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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

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Pityrosporum was used. In the 1980s the evidency 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*

	Growth with							
	No lipid supplement	High concentration of Tween 20	Tween 40	Low concentration of Tween 80	Cremo-phor	Splitting of Esculin	Catalase reaction	Growth > 37 °C
M. pachydermatis	+	+	+	+	v	v	v	+
M. furfur	−	+	+	+	v	−	+	+
M. sympodialis	−	−	+	+	−	+	+	+
M. slooffiae	−	+	+	−	−	−	+	+
M. globosa	−	−	−	−	−	−	+	−
M. restricta	−	−	−	−	−	−	−	−
M. obtusa	−	−	−	−	−	+	+	−

+ 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 praeputium 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 malassezias 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-tretament 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 demonsrating 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 erythematons 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 areuginosa*.

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 citology 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 statum 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

1. Guého,E., Midgley,G., Guillot,J.: The genus Malassezia with description of four new species. Antonie van Leeuwenhoek **69**, 337–355 (1996).
2. Guillot,J., Guého,E.: The diversity of Malassezia yeasts confirmed by rRNA sequence and nuclear DNA comparisons. Antonie van Leeuwenhoek **67**, 297–314 (1995).

3. Guillot,J., Bond,R.: Malassezia pachydermatis: a review. Med Mycol **37**, 295–306 (1999).
4. Bond,R., Anthony,R.M.: Characterisation of markedly lipid-dependent Malassezia pachydermatis isolates from healthy dogs. J Appl Bacteriol **78**, 537–542 (1995).
5. Bockhout,T., Kamp,M., Guého,E.: Molecular typing of Malassezia species with PFGE and RAPD. Med Mycol **36**, 365–372 (1998).
6. Weidman,F.D.: Exfoliative dermatitis in the Indian rhinoceros (Rhinoceros unicornis), with description of a new species: Pityrosporum pachydermatis. Fox,H. Ed. Rep Lab Mus Comp Zoo Soc Philadelphia 1925, pp 36–43
7. Bond et al.: Population sizes and frequency of Malassezia pachydermatis at skin and mucosal sites on healthy dogs. J Small Anim Pract **36**, 147–150 (1995).
8. Sierra,P., Guillot,J., Jacob,H., Bussiéras,S., Chermette,R.: Fungal flora on cutaneous and mucosal surfaces of cats infected with feline immunodeficiency virus or feline leukemia virus. Am J Vet Res **61**, 158–161 (2000).
9. Bond,R., Lloyd,D.H.: Studies on the role of carbohydrates in the adherence of Malassezia pachydermatis to canine comeocytes in vitro. Vet Dermatol **9**, 105–109 (1998).
10. Bond,R., Wren,L., Lloyd,D.H.: Adherence of Malassezia pachydermatis and Malassezia sympodialis to canine, feline and human corneocytes in vitro. Vet Rec **147**, 454–455 (2000).
11. Wagner,R., Schadler,S.: Qualitative study of Malassezia species colonisation in young puppies. Vet Rec **147**, 192–194 (2000).
12. Coutinho,S.D., Paula,C.R.: Proteinase, phospholipase, hyaluronidase and chondroitin-sulphatase production by Malassezia pachydermatis. Med Mycol **38**, 73–76 (2000).
13. Kesavan,S., Holland,K.T., Ingham,E.: The effects of lipid extraction on the immunomodulatory activity of Malassezia species in vitro. Med Mycol **38**, 239–247 (2000).
14. Bond,R., Elwood,C.M., Littler,R.M., Pinter,L., Lloyd,D.H.: Humoral and cell-mediated responses to Malassezia dermatitis. Vet Rec **143**, 381–384 (1998).
15. Morris,D.O., Olivier,N.B., Rosser,E.J.: Type-1 hypersensitivity reactions to Malassezia pachydermatis extracts in atopic dogs. Am J Vet Res **59**, 836–841 (1998).
16. Mayser,P., Hase,P., Papavassilis,C., Pickel,M., Gruender,K., Gueho,E.: Differentiation of Malassezia species: selectivity of cremophor EL, castor oil and ricinoleic acid for M. furfur. Br J Dermatol **137**, 208–213 (1997).
17. Weiss,R., Raabe,P., Mayser,P.: Sprosspilze der Gattung Malassezia: Taxonomische Einteilung und Bedeutung aus (veterinar)medizinischer Sicht. Mycoses **43**, supl. 169–172 (2000).