



MALASSEZIA VERSUS CANDIDA IN HEALTHY DOGS

Sihelská, Z., Pangráčová Piterová, M., Čonková, E.
Harčárová, M., Böhmová, E.

Department of Pharmacology and Toxicology,
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
Slovakia

zuzana.sihelska@uvlf.sk

ABSTRACT

The genera *Malassezia* and *Candida* include yeasts which are members of the normal mycobiota of the skin and mucosal sites of humans and other warm-blooded animals. These yeasts are associated with a variety of dermatological disorders and also systemic diseases in humans and other animals. This study confirms the occurrence of *Malassezia* and *Candida* species in healthy dogs. Samples were collected from different body sites: external ear canal, interdigital area, skin of the axilla and of the neck, and the oral and rectal mucosae. The isolates were identified using phenotypic methods (biochemical-physiological and morphological characteristics). The presence of yeasts were investigated in the specimens from 70 healthy dogs. *Malassezia* species were isolated in 44 dogs from which 84 *Malassezia* isolates were obtained. Only one *Candida* isolate was obtained from the dogs examined. It was found that *Candida* does not occur in dogs normally and *Malassezia* was the main colonizing yeast in healthy dogs.

Key words: *Candida*; dog; incidence; *Malassezia*; mycoses; skin

INTRODUCTION

The most frequent fungal infections in animals are dermatophytosis and dermatomycosis. Dermatophytes are keratinophilic and keratinolytic fungi, characterized by a high affinity to keratin-containing tissues. In dogs, dermatophytosis are most often caused by genera of *Microsporum* and *Trichophyton* [1]. Dermatomycosis are also very frequently diagnosed in veterinary medicine, especially those caused by yeast of the genera of *Malassezia* and *Candida*. The yeasts of these genera are part of an animal's normal flora. However, despite being saprobes, there have been many reports of infections caused by these microorganisms, which present different clinical manifestations.

In dogs, *Malassezia* spp. have been associated with *otitis externa* and *dermatitis* [27, 29, 31] and as a possible aggravating factor in the physiopathology of corneal ulcers [33]. The predisposing factors that determine the formation of *Malassezia* mycoses include: the alteration of the host's

immune system, greasy skin, high temperature, humidity, corticosteroid treatment and others [2]. Currently, the genus *Malassezia* includes 16 species: *M. globosa*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. furfur*, *M. sympodialis*, *M. japonica*, *M. yamatoensis*, *M. dermatis*, *M. pachydermatis*, *M. caprae*, *M. equina*, *M. nana*, *M. cuniculi*, *M. brasiliensis* and *M. psittaci* [7, 8].

Several reports have shown that *Candida* spp. are also important pathogens in dogs, being related to: urinary infections [32], endophthalmitis [26], cutaneous lesions [28], and systemic infections [6]. The group with the most relevant clinical species includes: *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. orthopsilosis*, *C. metapsilosis*, *C. tropicalis*, *C. viswanathii*, *C. krusei*, *C. guilliermondii*, *C. lusitanae*, *C. dubliniensis*, *C. pelliculosa*, *C. kefyr*, *C. lipolytica*, *C. famata*, *C. inconspicua*, and *C. rugosa* [13, 37]. The transition to pathogenicity in *Candida* species, mainly in *C. albicans*, occurs generally due to the host's immunological condition, leading to a microbiota unbalance, which is associated with important virulence factors, such as biofilm formation and hydrolytic enzymes production, and promotes fungal dissemination to other sites and organs. The clinical manifestations vary from a localized mucosal or skin infection to disseminated disease [23].

The purpose of this study was to compare the occurrence of *Malassezia* and *Candida* yeasts on the skin and the mucous membranes in healthy dogs.

MATERIALS AND METHODS

Animals and samples

The survey was carried out on 70 healthy dogs in good general health with no history of skin or ear diseases and with no antimicrobial treatments in the past 12 months. The ages of animals ranged from 4 months to 10 years. From each dog, four samples originating from four different anatomical sites of the body (external ear canal, interdigital area, skin of the axilla and of the neck) were collected by a standard swab method. A sterile cotton swab soaked in sterile saline was used to rub against the skin surface, with continuous rotation of the swab over at least a 10 seconds period. In addition, 20 dogs had samples taken also from oral and rectal mucosae.

Cultivation and microscopy

The samples were inoculated on specific media for the culturing of *Malassezia* and *Candida*: Sabouraud dextrose agar with chloramphenicol (SCH) (HiMedia Laboratories Pvt. Ltd., Mumbai, India), Modified Leeming & Notman agar medium (MLNA) [25], and Modified *Candida*-Chrom agar (HIT) with Tween 40 [21]. The plates were incubated at 32 °C for 7 days. All yeast cultures were identified to the genus level by using conventional mycological methods, including the examination of both macroscopic appearance of the colonies (colonies shape, texture and colour) and the microscopic cell morphology (cell size, shape and budding characteristics). In order to assess the lipid dependency, the colonies were subcultured on Sabouraud dextrose agar. Each isolate was stained by Gram and examined by microscopy for the presence of the typical *Malassezia* and *Candida* yeast cells. More detailed identification of *Malassezia* was performed according to Kaneko et al. [20]. Reference strains *M. pachydermatis* (CBS 1879, Utrecht, Holandsko) (Fig. 1) and *C. albicans* (CCM 8512, Brno, CR) (Fig. 2) were used as positive controls.

RESULTS

Forty four out of 70 examined dogs were scored positive for *Malassezia* spp. (62.9%). Out of all of the samples only one was identified as a *Candida* isolate which was obtained from the rectal mucosa and identified as *C. albicans*. No *Candida* yeasts were found on the skin. From the samples, a total of 84 *Malassezia* isolates were obtained. All 84 *Malassezia* isolates were identified as *M. pachydermatis* (Fig. 3). The information about the occurrence of yeasts on the skin in the dogs is presented in Table 1. Seventy three isolates were collected from the skin of the following body sites: 36 isolates — external ear canal (51.4%); 20 isolates — interdigital area (28.6%); 10 — axilla (14.3%); and 7 — neck (10.0%). Six *Malassezia* cultures (30.0%) were obtained from the oral mucosa and five (25.0%) from the rectal mucosa (Table 2).

DISCUSSION

In dogs, yeasts preferably colonize moist areas such as mucous membranes, mucocutaneous junctions, skinfolds,



Fig. 1. Cultivation of *M. pachydermatis* reference strain on HIT



Fig. 2. Cultivation of *C. albicans* reference strain on HIT

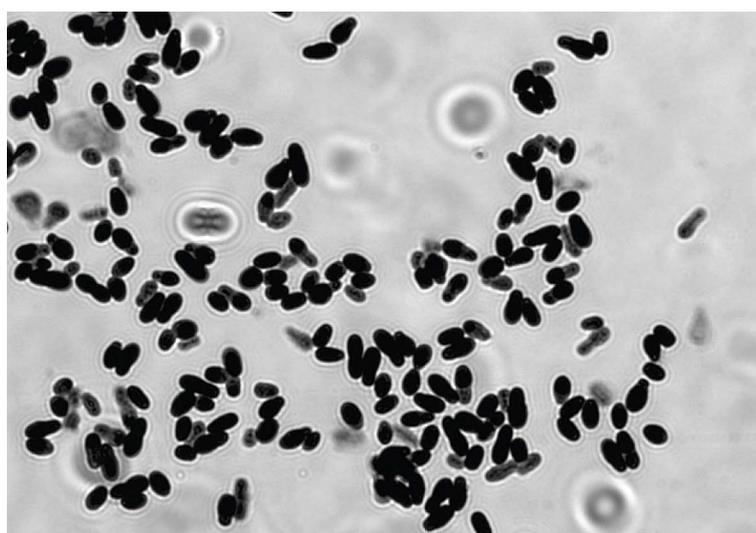


Fig. 3. Cells of *M. pachydermatis* isolate (Axio Observer Z.1, Magn. ×1000).

Table 1. Presence of *Malassezia* and *Candida* yeasts on the skin in dogs (70 animals)

Examined body sites	Number of <i>Malassezia</i> isolates	Number of <i>Candida</i> isolates
External ear canal	36 (51.4%)	0 (0%)
Interdigital area	20 (28.6%)	0 (0%)
Skin of the axilla	10 (14.3%)	0 (0%)
Skin of the neck	7 (10.0%)	0 (0%)
Total	73	0

Table 2. Presence of *Malassezia* and *Candida* yeasts on the mucous membrane in dogs (20 animals)

Examined body sites	Number of <i>Malassezia</i> isolates	Number of <i>Candida</i> isolates
Oral mucosa	6 (30.0%)	0 (0%)
Rectal mucosa	5 (25.0%)	1 (5.0%)
Total	11	1

interdigital areas and ears. *Candida* and *Malassezia* species are ubiquitous organisms existing almost exclusively as commensal organisms, which rarely become pathogenic [18]. A range of skin micro-environmental factors, such as the bacterial microbiota present, pH, salts, immune responses, biochemistry, and physiology, may play a role in the adherence and growth of yeasts, favouring distinct genotypes depending on the geographical area and/or the skin sites [17].

Currently, the results of scientific studies on *Candida* yeasts in animals vary. Brito et al. [5] and Cleff et al. [11] stated, that *Candida* genus is considered to be part of the microbiota of dogs. We did not identify *Candida* yeasts on the skin of our examined dogs. This give rise to the question about *Candida* presence/absence on the skin of dogs. Lee et al. [24] asserted that the presence of *Candida* in a dog is always the expression of a pathologic state and of its intrinsic pathogenicity. Our results indicate that *Candida* spp. is not a member of the normal skin flora. We identified one *Candida* isolate from the rectal mucosa. *Candida* yeasts colonize mostly mucous membrane [16, 34, 35] and their occurrence on the skin is not common and may be bound with outbreak of a disease.

The most *Malassezia* affected animal is the dog [31]. In general, *Malassezia* yeasts are associated with the skin and mucous membranes in healthy and diseased dogs [9, 10, 22, 30, 31]. Even 62.9% of the dogs are positive for *Malassezia* yeasts. In our study, *M. pachydermatis* was isolated as the sole species in all dogs examined. Generally, lipophilic *M. pachydermatis* is the main colonizing yeast in healthy and also in diseased dogs [31]. Only a few times lipid-dependent *Malassezia* yeasts (*M. furfur*, *M. nana* and *M. obtusa*) were identified [11, 12, 14, 15, 36].

In our group of dogs, the external ear canal was the most frequently colonized (51.4%). However, Nardoni et al. [30] appointed the frequency of *Malassezia* isolation: interdigital area (70.7%), ears (63.4%), axilla (23.8%), and perineum and anus (19.0%). Cafarchia et al. [9] stated that the perianal region is the most frequently colonized and in the ear canal is 12.1% of all *Malassezia*. But Kumar et al. [22] detected *Malassezia* yeasts in 39.39% of healthy ears and Campbell et al. [10] represented 17% prevalence of *Malassezia* in healthy ears. The occurrence of *Malassezia* on the different body sites varies and depends on: the group of examined dogs, season, type of hair and ear and other factors.

Malassezia yeasts have been isolated from different mucosal sites from healthy dogs. In our group, *Malassezia* occurred on the oral mucosa in 30.0% of the samples and on the rectal mucosa in 25.0% of the samples. Hajsig et al. [19] isolated this yeast also from the anus, anal sacs, and vagina of healthy dogs. Bond et al. [4] recovered *M. pachydermatis* from the anus and mouth of 27.5% of healthy dogs of various breeds. However, Bond and Lloyd [3] suggested that in some cases, mucosal colonization may be secondary to skin proliferation of the lipophilic yeasts.

In conclusion, *Candida* spp. does not occur on the skin in healthy dog normally. The presence of this yeast is linked rather with the mucosae. On the skin and mucosa is the highest occurrence of *Malassezia* yeasts and *M. pachydermatis* remains the most prevalent species in the healthy dog.

ACKNOWLEDGEMENT

This study was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0377.

REFERENCES

1. **Blaszowska, J., Wójcik, A., 2012:** Current problems concerning parasitology and mycology with regard to diseases of the skin and its appendages. *Pol. Parasitol. Soc.*, 58, 111–123.
2. **Bond, R., Ferguso, E. A., Curtis, C. F, Craig, J. M., Lloyd, D. H., 1996:** Factors associated with elevated cutaneous *Malassezia pachydermatis* populations in dogs with pruritic skin disease. *JSAP*, 37, 103–107.
3. **Bond, R., Lloyd, D. H., 1997:** Skin and mucosal populations of *Malassezia pachydermatis* in healthy and seborrheic Basset Hounds. *Vet. Dermatol.*, 8, 101–106.
4. **Bond, R., Saijonmaa-Koulumies, L. E. M., Lloyd, D. H., 1995:** Population sizes and frequency of *Malassezia pachydermatis* at skin and mucosal sites on healthy dogs. *J. Small Anim. Pract.*, 35, 147–150.
5. **Brito, E. H., Fontenelle, R. O., Brillhante, R. S., Cordeiro, R. A., Monteiro, A. J., Sidrim, J. J., Rocha, M. F., 2009:** The anatomical distribution and antimicrobial susceptibility of yeast species isolated from healthy dogs. *Vet. J.*, 182, 320–326.
6. **Brown, M. R., Thompson, C. A., Mohamed, F. M., 2005:** Systemic candidiasis in an apparently immunocompetent dog. *J. Vet. Diagn. Invest.*, 17, 272–276.

7. Cabañes, F.J., Coutinho, S.D., Puig, L., Bragulat, M.R., Castellá, G., 2016: New lipid-dependent *Malassezia* species from parrots. *Rev. Iberoam. Micol.*, 33, 92—99.
8. Cabañes, F.J., Vega, S., Castellá, G., 2011: *Malassezia cuniculi* sp. nov., a novel yeast species isolated from rabbit skin. *Med. Mycol.*, 49, 40—48.
9. Cafarchia, C., Gallo, S., Romito, D., Capelli, G., Chermette, R., Guillot, J., Otranto, D., 2005: Frequency, body distribution, and population size of *Malassezia* species in healthy dogs and in dogs with localized cutaneous lesions. See comment in PubMed Commons below. *Vet. Diagn. Invest.*, 17, 316—322.
10. Campbell, J.J., Coyner, K.S., Rankin, S.C., Lewis, T.P., Schick, A.E., Shumaker, A.K., 2010: Evaluation of fungal flora in normal and diseased canine ears. *Vet. Dermatol.*, 21, 619—625.
11. Cleff, M.B., Lima, A.P., Faria, R.O., Meinerz, A.R.M., Antunes, T.A., Araújo, F. et al., 2005: Isolation of *Candida* spp. from vaginal microbiota of healthy canine females during estrous cycle. *Braz. J. Microbiol.*, 36, 201—204.
12. Crespo, M.J., Abarca, M.L., Cabañes, F.J., 2000: Atypical lipid-dependent *Malassezia* species isolated from dogs with otitis externa. *J. Clin. Microbiol.*, 38, 2383—2385.
13. Diezmann, S., Cox, C.J., Schönian, G., Vilgalys, R.J., Mitchell, T.G., 2004: Phylogeny and evolution of medical species of *Candida* and related taxa: a multigenic analysis. *J. Clin. Microbiol.*, 42, 5624—5635.
14. Duarte, E.R., Lachance, M.A., Hamdan, J.S., 2002: Identification of atypical strains of *Malassezia* spp. from cattle and dog. *Can. J. Microbiol.*, 48, 749—752.
15. Duarte, E.R., Resende, J.C.P., Hamdan, J.S., 2009: Characterization of typical and atypical *Malassezia* spp. from cattle and dog by random amplified polymorphic DNA analysis. *Arg. Inst. Biol.*, 76, 157—164.
16. Fotos, P.G., Hellstein, J.W., 1992: *Candida* and candidiasis. Epidemiology, diagnosis and therapeutic management. *Dent. Clin. North. Am.*, 36, 857—878.
17. Gaitanis, G., Magiatis, P., Hantschke, M., Bassukas, I.D., Velegraki, A., 2012: The *Malassezia* genus in skin and systemic diseases. *Clin. Microbiol. Rev.*, 25, 106—141.
18. Greene, C.E., Chandler, F.W., 2006: Candidiasis, torulopso-sis, and rhodotorulosis. In Greene, C. E. (Ed.): *Infectious Diseases of the Dog and Cat*. 3. Philadelphia, Saunders Elsevier, 627—633.
19. Hajsig, M., Tadic, V., Lukman, P., 1985: *Malassezia pachydermatis* in dogs: significance of its location. *Vet. Arch.*, 55, 259—266.
20. Kaneko, J., Makimura, K., Abe, I., Shiota, R., Nakamura, Y., Kano, R. et al., 2007: Revised culture-based system for identification of *Malassezia* species. *J. Clin. Microbiol.*, 45, 3737—3742.
21. Kaneko, T., Makimura, K., Sugita, T., Yamaguchi, H., 2006: Tween 40-based precipitate production observed on modified chromogenic agar and development of biological identification kit for *Malassezia* species. *Med. Mycol.*, 44, 227—231.
22. Kumar, A., Singh, K., Sharma, A., 2002: Prevalence of *Malassezia pachydermatis* and other organisms in healthy and infected dog's ears. *Israel J. Vet. Med.*, 57, 145—148.
23. Lagunes, L., Rello, J., 2016: Invasive candidiasis: from myco-biome to infection, therapy, and prevention. *Eur. J. Clin. Microbiol. Infect. Dis.*, 35, 1221—1226.
24. Lee, H.A., Hong, S., Choe, H., Kim, O., 2011: Folliculitis and alopecia with cutaneous candidiasis in a Beagle dog. *Lab. Anim. Res.*, 27, 63—65.
25. Leeming, J.P., Notman, F.H., 1987: Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J. Clin. Microbiol.*, 25, 2017—2019.
26. Linek, J., 2004: Mycotic endophthalmitis in a dog caused by *Candida albicans*. *Vet. Ophthalmol.*, 7, 159—162.
27. Machado, M.L.S., Appelt, C.E., Ferreira, L., Guillot, J., 2003: Otites e dermatites por *Malassezia* spp. em cães e gatos. *Clínica Veterinária*, 44, 27—34.
28. Moretti, A., Posteraro, B., Boncio, L., Mechelli, L., Gasperis, E., Agnetti, F., Raspa, M., 2004: Diffuse cutaneous candidiasis in a dog. Diagnosis by PCR-REA. *Rev. Iberoam. Micol.*, 21, 139—142.
29. Morris, D.O., 1999: *Malassezia dermatitis* and otitis. *Vet. Clin. North. Am. Pract.*, 29, 1303—1310.
30. Nardoni, S., Dini, M., Taccini, F., Mancianti, F., 2007: Occurrence, distribution and population size of *Malassezia pachydermatis* on skin and mucosae of atopic dogs. *Vet. Microbiol.*, 122, 172—177.
31. Nardoni, S., Mancianti, F., Corazza, M., Rum, A., 2004: Occurrence of *Malassezia* species in healthy and dermatologically diseased dogs. *Mycopath.*, 157, 383—388.
32. Ozawa, H., Okabayashi, K., Kano, R., Watari, T., Watanabe, S., Hasegawa, A., 2005: Rapid identification of *Candida tropicalis* from canine cystitis. *Mycopath.*, 160, 159—162.
33. Prado, M.R., Brito, E.H.S., Giraño, M.D., Monteiro, A.J., Sidrim, J.J.C., Rocha, M.F.G., 2004: Higher incidence of *Malassezia pachydermatis* in the eyes of dogs with corneal ulcer than in healthy dogs. *Vet. Microbiol.*, 100, 115—120.
34. Radosavljevic, M., Koenig, H., Letscher-Bru, V., Waller, J.,

- Maloisel, F., Lioure, B., Herbrecht, R., 1999: *Candida catenulata* fungemia in a cancer patient. *J. Clin. Microbiol.*, 37, 475—477.
35. Santin, R., Mattei, A. S., Waller, S. B., Madrid, I. M., Cleff, M. B., Xavier, M. O. et al., 2013: Clinical and mycological analysis of dog's oral cavity. *Braz. J. Microbiol.*, 44, 139—143.
36. Sihelská, Z., Váczi, P., Čonková, E., 2016: Species composition of *Malassezia* yeasts in dogs in Slovakia. *Berl. Munch. Tierarztl. Wochenschr.*, 129, 351—354.
37. Yapar, N., 2014: Epidemiology and risk factors for invasive candidiasis. *Ther. Clin. Risk Manag.*, 10, 95—105.

Received November 22, 2016

Accepted January 18, 2017