Occurrence of *Malassezia* Species in Persian and Domestic Short Hair Cats with and without Otitis Externa

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ABSTRACT. The yeasts of the *Malassezia* genus are opportunistic microorganisms in the skin and auricular canal of human and animals, mainly cats, and can cause otitis externa and dermatitis disorders. The aim of this study was to evaluate the occurrence of different species of *Malassezia* in the external ear canal of cats with and without otitis externa. Thirty-one normal cats and 82 animals with otitis externa were clinically examined. Sterile cotton swabs were used to collect specimens from the external ear canal and streaked onto the surface of Sabouraud dextrose agar (SDA) and modified Dixon agar. *Malassezia* yeasts were isolated from 95.1% and 48.4% of the cats with and without otitis externa, respectively. The rate of isolation in affected animals versus normals was highly significant (P<0.05). Out of the 137 isolates obtained from cats with otitis, 57.7% were identified as *M. pachydermatis* (with significant frequency; P<0.05), 15.4% as *M. obtusa*, 11.4% as *M. globosa*, 7.3% as *M. sloffiae*, 4.1% as *M. sympodialis*, 2.4% as *M. furfur* and 1.6% as *M. restricta*. *Malassezia* species were frequently isolated from subjects with age range from 1to 4 years old (42.7%). Our finding of *Malassezia* isolates indicated that feline otitis externa can be associated with lipid-dependent *Malassezia* species in addition to the non lipid-dependent species *M. pachydermatis*.


Feline otitis externa is a very common and etiologically complex disease. Because of the availability of several predisposing factors, such as humidity, pH, allergies, foreign bodies and otoacarisis, the external ear canal of the cat provides an ideal environment for the growth of different microorganisms [2]. Other factors to be considered in regard to microbial infections include antimicrobial agents, corticoid therapy, hormonal/nutritional disorders, and pathological complexes, such as neoplasia and immunosuppressing diseases. Yeasts and bacteria are considered to be normal constituents of the feline ear microflora [6].

The yeasts of the *Malassezia* genus are common commensal organisms of the skin of humans, birds and many domestic and wild animal species [7, 10, 20]. The genus has been recently revised on the basis of molecular data [14] and lipid requirements [17] and today 10 lipid-dependent species are known (i.e. *M. dermatitis*, *M. equi*, *M. furfur*, *M. globosa*, *M. japonica*, *M. nana*, *M. obtusa*, *M. restricta*, *M. sloffiae* and *M. sympodialis*), together with *M. pachydermatis* which is not dependent on lipid supplementation for the growth [19].

Changes in the microclimate can induce the growth of *Malassezia* species, which may cause clinical diseases. The most common dermatological disorders of the human skin, such as atopic dermatitis, dandruff, folliculitis, pityriasis versicolor or seborrhoeic dermatitis and intravascular catheter-acquired infections [1, 21]. The pathogenic role of these yeasts has been recognized in various animals, mainly otitis externa and dermatitis disorders [9, 15]. In recent years some lipid dependent species, such as *M. furfur*, *M. globosa* and *M. sympodialis* have been isolated from the ears of healthy and diseased dogs and cats [8, 24]. Nevertheless, very little is known about the frequency and population size of *Malassezia* species in 2 predominant breeds of cats living in Iran including Persian and domestic short hair cats. The purpose of this study was to investigate the occurrence and identification of different *Malassezia* species in the acoustic meatus of cats with and without otitis externa.

MATERIALS AND METHODS

Animals: A sum of 113 domestic cats (75 females and 38 males) ranging from 8 months to 11 years old and from 2 different breeds, Persian cats and short hair cats, were presented at Small Animal Hospital, University of Tehran, Iran. The animals were divided into 2 groups:

A) Normal cats: 31 owned animals in good general health, with no history of ear diseases and no drug treatment in the preceding 6 months; B) Cats with otitis externa: 82 animals with clinical signs, such as external ear canal inflammation and ear discharge, thickening of the ear canal, head shaking and pruritis.

Sample collection: After each cat was immobilized, its external ear canal was disinfected with an alcohol:ether (1:1) solution. Cerumen or the secretion from one ear of each animal was collected using sterile cotton swabs. All chemicals used, unless otherwise stated, were obtained from Merck Company (Darmstadt, Germany).

Isolation and identification of *Malassezia* species: The swab with the sample was streaked onto the surface of Sab-
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Our aud dextrose agar (SDA) and modified Dixon agar. The plates were incubated at 32°C for 4–7 days. *M. pachydermatis* was identified by morphology microscopically and by the ability to grow on SDA. The identification of the lipid-dependent yeasts was based on the ability to use certain polyoxyethylene sorbitan esters (Tweens 20, 40, 60 and 80), as described by Gue’ho et al. [14], catalase reaction proposed by Guillot et al. [17]. The Cremophor EL assimilation test and the splitting of esculin described by Mayser et al. [22] and precipitate production on mDixon agar reported by Hammer and Riley [18] were used as additional tests (Table 1).

The tween test was carried out by a preparation of 2 ml of 10^5 cells/ml yeast suspension that was mixed with 16 ml of Mycosel agar at 40–50°C. The mixture was homogenized and poured into Petri dishes. After the medium solidified, 4 μl of Tween 20, 40, 60 and 80 (Sigma Co., St. Louis, MO, U.S.A.) was added to each plate at equidistant points and 4 μl of Cremophor EL was placed at the center. All cultures were incubated at 32°C for 7 days. Presence of catalase was determined on a glass slide; one drop of 10-volume hydrogen peroxide (H₂O₂) was added to a small inoculum of the yeast. The production of bubbles indicated a positive reaction. The identification method described by Guillot et al. [17] permits figure out some characteristics of the each *Malassezia* species isolated in the present work that favor a correct and security identification, to name a few, *M. pachydermatis* is the only *Malassezia* species that grows in a medium without the addition of lipid; *M. furfur* is the unique species able to assimilate Cremophor EL and to use all kind of tweens as a lipid source; *M. globosa* strains present an exclusive globose shape of its cells when visualized by common optical microscopy after Gram staining. Besides this, *M. globosa* is not able to assimilate any kind of tween as a lipid source. *M. sympodialis* presents a characteristic sympodial budding; it may be differentiated from *M. furfur* by its inability to grow on glucose/peptone agar with 10% tween 20.

### RESULTS

The present study was aimed to detect *Malassezia* species and identify the respective species isolated from the external ear canal of Persian (19) and domestic short hair (63) cats with otitis externa (totally; 82) referred to Small Animal Hospital of Tehran. *Malassezia* species positive cultures were obtained from 78 of 82 diseased animals (95.1%) and from 15 of 31 (48.4%) healthy cats, representing significant difference between 2 groups (P<0.05) (Fig. 1). A total number of 52 lipid-dependent and 71 non-lipid isolates were obtained from the present survey were analyzed by means of the chi-square test. A *P* value less than 0.05 was considered significant.

### Table 1. Characteristics of different *Malassezia* species.

<table>
<thead>
<tr>
<th>Characteristics</th>
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<th>b</th>
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<tr>
<td>Growth on SDA* at 32°C</td>
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<td>+</td>
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<tr>
<td>Growth on mDixon at 40°C</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<td>Catalase reaction</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
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<td>Use of lipid sources:</td>
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<tr>
<td>– Tween 20 (10%)</td>
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<td>– Tween 40 or 60 (0.5%)</td>
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<td>+</td>
<td>+</td>
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<td>–</td>
<td>+</td>
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<tr>
<td>– Tween 80 (0.1%)</td>
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<tr>
<td>– Cremophor EL</td>
<td>+</td>
<td>v</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Hydrolysis of esculin</td>
<td>–</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Precipitate production on mDixon agar</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>–</td>
<td>NT</td>
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* Sabouraud dextrose agar (SDA) without any lipid supplementation.

### Statistical analysis

Data obtained from the present survey were analyzed by means of the chi-square test. A *P* value less than 0.05 was considered significant.

![Fig. 1. Comparison of percentage of *Malassezia* yeasts isolated from external ear canal of cats with and without otitis externa in 2 different breeds.](image)

[Image of a bar graph showing the percentage of *Malassezia* yeasts isolated from external ear canal of cats with and without otitis externa in 2 different breeds.]
recovered from diseased animals during this study. From 78 cats with otitis externa presenting Malassezia species, 57.7% were *M. pachydermatis*, 15.4% were *M. obtusa*, 11.4% were *M. globosa*, 7.3% were *M. slooffiae*, 4.1% were *M. sympodialis*, 2.4% were *M. furfur* and 1.6% were *M. restricta* (Table 2). The identification of the different species gave the following results. *M. pachydermatis* was isolated as a pure culture in 33 cases and associated with other Malassezia species in 35 diseased cats; *M. pachydermatis* was associated with *M. obtusa* in 19 cases, with *M. sympodialis* in 5 cases, with *M. restricta* in 2 cases and with both *M. globosa* and *M. slooffiae* in 9 cases. *M. globosa* and *M. furfur* were also recovered from 5 and 3 animals as pure cultures, respectively.

Subjects with age range from 1 to 4 years old were more affected and represented 42.7% of the positive results. No statistically significant difference related to age and sex of subjects was observed, when analyzing all Malassezia-positive samples.

**DISCUSSION**

Malassezia species have been recognized as members of the microbiological flora of human and animal skin; they are also considered to be aetiological agents of otitis externa in different animals [10]. In our study, Malassezia species positive cultures were obtained from 95.1% of diseased animals and 48.4% of healthy cats, indicating significant difference between them. Data available in literature showed prevalence ranging from less than 10% to 23% in healthy subjects [3, 13, 16, 26], and from 19% to 41.2% in animals affected by otitis [9, 13]. The higher frequency of Malassezia yeasts reported in animals with otitis compared to healthy animals indicates that these yeasts overgrow in infection sites and play a role in the pathogenesis of otitis externa. The overgrowth of these yeasts in infection sites has been demonstrated to be an important factor in inducing disease as well as the production of phospholipase enzymes [4–6]. Our results showed higher frequency of Malassezia species compared to other investigations and this fact could be associated with the sampling technique used, the sampling period, the choice of culture media and the use of 2 different media to allow the isolation of lipid dependent species.

Interestingly, *M. pachydermatis* was the species more isolated from acoustic meatus of both cats with otitis (86.6%) and normal cats (13.4%). This species has an opportunistic nature, and it may become pathogenic with any alteration in the skin surface microclimate or in host defense as well as can play an important role in chronic dermatitis and otitis externa in carnivores, especially in cats [13, 24, 25]. The frequency of isolation of this species from the external ear canal was reported to be 15% to 49% for healthy animals and may increase up to 50% to 83% for subjects with otitis externa [13], which are in close consistent with our findings. Skin of different animals can be colonized by lipid-dependent species in addition to *M. pachydermatis* [16]. The following lipid-dependent species were isolated from diseased cats: *M. globosa*, *M. restricta*, *M. furfur*, *M. slooffiae*, *M. obtusa* and *M. sympodialis*. After investigation, mixed yeast flora was determined. The isolation of *M. pachydermatis* together with lipid-dependent species from different animals with otitis externa were previously reported by several investigators; from cats and dogs by Crespo et al. [8, 9], from cattle by Duarte et al. [11], from lions by Coutinho et al. [7], from horses and goats by Crespo et al. [10] and from bats by Gandra et al. [12]. *M. slooffiae* seems to represent a large proportion of the cutaneous yeast flora in pigs, and it has been isolated from normal skin in sheep and goats as well [14]. In our study, this spe-
cies was isolated from 9 cases of diseased cats, which has not been demonstrated in cats in previous reports. The isolation of lipid-dependent species from cats might suggest a potential role of these animals as carriers for humans. Another characteristic of subjects, such as age, was also evaluated with regards both to the prevalence of lipophilic yeasts and to the species of Malassezia isolated, but no significant correlation was found.

Our finding of lipid-dependent isolates indicated that feline otitis externa can be associated with lipid-dependent Malassezia species in addition to the non lipid-dependent species M. pachydermatis. Finally, the data acquired in this study provide helpful insights into the occurrence of Malassezia species in cats with and without otitis and confirm the importance of both microscopic examination of stained smears from otic swabs and different culture media with lipid sources, such as SDA supplemented with olive oil and mDixon agar for the diagnosis of Malassezia infections.

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REFERENCES