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Original Scientific Article

MALASSEZIA, MITES AND BACTERIA IN THE EXTERNAL EAR CANAL OF DOGS AND CATS WITH OTITIS EXTERNA

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Summary: Occurrence of *Malassezia*, mites and bacteria, was evaluated through cytology, culture and microscopical analysis of auricular cerumen collected from 115 cats and 203 dogs with otitis externa. For the identification of *Malassezia* species, a PCR-based technique was also used. All the patients enrolled in the study were examined for *Malassezia* and all cats and 101 dogs were also investigated for mites. Bacteriological examination was performed on 16 cats and 60 dogs. The associations between *Malassezia* and the other pathogens and the correlations between *Malassezia* and season, gender and ear conformation, were evaluated. *Malassezia* pachydermatis was isolated from 58.2% cats and 52.7% dogs, while *Otodectes cynotis* was identified in 66.1% cats and in 5.9% dogs. Bacteria were detected in 18.7% cats and 36.7% dogs and *Staphylococcus pseudointermedius*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus canis*, *Escherichia coli* and *Bacillus* sp. were identified. *M. pachydermatis* was isolated in pure culture from 28.3% cats and from 87.1% dogs, while it was associated with *O. cynotis* in 70.1% of cats and in 5% of dogs, and with bacteria in 1.5% of cats and 23.3% of dogs. Mixed infections caused by *M. pachydermatis*, bacteria (*S. pseudointermedius*) and mites occurred in 1 cat and 1 dog. Our results suggest that ear conformation is an important individual predisposing factor for *Malassezia* otitis in dogs and indicated the influence of the season on onset of *Malassezia* infections in cats.

Key words: *Malassezia pachydermatis*; *Otodectes cynotis*; bacteria; otitis externa; dog; cat

Introduction

Otitis externa is a common presenting complaint in veterinary medicine, both in general and referral practices. It is also one of the more frustrating entities practitioners are called on to deal with (1). Yeasts belonging to *Malassezia* genus, mites such as *Otodectes cynotis* and bacteria, mainly *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Escherichia coli*, are frequently involved in the occurrence of otitis externa (2, 3, 4). Mites are regarded as primary causes and are reported to occur in up to 50% of cats and 5-10% of dogs (2), while yeasts and bacteria are not considered primary pathogens, mainly acting as perpetuating or predisposing factors (2, 3, 5, 6, 7). However, in the literature data about a possible association among these pathogens are scarce.

Successful management of otitis externa mainly depends on the understanding of the primary, predisposing, and perpetuating factors involved in its pathogenesis (2, 5). These factors cannot be grossly distinguished from each other and are frequently involved in mixed infections

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(2, 5, 7). Individual predisposing factors could also play an important role, working in concert with the primary or perpetuating causes of otitis externa in causing clinical disease (2).

The aim of this retrospective study was to assess the occurrence of yeasts, mites and bacteria in cats and dogs affected by otitis externa, evaluating associations among the occurrence of *Malassezia* and other pathogens, and possible correlations of *Malassezia* infections with season and individual factors, such as gender and ear conformation.

Material and methods

Adult feline and canine patients (more than 1 year old) referred for otitis externa to private veterinary practitioners were enrolled in the study. Patients that received specific otologic drugs 2 months prior to the referral were excluded. Diagnosis had to be accomplished on the basis of clinical signs such as head shaking, ear scratching, auricular discharge, malodour, erythema, ear swelling and pain (20). Breed, gender and season in which otitis presented had to be available for data analysis.

Table 1: Number and breed of dogs with otitis externa examined for *Malassezia*, mites and bacteria

Dog breed	N. animals
Alan	1
Basset Hound	1
Border Collie	1
Boxer	7
Cocker Spaniel	6
Course	1
Dalmatian	1
English Setter	7
Espagneul Breton	1
German Sheperd	5
Golden Retriever	1
Italian Pointer	1
Italian Bloodhound	1
Labrador Retriever	32
Maremma Shepherd	1
Newfoundland	1
Pekingese	2
Pug	3
Puli	1
Shar-pei	1
Springer Spaniel	1
St. Bernard	1
West Highland White Terrier	1
Cross-breed	125
Total	**203**

All cats were European shorthair, while cross-bred dogs represented the majority (125/203) as reported in Table 1. Ear conformation was recorded for 178 dogs (29 straight and 149 pendulous). Gender was recorded in 49 (24 females, 25 males) cats and in 198 (97 females, 101 males) dogs. The season of onset of clinical signs was reported in 76 cats and 203 dogs.

All the animals were examined for *Malassezia*, all cats and 101 dogs were also investigated for *O. cynotis*. Bacteriological examinations were performed on 16 cats and 60 dogs, respectively. From both ears of all examined animals, samples of ear wax and secretions were collected by means of sterile ear swabs. All samples were examined for *Malassezia* by cytology. Otologic smears were stained by Diff Quick® (Diff-Quik, Medion Diagnostics AG, Düdingen, Switzerland) and examined at 400X and 1000X. Diagnosis of *Malassezia* otitis was achieved when more than 10 blastospores/microscopic field (mean number of 10 fields) at 400X were observed (8). On positive samples, culture onto m-Dixon medium was performed as previously described (9, 10). Briefly, after collection, the specimens were promptly seeded onto Sabouraud dextrose agar added with 0.5% of chloramphenicol and cycloheximide (Sabouraud Agar + Actidione®, Liofilchem, Italy) and mDixon Agar (3.6% malt extract, 0.6% peptone, 2% desiccated ox-bile, 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 1.2% agar, 0.5% chloramphenicol and 0.5% cycloheximide). All the plates were incubated at 30 °C for about 7 days, and daily inspected for *Malassezia* growth from day 4 post-inoculation.

Preliminary identification of yeasts was based both on macroscopic appearance of colonies and microscopic cell morphology. Bond and Anthony (11) demonstrated the possible lipid dependence of some isolates of *M. pachydermatis*, so strains referable to this species apparently lipid-dependent were identified by serial transfers on a lipid-free culture medium. The Tween assimilation test as described by Guillot et al. (12) and catalase activity were performed as additional tests to both to confirm the identification, and to exclude the presence

of other *Malassezia* species. Morphological and biochemical identification was confirmed by means of a PCR-based technique using restriction enzyme digestion, specific for the discrimination of 11 *Malassezia* species, as described by Mirhendi et al. (13). In order to achieve pure cultures, five colonies of each positive sample were subcultured onto mDixon Agar and stored at −20 °C until analysis. The cell walls were mechanically disrupted by freeze–thawing and genomic DNA was extracted and purified according to the DNeasy™ protocol for animal tissue (QIAGEN Inc., Valencia, CA, USA). The primers selected for this protocol amplify the target part of 26S rDNA, providing a single PCR product of an expected size of 580 bp. The PCR products were subjected to REA using CfoI and BstF51, separately, according to the manufacturer's instructions (Fermentas International Inc., Burlington, Ontario, Canada). Digested fragments were analyzed by electrophoresis in 2% agarose gel.

Otologic smears from 16 cats and 60 dogs were examined also for bacteria, both by cytology and culture. At cytology, bacterial population (rods and cocci) was considered overrepresented when more than 6 organisms at 1000X (mean number of 10 fields) were detected in Diff-Quik stained otologic smears (3). Samples were cultured on Blood Agar and incubated aerobically at 37°C for 24 hours. If bacterial growth occurred, the different colonies were submitted to Gram staining, then cultured on plates containing the following selective media: Tryptone Bile X-glucoronide Medium (Oxoid LTD, Basingstoke, Hampshire, England), *Pseudomonas* CFC Selective medium (Oxoid), Baird-Parker Medium + Egg Yolk Tellurite Emulsion (Oxoid), Violet Red Bile Agar (Oxoid). Plates were incubated aerobically at 37°C for 24 hours. The isolates obtained were typed by API System 20E, API System 20NE, API System Staph, API System 20 Strep (BioMerieux, Marcy-l'Etoile, France). For the search of mites, ear wax from all cats and 101 dogs was microscopically examined at 100X and 400X.

Data were statistically elaborated with χ^2 and ANOVA tests (14) in order to evaluate correlations of *Malassezia* occurrence with season, gender, ear conformation and the possible association among the different pathogens examined (significativity $P<0.05$).

Results

Cytological examinations allowed to detect more than 10 yeast cells/field from 67/115 (58.2%) cats and 107/203 (52.7%) dogs. On the basis of culture results, *Malassezia pachydermatis* was identified in pure culture in all positive specimens. *O. cynotis* was identified in 76/115 (66.1%) cats and in 6/101 (5.9%) dogs. Bacteria were detected in 3/16 (18.7%) cats and from 22/60 (36.7%) dogs and more than 6 organisms at 1000X were found in all 25 positive samples. More detailed results are presented in Table 2. Cultures allowed the identification of *Staphylococcus pseudointermedius* (1 cat and 10 dogs), *Staphylococcus epidermidis* (6 dogs), *Pseudomonas aeruginosa* (3 dogs), *Streptococcus canis* (1 cat and 1 dog), *E. coli* (1 cat and 1 dog) and *Bacillus sp.* (1 dog) (Table 3).

Table 2: Prevalence of *Malassezia pachydermatis*, *Otodectes cynotis* and bacteria in 203 dogs and 115 cats with otitis externa

Malassezia pachydermatis			
	Examined	Positive	Prevalence
Dogs	203	107	52.7%
Cats	115	67	58.2%
Otodectes cynotis			
	Examined	Positive	Prevalence
Dogs	101	6	5.9%
Cats	115	76	66.1%
Bacteria			
	Examined	Positive	Prevalence
Dogs	60	22	36.7%
Cats	16	3	18.7%

Table 3: Prevalence of bacteria species isolated from 60 dogs and 16 cats with otitis externa

	Prevalence (positive/examined) in dogs	Prevalence (positive/examined) in cats
Staphylococcus pseudointermedius	16.7% (10/60)	6.3% (1/16)
Staphylococcus epidermidis	10% (6/60)	0/16
Pseudomonas aeruginosa	5% (3/60)	0/16
Streptococcus canis	1.7% (1/60)	6.3% (1/16)
Escherichia coli	1.7% (1/60)	6.3% (1/16)
Bacillus sp.	1.7% (1/60)	0/16

M. pachydermatis was isolated as unique agent from 19/67 (28.3%) cats and from 88/101 (87.1%) dogs, while it was associated with *O. cynotis* in 47/67 (70.1%) cats and in 5/101 dogs (5%). Yeasts and bacteria were associated in 1/16 (6.2%) cats and in 14/60 (23.3%) dogs. *O. cynotis* was isolated as unique agent in 29/76 (38.2%) cats, while mites alone were never recovered from dogs. Bacteria as sole causative agents were observed in 7/22 (31.8%) dogs and 0/3 cats. Mixed infections caused by yeasts, bacteria (*S. pseudointermedius*) and mites occurred in 1 cat (6.2%) and 1 dog (1.7%), respectively.

Significant statistical values were found when comparing dog ear conformation and occurrence of *Malassezia* (χ^2=4.69, $P < 0.05$), with significantly frequent infections observed in dogs with pendulous ears. A seasonal dependence ($P < 0.05$) was also observed in the onset of *Malassezia* infection in cats that was mostly recorded in summer and winter (evaluated by ANOVA test).

Discussion

One of the aims of the present study was to investigate the occurrence as well as correlation between *Malassezia* infection and the presence of other agents of otitis externa in cats and dogs, such as bacteria and mites. High rates of isolation of *Malassezia* were recorded in domestic carnivora affected by otitis externa, as reported in previous studies (15). *M. pachydermatis* was identified in pure culture in all samples resulted positive at cytological examination, suggesting the role of this fungal species in the aetiology of otitis externa in dogs and cats (16).

Among the perpetuating factors, bacteria and yeasts are believed to be less frequent in feline than in canine otitis cases (5). However, in the present study the occurrence of *M. pachydermatis* infection was slightly higher in cats (58.2%) than in dogs (52.7%). As found by other authors (17, 18, 19), staphylococci were the most commonly isolated bacteria. In addition, all bacterial species/genera here isolated were already reported in dogs and cats with otitis externa (3, 18, 20).

The presence of *O. cynotis*, a mite considered a primary cause of otitis externa, was significantly higher in cats than in dogs (66.1% and 5.9%, respectively), as reported in literature (2). According to some authors (5, 7), when bacteria or *Malassezia* yeast are present in cats with otitis externa, systemic medications should be considered, even if the middle ear is not involved.

It is important to note that bacteria or yeasts associated with cases of otitis externa are only opportunists and are not primary pathogens (2, 3, 5); in fact they are normally present in low numbers in the external ear canal. When a primary disease damages this anatomic component of the ear, the normal microflora can proliferate and exacerbate or perpetuate inflammatory reactions (2). Although information about other primary factors as allergies and endocrinopathies was not available, in cats *O. cynotis* infection was probably one of the main factors for the *Malassezia* overgrowth, especially considering the high prevalence (about 70%) of cats colonised by *O. cynotis*. However, from the analysis of data obtained in this study, there was no significant association between *Malassezia* and the other primary and/or perpetuating pathogens which could lead to otitis externa in cats and in dogs. Nevertheless, expecially in case of bacteria, the existence of a possible correlation with *Malassezia* could have been masked by the relatively low number of examined animals and further studies are needed. Statistical analysis

confirmed that ear conformation ($P < 0.05$) is an important predisposing feature in pathogenesis of *Malassezia* otitis in dogs (2, 3, 6, 21). Other anatomical and conformational factors, such as seborrhea, high moisture levels in ear canals, hypoplastic and stenotic ear canals and a high density of hair in ear canals, are known to be predisposing factors to otitis externa (2, 3, 7, 9). However, in this retrospective study it was not possible to evaluate these features because information about these data were lacking in examined animals. The influence of the season on onset of *Malassezia* infections in cats appeared also significant. This latter correlation has not been extensively investigated before; however, winter seems a predisposing season for ear *Malassezia* infections in this animal species (16).

In conclusion, in both dogs and cats, emphasis should be placed on establishing a diagnosis of otitis externa through physical, parasitological and cytological examinations, followed by culture and molecular diagnosis (1, 7, 9, 22), allowing a more correct etiological diagnosis and a specific treatment schedule, and to overcome recurrences due to an improper management.

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MALASEZIJA, PRŠICE IN BAKTERIJE V UŠESNEM KANALU PRI PSIH IN MAČKAH OB VNETJU ZUNANJEGA SLUHOVODA

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Povzetek: S citološko, gojitveno in mikroskopsko analizo ušesnega masla smo določili pojavnost kvasovk iz rodu *Malassezia*, pršic in bakterij pri 115 mačkah in 203 psih ob vnetju zunanjega sluhovoda. Identifikacija malasezije je bila opravljena tudi z metodo PCR. Analizo na prisotnost malasezije smo opravili pri vseh bolnikih, na prisotnost pršic pa pri vseh mačkah in 101 psu. Bakteriološka analiza je bila opravljena pri 16 mačkah in 60 psih. Analizirali smo povezavo med pojavnostjo malasezije in ostalimi patogeni ter korelacijo med pojavnostjo malasezije in letnim časom, spolom ter obliko ušesa. *Malassezio pachydermatis* smo izolirali iz ušesnega masla 58,2 % mačk in 52,7 % psov, *Otodectes cynotis* pri 66,1 % mačkah in 5,9 % psov. Bakterije smo odkrili pri 18,7 % mačk in 36,7 % psov. Med bakterijami smo ugotovili vrste *Staphylococcus pseudointermedius*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus canis*, *Escherichia coli* in *Bacillus sp*. Vrsta *M. pachydermatis* je bila izolirana v čisti kulturi pri 28,3 % mačk in 87,1 % psov, skupaj z O. cynotis pri 70,1 % mačk in 5 % psov, skupaj z bakterijami pa pri 1,5 % mačk in 23,3 % psov. Pri 1 psu in 1 mački smo določili mešano okužbo z *M. pachydermitis*, bakterijami (*S. pseudointermedius*) in pršicami. Naši rezultati tudi kažejo, da je pri psih oblika ušesa pomemben preddispozicijski dejavnik za pojavnost vnetja ušesa z malasezije. Pri mačkah pa se malasezije pojavlja sezonsko.

Ključne besede: *Malassezia pachydermitis*; *Otodectes cynotis*; bakterije; *otitis externa*; pes; mačka