



MALASSEZIA PACHYDERMATIS – ETIOLOGY AND CLINICAL FINDINGS IN CANINE EXTERNAL OTITIS – THERAPEUTIC APPROACHES

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ABSTRACT

In 2007, 48 swab samples obtained from dogs with otitis externa were investigated by means of parallel cultivation on blood agar and Sabouraud dextrose agar, supplemented with chloramphenicol and cycloheximide.

Thirty four plasmocoagulase-positive *Staphylococcus* spp., 12 *M. pachydermatis*, 7 *Streptococcus* spp., 4 *Escherichia coli*, 4 *Proteus mirabilis*, and 3 *Pseudomonas aeruginosa* strains were isolated.

Association between: *M. pachydermatis* and *Staphylococcus* spp. (in 9 samples), *M. pachydermatis* and *Streptococcus* spp. (2 samples) and *M. pachydermatis* and *Escherichia coli*, (in one sample) were determined. There were not coinfections of *M. pachydermatis* with either *Pseudomonas aeruginosa* or *Proteus mirabilis*.

Three therapeutic protocols were tested. The best clinical effect was achieved in the combined local application of an antibacterial and an antifungal drug.

INTRODUCTION

During the last years, the interest of keeping dogs as pets, for hunting or as guards is continuously increasing. The owners are more and more concerned about their health. The inflammation of external ear induced by foreign bodies, microbial or parasitic agents is not a life-threatening condition, but causes discomfort in the dog and worries the owners. Very often, the untimely, improper treatment or the use of inappropriate drugs results in chronification of the disease and delays the complete healing of the patient. In most cases of external otitis, veterinarians apply antibacterial medications without laboratory examinations – isolation, identification of the etiological agents and testing of their sensitivity to chemotherapeutics. The treatment of otitis in dogs could be inefficient, if the possible involvement of yeasts is not taken into consideration.

In healthy dogs, *M. pachydermatis* (*Pityrosporum pachydermatis*) could be isolated from the ear canal or the skin (1, 2, 3). This organism is however very frequently detected in otitis or dermatitis, independently or associated with other microorganisms as *Staphylococcus* spp, *Streptococcus* spp., *Escherichia coli* and others. (4, 5).

This information focused our attention to investigating the frequency of isolation of *M. pachydermatis* and its role in the etiology and the clinical manifestation of external otitis in dogs with regard to the optimization of therapeutic approaches.

The purpose of this study was to establish the prevalence of *Malassezia pachydermatis* and its involvement in the etiology of canine external otitis.

MATERIAL AND METHODS

Animals:

Dogs with clinical signs of otitis were selected for the study. We paid attention to patient with unilateral or bilateral ear droop, pruritus, pain at palpation, reddening, thickening of the ear mucosa and increased amount of ear canal discharge.

Samples:

Forty eight samples of ear secretions were obtained from dogs with signs of external otitis, by means of sterile cotton swabs.

The samples were cultivated parallelly on blood agar containing 8-10 % defibrinated sheep blood (Bul Bio, National Centre of Infectious and Parasitic Diseases) under aerobic conditions at 37°C for 24 hours and on Sabouraud dextrose agar supplemented with

chloramphenicol (0.4 g/l) and cyclohexamide (0.5 g/l) (Difco), under the same conditions for 2-7 days. After the incubation, grown colonies were identified using the routine microbiological methods. The sensitivity of all bacterial isolates was tested to antibacterial drugs using the disk diffusion method. The colonies grown on Sabouraud dextrose agar were identified as *M. pachydermatis* on the basis of colonies' morphology and the microscopic features of Gram and Loeffler-stained smears.

Therapeutic protocols

1. Local treatment with selected antibacterial drugs

This protocol was applied to 11 dogs, whose microbiological results did not show involvement of *M. pachydermatis*. The treatment lasted 5-10 days depending on the severity of the clinical signs. Amoxicillin/clavulanic acid (Synulox), neomycin/bacitracin (Bivacyn) or

chloramphenicol/prednisolone (Berlicitin) were used, applied once daily at 0.5-1 ml.

2. Local and parenteral treatment with chemotherapeutics, selected according to the results of antibiogrammes.

This protocol was used in 30 patients, whose isolates were sensitive to the combination amoxicillin/clavulanic acid. Synulox (Pfizer) was used as tablets (at 12.5 mg/kg/day) and as intramammary syringes (0.5-1 ml once daily). The duration of treatment was 5-7 days.

3. Local treatment with an antibacterial drug (according to antibiogrammes – amoxicillin/clavulanic acid: Synulox, neomycin/bacitracin: Bivacyn or chloramphenicol/prednisolone: Berlicitin; once daily at 0.5-1 ml) and an azole derivative (enilconazole).

Table 1. Isolated microbial species and their incidence in dogs with external otitis.

Microbial species	Number of analysed samples	Number of isolates	%
<i>Staphylococcus spp.</i>	48	34	70.8
<i>Malassezia pachydermatis</i>	48	12	25
<i>Streptococcus spp.</i>	48	7	14.6
<i>Escherichia coli</i>	48	4	8.3
<i>Proteus mirabilis</i>	48	4	8.3
<i>Pseudomonas aeruginosa</i>	48	3	6.3

Note: The total incidence is over 100% because of the isolation of more than one etiological agent in some samples.

Table 2. Etiological agents in cases of coinfections and their incidence in dogs with external otitis.

Microbial species	Number of analysed samples	Number of isolates	%
<i>Staphylococcus spp.</i> <i>Malassezia pachydermatis</i>	48	9	18.8
<i>Staphylococcus spp.</i> <i>Proteus mirabilis</i>	48	4	6.3
<i>Streptococcus spp.</i> <i>Malassezia pachydermatis</i>	48	2	4.2
<i>Escherichia coli</i> <i>Malassezia pachydermatis</i>	48	1	2.1

The Imaverol (10% enilconazole, Janssen) was used as 2% solution, applied by rubbing of the ear mucosa with a cotton swab. This course of treatment was used in 7 animals with *M. pachydermatis* isolates combined

with other bacterial agents. The treatment lasted for 7 to 10 days.

In cases when a local treatment was applied, the ear mucosa and the ear canal were cleaned from the available discharge prior to each

procedure.

In all used therapeutic protocols, control samples were obtained and analyzed on the 5th day of the treatment.

RESULTS

Out of the studied 48 samples, 12 *M. pachydermatis* strains were isolated. In nine cases, there was a coinfection with plasmocoagulase-positive staphylococci; in 2 samples, a *Streptococcus* spp. strain was also isolated and in one sample – a representative of the *Enterobacteriaceae* family (*E. coli*). *M. pachydermatis* was not isolated as a single microbial species in dogs, untreated with antibacterial drugs.

Furthermore, 34 plasmocoagulase-positive staphylococci were isolated. In 4 samples, *Proteus mirabilis* was also found out, these samples being obtained from dogs with chronic otitis.

As single etiological agents, 5 *Streptococcus* spp., 3 *Pseudomonas aeruginosa* and 3 *E. coli* isolates were present.

The data about the involved microbial species and their frequency of isolation in dogs with external otitis are presented in Table 1.

Table 2 presents the involved microbial agents in samples with more than one etiological agent and their frequency of isolation in canine external otitis.

Therapeutic protocols

The first therapeutic protocol (only local treatment) achieved clinical healing in 7 out of 11 treated animals (63.6%). The control microbiological examination revealed the presence of *M. pachydermatis* in 2 samples obtained from dogs with unsatisfactory effect of the therapy. The other two dogs where this protocol failed were with chronic otitis with coinfection with *Proteus mirabilis* and *Staphylococcus* spp. and in them, a *Proteus* strain was still isolated throughout the control inoculation.

The dogs treated according to the second protocol (locally and parenterally with selected chemotherapeutics) showed clinical healing in 26 patients (86.6%). In 4 dogs, the therapy was not successful. The control microbiological examination in 3 dogs revealed the presence of *M. pachydermatis* in pure culture and in one patient, there was again a coinfection with *Proteus mirabilis* and *Staphylococcus* spp.

In third therapeutic protocol (local application of an antibacterial drug and an azole derivative), all 7 patients were healed (100%).

DISCUSSION

The yeasts from the *Malassezia* genus, where *M. pachydermatis* belongs, are commensals, inhabiting the skin of mammals and birds, including the ear canal of the dog (1, 2, 3). In 1955, Gustafson (6) isolated budding yeasts from dogs with external otitis. Initially this species was called *Pityrosporum pachydermatis*, and subsequently – *Malassezia pachydermatis*. The etiological role of this microorganism in dermatitis was first reported by Dufait in 1983 (7). Numerous species have been described, but only several are incriminated as causative agents of diseases – *M. pachydermatis*, *M. furtur*, *M. globosa*, *M. sympodialis* (8). The last three species are part of the resident microflora of human skin (8).

Although *M. pachydermatis* is isolated from the skin or the ear canal in healthy dogs, its importance as a member of the microbial association responsible for the clinical manifestation of otitis externa should not be underestimated. The number of yeast cells upon the healthy mucosa is many times lower than on the damaged mucous coat. The inflammation of the auditory meatus helps the proliferation of yeasts (9, 10).

Our data about the frequency of isolation of *M. pachydermatis* are similar to the data reported by others (9,10)

In cases of proved associated infection between *M. pachydermatis* and other bacterial agents, the clinical manifestation of the otitis was more severe.

The inadequate therapeutic approaches (lack of laboratory diagnosis and empirical application of antibacterial drugs) often result in chronification of otitis. In 7 dogs included in our survey, a treatment has been performed but without clinical effect. In 4 of them *M. pachydermatis* and *Staphylococcus* spp., was found out, and in the other three – association of *Proteus mirabilis* of *Staphylococcus* spp.

In this study, the used therapeutic approaches were not successful in 7 dogs. The control examination of 2 samples showed the involvement of *M. pachydermatis*, and in these animals, the therapeutic protocol was changed. The inclusion of Imaverol was advised. In the other 5, the failure to heal was most probably due to the non-observance of recommended treatment modes from the part of the owners.

These clinical cases and the tested various approaches for treatment of external otitis support the thesis that when possible, laboratory examinations should be performed

prior to the treatment (not after a failure). In the practice, when the laboratory examination is not an available option, we recommend a treatment with preparations containing antibacterial and antifungal substances. At this time, the following medications responding to this condition are available at the Bulgarian market – Mitex (Richterpharma), Surolan (Janssen), and Dexoryl (Virbac).

Cases of a special interest were the five dogs whose initial microbiological test was negative for *M. pachydermatis*, but the second examination made to monitor the effect of the treatment, the test for this organism was positive. The explanation of this fact in our view is that throughout the inoculation, the bacterial flora grows faster and competes with yeasts. Furthermore, some staphylococcal strains, resistant to chloramphenicol were isolated, and these strains grew on Sabouraud dextrose agar as well. This was the probable reason why in these 5 dogs, the monitoring of the treatment effect showed the presence of yeasts.

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