Isolation of Malassezia spp. from cerumen of wild felids

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The objective of this study was to determine the presence of different species of the genus Malassezia in the healthy external auditory canal of wild felids maintained in captivity. One hundred and thirty-two adult animals (264 samples of cerumen), 77 males (58.3%) and 55 females (41.7%), were studied: large felids (55 animals) – 26 lions (Panthera leo), 13 tigers (Panthera tigris), 6 leopards (Panthera pardus), 6 jaguars (Panthera onca), 2 cheetahs (Acinonyx jubatus), 2 pumas (Puma concolor); small felids (77 animals) – 29 tiger cats (Leopardus tigrinus), 19 jaguarundis (Herpailurus yagouaroundi), 10 margays (Leopardus wiedii), 9 pampas cats (Oncifelis colocolo), 6 Geoffroy’s cats (Oncifelis geoffroyi), and 4 servals (Leptailurus serval). Samples were obtained by the introduction of a sterile swab into the ear canal after cleaning the auricle with an alcohol-ether solution. The swabs were seeded onto Petri dishes containing modified Mycosel agar and sterile olive oil was added to the surface of the medium before specimen seeding. The plates were incubated at 35°C for two weeks. The isolates were analyzed regarding macro- and micromorphology and identified through catalase tests and growth on Tween 20, 40, 60 and 80. Malassezia spp. were isolated from 58 of the felids studied (43.9%) and from 102 samples of cerumen (38.6%). Malassezia sympodialis was isolated exclusively in large felids (33 animals – 56.9%), and Malassezia pachydermatis exclusively in smaller varieties (25 animals – 43.1%). The incidence of fungi was higher in lions, with yeast being isolated in 25 of 26 animals (96.2%). Forty-eight strains (47.1%) were isolated from the right ear canal and 54 (52.9%) from the left. Although M. pachydermatis is the species considered a member of the microbiota of the mammalian external ear canal these results suggest that M. sympodialis participates in the microbiota of large felids.

Keywords Malassezia pachydermatis, Malassezia sympodialis, wild felids, microbiota, ear canal.

Introduction

Malassezia pachydermatis is part of the microbiota of the mucosae and skin of mammals, and is frequently isolated in the auditory canal and hair coat of healthy dogs and cats [1–5] and of many domestic and wild animal species [3,6,7].

Although M. pachydermatis is a commensal microorganism, some conditions lead to its exacerbated growth, causing clinical disease. The disease reflects alterations in the physical, chemical and/or immunological mechanisms that usually restrict the colonization of this yeast. Thus, conditions such as antibiotic and corticosteroid therapy, allergies, seborrheic dermatitis, nutritional or hormonal disorders, and complex diseases such as neoplasias are considered to be predisposing factors for Malassezia dermatitis [5,8–10].
Yeast isolates of the genus *Malassezia* are able to utilize lipids as a carbon source (lipophilic) and require the presence of long-chain fatty acids for their growth (lipodependent). One exception is *M. pachydermatis*, which is able to grow on media without the addition of lipids [11–13].

In 1996, Gueho et al. [11] revised the genus *Malassezia* using morphological, ultrastructural, physiological and molecular biological methods, and added four new species (*M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*) to the three previously established members (*M. furfur*, *M. pachydermatis* and *M. sympodialis*). Therefore, the genus consists of seven recognized species, all of them lipodependent, except for *M. pachydermatis*. In recent years, authors have suggested that three other species, *M. dermatis*, *M. japonica* and *M. nana* [14–16], might be included in the genus.

Since *M. pachydermatis* is considered to be zoophilic and is the only non-lipodependent species, veterinary diagnostic laboratories usually employ common mycology culture media, such as Sabouraud dextrose agar, for the isolation of this microorganism and not media supplemented with lipids as required by the other species. However, at the research level the other species have been isolated in both healthy (microbiota) and diseased animals.

A study carried out in the State of Minas Gerais, Brazil, investigating the presence of these yeasts in the external ear canal of cattle, demonstrated not only *M. pachydermatis*, but also other species of the genus in healthy animals and in those with otitis [17].

*M. furfur*, *M. obtusa* and *M. sympodialis* were recently isolated in dogs and cats with otitis [18,19], and the presence of *M. furfur*, *M. globosa* and *M. sympodialis* in the microbiota of the skin, mucosae and ear canal of domestic felines has been reported [20–22]. Dizotti and Coutinho (2004) [23], investigating the external ear canal of 45 cats (20 with otitis and 25 healthy animals), isolated *M. pachydermatis* and *M. sympodialis* in the two groups, where the isolation frequency of the two species was significantly higher in the group of animals with otitis.

These observations broaden the perspective that not only *M. pachydermatis* but also other, formerly unrecognized species of the genus, are members of the microbiota of domestic and wild mammals. It would therefore be of interest to determine which *Malassezia* species are really part of the microbiota of domestic and wild mammals [12,20,21], since this knowledge would permit a clearer understanding of the detection of these yeasts in veterinary clinical practice, considering that the pathogenic mechanisms of lipodependent species are probably similar to those of *M. pachydermatis*, with these species becoming pathogenic in response to the same predisposing factors [19].

Since no studies are available in the literature determining the presence of these yeasts in the external ear canal of wild felids and the studies were successful in isolating different species in domestic felines, the objective of this investigation was to identify *Malassezia* spp in the microbiota of the external ear canal of wild felids maintained in captivity.

### Materials and methods

#### Animals

One hundred and thirty-two adult animals (264 samples of cerumen – left and right ear canal), 77 males and 55 females, maintained by the Fundação Parque Zoológico de São Paulo, were studied: large felids (55 animals) – 26 lions (*Panthera leo*), 13 tigers (*Panthera tigris*), 6 leopards (*Panthera pardus*), 6 jaguars (*Panthera onca*), 2 cheetahs (*Acinonyx jubatus*), 2 pumas (*Puma concolor*); small felids (77 animals) – 29 tiger cats (*Leopardus tigrinus*), 19 jaguarundis (*Herpailurus yagouaroundi*), 10 margays (*Leopardus wiedii*), 9 pampas cats (*Oncifelis colocolo*), 6 Geoffroy’s cats (*Oncifelis geoffroyi*), and 4 servals (*Leptailurus serval*).

#### Samples

The anesthetic protocol was the same as that used for routine handling of felids in this zoo. The animals were sedated using anesthetic darts containing 1.0 mg/kg weight xylazine and 10.0 mg/kg weight ketamine. If more prolonged relaxation was required, 0.5 mg/kg diazepam was applied.

Samples were obtained by the introduction of a sterile swab into the auditory canal after cleaning the auricle with an alcohol-ether solution.

#### Yeast isolate

The swabs were seeded onto Petri dishes containing Mycosel agar modified by the addition of glucose at 4% final concentration, 2% malt extract, 0.5% glycerol, and 500 mg/L of chloramphenicol. Sterile olive oil was added to the surface of the medium before specimen seeding. The plates were incubated at 35°C for two weeks.

#### Colony identification

Colonies were studied for macro- and micromorphology to confirm genus characteristics [11,24]. Catalase reactions and Tween 20, 40, 60, and 80 utilization were
performed to identify lipodependent colonies [11,24]. In the Tween test, the medium recommended by the present authors was used [11,24]; however, these substances were directly placed on the agar surface (4 μL) at four equidistant points on the plate. *M. pachydermatis* strains were identified by macro- and micromorphology and their ability to grow on Sabouraud dextrose agar, without the addition of lipids [11,24].

**Statistical analysis**

In order to analyze the results obtained, the χ² test was applied [25]; the rejection level of the null hypothesis was fixed at 0.05 or 5% (α ≤0.05).

**Results**

Table 1 shows the results of the isolation of *Malassezia* spp. from the external ear canal of wild felids. *Malassezia* spp. were isolated in 58 (43.9%) animals and 102 (38.6%) of the samples studied. *M. sympodialis* (Fig. 1) was isolated in 33 (56.9%) from positive animals and *M. pachydermatis* in 25 (43.1%), with no significant difference in the frequency of isolation between the two species.

*M. sympodialis* was isolated from 25 lions (43.1%), four jaguars (6.9%), two tigers (3.4%), and two leopards (3.4%). The frequency of *M. sympodialis* was significantly higher in the lions group, with the microorganism being isolated in 96.2% of these animals. *M. pachydermatis* was isolated in 17 tiger cats (29.3%), four pampas cats (6.9%), two margays (3.4%), and two servals (3.4%). All *M. sympodialis* strains were isolated in large felids and all *M. pachydermatis* in small varieties.

No difference in the frequency of the *Malassezia* spp. was observed between genders or the ear canal analyzed (right or left).

**Discussion**

The minor modifications of the Tween test, placing the substances directly on the agar surface, simplified the technique and did not compromise the readings.

The percentage of *Malassezia* spp. isolated from the microbiota of wild felids (43.9%), except for the lions group, was close to that reported for several domestic animal species, in which these frequencies show wide variations but never exceed 50–60% [2,17,26–28]. The high frequency of *Malassezia* spp. from the microbiota of the external ear canal of lions (96.2%) is notable, because this percentage is significantly higher than that reported for the other wild felids, including both large (27.6%) and small varieties (32.5%), and even higher than that reported in surveys of animals with external otitis [17,27,28]. However, it is not possible to compare the results obtained here as no studies like the present one performed on wild felids are available in the literature.

In this investigation, *M. sympodialis* was exclusively isolated from large felids and *M. pachydermatis* from small varieties. The fact that *M. pachydermatis* is the main species found in the microbiota of the ear canal of domestic felines [2–4] and that cats are the closest phylogenetic relatives of small wild felids [29], might explain the isolation of exclusively *M. pachydermatis* in this group.

With respect to the exclusive isolation of *M. sympodialis* from large felids, suggests that this species is an integrated part of the microbiota of the ear canal of...
these animals. Although all felids examined in the present survey belonged to the same institution, they did not share the same physical space. The animals were kept in separate pens, distant from one another, with concrete or soil floors, thus reducing the acquisition of a particular microbiota due to the absence of social contact. However, it would be interesting to perform a similar study on animals not held in captivity, in order to determine whether the same occurs in nature.

The 43.9% frequency of *Malassezia* spp. in the ear canal of wild felids is significant since, although the animals studied showed no clinical signs of external otitis, it is believed that any species of the genus may exacerbate their multiplication in situations of imbalance and play a pathogenic role in infections [17,19].

Until a few years ago, *M. pachydermatis* was considered to be the only species of the genus belonging to the microbiota of both domestic and wild animals [3,9] and also the etiological agent of both the otitis and dermatitis processes [2,3,9]. This aspect is important because *Malassezia* lipodependent species have been isolated in research laboratories, in different studies conducted on both healthy and diseased animals [18–20,22,27]. The present investigation clearly demonstrates the urgent need for the adaptation of *Malassezia* spp. isolation techniques for commercial laboratory diagnosis, since the use of media without the addition of lipids for the determination of *Malassezia* spp. might yield false-negative results in veterinary clinical samples.

Although the final characterization among some *Malassezia* species is determined through the use of genetic studies, the objective of this work was to employ isolation and identification techniques that are feasible in clinical diagnostic laboratories and not only at the level of research. The phenotypical identification methods have been used by several researchers because they permit a satisfactory identification scheme for *Malassezia* species and current publications have cited these methods in scientific journals, including *Medical Mycology*, attesting to the validity of these techniques.

Finally, we should emphasize the importance of studies like the present one which, in addition to clarifying unknown aspects of *Malassezia* spp., open perspectives for new questions and projects aimed at a clearer understanding of the different characteristics of these yeasts, may help the veterinarian in the diagnosis of, and therapeutic approach, to this disease.

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References
