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A comparison of different kinds of *Malassezia* species in healthy dogs and dogs with otitis externa and skin lesions

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Abstract: *Malassezia* yeasts are commensal organisms on the skin of warm-blooded vertebrates. The lipid-dependent *Malassezia* species have recently been cultured from veterinary specimens. The present study investigated and compared different *Malassezia* species in the skin and external ear canal of healthy and diseased dogs. The sampling in the study was carried out on 152 animals, comprising 90 samples from the diseased group and 62 samples from the healthy group. All of the samples were determined by cytological examination and fungal culture. The isolated yeasts were identified by their morphological features as well as their physiological characteristics.

The culture results were positive in only 32.2% samples, including 75.5% samples from the diseased group, and 24.5% samples from the healthy group. A total of 75 strains from 6 *Malassezia* species isolated from both groups were detected with a frequency rate as follows: *M. pachydermatis* (56%), *M. sympodialis* (28%), *M. furfur* (8%), *M. obtusa* (5.4%), *M. globosa* (1.3%), and *M. restricta* (1.3%).

The present work confirms both the presence of *M. pachydermatis* as the most prevalent species in both groups, and the presence of some lipid- dependent species of *Malassezia*.

Key words: Dogs, yeasts, skin lesions, otitis externa, *M. pachydermatis*

Introduction

Malassezia yeasts are commensal skin organisms of warm-blooded vertebrates. They are considered to be opportunistic pathogens that may cause human and animal skin disorders (1,2). Currently, 13 species are included in the *Malassezia* genus: 12 lipid-dependent species (*M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. slooffiae*, *M. restricta*, *M. nana*, *M. dermatis*, *M. japonica*, *M. yamatoensis*, *M. caprae*, and *M. equina*) and only 1 non-lipid-dependent species (*M. pachydermatis*) (3-10). Traditionally *M. pachydermatis* has been considered to be zoophilic, and is usually associated with otitis externa and various clinical forms of dermatitis in domestic animals, particularly in dogs (5,11). In contrast, lipid-dependent *Malassezia* species yeasts were considered to be strictly anthropophilic. However, several authors have recently cultured these species from animal specimens (12-16). Several differentiating systems have been published based on biochemical and physiological differences and suitable for routine

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diagnosis. The purpose of the present study was to investigate the occurrence, identification and comparison of different *Malassezia* species from the skin and external ear canal in healthy and diseased dogs.

Materials and methods

Dogs and sampling procedures

From November 2007 to March 2009, 152 dogs were clinically examined for *Malassezia* spp. and grouped as follows:

Healthy dogs: 62 privately owned dogs were in good general health with no history of skin or ear diseases in the preceding 5 months. These dogs had not been administered any medication during the same period. A total of 43 and 19 samples were collected from the ear canal and skin of healthy dogs, respectively. Dogs in this group were aged between 1 month and 12 years (median 1.7 years): 33 were females and 29 males.

Diseased dogs: 90 privately owned dogs with otitis externa (n = 66) or skin lesions (n = 24) localized on only 1 anatomical site were included in this study. Dogs in this group were aged between 1 month and 2.9 years (median 3 years): 44 were females and 46 males.

All the dogs included in the study came from the Small Animal Clinic of the Faculty of Veterinary Medicine, the University of Tehran. Samples were collected from 8 anatomical sites (i.e. scalp, periorbital, perioral, back, trunk, groin, interdigital, and external ear canal) using sterile cotton swabs moistened with sterile saline solution (0.9% NaCl) for the external ear canal, and scraping with a scalpel for other body sites.

Cytological examination and mycological culture

The number of *Malassezia* yeasts was evaluated by cytological examination staining with May-Grunwald Giemsa for microscopic examination. The results were considered positive if more than 5 cells and more than 10 *Malassezia* species for skin sites and the ear canal, respectively, were observed in 5 random fields at 40× magnifications (2,17-19).

The samples were cultured on modified Dixon agar and Sabouraud dextrose agar (SDA), containing 0.05% chloramphenicol (Merck, Darmstadt, Germany) and 0.05% cycloheximide (Sigma, St Louis, MO, USA). All the plates were incubated at 31 °C for 10 days and were monitored daily.

Preliminary identification of yeasts was based on the macroscopic appearance of colonies and microscopic cell morphology. When growth was detected on solid media, a maximum of 5 different colonies were selected from modified Dixon agar, and processed to obtain the identification of the species. *M. pachydermatis* was identified by macro- and microscopic features, and by its ability to grow when subcultured onto a lipid-free medium (3,14). Various Tween (i.e. 20, 40, 60, 80) assimilation tests, as previously described (15,20), catalase activity (3,20,21), the splitting of esculin (3,21), growth ability at 40 °C (21) and the tryptophan assimilation test (22) were used as additional tests for the differentiation of lipid-dependent yeasts.

Statistical analysis

Quantitative data were analyzed by Student's t test. The data of the diseased and healthy groups were analyzed by chi-square test. A P-value of ≤ 0.05 was considered statistically significant. The software program used was SPSS (Version 16).

Results

The highest percentage of animals carrying *Malassezia* species in the diseased and healthy groups was found in the age group 1-5 followed by the less than 1 year group. In the healthy group, roll smear cytology and cultural results were positive in 27.42% and 19.35% of the samples, respectively, whereas in the diseased group, the results were positive in about 56.66% and 41.11% of the samples. The performance of cytological examination compared with fungal culture, in terms of agreement, Se and Sp calculated on 152 samples, showed relative specificity of around 82% and sensitivity of 100% (coefficient of concordance was 0.72). Tables 1 and 2 show the results of the samples concerning different physiological tests in the diseased and the healthy groups, respectively. From 37 culture-positive samples in the diseased group, 20 samples (54.1%) represented mixed cultures, and a single species was demonstrated in 17 samples (45.9%). From 12

Table 1. Identification of different species of *Malassezia* based on physiological tests in the disease group.

Isolate	Source	Growth without lipid	Catalase reaction	Splitting of esculin	Tryptophan assimilation	Growth at 40 °C	Tween assimilation				Identifications
							20	40	60	80	
1	Dog Skin	+	+	+	−	+	+	+	+	+	1, 2
2	Dog Ear	−	+	+	+	+	+	+	+	+	2, 4
3	Dog Ear	+	−	−	−		+	+	+	+	1
4	Dog Skin	+	+	−	−		+	+	+	+	1
5	Dog Ear	+	+	−	−		+	+	+	+	1
6	Dog Skin	+	+	+	−	+	+	+	+	+	1, 2
7	Dog Ear	+	+	−	−		+	+	+	+	1
8	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
9	Dog Skin	−	+	+	−	+	+	+	+	+	2, 4
10	Dog Ear	+	+	(+)	+		+	+	+	+	1, 4
11	Dog Ear	+	−	−	−		+	+	+	+	1
12	Dog Ear	+	−	−	−		+	+	+	+	1
13	Dog Skin	+	−	+	−	−	+	+	+	+	1, 3
14	Dog Ear	+	+	−	−		+	+	+	+	1
15	Dog Ear	+	−	−	−		+	+	+	+	1
16	Dog Skin	+	+	+	+	+	+	+	+	+	1, 2, 4
17	Dog Skin	+	+	+	+	+	+	+	+	+	1, 2
18	Dog Ear	+	+	−	−		+	+	+	+	1
19	Dog Skin	+	+	(+)	+	−	+	±	±	+	1, 4
20	Dog Skin	−	+	(+)	+	+	+	+	+	+	4
21	Dog Ear	+	+	−	−		+	+	+	+	1
22	Dog Ear	−	+	+	−	+	−	+	+	+	2
23	Dog Ear	+	+	+	−	−	−	−	−	−	1, 3
24	Dog Ear	+	−	−	−		+	+	+	+	1
25	Dog Ear	+	−	−	−		+	+	+	+	1
26	Dog Ear	+	+	−	−		+	+	+	+	1
27	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
28	Dog Ear	+	+	+	−	−	−	−	−	−	1, 3
29	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
30	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
31	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
32	Dog Ear	+	+	+	−	+	−	+	+	+	1, 2
33	Dog Ear	+	−	−	−		−	−	−	−	1, 5
34	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
35	Dog Ear	−	+	−	−		−	−	−	−	6
36	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
37	Dog Ear	+	+	−	(after 4^{W})+		+	+	+	+	1

1 = *M. pachydermatis*, 2 = *M. sympodialis*, 3 = *M. obtusa*, 4 = *M. furfur*, 5 = *M. restricta, 6 = M. globosa*
+: positive, −: negative, (+): weak positive

Table 2. Identification of different species of *Malassezia* based on physiological tests in healthy group

Isolate	Source	Growth without lipid	Catalase reaction	Splitting of esculin	Tryptophan assimilation	Growth at 40 °C	Tween assimilation				Identifications
							20	40	60	80	
1	Dog Ear	+	+	−	−		+	+	+	+	1
2	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
3	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
4	Dog Ear	+	+	+	−	−	−	−	−	−	1, 3
5	Dog Ear	+	+	+	−	+	+	+	+	+	1, 2
6	Dog Ear	+	−	+	−	+	+	+	+	+	1, 2
7	Dog Ear	+	+	−	−		+	+	+	+	1
8	Dog Ear	−	+	+	−	+	−	+	+	+	2
9	Dog Ear	+	−	−	−		+	+	+	+	1
10	Dog Ear	+	+	−	−		+	+	+	+	1
11	Dog Ear	−	+	+	−	+	−	+	+	+	2
12	Dog Ear	+	−	−	−		+	+	+	+	1

1=*M. pachydermatis*, 2 = *M. sympodialis*, 3 = *M. obtusa*
+: positive, −: negative, (+): weak positive

culture-positive samples in the healthy group, mixed cultures and single species were found in 5 samples (41.7%) and 7 samples (58.3%), respectively.

Using the combination identification system, the most commonly isolated species in the diseased group with culture-positive results was *M. pachydermatis* (55.2%), followed by *M. sympodialis* (25.9%), *M. furfur* (10.3%), *M. obtusa* (5.2%), *M. globosa* (1.7%), and *M. restricta* (1.7%), and in the healthy group with culture-positive results, the most commonly isolated species was *M. pachydermatis* (58.8%), followed by *M. sympodialis* (35.3%) and *M. obtusa* (5.9%) (Table 3). A total of 75 strains from 6 *Malassezia* species isolated from both groups and their species were detected with a frequency rate as follows: *M. pachydermatis* (56%), *M. sympodialis* (28%), *M. furfur* (8%), *M. obtusa* (5.4%), *M. globosa* (1.3%), and *M. restricta* (1.3%).

Table 3. Frequency of different species of *Malassezia* in disease and healthy groups.

Malassezia species	Skin lesions		Otitis externa		Healthy skin and ear	
	Number	%	Number	%	Number	%
M. pachydermatis	7	41.2	25	61	10	58.8
M. sympodialis	5	29.4	10	24.4	6	35.3
M. furfur	4	23.5	2	4.9	0	0
M. obtusa	1	5.9	2	4.9	1	5.9
M. globosa	0	0	1	2.4	0	0
M. restricta	0	0	1	2.4	0	0
Total	17	100	41	100	17	100

Discussion

The importance of *M. pachydermatis* in dogs has been extensively reported. This species can play an important role in chronic dermatitis and otitis externa in carnivores, and in dogs in particular (5,11). With regard to culture in the present study, samples were positive in 41.1% and 19.4% from the diseased and healthy groups, respectively. Data available in literature show the prevalence of different *Malassezia* species ranging from less than 10% (13) to 20%-23% (14) in healthy subjects, and from 19% (14) to 41.2% (23) in animals affected by otitis (11). The nature of the animal populations that were 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 all be factors that might explain these differences. Regarding the species isolated, *M. pachydermatis* was most commonly cultured from the skin and external ear canal both in healthy and diseased dogs as the prevalence of *M. pachydermatis* in the ears of healthy dogs, and in those with otitis externa was 58.8% and 61%, respectively. *M. pachydermatis* was isolated from skin lesions with a prevalence of 41.2%. In the present study, the results of the prevalence of *M. pachydermatis* in healthy ears and otitis externa are not significant; and the high percentage of this species in the healthy group (ear and skin) as compared with the diseased group may be caused by the following reasons: 1) The numbers of animals studied (the number of the diseased group in comparison with the healthy group), 2) The overgrowth with saprophytic molds before yeast colonies had grown, and 3) Bacterial populations overcame yeast populations. The presence of lipid-dependent species in carnivores was recently suggested by their presence on the ear and skin, initially in cats and later in dogs (12,14-16,24). The isolation of lipid-dependent species from dogs in this study suggests a potential role of these animals as carriers for humans. Furthermore, it suggests that culture media such as mDixon agar should be used in addition to media without lipid supplementation. On the other hand, lipid-dependent species have been reported in mixed cultures from canine and feline samples (13,15,22,23). In the present study, from 37 samples in the diseased group, 58 isolates were obtained, including 32 isolates (55.2%) related to *M. pachydermatis* and 26 isolates (44.8%) related to lipid-dependent *Malassezia* species. Furthermore, from 12 samples in the healthy group, 17 isolates were obtained, including 10 isolates (58.8%) related to *M. pachydermatis* and 7 isolates (41.2%) related to the lipid-dependent *Malassezia* species. A point of further interest is that one sample may contain more than one species of *Malassezia*; consequently, different identification techniques should be used. For this reason, we used the above-mentioned techniques in the present study. In doing so, we were able to isolate mixed cultures from 58.8% and 70.68% of the healthy and diseased groups, respectively (Tables 1-3). One of the problems encountered in recent studies has been that the results of the rate of culture vary from reporter to reporter. This is because culture-based methods can be biased by different growth rates and culture requirements in different species and *M. restricta* may not be detected in samples in which a catalase reaction-positive *Malassezia* species is mixed. On the other hand, the negative response of catalase reaction alone is not sufficient for the isolation of *M. restricta*, because the *M. pachydermatis* strains can exhibit variable reactions (25). In this study, we were able to identify only one isolate of *M. restricta*. Therefore, in order to resolve these problems, and to examine the exact distribution of different lipid-dependent species in the skin/ear of animals/humans, the use of molecular confirmation is recommended. In conclusion, the present work confirms both the presence of *M. pachydermatis* as the most prevalent species in the skin and external ear canal of healthy and diseased dogs, and the presence of some lipid-dependent species of *Malassezia*.

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