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Isolation of *Malassezia* species from healthy cats and cats with otitis

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Lipid-dependent *Malassezia* species have recently been cultured from veterinary specimens. The identification of *Malassezia* species isolates from animals is important to clarify the epidemiology of these lipophilic yeasts. *Malassezia* species were cultured from the external ear canals of 63 out of 99 cats with otitis and 12 of 52 (23%) healthy control cats. The rate of isolation in affected animals versus controls was highly significant ($P < 0.01$). *Malassezia pachydermatis* was isolated as a pure culture in 33 (45.2%) cats, associated with *Malassezia globosa* and *Malassezia furfur* in 20 (50%) and 17 (42.5%) animals, respectively. Three different species were isolated simultaneously in three cats (two cats with *M pachydermatis*, *M globosa* and *M furfur*, one subject with *M pachydermatis*, *M furfur* and *Malassezia sympodialis*). *M globosa* was isolated as the sole species in two animals. The present work confirms the presence of some lipid-dependent species of *Malassezia* in both healthy and otitic cats.

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The basidiomycetous yeast genus *Malassezia* includes lipophilic fungi which are an integral part of the commensal skin microbiota of humans and warm-blooded animals. They are also medically important organisms associated with a variety of diseases in man (Guého et al 1998) and animals (Scott et al 2001, Matousek and Campbell 2002) and the skin inflammatory response is a possible consequence of the reaction and/or hypersensitivity to yeast metabolites. Malasseziosis is also considered a perpetuating factor of external otitis in dogs and cats and may be associated with pruritus, minimal pathological changes and the presence of black and waxy cerumen in the ear canal (Scott et al 2001, Mauldin et al 2002).

Malassezia species can be identified by morphological, ultrastructural, biochemical and molecular patterns. *Malassezia pachydermatis* is the only non-lipid-dependent species, its in vitro growth being enhanced but not dependent on lipid supplementation (Ahearn and Simmons 1998). The other six species *Malassezia furfur*, *Malassezia sympodialis*, *Malassezia globosa*, *Malassezia slooffiae*, *Malassezia obtusa* and *Malassezia restricta* require an exogenous lipid supply for growth (Guého et al 1996, Guillot et al 1996).

M pachydermatis was originally thought to be zoophilic, because of its frequent isolation from a broad spectrum of animals, in particular wild and domestic carnivores, such as dogs, cats, bears, ferrets and foxes. Subsequently, this species was identified in life-threatening fungal septicaemia in low-birth-weight neonates (Van Belkum et al 1994). On the contrary, lipid-dependent *Malassezia* species yeasts were considered to be strictly anthropophilic; recently, however, several authors have cultured these species from animal specimens: *M furfur*, *M sympodialis* and *M globosa* have been cultured from the skin and the external ear canals of healthy cats and cats with otitis (Bond et al 1996, Bond et al 1997, Crespo et al 1999, Crespo et al 2000). *M furfur*, *M sympodialis*, *M globosa*, *M restricta* and *M obtusa* were cultured from healthy ruminants in Spain (Crespo et al 2002): in the same paper the occurrence of *M furfur*, *M slooffiae*, *M obtusa*, *M globosa* and *M restricta* in horses free of skin disease is also described.

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On the basis of these data, the identification of *Malassezia* species isolates from animals in order to clarify their epidemiology is well established.

The aim of the present paper was to investigate the occurrence and identification of the different *Malassezia* species from the external ear canal of cats affected by otitis versus healthy controls.

Materials and methods

Cats

The survey was carried out on 151 cats, including 147 domestic shorthair cats, two Persian cats and two Chartreux cats. Physical examination of all animals was conducted before sampling. Fifty-two subjects were randomly chosen among cats of different source with no sign of cutaneous nor other disease to be employed as healthy controls: 99 cats were referred to different practitioners with signs of external otitis, such as pruritus, head shaking, pain, with accumulation of exudate and/or cerumen in the external ear canals and mild to moderate inflammation. *Otodectes cynotis* was observed by microscopic examination of cerumen in 69 cats affected by otitis. The age of animals ranged from 3 months to 17 years (mean = 4.88; $\sigma = 3.28$). Seventy-three cats (48.4%) were male, 78 (51.6%) were female. None of the cats examined had been treated with otic or systemic drugs in the preceding 5 weeks. The animals' source was recorded, and they were classified as follows: 63 cats kept in four different catteries and 88 subjects from private owners. In this last group, 21 cats had an indoor lifestyle, 25 were kept outdoors, 42 could freely roam indoors and outdoors. Detailed data on the animals included in the survey are reported in Table 1.

Mycology

Samples were collected from the right and left external ear canals of each animal using sterile cotton swabs. The samples were inoculated on to Mycobiotic agar (Difco, USA) and modified Dixon medium, and incubated at 30 °C: the plates were checked on the fourth day for growth. If any fungal development was observed on the 10th day of incubation, the plates were discarded as negative. When growth was detected on solid media, a maximum of five different colonies were selected from modified Dixon and/or Mycobiotic agar, and processed to obtain the identification of species. *M pachydermatis* was identified by macro- and microscopic features, and by its ability to grow when subcultured on to lipid-free medium. Lipid-dependent species were identified by checking morphological and biochemical features, as described by Guého et al (1996). The presence of *M furfur*, *M sympodialis* and *M slooffiae* was confirmed by the Tween diffusion test, which allows differentiation between these species, based on their ability to assimilate various polyoxyethylene sorbitan esters (Guillot et al 1996). Bond and Anthony (1995) demonstrated the possible lipid-dependence of some isolates of *M pachydermatis*: also, the presence of some lipids in the primary culture from the specimen might allow the growth of *M furfur* in a lipid-free medium. For these reasons, subsequent transfers were performed to distinguish clearly between the two species. The identification of *M furfur* was confirmed using the Cremophor EL (Sigma) assimilation test, as reported by Raabe et al (1998). The splitting of esculin was also performed as an additional key to identify both *M furfur* and *M sympodialis* (Mayser et al 1997). *M globosa* was identified by its inability to utilise any of the four Tween compounds, and by its unique morphology within the genus. The lack of catalase activity, which is a specific feature of *M restricta*, was utilised to demonstrate the presence of this species. To confirm the identification, each test was performed in duplicate. Each sample was stained with Diff-Quick, and examined by microscopic morphology for the presence of typical *Malassezia* species yeast cells.

Statistical analysis

Data obtained from the present survey were analysed by means of the chi-square test. A P value of <0.05 was considered significant.

Results

Malassezia species positive cultures were obtained from 75 subjects (49.6%): from 12 of 52 (23%) healthy animals and from 63 of 99 (63.6%) cats with otitis. Malassezia occurred in 44 out of 69 (63.7%) animals affected by otodectic mange, and in 19 out of 30 (63.3%) cats with no evidence of *O cynotis*. Microscopic observation of samples was positive in 78 animals (51.6%): the three subjects from which in vitro growth was not obtained were healthy, with a low number of blastoconidia for microscopic field (average of

Table 1. Details of the animals included in the survey and of *Malassezia* species isolated

	M pachydermatis	*M pachydermatis* + lipid-dependent species				Lipid-dependent species	Negative	Total
		a	b	c	d	e		
Clinical status								
Otitis	13	5	0	1	0	0	11	30
Otitis and otodectic mange	13	8	2	0	20	1	25	69
Healthy	7	4	0	0	0	1	40	52
Lifestyle								
Cattery	9	5	2	0	20	2	25	63
Indoors	2	4	0	1	0	0	14	21
Outdoors	8	6	0	0	0	0	11	25
Indoors and outdoors	14	2	0	0	0	0	26	42
Age								
≤1 year	8	4	0	1	3	0	10	26
2–5 years	15	7	1	0	7	2	39	71
> 6 years	10	6	1	0	10	0	27	54
Sex								
Male	19	10	1	0	9	1	33	73
Female	14	7	1	1	11	1	43	78
Total	33	17	2	1	20	2	76	151

a, *M pachydermatis* + *M furfur*.
b, *M pachydermatis* + *M globosa* + *M furfur*.
c, *M pachydermatis* + *M furfur* + *M sympodialis*.
d, *M pachydermatis* + *M globosa*.
e, *M globosa*.

five or less *Malassezia* species organisms per 400× field) (Scott et al 2001).

The identification of the different species gave the following results. *M pachydermatis* was isolated in 73 animals: as a pure culture in 33 cases (45.2%), associated with other *Malassezia* species in 40 (54.8%) cats. *M pachydermatis* was associated with *M globosa* in 20 (50%) cases, with *M furfur* in 17 (42.5%) cases, with both *M globosa* and *M furfur* in two (5%) cases, and with *M furfur* and *M sympodialis* in one (2.5%) case. *M globosa* was recovered from two animals as a pure culture.

M pachydermatis was isolated as the sole species in seven (13.4%) healthy cats and from 26 (26.3%) cats with otitis, including eight subjects without signs of otocariosis, and 13 with otodectic mange. *M furfur* and *M pachydermatis* were isolated from 4 (7.7%) healthy cats and 13 (13.2%) cats with otitis, including five subjects with otodectic mange.

The cats harbouring *M pachydermatis* and *M globosa* showed signs of otitis, all of them had otocariosis and all of them were living in the same cattery.

One of the two animals from which *M globosa* was the only cultured species was healthy, while the other one was affected by otodectic mange.

M pachydermatis, *M globosa* and *M furfur* were isolated together from two animals with otodectic mange kept in the cattery.

The association *M pachydermatis*, *M furfur* and *M sympodialis* was present in one house-pet cat with otitis.

The rate of isolation in healthy versus diseased animals appeared to be very significant ($P < 0.01$). No statistically significant difference related to age, sex or lifestyle of animals was observed, when analyzing all *Malassezia*-positive samples.

Discussion

Malassezia species were cultured from nearly one half of the cats examined and *M pachydermatis*

was the most representative species from both healthy cats and cats with otitis. *M furfur* was isolated as a pure culture by Crespo et al (2000) during a survey on the occurrence of lipophilic yeasts in healthy cats. In our work, it was never isolated as a sole species, being always associated with *M pachydermatis*. *M pachydermatis* and *M sympodialis* had been previously isolated from two cats with otitis externa by Crespo et al (2000), and from feline skin samples by Bond et al (1996). This last species was isolated also in the present survey, confirming its presence in feline species. In our study, *M sympodialis* was isolated from only one diseased cat, associated with *M pachydermatis* and *M furfur*. *M globosa* had been isolated together with *M sympodialis* from healthy cat skin (Bond et al 1997) and was also recovered by us from both healthy and diseased cats sharing the same cattery, mostly associated with *M pachydermatis* and in two cases with *M furfur*.

Data available in literature show prevalences ranging from less than 10% (Bond et al 1997, Rosychuck and Luttgen 2000) to 20–23% (Guillot et al 1994, Greene 1998) in healthy subjects, and from 19% (Greene 1998) to 41.2% (Crespo et al 2002) in animals affected by otitis. The unhomogeneous nature of the animal populations examined, the strict lipid requirements of these yeast species, the difficulty in maintaining them in culture, the sampling technique and the choice of culture media could be possible factors to explain these differences. In this survey, the rate of *Malassezia* species isolation in cats with otitis is high if compared with the above-mentioned studies. The differences might be explained by the fact that in our study, 130 of the 151 cats examined were living in a cattery or having contact with the outdoors, where direct transmission of yeasts among animals is possible. Furthermore, predisposing factors for *Malassezia* species overgrowth in feline species have still not been elucidated and the association with allergic disorders is not as well established as in dogs (Scott et al 2001). The same predisposing factors proposed for canine species are hypothesised: changes in the cutaneous microenvironment, alterations in defence mechanisms, systemic disorders, keratinisation defects, allergic skin diseases and medications (Scott et al 2001, Matousek and Campbell 2002). In our study, otocariosis was diagnosed in 69.9% of subjects with external otitis and *Malassezia* species was isolated from 44 (63.8%) of them. The percentage of isolation in cats with no evidence of *O cynotis* infestation was quite similar (63.3%), so any difference between the two groups of animals could be drawn. Other characteristics of the animals such as lifestyle, age and sex were also evaluated with regards both to the prevalence of lipophylic yeasts and to the species of *Malassezia* isolated, but no significant correlation was found. For this reason, lipid-dependent species could be relatively common in the cutaneous microenvironment of this species, and they could act as *M pachydermatis* in otitis externa.

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