PATHOLOGICAL AND CLINICAL ASPECTS OF THE DISEASES CAUSED BY MALASSEZIA SPECIES

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From veterinary point of view Malassezia pachydermatis has the greatest significance. It has been standing in the focus of interest since the early 1990s, mostly because of the frequency of otitis externa and dermatitis caused by this yeast in dogs. This is the only lipid-independent species in the genus Malassezia. It can be found in very large proportion on the skin of healthy animals, but can be isolated in much greater number from diseased dogs. It often causes illness together with other pathogens (e.g. Staphylococcus intermedius). Some breeds are predisposed. In addition to the treatment of the accidental concurrent diseases, therapy consists of systemic and/or topical antimicrobial treatment. Ketoconazole is used most frequently. Malassezia pachydermatis plays also a role in the skin disorders of other carnivores. It has little zoonotic potential, it can be dangerous to immunocompromised humans. The other Malassezia species have little veterinary importance, although M. sympodialis and M. globosa were isolated from asymptomatic animals (mostly cats) and from mixed infections.

Keywords: Malassezia yeasts, animal infections

Microbiology

Taxonomy, culture

The lipophilic yeasts of the cutaneous microflora are well-known for a long time, but their exact taxonomic classification was made only in the middle of 1990s [1, 2]. Formerly there was a large nomenclatural confusion, mainly the name
Pityrosporum was used. In the 1980s the evidence of the synonymy of *Malassezia* and *Pityrosporum* was accepted. On the basis of genetic studies in the genus *Malassezia* there are three old (*M. furfur, M. sympodialis* and *M. pachydermatis*) and four new species (*M. globosa, M. obtusa, M. restricta, M. slooffiae*). As a human pathogen, *M. furfur*, the causative agent of pityriasis versicolor and other skin and systemic disease has the greatest significance, while in the animals *M. pachydermatis* is the most important. Latter is the only species that does not need long-chain fatty acids for growing (it is not lipid-dependent). The cell wall of malassezias is thick, built up from more layers; outside there is a thin layer, what can easily disappear during the histological procedure. The inner surface of the cell wall is corrugated. This cell wall structure together with the staining ability with diazonium blue and enteroblastic budding is characteristic of the fungi *Basidiomycetes*. For the culture different media are used: Sabouraud agar supplemented with olive oil or the modified Dixon agar. Incubation at 37 °C is optimal [3]. In addition to the genetic methods, the identification of the species is possible on the basis of the probes shown in the Table [3, 16, 17].

**Scheme for the identification of Malassezia isolates**

<table>
<thead>
<tr>
<th>Growth with</th>
<th>No lipid supplement</th>
<th>High concentration of Tween 20</th>
<th>Low concentration of Tween 80</th>
<th>Cremophor</th>
<th>Splitting of Esculin</th>
<th>Catalase reaction</th>
<th>Growth &gt; 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pachydermatis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td><em>M. furfur</em></td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>M. sympodialis</em></td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. slooffiae</em></td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>M. globosa</em></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>M. restricta</em></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>M. obtusa</em></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>v</td>
<td>−</td>
<td>v</td>
</tr>
</tbody>
</table>

+ positive; − negative; v variable reaction

*M. pachydermatis* is the most important member of the *Malassezia* genus for the veterinarian. Within the species 7 sequence types were separated on the basis of the examination of the large ribosomal subunit [2]. Some of them seem to be host specific. *M. pachydermatis* is a lipophilic species, but unlike the other malassezias, the lipid supplementation with long-chain fatty acids is not essential, therefore it can be more
easily isolated. Besides the Sabouraud’s dextrose agar it grows well on blood agar plates. However, there were some types described, that do not grow at all or only in very small colonies in primoculture on Sabouraud agar. After some transfers these isolates, belongig to the sequence type ‘Id’, form small colonies, but usually loose their lipid requirements [4]. On Sabouraud agar M. pachydermatis strains form dry, convex colonies with smooth surface. Initially they are white, many times with pinkish shade, later brown. Morphologically two types are distinguished, but both are oval or elliptic, the cells are 2–3×5–6 μm large. The unipolar budding is characteristic of them. Random amplified DNA typing (RAPD) can be a useful method for epidemiological studies within the species [5].

Ecology

_Malassezia_ yeasts can be isolated mostly from the skin of various animals and humans. There are mainly lipid-dependent species on humans, and they exist on certain animals (rhinos, pigs, cats) together with _M. pachydermatis_. Latter was isolated first time from the dermatitis of an Indian rhinoceros by Weidman in 1925 [6], and later it was detected on various wild and domesticated animals, dominantly carnivores. They can be found most frequently in the ear and on the skin of dogs; the proportion of carriage can be over 50 percent. They are in large number principally on the perioral, perianal, inguinal, axillar and interdigital area, and also in the oral cavity, perianal glands, on the vulva and the prepuce and in the skin folds [7]. Certain breeds are predisposed to develop illnesses caused by _M. pachydermatis_ (e.g. Basset Hound, American cocker spaniel, West Highland white terrier), but there is no gender or age predilection. Studies confirmed that the frequency of _Malassezia_ carriage of the cats infected by the viruses causing immunodeficiency (feline immunodeficiency virus, feline leukemia virus) is many times higher than in the seronegative control groups. Contrary to the saprophyte fungi (e.g. _Aspergillus_ and _Penicillium_ spp.) and dermatophytes this cannot be attributed to the common risk factors arised from living outdoor, because malassezs do not occur free in the environment [8].

There is no close relation between the frequency of the carriage and the symptoms, although the yeasts can be isolated in significantly greater number from the diseased animals.

Pathomechanism

Adherence, the specific attachment of microorganisms to cells plays an important role in the colonisation of _Malassezia_ yeats and in the infection. The adherence of _M. pachydermatis_ to the canine corneocytes in vitro is dose and time-dependent, and is maximal after 2 hours. It increases with growing of the temperature,
but not influenced by the presence of staphylococci. The pre-treatment of the yeast cells and the corneocytes with trypsin decreased the adherence in a minor part of the strains, suggesting that trypsin-soluble proteins and glycoproteins take part in the process. On the other hand incubation with mannose, sucrose and N-acetyl-D-glucosamine was ineffective. From the 4 lectins examined with the purpose of demonstrating the suspected carbohydrate adhesins on the surface of the yeast cells and such ligands on the corneocytes, only the treatment of canine cells with concanavalin-A decreased the adherence in a minor part of the isolates. This effect was abrogated by pre-incubating concanavalin-A with its hapten inhibitor, suggesting that mannosyl-bearing residues on the epithelial cells serve as ligands by that strain [8].

For the moment it is unclear which factors influence the affinity of Malassezia species to the various hosts. The results of a study directed to the adherence of some M. pachydermatis and M. sympodialis strains to human, canine and feline corneocytes in vitro do not correspond with the in vivo frequency data [9]. It is known, however, that M. pachydermatis colonises the skin and mucosae of puppies on the first days of their lives [10].

From the exoenzymes as possible pathogenity factors M. pachydermatis strains produce proteinase, phospholipase, hyaluronidase and chondroitin-sulphatase almost without exception, and there were no significant differences in the enzyme production between the strains from the ears or the skin of dogs [11].

Investigating the immunomodulatory effect of Malassezia species it was found that they decrease the cytokin (IL-1β, IL-6, TNF-α) production of peripheral blood mononuclear cells in vitro. After extraction of lipids from the cell wall of yeasts, the inhibition of these pro-inflammatory cytokines stops. It is supposed that the thin lipid layer covering the outer surface of Malassezia cells masks carbohydrate domains, and their effect to stimulate cytokine production can not be realized [12]. The M. pachydermatis population is significantly higher in number on the skin of dogs with seborrhoeic dermatitis than on healthy dogs, but the general proliferative skills of peripheral blood lymphocytes (investigated by stimulation with phytohaemagglutinin) were not different in the two groups, while the reaction of the lymphocytes from the diseased dogs was lower to Malassezia antigens. Significantly higher specific IgG and IgA levels were detected in sick dogs, although it has small protective significance [13]. The reports on human M. pachydermatis infections support the importance of cell-mediated immunity, because such infections occur only in patients suffering from the disorders of cell-mediated immunity. Malassezias are able to cause type I hypersensitivity reactions, that can be detected by intradermal tests, and probably takes part in the pathogenesis of Malassezia dermatitis [14]. The clinical importance can be considerable in pruritic cases.
Clinical aspects

Symptoms

From veterinary point of view diseases of dogs caused by *M. pachydermatis* have the greatest significance. The two main types are otitis externa and *Malassezia* dermatitis.

Fungal otitis externa is a well-known entity for a long time, but it is almost impossible to give exact morbidity data, because asymptomatic carriage of *M. pachydermatis* in the ear canal of dogs is common and it is very difficult to create definitive diagnostic criteria. In case of heavy monoinfection the skin of ear canal is erythematous and pruritic, covered by dark ceruminous discharge. It is observed mainly in dogs with long, hanging ears. Mixed infections are frequent, the commonest bacteria are *Staphylococcus intermedius* and *Pseudomonas aeruginosa*.

Inflammation of other skin areas is often associated with primary diseases (hypothyreoditis, hyperadrenocorticism, diabetes mellitus, atopia, etc.). The judgement of previous antibiotic and glucocorticoid therapy is controversial. Generally alopecia, erythema and scaling appears on the face, trunk, perianal, interdigital and periungual area and in skin folds. In chronic cases hyperpigmentation and other secondary lesions due to scratching and licking (hyperpigmentation, erosions, lichenification) can be often seen. The clinical diagnosis is many times seborrhoea sicca or oleosa. In cases of a generalized lesion there are intensive pruritus and offensive odour. *M. pachydermatis* rarely induce folliculitis in dogs, looks like bacterial folliculitis caused by *Staphylococcus intermedius*.

Although *Malassezia* yeast can be isolated from the mucosae, there are only a few pathogens on these areas. The inflammation of oral cavity, pharynx and perianal glands were reported.

Cats are often carriers of *M. pachydermatis*, but not so frequently as dogs. It was isolated more times from the ear of healthy cats alone or together with *M. sympodialis* and *M. obtusa*.

Individual otitis cases caused by *M. pachydermatis* were reported in other carnivores, pigs, camels, dermatitides in rhinos and sea lion.

Diagnosis

Beyond the informative anamnesic data (predisposed breed, diagnosed endocrinopathy, atopy, previous problems due to *Malassezia*, parasitoses, keratinization disorders, etc.) and characteristic symptoms, for the diagnosis is necessary to detect the yeast. The methods are cytology, culture and histopathology. For the cytology swab and tape samples or direct touch preparations are examined,
stained with methylene blue, Gram or Diff-Quik. For culture traditional or contact plates are used (Sabouraud dextrose or modified Dixon agar, 32 or 37°C). Because of the frequency of asymptomatic carriage, the interpretation of the results of these methods needs cautiousness. Histopathology is rarely used in the practice; false negative results can arise because of the detachment of stratum corneum during preparation. There are no generally accepted quantitative diagnostic criteria for these methods.

**Therapy**

*Malassezia* dermatitis needs an effective antimicrobial therapy, in addition to the treatment of the occasional primary disorder. During this the presence of other pathogens must be taken into account. In case of an extended lesion, systematic treatment is necessary; the most frequently used drug is ketoconazole in dose 5–10 mg/kg body weight. Dogs tolerate ketoconazole well, during the 10–14 days period of an average treatment, side effects (anorexia, vomitus, diarrhoea) seldom occur. In the case of a long-term therapy or a risk patient the control of liver enzymes is advisable.

Ketoconazole cannot be given to pregnant animals and patients with liver disease, that time the literature recommends itraconazole. Cats are more sensitive to these drugs, in that case the careful dosage and laboratory control is essential.

In the topical treatment mainly shampoos are used with ketoconazole, miconazole, itraconazole, chlorhexidine (the latter two also in combination), selenium disulphide or lime sulphur as active agent. Their effectivity depends on the proper application. According to the experiences the simultaneous systemic and topical therapy is more effective.

Otitides are mainly topically treated with preparations containing antibiotic, antifungal and antiinflammatory agents, supplemented with ketoconazole given per os when it is necessary.

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**References**