



# A survey of mycotic otitis externa of dogs in Lisbon

Fernando Manuel Bernardo<sup>1</sup>, Hermínia Marina Martins<sup>2</sup> and Maria Lígia Martins<sup>2</sup>

<sup>1</sup>Faculdade Medicina Veterinária and <sup>2</sup>Laboratório Nacional de Investigação Veterinária, Lisboa, Portugal

## Summary

One hundred and thirteen dogs of different breeds and with different clinical forms of external otitis were mycologically and bacteriologically examined. Forty six of those dogs showed abnormal cerumen with a high yeasts contamination. These yeasts belong to four species: *Malassezia pachydermatis* (80.4%), *Cryptococcus laurentii* (13.1%), *Candida parapsilosis* (4.3%) and *Trichosporon cutaneum* (2.1%). All strains, excepting *C. laurentii* were highly lipolytic. Most of the clinical cases associated with those yeasts were chronic, with hyperkeratosis and lichenification, and most of them were relapsed otitis (91,3%). The most affected dogs were a pendulous ears breeding (65,7%) and males (86,8%). Some dogs had other cutaneous disorders (seborrhoeic dermatitis, pemphigus). *In vitro* tests, using seven different antifungal drugs were systematically performed. All strains revealed to be 5-fluorocytosine-resistant and 32% of them were also resistant to nystatin. One *M. pachydermatis* isolated was resistant to all of the tested antifungal drugs.

## Key words

Otitis, yeasts, *Malassezia pachydermatis*, dogs

## Estudio de otitis micótica externa en perros de la región de Lisboa

## Resumen

Fueron examinados micológica y bacteriológicamente 113 perros de diferentes razas y con distintas formas clínicas de otitis externa. Cuarenta y seis de estos perros presentaban un cerumen anormal altamente contaminado con levaduras. Estas levaduras pertenecían a cuatro especies: *Malassezia pachydermatis* (80,4%), *Cryptococcus laurentii* (13,1%), *Candida parapsilosis* (4,3%) y *Trichosporon cutaneum* (2,1%). Todas las especies, con excepción de *C. laurentii*, eran altamente lipolíticas. La mayoría de las formas clínicas eran una otitis crónica o reincidente (91,3%). Los perros más afectados tenían las orejas péndulas (65,7%) y la mayor parte de ellos eran machos (86,8%). Algunos perros tenían otros trastornos cutáneos. Fueron realizados sistemáticamente varios ensayos *in vitro*, usando siete diferentes antifúngicos. Todas las especies fueron resistentes a la 5-fluorocitosina y un 32,6% de ellas lo fueron también a la nistatina. Una de las cepas de *M. pachydermatis* fue resistente a todos los antifúngicos ensayados.

## Palabras clave

Otitis, Levaduras, *Malassezia pachydermatis*, Perro

The inflammation of external auditory canal meatus of dogs is one of the most common cutaneous diseases, and is frequently difficult to cure [1]. Otitis has many predisposing factors such as excessive ear wax secretion or discharge, trauma associated with faulty cleaning to the ears, foreign bodies, excessive moisture, or parasites within the external auditory canal.

Acute and suppurate otitis of dogs are predominantly cause by bacteria (*Pseudomonas*, *Staphylococcus* spp, *Proteus* spp) [2,3]. Chronic otitis is common in dogs with pendulous ears or in animals with other cutaneous disorder like seborrhoea or atopy and in those treated with antibacteria drugs. Most of chronic otitis in dogs is characterized by erythema, hyperkeratosis and lichenification of the internal face of the ear, cerumen abundant, brownish, thicker and with characteristic sour smell (wet dog).

In normal conditions, many yeasts species can be sporadically isolated from the ear but always in few numbers. In chronic situations, some yeasts species, and particularly *Malassezia pachydermatis* are predominantly found; its excessive population can lead to inflammation [4].

Despite antifungal therapy, many cases do not respond and remain unresolved for long periods, till they may cure spontaneously or became a more serious pro-

## Dirección para correspondencia:

Dr. Hermínia Maria Martins  
Laboratório Nacional de Investigação Veterinária,  
Est. Benfica 701, 1500 Lisboa, Portugal  
Tel.: +351-1 716 3964; Fax: +351-1 716 2075

Aceptado para publicación el 27 de mayo de 1998

blem.

Massive antibiotherapy predispose to subsequent mycotic infections. Otitis externa in dogs have merited worldwide attention, but insufficient has been reported on the microbiology aspects of the disease. This work reports a study of some of the mycotic agents found in these otitis, and *in vitro* testing of its antifungal sensivity.

## MATERIAL AND METHODS

**Samples.** One hundred and thirteen dogs were examined in the Hospital of the Veterinary Faculty of Lisbon as part of a designed survey to extend our knowledge to the effects of age and breed as predisposing factors to otitis. Dogs were three months to 15 years old. Clinical signs were characterised and registered.

Samples were obtained using sterile swabs, to collect cerumen from the external ear canal. Swabs were processed in a maximum delay of 2 h.

**Yeasts research.** A small fraction of the clinical material was microscopically observed after being Gram-stained. The 113 samples were inoculated on Yeast Malt Agar (YMA) (Difco, USA) with antibiotic addition (Chloramphenicol 25 mg/ml) (Sigma, Germany) and incubated at 37°C for five to ten days.

Yeast isolates were first examined microscopically in a mount preparation in Lactophenol Cotton Blue (Diagnostica Merck, Germany) and further biochemical identified by using API ID 32 C (Bio-Mérieux, France) complemented with other tests: Christensen's Urea agar, KNO<sub>3</sub> assimilation, Rice Tween Agar (RAT)(Bio-Mérieux).

All isolates were finally cultured on Tributyrin agar (Oxoid, USA) to verify lipolytic activity.

**Fungistatic sensivity tests.** Antimycotic activity of seven different commercial fungistatic was performed using disks of (Diagnostic Pasteur Sanofi, France) clotrimazole (CTR)-50 µg, amphotericine (Ab)- 100 µg, micotriazole (MCZ)- 50 µg, ketoconazole (KET)-50 µg, nystatin (NY)-100 UI, econazole (EC)- 50 µg, and 5-fluorocytosine (5FC)-10 µg.

Antimicrobial activity was performed using two different media: Casitone Agar (Sanofi) and Semisynthetic Agar (Sanofi). The optical density of yeasts suspensions swab spread on media were equivalent to McFarland Standard number 2 (Bio-Mérieux). Results were obtained after 48-72 h of incubation at 37°C. Diameter of inhibition zones were taken at 72 h.



Figure 1. *Malassezia pachydermatis* culture in Y.M.A.

## RESULTS

All the otitis cases were clinically detected, with abnormal exudation, and inflammatory signals. Gram stained of scrubbings with a dominant population of yeast were considered significant, as a possible source of infection.

Mycological analysis which reveal an exuberant cultures of yeast and without significant or dominant bacterial flora were considered to be of fungal aetiology. Forty six cases of otitis externa (40.7%) were considered to be caused by fungi (Table 1). The remaining otitis, 67 cases, mostly acute forms, had a bacterial aetiology.

Table 1. Occurrence of yeasts in external otitis of dogs.

Isolated Yeasts	n/N	%
<i>M. pachydermatis</i>	37 / 46	80.4%
<i>C. laurentii</i>	6 / 46	13.1%
<i>C. parapsilosis</i>	2 / 46	4.3%
<i>T. cutaneum</i>	1 / 46	2.2%

n/N: number of dogs with positive test results / number of dogs with mycotic otitis.

The most prevalent agent in external mycotic otitis was *M. pachydermatis* (80.4%) (Figures 1 & 2). But other yeasts were found as otitis cause- *Cryptococcus laurentii* (13.1%), *C. parapsilosis* (4.3%) and *Trichosporon cutaneum* (2.2%).

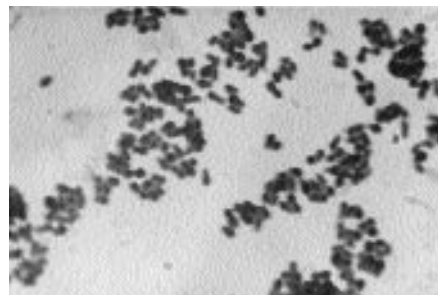


Figure 2. GRAM stain of *Malassezia pachydermatis*

With the exception of *C. laurentii* all the other agents had a high lipolytic activity (Figure 3).

Of the 46 dogs, with mycotic otitis, most were adults (73.7%), 26.3% were young, less than one year of age.

The majority of affected dogs were male (86.8%) and most of them were of breeds with pendulous ears (65.7%). We also verified that, either in acute cases (18.7%) or in chronic one's mostly with lechenification

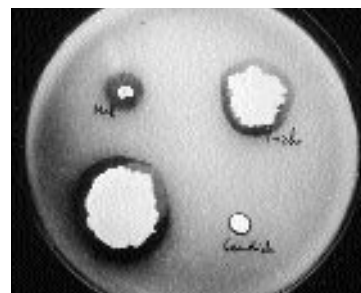


Figure 3. Lipolytic activity of etiological agents of mycotic otitis.

**Table 2.** Resistance profiles of isolated yeasts.

Yeasts	Resistance profiles							Total
	NY <sup>R</sup> + 5FC <sup>R</sup>	CTR <sup>R</sup> +NY <sup>R</sup> +5FC <sup>R</sup>	KET <sup>R</sup> +NY <sup>R</sup> +5FC <sup>R</sup>	Ab <sup>R</sup> +MCZ <sup>R</sup> +NY <sup>R</sup> +5FC <sup>R</sup>	CTR <sup>R</sup> +Ab <sup>R</sup> KET <sup>R</sup> +NY <sup>R</sup> +5FC <sup>R</sup>	CTR <sup>R</sup> +Ab <sup>R</sup> +MCZ <sup>R</sup> +NY <sup>R</sup> +5FC <sup>R</sup>	CTR <sup>R</sup> +Ab <sup>R</sup> +MCZ <sup>R</sup> +NY <sup>R</sup> +KET <sup>R</sup> +Ec <sup>R</sup> +5FC <sup>R</sup>	
<i>M. pachydermatis</i>	5	1	1	1	0	1	1	10
<i>C. laurentii</i>	1	0	1	0	1	0	0	3
<i>C. parapsilosis</i>	1	0	0	0	0	0	0	1
<i>T. cutaneum</i>	0	0	1	0	0	0	0	1
Total	7	1	3	1	1	1	1	15

(91.3%), there was always a very large amount of thick wax or macerated tissues in the ear canal. Some animals (26%) presented other cutaneous problems associated (seborrhoeic dermatitis and pyoderma).

Concerning the *in vitro* antimycotic activity of fungistatic, all strains were resistant to 5FC (10mg). Fifteen strains showed multiple resistance (Table 2). Twenty seven percent of *M. pachydermatis* isolated revealed multiple fungistatic resistance.

## DISCUSSION

The survey was primarily exploratory and associated with disease in external ears of dogs.

These results seem to confirm the relevance of *M. pachydermatis* as an important fungal agent of external mycotic otitis of dogs [5,6].

Mycotic otitis of dogs seems to be more frequent in chronic situations probably due to the fact that acute ones are submitted to excessive pressure of therapeutic agents, for long periods of corticoid and antibacterial topic applications.

Results confirm that mycotic otitis is more common in adults dogs, belonging to breedings with pendulous ears. Most of the affected dogs had chronic otitis and many of them shows hyperkeratinization and lichenification of the ear epithelium.

Antimycotic resistances found on *in vitro* tests, are probably the expression of some excessive topic therapeutic pressure, but, in clinical terms, they may not have special relevance, because the minimal inhibition concentration (MIC's) is largely exceeded in topic applications. For the therapy of dog otitis, many emollients are used topically, like polyenes and imidazoles, associated with bacteriostatic, acaricides and corticoids. These applications do not allow accurate dosaging.

*The authors are grateful to Mr. Jorge Pousadas for the graphic composition and to Mrs. Anabela Ramos for her technical assistance.*

## References

- Fraser G. *Pityrosporum pachydermatis* Weidman of canine origin. Trans Br Mycol Soc 1961;44:441-448.
- Baxter M, Lawler DC. The incidence and microbiology of otitis externa of dogs and cats in New Zealand. N Z Vet J 1972;20: 29-32.
- Bernardo F M A, Martins HML, Gonçalves S. Otitis externascaninas. I Congresso de Dermatologia de Cão e Gato, FMV, Lisboa, 1995.
- Muller G H, Kirk RW, Scott DW. Dermatologia dos pequenos animais (3 Ed). S. Paulo, Editora Manole, 1985: 255-309.
- Abou-Gabal M, Chastain CB, Hogle RM. *Pityrosporum pachydermatis* "canis" as a major cause of otitis externa in dogs. Mykosen 1979;22:192-199.
- Sharma VD, Rhoades HE. The occurrence and microbiology of otitis externa in dog. Journal of Small Animal Practice 1975;16: 241-247.