

OCCURENCY OF *MALASSEZIA PACHYDERMATIS* AND OTHER INFECTIOUS AGENTS AS CAUSE OF EXTERNAL OTITIS IN DOGS FROM RIO GRANDE DO SUL STATE, BRAZIL (1996/1997)

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ABSTRACT

The purpose of this work was to evaluate the frequency of *Malassezia pachydermatis* infection and other infectious agents in dogs with external otitis and with healthy auditory tubes. Clinical manifestations of external otitis were evaluated. Samples from the auditory tube of 102 dogs with otitis and from 32 healthy dogs were submitted to direct microscopic examination and cultured in blood agar and Sabouraud dextrose agar with chloramphenicol and cycloheximide. Direct examination showed more than ten cells of *M. pachydermatis* in 52.0% of the samples from dogs with otitis, but in only 21.8% of the healthy auditory tube samples. *M. pachydermatis* was isolated in 37.5% of the samples from dogs with healthy auditory tube and 76.5% ($p < 0.01$) of the samples from dogs with otitis. There was an association between *M. pachydermatis* and *Staphylococcus aureus* ($p < 0.01$), but not with *Pseudomonas aeruginosa* ($p > 0.05$). Infection by *M. pachydermatis* was prevalent in the following breeds: Cocker Spaniel, German Shepherd and Brazilian Fila. No differences were found in frequency of the infection in relation to age, sex and ear anatomy of the dogs. Otitis were predominantly ceruminous and erythematous. *M. pachydermatis* was the most frequent agent in external otitis.

Key words: *Malassezia pachydermatis*, *Staphylococcus aureus*, otitis, dog

INTRODUCTION

The genus *Malassezia* belongs to the family *Cryptococcaceae*, order *Cryptococcales* class of *Blastomycetes* division *Deuteromycotina* (14). It is composed by yeasts with a thick cellular membrane, with multiple layers. The reproduction is asexual, with production of blastoconidia through a repetitive single polar process or budding, forming a round, ovoid or cylindrical cell when it separates from the mother cell. *M. pachydermatis* is the only species of the lipophilic-non-dependent genus. Morphologically it comes as an isolated or a grouped cell, with oval format or with single polar budding of wide base, acquiring the format of a "bottle". Hypha and pseudohypha are usually absent (2,12).

M. pachydermatis is a common component of the microbiota of the domestic carnivores skin. It is also isolated in external otitis and dermatitis. The cellular multiplication is facilitated when there is a change in the microenvironment, excessive production of earwax, pH alteration, after antibiotics therapy or in allergic cases (11).

The pathogenicity of *M. pachydermatis* was confirmed through experimental inoculation of dogs with healthy auditory tubes. They showed clinical signs 21 days after inoculation, histopathological lesions and presence of yeast. The control group was negative (19).

The aims of the present work were to study the occurrence of *M. pachydermatis* and other etiological agents in dogs with external otitis and their possible associations; and to characterize

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the otomycosis in relationship with breed predisposition, conformation of the auricular pavillion, sex, age, evolution, clinical signs and lesions.

MATERIALS AND METHODS

From May 1996 to April 1997, the presence of *Malassezia* sp was searched in the ear canal from dogs with external otitis and from healthy dogs. Dogs from several breeds and ages were examined (Table 1). A total of 134 samples was analyzed and divided in two groups. Group I was composed by 32 samples from dogs with healthy auditory tubes and group II by 102 samples from dogs with external otitis. The samples were collected from dogs that were taken to the Veterinary Hospital, Federal University of Pelotas (Brazil) and in private clinics of towns from the South and Midwest of Rio Grande do Sul state (Brazil).

In Group I, all dogs were previously evaluated by otoscopy and showed no clinical signs or otitis lesions: 12 samples came from pendular ears, eight from semi-pendular and 12 from erect ears. The samples belonged to the Group II were collected from 72 cases of external otitis with chronic evolution (more than one month), six from external otitis whit acute evolution (up to one month) and 24 without history: 67 sample belonged to dogs pendular ears, six from semi-pendular, 26 from erect ears and three with no information. In 93 cases the otitis was unilateral and in eight unilateral.

The samples were taken with the aid of a sterile swab, moistened in saline solution and rubbed in the anterior ear canal. The detection of *M. pachydermatis* in the ear discharge was performed by direct examination of the smear. Slides were stained with Gram or Loeffler for cytologic examination. According to the number of yeast cells per microscopic field it was applied the following score: - negative; + from one to five cells; ++ from six to ten cells and +++ more than ten cells.

The samples were cultivated in Sabouraud dextrose agar with chloramphenicol (0.4 g/l) and cycloheximide (0.5 g/l) kept at 37°C for up to ten days, and examined macroscopic and microscopic evaluation. For bacterial isolation all the samples were cultivated in agar with 8% of defibrinated sheep blood and incubated for up to 72 hours. Morphological and biochemical characterization were performed.

The results were analyzed by the Epi Info version 5.01b. Afterwards the variables were codified and crossed in two by two table, using the Qui-square and Fischer's tests.

RESULTS AND DISCUSSION

Direct examination

Twenty-five samples from the Group I (78.1%) were negative for *M. pachydermatis*. One (3.1%) had from one to five cells per field (++) and six (18.7%) had one to five cells (+) (Table 2).

Table 1. Distribution of dogs with healthy auditory tubes and external otitis regarding breed, age and sex in samples from Southern and Midwest regions of Rio Grande do Sul, Brazil (1996 - 1997).

GROUP I									
BREEDS	n	AGE (years)					SEX		
		≤1 n°	2-3 n°	4-5 n°	6-7 n°	≥8 n°	M n°	F n°	
Cocker Spaniel	2	-	-	2	-	-	2	-	
Collie	2	-	-	-	-	2	2	-	
Danish	3	-	3	-	-	-	2	1	
Brazilian Fila	4	2	2	-	-	-	2	2	
Labrador	2	-	2	-	-	-	2	-	
German Shepherd	2	-	-	-	2	-	-	2	
Miniature Pinscher	2	-	-	-	-	2	-	2	
Poodle	5	2	3	-	-	-	2	3	
Schnauzer	2	2	-	-	-	-	-	2	
Brazilian Terrier	2	-	-	-	2	-	2	-	
WDB	6	-	-	6	-	-	2	4	
Total	32	6	10	8	4	4	16	16	

GROUP II									
BREEDS	n	AGE (years)					SEX		
		≤1 n°	2-3 n	4-5 n°	6-7 n°	≥8 n°	NI n°	M n°	F n°
Bassethound	5	4	1	-	-	-	-	3	2
Beagle	6	-	4	-	-	2	-	-	6
Cocker Spaniel	21	2	2	4	9	4	-	15	6
Collie	3	-	3	-	-	-	-	1	2
Dachshund	2	-	2	-	-	-	-	-	2
Brazilian Fila	n°12	3	2	-	5	2	-	8	4
Labrador	1	1	-	-	-	-	-	1	-
Mastim Napolitano	2	-	2	-	-	-	-	2	-
Old English Sheepdog	2	-	2	-	-	-	-	-	2
German Shepherd	13	-	4	3	2	2	2	9	4
Belgium Shepherd	4	-	-	4	-	-	-	4	-
Poodle	9	4	-	1	-	2	2	5	4
Rottweiler	2	-	2	-	-	-	-	2	-
Saint Bernard	1	-	-	1	-	-	-	1	-
Brazilian Terrier	2	-	-	-	-	2	-	-	2
Yorkshire Terrier	1	-	1	-	-	-	-	1	-
WDB	14	-	6	-	3	5	-	7	5
no information	02	-	-	-	-	-	2	-	2
Total	102	14	31	13	19	19	6	61	41

Group I = samples of healthy auditory tubes; Group II = samples of external otitis; n° = number of samples; NI = no information; M = male; F = female; WDB = Without defined breed.

In Group II (external otitis), more than 10 cells of *M. pachydermatis* per microscopic field was observed in 53 cases (52.0%). The remaining samples presented the following results: 20 (19.6%) between six and ten cells per field, 16 (15.7%) from one to five cells and 13 (12.7%) showed no cells

Table 2. Results of direct exam and isolation of *M. pachydermatis* in healthy auditory tubes and external otitis from Southern and Midwest regions of Rio Grande do Sul, Brazil (1996 - 1997).

DIRECT EXAM	ISOLATION <i>M. PACHYDERMATIS</i>					
	GROUP I			GROUP II		
	Pos n°(%)	Neg n°(%)	Total n°(%)	Pos n°(%)	Neg n°(%)	Total n°(%)
(+++)	-	-	-	52(51.0)	1(1.0)	53(52.0)
(++)	-	1(3.1)	1(3.1)	17(16.7)	3(2.9)	20(19.6)
(+)	5 (15.6)	1(3.1)	6(18.7)	6(5.9)	10(9.8)	16(15.7)
(-)	7 (21.9)	18(56.3)	25(78.2)	3(2.9)	10(9.8)	13(12.7)
TOTAL	12 (37.5)	20(62.5)	32(100)	78(76.5)	24(23.5)	102(100)

Group I = samples of healthy auditory tubes; Group II = samples of external otitis; n° = number of samples; %= percent; Pos = positive; Neg = negative; (+++) = more than 10 cells de *M. pachydermatis*; (++) = from 6 to 10 cells de *M. pachydermatis*; (+) = from 1 to 5 cells de *M. pachydermatis*; (-) = negative.

(Table 2). Statistical significant difference was demonstrated ($p < 0.01$), in relation to a greater number (more than ten) of cells of *M. pachydermatis* for Group II than the Group I. Our results were not in accordance with Ribeiro *et al.* (21), who found higher number of cells of *M. pachydermatis* in infected (40.0%) and healthy (37.5%) animals. However, our results were close to Kiss *et al.* (13), who analyzed dogs with external otitis by the fluorescent technique, and observed larger numbers of yeast cells, proposing that more than ten cells per microscopic field is indicative of pathogenicity (18).

Isolation of *M. pachydermatis*

The isolation of *M. pachydermatis* was larger ($p < 0.01$) in dogs with external otitis (Group II) in relation to the healthy one (Group I). *M. pachydermatis* was isolated in 12 (37.5%) out of 32 samples of Group I and in 78 (76.5%) of the samples of Group II (Table 2). This difference was larger than that observed by Feigl *et al.* (4) in otitis (50.6%) and healthy auditory tubes (43.8%). The presence of yeast in healthy auditory tubes was also found by Fraser (7) and Langoni *et al.* (15) (36.0% and 43.0% respectively). On the other hand, Nunes and Hamdan (20) reported the isolation of *M. pachydermatis* in 91% of samples from healthy dogs. However, these authors did not mention if the dogs were examined for subclinical otitis.

M. pachydermatis was the cause of otitis in 76.5% of the affected dogs. Similar results are reported by Kiss *et al.* (13) and Szykiewicz *et al.* (24) who mentioned that the yeast was responsible for 76.3% and 71% of the cases of otitis, respectively. In other reports, the frequency of *M. pachydermatis* as the cause of otitis ranged from 8% to 44% (5,7,8,9). In 51.3% (40 samples) of the infections by *M. pachydermatis*, the otitis was waxy and erythematous as mentioned by other authors (8,9,10,13,16). In 15.4% (12 samples) of the cases, the otitis was purulent. 3.8% (three samples) of the dogs had ulcerations, and in 29.5% of them (23 samples) the otitis was a combination of clinical signs and lesions.

The number of samples isolated with more than ten cells per field was larger ($p < 0.01$) comparing to the other scores, what justifies the use of the direct examination with cell counting for the initial diagnosis of the malasseziosis. In the samples of Group II, which presented more than ten cells in the direct examination, the isolation occurred in 52 (98.1%), while the 13 samples with negative result in the direct examination, *M. pachydermatis* was isolated in three (23%). Of the samples of Group I, *M. pachydermatis* was isolated in five (22.7%) that presented from one to five cells per field in the direct examination and in seven (18.4%) of the 25 negative samples (Table 2).

There were no statistical differences for sex, breed, age and ear conformation in relation to the isolation of *M. pachydermatis*. The otitis externa was much more frequent in Cocker Spaniel (14 - 21.9%), German Shepherd (13 - 20.3%) and Brazilian Fila (8 - 12.5%) (Table 3). The first two breeds were also mentioned by Kiss *et al.* (13) and Giorgi *et al.* (9).

M. pachydermatis was isolated in only 50.0% (six samples) of pendular ears and 33.3% (four samples) of erect ones in Group I, but in the group II 71.6% (48 samples) of the pendular ears and 92.3% (24 samples) of the erect ones (Table 4). The results obtained were similar to Baxter (1), who obtained in healthy pendular ears 59.0% of *M. pachydermatis* and 23.0% in the erect ones. Our data from pendular ears of group II was also similar to Baxter (1) in 71.0% of samples. In spite of the great frequency of isolation in dogs with erect ears, the majority of the otitis samples (48 out 67) came from dogs with pendular ears, reinforcing the affirmative of Stewart (23) who indicated the pendular ears as a predisposing factor for the establishment of otitis. The results obtained also agree with Lobell *et al.* (17), who considered the pendular ears a predisposing condition for otitis, although dogs with erect ears, mainly the German Shepherd, are also susceptible.

Most of the samples coming from dogs with otitis were of chronic evolution (72 samples), in which *M. pachydermatis* was isolated in 61 (84.7%), but was also cultured from 50% of six

cases of acute evolution. The great majority of cases (93 samples) were bilateral otitis, and *M. pachydermatis* was isolated in 78.4% of the cases. Similar results were reported by Gentilini *et al.* (8) and Smith (22).

It is quite interesting to mention that during the same period of this study (96/97), Ferreiro *et al.* (6) notified an increased incidence (3.5% of 1399 samples) of this yeast among other agents of dermatomycoses in a different region of the Rio Grande do Sul state, in comparison with previous data (79/83).

Other etiological agents

From 32 positive samples for *M. pachydermatis* (Group I), only two (6.3%) presented *Staphylococcus intermedius*. In samples of group II, besides *M. pachydermatis*, it was also isolated the yeast *Candida albicans* (2.0%), and the following bacteria: *Staphylococcus intermedius* (25.5%), *Staphylococcus aureus* (17.6%), *Pseudomonas aeruginosa* (15.7%), *Proteus* sp (10.8%), *Streptococcus* sp (6.9%), *Staphylococcus epidermidis* (5.9%), *Actinomyces* sp (5.9%). There was no bacterial growth in 11.8% samples. The most frequent agents isolated in samples from Group II were *M. pachydermatis* and *Staphylococcus* sp, similar to the results found by other authors (3,6,10,14,25). The

Table 3. Distribution of breeds with isolation of *M. pachydermatis* from healthy (Group I) and with external otitis (Group II) from Southern and Midwest regions of Rio Grande do Sul, Brazil (1996 - 1997).

BREEDS	ISOLATION <i>M. PACHYDERMATIS</i>		
	GROUP I n°(%)	GROUP II n°(%)	TOTAL n°(%)
Basset hound	...	4 (6.2)	4 (5.2)
Beagle	...	6 (9.4)	6 (7.9)
Cocker Spaniel	2 (16.7)	14 (21.9)	16 (21.0)
Collie	-	02 (3.1)	2 (2.6)
Dachshund	-	02 (3.1)	2 (2.6)
Brazilian Fila	-	08 (12.5)	8 (10.5)
Labrador	02 (16.7)	01 (1.7)	3 (3.9)
Mastim Napolitano	-	02 (3.1)	2 (2.6)
Old English Sheepdog	...	02 (3.1)	2 (2.6)
German Shepherd	02 (16.7)	13 (20.3)	15 (19.7)
Belgium Shepherd	...	04 (6.2)	4 (5.3)
Miniature Pinscher	02 (16.7)	...	2 (2.6)
Poodle	02 (16.7)	02 (3.1)	4 (5.3)
Rottweiler	...	02 (3.1)	2 (2.6)
Saint Bernard	-	01 (1.7)	1 (1.3)
Schnauzer	02 (16.7)	...	2 (2.6)
Brazilian Terrier	-	01 (1.7)	1 (1.3)
Without defined breed	-	14 (17.9)	14 (15.6)
Total	12 (100)	78 (100)	90 (100)

Group I = samples of healthy auditory tubes; Group II = samples of external otitis; n° = number of samples; % = percent.

genera *Streptococcus* (6.9%) and *Proteus* (10.8%) presented a frequency not considered as high for these two agents of external otitis, in accordance with other authors (3,5,13). Other secondary agents were also isolated apart from *M. pachydermatis*, as reported majority of authors (3,5,8,9,10,13,24).

In samples from Group II the most frequent interaction was *M. pachydermatis* and *Staphylococci* (Table 5), which was also described by Fernandes and Gomes (5). The concomitant occurrence of *Staphylococcus intermedius* (27.0%) and *Staphylococcus aureus* (23.1%) with *Malassezia pachydermatis*, was very close, to that reported by these

Table 4. Results of isolation of *M. pachydermatis* from pendular, semipendular and erect ears, in healthy dogs (Group I) and with external otitis (Group II) from Southern and Midwest regions of Rio Grande do Sul state, Brazil (1996 - 1997).

CONFORMAT ION OF EARS	ISOLATION OF <i>M. PACHYDERMATIS</i>		
	POSITIVE n°(%)	NEGATIVE n°(%)	TOTAL n°(%)
GROUP I			
Pendular	6 (50.0)	6 (50.0)	12 (100)
Semi- pendular	2 (25.0)	6 (75.0)	8 (100)
Erect	4 (33.3)	8 (66.7)	12 (100)
GROUP II			
Pendular	48 (71.6)	19 (28.4)	67 (100)
Semi- pendular	5 (83.3)	1(16.7)	6 (100)
Erect	24 (92.3)	2(7.7)	26 (100)
No information	1 (33.3)	2 (66.7)	3 (100)
Total	90 (67.2)	44 (32.8)	134 (100)

Group I = samples of healthy auditory tubes; Group II = samples of external otitis; n° = number of samples; % = percent.

Table 5. *Malassezia pachydermatis* in monoculture and in interaction with bacteria in the 78 otitis samples (Brazil, RS, 1996- 1997).

ISOLATIONS	GROUP II n°(%)
Monoculture	25 (32.1)
Mixed infection	53 (67.9)
<i>M. pachydermatis</i> + <i>Staphylococcus aureus</i>	16 (20.5)
<i>M. pachydermatis</i> + <i>Staphylococcus intermedius</i>	15 (19.2)
<i>M. pachydermatis</i> + <i>Proteus</i> sp	9 (11.5)
<i>M. pachydermatis</i> + <i>Actinomyces</i> sp	3 (3.8)
<i>M. pachydermatis</i> + <i>S. aureus</i> + <i>S.intermedius</i>	2 (2.6)
<i>M. pachydermatis</i> + <i>Pseudomonas aeruginosa</i>	2 (2.6)
<i>M. pachydermatis</i> + <i>S. intermedius</i> + <i>P. aeruginosa</i>	2 (2.6)
<i>M. pachydermatis</i> + <i>S. intermedius</i> + <i>Actinomyces</i> sp	2 (2.6)
<i>M. pachydermatis</i> + <i>Streptococcus</i> sp	2 (2.6)
Total	78 (100)

authors. The interaction between *M. pachydermatis* and *Staphylococcus aureus*, was significantly bigger, since *M. pachydermatis* was found together with the yeast in all the 18 isolations ($p < 0.01$), while the association with *S. intermedius* was found in 21 out of 26 cultures. Other authors described association with *S. intermedius* or *S. aureus* as being more frequent (3,4,13). The concomitant culture of *Malassezia pachydermatis* and *Pseudomonas aeruginosa* occurred in only four (5.0%) cases of otitis, demonstrating no interaction ($p > 0.05$), which was already observed previously (9,16,23).

RESUMO

Ocorrência de *Malassezia pachydermatis* e de outros agentes etiológicos de otite externa em cães no estado do Rio Grande do Sul, BR (1996-1997)

Este trabalho teve como objetivos estudar a ocorrência da *Malassezia pachydermatis* e de outros agentes etiológicos em cães com otite externa e com conduto auditivo externo hígido; caracterizar a otomicose por *M. pachydermatis*. Foram analisadas 32 amostras de cães com o conduto auditivo hígido e 102 de otite externa. Todas as amostras foram submetidas ao exame direto, cultivo em ágar sangue e em ágar Sabouraud dextrose acrescido de cloranfenicol e cicloheximida. Ao exame direto 52.0% das amostras de otite apresentaram mais de dez células de *M. pachydermatis*. Somente 21.8% das amostras de orelhas hígidas apresentaram no exame direto células com esta morfologia (uma a dez células). *M. pachydermatis* foi isolada em 37.5% das amostras do conduto auditivo hígido e em 76.5% das amostras de otite ($p < 0.01$). Foi frequente a associação entre *Malassezia pachydermatis* e *Staphylococcus aureus* ($p < 0.01$) enquanto a associação da levedura com *Pseudomonas aeruginosa* não foi comum ($p > 0.05$). A infecção por *M. pachydermatis* foi prevalente em cães da raça Cocker Spainel, Pastor Alemão e Fila Brasileiro, não tendo sido demonstradas diferenças para idade, sexo e conformação do pavilhão auricular. A otomicose foi predominantemente ceruminosa e eritematosa. *M. pachydermatis* foi o agente com maior prevalência nas otites externas.

Palavras-chave: *Malassezia pachydermatis*, *Staphylococcus aureus*, otites, cães

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