophilic [5,6]. Zoophilic fungi are primarily isolated from animals and can cause disease in humans via direct contact with cats, dogs, horses, and cattle, among others. The geophilic fungi have as a reservoir the soil and only occasionally infect humans and animals, while the anthropophilic fungi are restricted to humans and rarely infect animals [2,7].

The genera *Microsporum* and *Trichophyton* are often associated with dermatophytoses in pet animals [5,8]. The most common agents in dogs and cats are *Microsporum canis* (*M. canis*), *Microsporum gypseum* (*M. gypseum*) and *Trichophyton mentagrophytes* (*T. mentagrophytes*) [9]. *Microsporum canis* is responsible for 90% of dermatophytosis cases in cats [5] and 40 to 90% in dogs [1,10-12]. *M. gypseum* and *T. mentagrophytes* are less frequently isolated [8,13,14]. Although *M. canis* is not part of the regular microbiota of the integument of cats, these are considered natural reservoirs of this fungus species [15]. Transmission occurs by direct contact with contaminated fomites or by exposure to environments that host spores [16,17].

The genera *Candida* and *Malassezia* may also be isolated from the integument of healthy dogs and cats, however, they may cause diseases when there are immune system or host integument microenvironment changes. *Malassezia* species may be isolated from otitis in dogs and cats, and *Candida* from otitis externa and dermatomycosis in dogs [2]. This research aims to determine the prevalence of dermatophytes and yeasts in the integument of healthy dogs and cats.

2. Material and Methods

The research project that has given rise to this study was approved by the Ethics Committee on

Animal Use - CEUA/Unicastelo, under protocol no. 1-00001/2012. The present research was developed in the Microbiology laboratory of the Universidade Brasil (UB), Campus de Fernandópolis, SP.

Samples of hair and shedding from dogs and cats were collected from 30 animals of each species of both genders, of various breeds and ages. The samples were collected aseptically from veterinary clinics registered at the Veterinary Hospital of UB. During collection, the brushing technique was used; for such purpose, the animals were immobilized with their hands and the brush was gently applied.

Hair and skin shedding were examined for fungal elements by direct microscopic examination with 20% potassium hydroxide and slightly heated to check for the presence of hyphae and arthrospores.

For the isolation of dermatophytes, the samples were inoculated in dermatophyte test medium (DTM - Oxoid®) and Sabouraud Dextrose agar (ASD - Oxoid®) enriched with yeast extract, thiamine antibiotics (streptomycin and chloramphenicol) supplemented with cycloheximide and incubated at 25°C and 35°C for 10 days, being evaluated from the third day of incubation to verify the establishment of culture, although the diagnosis was made on the tenth day. The identification of dermatophytes and yeasts was based on the macroscopic examination of the colonies and by microscopic examination using lactophenol with cotton blue. Only those samples that in culture developed dermatophytes and/or *Candida* or *Malassezia* were considered positive, regardless of the results obtained in the direct microscopic examination.

Microscopic examination was carried out using the technique described by Kurnatowska et al. [18] and Kozel et al. [19]. A drop of cotton blue lactophenol was added to a plate. Afterward, a fragment was removed from the edges of the culture and deposited on the drop of dye, and subsequently a coverslip was deposited. Following this procedure, the structures were observed under the optical microscope (400x). For macroscopic identification, the characteristics of the colonies were evaluated by considering the face and reverse side of the colonies [18-21]. Whenever necessary, complementary tests were performed, such as the urease test for identification.

The yeasts, with presumptive identification of the genus *Candida*, were seeded in CHROMAGAR (Difco®) and subsequently identified by germ tube formation, urease tests, and carbohydrate fermentation (maltose, sucrose, lactose, galactose, xylose, and dextrose).

The colonies that presented glabrous texture, rough appearance, and creamy-yellow coloration, both face or reverse, were identified by the methodology described by Kindo et al. [22] and Hamdino et al. [23]. It was evaluated the growth in lipid-free media, the production of catalase, the ability to grow in the absence of lipids, the production of the enzyme catalase and the ability to assimilate different concentrations of Tween (20, 40, 60 and 80) in Sabouraud Dextrose agar (Oxoid®) supplemented with chloramphenicol and cycloheximide, and incubated at 32°C for 7 days, when growth was observed indicating assimilation of the substrate, suggesting a positive result.

The obtained data were tabulated for analysis of results by the F test in the analysis of variance and the means compared by the Tukey test with 5% probability, using the ASSISTAT software [24].

3. Results and Discussion

The present study included a total of 60 animals (30 cats and 30 dogs) with no typical lesions of dermatophytosis. All samples collected from those animals were positive for dermatophytes on direct microscopic examination, with the presence of hyaline hyphae and arthroconids. All samples were

cultured and showed growth of dermatophytes; yeasts were found in a smaller proportion (Tables 1 and 2).

It was found that 100% of cats had a positive mycological culture for *M. canis*, 10 (33.33%) for *M. gypseum* and 15 (50%) for *T. mentagrophytes*, verifying the prevalence of *M. canis* (P>0.001, Table 1). The proportions of dermatophyte isolation were considerably variable between different surveys, however, *M. canis* was isolated more frequently from pet animals [5,17]. The predominance of *M. canis* found in the present research is consistent with the results obtained by Mancianti et al. [13] and Beraldo et al. [14], who reported that 97% and 67.8% of the cats evaluated, respectively, were carriers of this dermatophyte. Some research highlights the importance of pet cats as carriers of dermatophyte spores, transmitting mainly *M. canis,* being a source of environmental contamination and infection to other animals and humans [1,25,26].

In dogs, out of thirty samples, twenty-six (86.66%) were positive for *M. canis*, showing prevalence over the other fungal species (P<0.001, Table 1). Cabañes [5] evaluated dogs with dermatophytosis, verifying the prevalence of *M. canis* (77.8%) over the other dermatophytes, similar results were obtained by Cardoso et al. [12]. These authors evaluated symptomatic and asymptomatic dogs for dermatophytosis and found that 78% of the animals with characteristic lesions were positive for *M. canis*, while 12% of the healthy ones had this dermatophyte. Frias and Kozusny-Andreani [10] evaluated 200 dogs, all without characteristic lesions of dermatophytosis, but found that 51% were positive for *M. canis*, 26% for *M. gypseum* and 2.1% for *T. mentagrophytes*. While Gangil et al. [8] found a prevalence of *M. gypseum* (57,83%) over *T. mentagrophytes* (18.3%) in dogs evaluated in India. Smaniotto et al. [27] and Romani et al. [28] found divergent results when evaluating samples collected from different body regions of dogs and found that most were negative for dermatophyte culture, with only *M. canis* isolated.

Table 1. Identification, number and percentages of dermatophytes and yeasts isolated from healthy cats.

	Cats		Dogs	
Microorganisms	**Number**	**%**	**Number**	**%**
Micosporum canis	30 a*	100,00	26 a	86,66
Microsporum gypseum	10 c	33,33	8 c	26,66
Trichophyton mentagrophytes	15 b	50,00	10 b	33,33
Candida albicans	8 c	26,66	10 b	33,33
Malassezia spp	2 d	6,66	8 c	26,66
Malassezia pachydermatis	0 d	0,00	15 b	50,00

*Similar letters in the same column do not differ statistically by Tukey's test at 5% probability. (CV%) coefficient of variation.

Candida and *Malassezia* yeasts were isolated in dogs and cats, though in lower proportions than dermatophytes in samples from cats (Tables 1 and 2). *Malassezia pachydematis* is a zoophilic microorganism, found on the skin surface and ear canal of mammal animals, occasionally isolated from the healthy skin of humans [3,17]. In the present study, this yeast was isolated in 50% (15) of the dogs evaluated, but it was not found in cats (Table 1), whereas positive cultures for *Malassezia spp* were found in 2 cats (6.6%) and in 8 dogs (26.66%). *Candida albicans (C. albicans)* was isolated in samples from 10 dogs and 8 cats (26.66% and 33.33%, respectively).

Table 2 shows the results for each dermatophyte and yeast compared to the animals tested. The prevalence of *M. canis* and *M. gypseum* and *T. mentagrofites* was observed in cats (P<0.001), while *C. albicans* and *Malassezia* species were more frequent in dogs (P<0.001). Isolation of *Malassezia* from hair and integument scales of healthy dogs is frequent since they are commensals of animal skin [17,27,28].

Table 2. Proportion of each dermatophyte and yeast in healthy dogs and cats.

	Cats	Dogs	CV%
Microorganisms	**Number**	**Number**	
Micosporum canis	30 a*	26 b	4,37
Microsporum gypseum	10 a	8 b	8,61
Trichophyton mentagrophytes	15 a	10 b	7,71
Candida albicans	8 b	10 a	8,61
Malassezia spp	2 b	8 a	14,14
Malassezia pachydermatis	0 b	15 a	14,91

*Similar letters in the same column do not differ statistically by Tukey's test at 5% probability. (CV%) coefficient of variation.

The results of the culture of samples collected from dogs and cats showed the occurrence of associations between dermatophytes and yeasts (Figures 1 and 2), except for three cats and two dogs, from which only *M. canis* was isolated. The prevalence of the association between *M. canis* and *T. mentagrophytes* was observed in cat samples (P<0.001, Figure 1), while in dogs, the associations between *M. canis, T. mentagrophytes* and *Malassezia pachydermidis* and between *M. canis, T. mentagrophytes* and *C. albicans* prevailed (Figure 2), expressively significant when compared to the other associations (P<0.05).

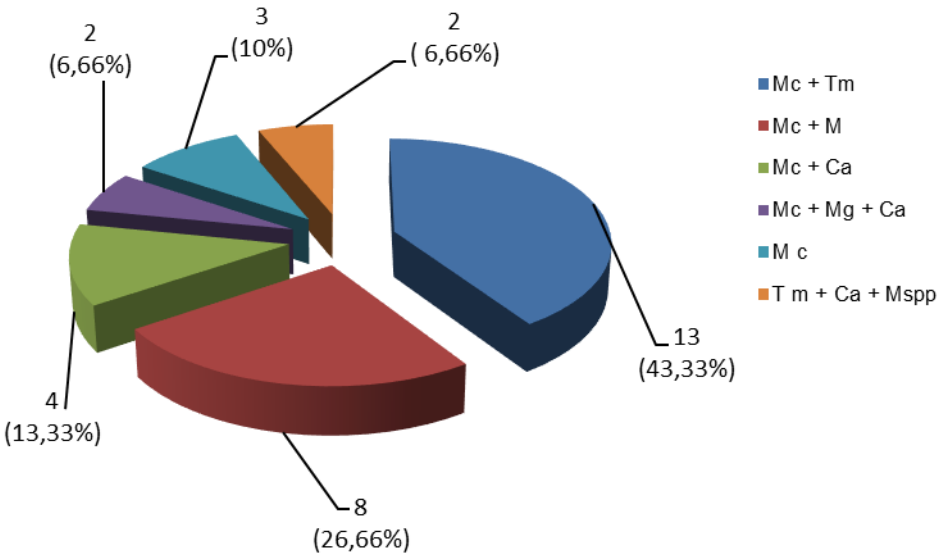

Figure 1: Frequency of dermatophyte and ledure isolates on the integument of healthy cats. (Mc) *Microsporum canis*; (Mg) *Microsporum gypseum*; (Tm) *Trichophyton mentagrophytes*; (Ca) *Candida albicans*; (Mp) *Malassezia pachydermidis*; (Mssp) *Malassezia spp.*

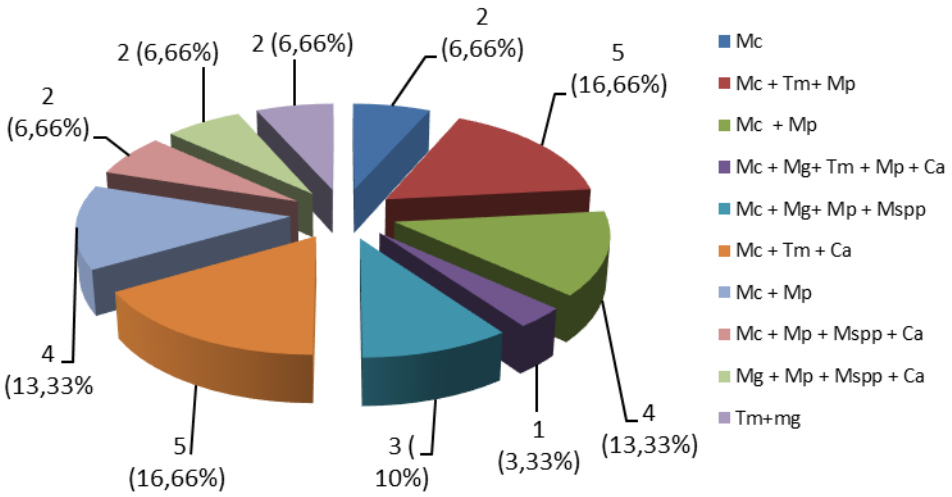

Figure 2: Frequency of dermatophyte and yeast isolations from tegument of healthy dogs. (Mc) *Microsporum canis;* (Mg) *Microsporum gypseum* (Tm) *Trichophyton mentagrophytes*; (Ca) *Candida albicans*; (Mp) *Malassezia pachydermidis*; (Mssp) *Malassezia spp.*

In most studies on dermatophytes conducted in cats and dogs in different countries, the most frequently isolated species are *M. canis, M. gypseum* and *T. mentagrophytes* [1,8,10-12,14]. The prevalence of dermatophytes and dermatophytosis in dogs and cats, presumably is related to temperature and humidity, varying with location, season, and climate variations [11].

The results of this study showed that asymptomatic dogs and cats are carriers of dermatophytosis and dermatomycosis agents and may be important sources of environmental contamination and intra and interspecific infection [1], requiring efficient prophylactic measures to prevent the spread of these microorganisms.

4. Conclusion

The study revealed the prevalence of *M. canis* on the integument of healthy dogs and cats. *M. canis*, as well as the isolated species *M. gypseum, T. mentagrophytes, C. albicans, Malassezia pachydermidis*, and *Malassezia spp* are associated with the integument of dogs and cats, and the integument of these animals also shows associations of dermatophyte species and yeast.

Authors' Contributions

Surpilli FO.: participation in the development of the paper, drafting and writing of the textual chapters, formatting and final proofreading; *Kozusny-Andreani DI*.: drafting and writing of the textual chapters, formatting, final proofreading, and author approval; *Sousa UR*.: conception and design; *Ramos RR*.: conception and design and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflictsof interest.

Ethics Approval

Approved by the Ethics Committee on Animal Use - CEUA/Unicastelo, state of São Paulo, Brazil, (Protocol 1-00001/2012).

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Not applicable.

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