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# OCCURRENCE OF PARASITES, BACTERIA, FUNGI IN THE EXTERNAL EAR CANAL OF HEALTHY CATS

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Otitis externa occurs frequently in small animals. The microorganisms which have been reported to occur most frequently are *Malassezia pachydermatis, Staphylococcus* spp. and different species of Pseudomonas and Proteus. *Otodectes cynotis* has also been cited as a primary cause of otitis infection. In our study the prevalence of ectoparasites, bacteria and fungi in the non – otitic external ear canals of healthy cats has been investigated.

68.75% of cats were positive. 42.5% (+ 34/80) of cats were found positive for *O. cynotis,* usually in association with others microorganisms. In reference to bacterial patterns observed *Staphylococcus aureus* and *Staphylococcus epidermidis* provided the highest prevalence (8.7% and 7.5%, respectively).

The bacteria in 13.7% of cases (+11/80) were isolated without association with other agents. *M. pachydermatis* yeast organisms were found in 8.7% of cats (+7/80). Associative relationships and biological aspects were underlined.

**Key words**: healthy cat, external ear canal, parasites, bacteria, fungi.

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## INTRODUCTION

Otitis externa is one of the most frequent reasons for small animal veterinary consultation and, particularly in dogs it is frequently found (occurring up to 20% of dogs and in 2-7% of cats) (Bollier *et al.*, 1996; Gotthelf, 2000).

Otitis externa is an aetiologically complex disease, whose primary causes include, among others, ectoparasites (*Otodectes cynotis* mite primarily responsible for 50% external otitis in the young cat and for 5-10% in the dog), allergic diseases, endocrine disorders and foreign bodies.

Yeasts and bacteria are considered to be normal constituents of the domestic carnivores ear microflora. However, changes in the microclimate can turn the stratum corneum into a good medium for the multiplication of microorganisms that may cause clinical cases (Kiss *et al.*, 1996).

The microorganisms most commonly isolated from otitis externa are the *Malassezia pachydermatis* yeast (causal organism of 50-80% of clinical cases of otomycosis in dogs and of 15-20% in cats) followed by *Staphylococcus intermedius* (Baxter, 1976; Guillot and Guèho, 1995). The Malassezia genus has recently been revised by using morphology and molecular biology: the lipid-dependent yeasts which require lipid supplementation for growth, correspond now to six species, particularly linked to human species. These are *Malassezia furfur, Malassezia sympodialis, Malassezia globosa, Malassezia obtusa, Malassezia slooffiae, Malassezia restricta,* while the non-lipid-dependent yeasts, able to grow on routine laboratory media, were assigned to the unique species *M. pachydermatis*, mainly isolated from animals, especially domestic carnivora, which may cause chronic dermatitis and otitis externa (Guillot, 1997). For the clinical resolution of the otitic pathology, knowledge of the aetiology is the basic step required to establish successful therapy (Spaterna et *al.*, 1995). One of the major considerations that must be made in diagnosing and treating ear infections is to determine whether the infection is a monoetiologic infection or, as often occurs, are yielding multiple isolates (this is especially true of animals with chronic infection) (Kowalski, 1988).

Therefore the authors believe it is interesting to evaluate the occurrence of parasites, bacteria and fungi, by direct microscopic and cultural examinations of earwax samples belonging to external auditory canal of healthy cats in order to define the normal ear flora and the possible biological associations.

## MATERIALS AND METHODS

- Animals: n. 80 kennelled european race cats, of different age (≤ 1 year, 1-3 years, 3-5 years) and sex in good general health were examined. Samples of earwax from each ear belonging to cats for parasitological, bacteriological, mycological and cytological examination were collected;

- Parasitological examination: a direct microscopic examination of cerumen, by rolling the ear swab sample in 1-3 drops of paraffin oil or lactophenol on a slide, to evidence the presence of ectoparasites was performed;

- Bacteriological examination: the swab specimens were inoculated onto Trypticase Soy Broth (TSB-Oxoid) and incubated at 37°C for 12h; after incubation, 1 ml of TSB was spread on Nutrient Agar added with 5% of sheep blood, Mannitol Salt Agar, KF Streptococcus Agar and Mac

Conkey Agar (Oxoid). The plates were incubated at 37°C for 48h. The microorganisms isolated were identified using biochemical tests, API System (Bio-Mèrieux) and Sceptor System (Bekton Dicknson) and, when necessary, using serological tests;

- Mycological examination: the samples were cultured on Sabouraud's Dextrose Agar and on modified Dixon's agar for 2 weeks. In order to carry out the identification of Malassezia yeasts, the method described by Guillot *et al.*, (1996) was considered;

- Cytological evaluation: smears of swab were heat-fixed, stained (May-Grunwald-Giemsa or Gram stain reaction) and were examined microscopically.

## RESULTS

The results of parasitological, bacterial and mycological investigations in the samples of swab belonging to healthy cats are shown in Table 1.

68.75% of cats were positive. 34 cats were positive for *O. cynotis* (42.5%) in combination with other organisms or alone isolated (in n° 15 specimens). 29 samples were positive for bacteriological examination (36.2%). The strains belonged to different species and *S. aureus* and *S. epidermidis* provided the highest prevalence (8.7% and 7.5%, respectively). A lot of samples gave an association of two or more bacteria while in 23 cases a single microorganism was isolated.

Table 1 - Results of parasitological, bacterial and mycotic investigations from cerumen of healthy cats.

| N. | Sex | Age (Range of years) | Parasites* | Bacteria | Fungi |
|---|---|---|---|---|---|
| 1 | M | 3-5 | + *O. cynotis* | – | *Mucor* spp. |
| 2 | M | 3-5 | + *O. cynotis* | – | *Mucor* spp. |
| 3 | F | 3-5 | + *O. cynotis* | *Staphylococcus epidermitis* | *Penicillium* spp., *Aspergillus* spp. |
| 4 | F | 3-5 | +*O. cynotis* | – | *Trichophyton mentagrophytes* |
| 5 | M | ≤ 1 | + *O. cynotis* | – | – |
| 6 | F | ≤ 1 | + *O. cynotis* | – | – |
| 7 | M | 3-5 | + *O. cynotis* | – | *Microsporum canis* |
| 8 | M | *1-3* | – | *Leuconostoc* spp | *Geothricum* spp. |
| 9 | M | *3-5* | – | – | *Penicillium* spp. |
| 10 | M | *1-3* | ++ *O. cynotis* | *Staphylococcus aureus* | *Malassezia pachydermatis* |
| 11 | F | *1-3* | ++ *O. cynotis* | *Slaphylococcus epidermitis* | – |
| 12 | M | ≤ 1 | + *O. cynotis* | – | – |
| 13 | F | *1-3* | + *O. cynotis* | *Staphylococcus aureus* | *Malassezia pachydermatis* |
| 14 | M | *3-5* | + *O. cynotis* | – | – |
| 15 | F | *1-3* | – | *Proteus vulgaris* | – |
| 16 | M | ≤ 1 | – | *Escherichia coli*, *Staphylococcus warneri* | – |
| 17 | M | *1-3* | ++ *O. cynotis* | – | – |
| 18 | F | 1-3 | + *O. cynotis* | – | – |
| 19 | M | 1-3 | ++ *O. cynotis* | – | – |
| 20 | F | *3-5* | – | *Arcanobacterium pyogenes*, *Staphylococcus auricularis* | – |

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|---|---|---|---|---|---|
| 21 | F | ≤ 1 | – | – | *Penicillium spp.* |
| 22 | F | 1-3 | +++ *O. cynotis* | *Staphylococcus aureus* | *Microsporum canis* |
| 23 | F | 1-3 | + *O. cynotis* | – | *Microsporum canis* |
| 24 | F | 1-3 | + *O. cynotis* | – | – |
| 25 | F | 1-3 | – | *Staphylococcus xylosus* | *Malassezia pachydermatis* |
| 26 | F | 3-5 | – | *Aerococcus viridans* | *Malassezia packydermatis* |
| 27 | F | ≤ 1 | – | *Leuconostoc spp, Staphylococcus epidermidis* | – |
| 28 | F | 3-5 | +++ *O. cynotis* | *Staphylococcus xylosus, Aerococcus viridans, Escherichia coli* | – |
| 29 | F | 3-5 | – | *Staphylococcus intermedius, Staphylococcus dysgalactiae Escherichia coli* | *Malassezia pachydermatis* |
| 30 | M | 3-5 | + *O. cynotis* | – | – |
| 31 | M | 3-5 | + + *O. cynotis* | – | – |
| 32 | F | ≤ 1 | – | *Staphylococcus sciuri, Escherichia coli* | – |
| 33 | F | 3-5 | + *O. cynotis* | *Staphylococcus intermedius* | – |
| 34 | F | 3-5 | – | *Bacillus cereus* | – |
| 35 | F | 3-5 | – | *Staphylococcus epidermidis* | – |
| 36 | F | 3-5 | + *O. cynotis* | – | – |
| 37 | M | ≤ 1 | – | *Staphylococcus epidermidis* | – |
| 38 | F | 3-5 | + *O. cynotis* | *Staphylococcus xylosus* | – |
| 39 | F | 3-5 | – | *Staphylococcus epidermidis* | – |
| 40 | M | 3-5 | + *O. cynotis* | *Staphylococcus xylosus* | – |
| 41 | F | 3-5 | – | *Staphylococcus sciuri* | *Penicillium* spp. |
| 42 | F | 3-5 | + *0. cynotis* | – | *Penicillium* spp, *Candida* spp |
| 43 | M | 1-3 | + *O. cynotis* | *Staphylococcus aureus* | *Penicillium* spp, *Candida* spp |
| 44 | F | 3-5 | – | – | *Penicillium* spp. |
| 45 | M | 1-3 | + *O. cynotis* | – | *Penicillium* spp. |
| 46 | M | 3-5 | ++ *O. cynotis* | *Staphylococcus aureus* | – |
| 47 | M | 1-3 | ++ *O. cynotis* | – | – |
| 48 | F | 1-3 | – | *Staphylococcus intermedius* | *Malassezia pachydermatis* |
| 49 | M | 1-3 | + *O. cynotis* | – | – |
| 50 | F | 3-5 | + *O. cynotis* | *Staphylococcus aureus* | – |
| 51 | F | 1-3 | – | – | *Penicillium* spp. |
| 52 | F | 1-3 | ++ *O. cynotis* | – | – |
| 53 | M | 1-3 | – | *Staphylococcus intermedius* | *Malassezia pachydermatis* |
| 54 | F | 1-3 | + + *O. cynotis* | – | – |
| 55 | M | 1-3 | – | *Staphylococcus aureus* | – |

* + = 4-5 mites (only adult);
++ = 5-15 mites (particularly nymphs and adults);
+++ = 15-40 mites (eggs, larvae, nymphs and adults).

By mycological examination, the most commonly isolated moulds were *Penicillium* spp. (11.2%) and dermatophytes (5%) (particularly, *Microsporum canis* and *Trychophyton mentagrophytes*). The *M. pachydermatis* yeast was isolated in 7 animals (8.7%) and always associated with bacteria (in 2 cases with *O. cynotis* also). The quantity of organisms recovered from samples of swab in cats by cytological examinations showed a normal commensal role of *M. pachydermatis* (Bollier *et al.*, 1996).The negative results (31.25%) concern cats of very young age (about 2 months).

## DISCUSSION

Mycotic agents associated with clinical cases of otitis externa in domestic carnivores are yeasts firstly (*Malassezia* spp., *Candida* spp., *Rhodotorula* spp., *Cryptococcus* spp.) and moulds secondly (*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., Dermatophytes, *Scopulariopsis* spp.).

The presence of a fungal species in the auricular canal may be justified by considering either the biological relations that the organism develops with the host or the milieu where the animal lives as a source of contamination. The potentiality of growth, then, for each organism will depend on favourable predisposing conditions present in hosts, on the pathogenic power of the strain and on the capacity of the fungus to change from a saprophytic condition to a parasitic modus vivendi.

In recent years there has been an increasing interest in gaining a better understanding of the epidemiology of Malassezia spp. infections, now considered to be emerging and opportunistic pathogens, responsible for a variety of pathological conditions in humans and in domestic and wild animals (Guillot, 1997; Bensignor *et al.*, 2002).

The results obtained allow us to draw the following conclusions:

1. the species of the Malassezia genus isolated in all the samples examined was *M. pachydermatis.*

The prevalence obtained in healthy cats (8.7%) is similar and comparable to the values obtained in small domestic carnivora by Manktelow (1960) in the dog (9%), by Nicklas (1979) in healthy cats (11.5%), by Lorenzini and Sala (1983) in the dog (8.9%), by Matsuda *et al.*, (1984) (7% of nonidentified yeasts in external ears of normal dogs), by Hajsig *et al.*, (1990) in healthy cats (8.0%) (the AA. also found *M. pachydermatis* in the contents of anal sacs more frequently, 16.7%), by Bond *et al.*, (1995) in pet dogs (10%).

It may be presumed that *M. pachydermatis* is similarly present under normal conditions in the two animal species but, then are the host-related factors that contribute to mycotic overgrowth. Because of the availability of several predisposing factors (humidity, pH, allergies, foreign bodies, otoacariasis, etc.) and owing to the anatomy of the canine ear, the external auditory canal of the dog provides an ideal environment for the growth of different microorganisms (Kiss *et al.*, 1996).

It should be emphasized that, regarding the presence of *M. pachydermatis* in specimens of earwax belonging to healthy cats and dogs, other authors obtained higher values: Baxter (1976) isolated the yeast from 49% of 200 healthy ears of dogs, Bollier *et al.*, (1996) isolated *M. pachydermatis* from 20-50% of dogs and

from 23% of cats, Crespo *et al.*, (2002) found a frequency of 17.6% and of 50% in cats and dogs respectively, and for Boncio *et al.*, (2003) the frequencies of isolation of *M. pachydermatis* in healthy pets were 25% in dogs and 16% in cats.

The environment where the animals live, the modus vivendi of the animal, the geographical and seasonal variations are important factors influencing the ear flora ecosystem with consequent microbic dynamism. The interpretation of the literature concerning the most prevalent organisms isolated from ear disease (and from normal conditions) may therefore need to be regionalized. In addition, prevalent organisms may be determined by breed susceptibility to certain primary disease states that result in otitis: for example, Bond *et al.*, (1995) isolated *M. pachydermatis* from the external car canal in 55% of beagles with a significantly greater population size (colony forming units per swab) and showed an acute otitis externa or otitis media in German shepherds frequently perpetuated by a secondary Pseudomonas infection (Gotthelf, 2000).

The results obtained in an epidemiological investigation of 120 canine otitis externa cases by Masuda *et al.*, (2000) are interesting because suggest that *M. pachydermatis* prefers the auditory canal of dogs with lipid- rich earwax (palmitic and oleic acids) as Shetland sheep and Siberian husky dogs, which have a high incidence of otitis externa with *M. pachydermatis* in spite of being erect ear types;

2. regarding the presence of dermatophytes, *M. canis* and *T. mentagrophytes* are the species isolated by us. The implication of dermatophytes in ceruminous otitis (and the alterations of the cutaneous ecosystem are important) is noteworthy (Gotthelf, 2000). Remaining in the mycological area, we isolated *Candida* spp. in 2 samples (2.5%), in association with *Penicillium* spp.. The study of McKellar *et al.*, (1990) is important because it showed that *C. albicans* and *M. pachydermatis* may be associated with otitis externa in a fox hound pack. The association of *C. albicans* or *C. krusei* with *M. pachydermatis* is described also by Kuttin and Glas (1984), by inducing a pathogen synergism. The authors reported also the additional responsibility, in single cases, of *Aspergillus fumigatus, A. niger* and *A. flavus*. Fraser (1961) described 3 cases of acute mycotic otitis, one of which was caused by *C. albicans* and the other two by *C. tropicalis*. Animals had received a prolonged antibiotic therapy;

3. with regard to parasitological investigations, the *O. cynotis* mite was the only ectoparasite found (42.5%). Samples reported a parasitic population variable both in numbers and/or in biological phases; when few mites are present generally they are adult forms and they are an expression of a recent infestation. The animals associated with many ectoparasites with various development phases did not present any clinic signs or suspicious behaviour. It is the primary cause of otitis and it represents an aetiological symbolical agent. The adult mites live on the skin surface of the horizontal ear canal covered by a layer of debris. Mechanical irritation by mites causes the production of a waxy, brown cerumen. Epidermal scales and debris combine with cerumen to form a favourable medium for the growth of secondary bacteria and Malassezia species (Gotthelf, 2000). *O. cynotis* is present in two cases with *M. pachydermatis* and with *S. au-*

*reus*; clinical cases of external otitis with *M. pachydermatis* in association with *O. cynotis* infestations were described (Mason, 1997b). *Sarcoptes scabiei, Notoedres cati, Demodex canis* can also cause ceruminous otitis;

4. the synergism occurring among yeasts and bacteria and also among bacteria is of great interest. *Malassezia* spp. has a symbiotic relationship with the commensal staphylococci even if there is no dependence since the inhibition of the first one does not interfere with the growth of the other (Mason, 1997a). In the external ear of healthy dogs, bacteria, in an epidemiological study, were found in 46% of the total samples and staphylococci (19%) were the main isolates (Matsuda *et al.*, 1984). *S. aureus,* isolated by us in healthy cats in 7 different samples, was often isolated from the skin of animals and men because it represents the natural habitat and is often involved in skin infections in pigs and other animals and food contamination (Scott *et al.*, 1988). The association between *Malassezia* yeast and S. *intermedius* is very interesting because are able to utilize each others's metabolic products (Matousek *et al.*, 2003). Kiss *et al.*, (1996) found that the growth of some species of Malassezia yeast is dependent on the presence of nicotinic acid produced by S. *intermedius.* Staphylococci and *Malassezia* spp. also produce lipase; it has been assumed that this enables the utilization of host sebum as a source of nutrient and production of substances that inhibit non commensal organisms.

Most normal dogs are colonized by *S. intermedius* at oral and nasal cavities and around the perineum. It is therefore likely that staphylococci are resident at these sites which then form a reservoir for transmission to the general skin surface (Mason *et al.*, 1996).

Dworecka-Kaszak (1998) observed, in 3 different periods (1993/94; 1995; 1996), the association of *M. pachydermatis* and coagulase-positive Staphylococci, like *S. aureus* and *S. intermedius,* in cats with otitis externa: particularly, mixed infections in 19.1%. 21% and 22% of clinical cases were reported while *M. pachydermatis* was isolated as a single aetiologic agent in 32.5%, 15.7% and 0% of cases.

The coagulase-negative staphylococci are commonly present on the skin of numerous animal species and cats carried them more frequently: 70% of ears from cats with otitis externa were affected (Marshall *et al.*, 1974). *Arcanobacterium pyogenes* is naturally associated with the mucous membranes of different animals (Mandell *et al.*, 2000). It was isolated from dogs with chronic otitis refractory to the therapy: the seriousness of lesions is linked to destructive and invasive abilities of the microorganism (Lorenzini and Sala, 1983).

The bacteria classified as *Leuconostoc* spp. and *Aerococcus*, isolated in some samples, are not generally considered as pathogens even if they may be isolated from cases of meningitis or otitis (Mandell *et al.*, 2000). *Escherichia coli* and *Proteus vulgaris* present in human and animal intestinal tract are considered as optional parasites. *Pseudomonas* spp. and *Proteus* spp. are part of the normal flora of the ear. The most prevalent microorganisms isolated from ear swab specimens from dogs with otitis externa were *S. aureus, P. aeruginosa, Proteus spp.* and also *S. intermedius, E. coli, Klebsiella spp., S. epidermidis* (Marshall *et al.*, 1974; Sanguinetti *et al.*, 1983; Kowalsky, 1988; Scarampella, 2001);

5. the recent isolation of *M. sympodialis, M. globosa* and *M. furfur* from auricular canal of healthy pet cats (Bond. *et al.*, 1997; Crespo *et al.*, 1999) and *M. obtusa* from dogs affected by external otitis (Crespo *et al.*, 2000) showed that these animals species can be colonized by lipid-dependent species of Malassezia, commensal organisms of human skin, in addition to *M. pachydermatis,* thus bearing witness to a host-parasite relationship constantly in progress (Chang *et al.*, 1998; Guého *et al.*, 1998). This is a very interesting aspect for eventual zoonotic relationship and therefore of remarkable medical-veterinary importance. Further epidemiological studies are also necessary to examine the diffusion of the Malassezia genus in nature involving different domestic and wild animals.

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