İstanbul Üniversitesi
Veteriner Fakültesi Dergisi

Journal of the Faculty of
Veterinary Medicine Istanbul University

İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ., 42 (2), 117-121, 2016
doi: 10.16988/iuvfd.2016.93199

Araştırma Makalesi ***Research Article***

Occurrence of *Malassezia pachydermatis* in Dogs with Otitis Externa

Kemal METİNER, Baran ÇELİK, Beren BAŞARAN KAHRAMAN, Belgi DİREN SIĞIRCI, Zehra Seda MAVİLİ, Seyyal AK*

Department of Microbiology, Veterinary Faculty, Istanbul University, Avcılar, 34320, Istanbul, Turkey

****Sorumlu Yazar / Corresponding Author:***
Kemal METİNER
e-mail: kmetiner@hotmail.com

Geliş Tarihi / Received:
07 July 2015

Kabul Tarihi / Accepted:
23 September 2015

Anahtar Kelimeler:
Köpek, otitis eksterna, *Malassezia pachydermatis,* izolasyon

Key Words:
Dog, otitis externa, *Malassezia pachydermatis,* isolation

Abstract

Swab samples taken from a total of 100 dogs which were suspected about otitis externa symptoms such as ear discharge, skin rash (exanthema), flaking off, inflammation, rash and stink were mycologically examined. The swabs were inoculated onto Sabouraud Dextrose Agar (SDA) containing 0.05% chloramphenicol and 0.05% cycloheximide, and were incubated at 37 °C for 7 days. After the incubation, isolates were evaluated by morphological, physiological and biochemical characteristics. Thirty six out of these isolates were identified as *Malessezia pachydermatis*, six as *Candida* spp., and two of them were identified as *Aspergillus* spp. *M. pachydermatis* was isolated from 15 of 33 (42.4%) one to three years old, 10 of 30 (33.3%) four to six years old, 8 of 19 (42.1%) seven to ten years old, 1 of 8 (12.5%) ten to twelve years old and 2 of 9 (22.2%) older than twelve years old dogs. There has been no isolation occured under one year old dog. The agent was isolated 17 of 46 (36.9%) females, while 19 of 54 (35.1%) males. Crossbred animals and the terriers have the highest occurrence rates (25 and 22.2% respectively) if the percentage of *M. pachydermatis* isolation from a breed verses all affected dogs was evaluated. Though, German Shepherds have the highest occurrence rate (57.1%) when evaluated within the breeds. Differences according to age, gender and breed characteristics were not statistically significant (P>0.05).

Özet

Otitis eksternalı köpeklerde *Malassezia pachydermatis*'in varlığı

Kulak akıntısı, deride kızarıklık (egzantem), kabuklanma, yangı, kızarıklık ve kötü koku gibi semptomlara sahip olan otitis eksterna şüpheli toplam 100 köpekten kulak svab örneği mikolojik yönden incelendi. Svablar, %0,05 kloramfenikol ve %0,05 sikloheksimid içeren Sabouraud Dextrose Agara (SDA) ekildi ve 37 °C de 7 gün inkübe edildi. İnkübasyonun ardından, izolatlar morfolojik, fizyolojik ve biyokimyasal özelliklerine göre değerlendirildi. İzolatların 36 adeti *Malassezia pachydermatis*, altısı *Candida* spp. ve iki adeti *Aspergillus* spp. olarak identifiye edildi. *M.pachydermatis*; bir üç yaş arası 33 köpeğin on beşinde (%42,4), dört altı yaş arası 30 köpeğin onundan (%33,3), yedi on yaş arası 19 köpeğin sekizinden (%42,1), on 12 yaş arası sekiz köpeğin birinden (%12,5) ve 12 yaşından büyük dokuz köpeğin ikisinden (%22,2) izole edildi. Bir yaş altında olan tek köpekte üreme olmadı. Etken, 46 dişi köpeğin 17'sinden (%36,9) ve 54 erkek köpeğin 19'undan (%35,1) izole edildi. Karışık köpek ırkları ve terrierler (%25 ve 22,2), *M. pachydermatis* izolasyonu incelenen bütün hayvanlar ile karşılaştırılırsa en yüksek orana sahipti. Bununla birlikte, ırklar içinde değerlendirildiğinde German Shepherd köpeklerin (%57,1) en yüksek izolasyon oranına sahip olduğu belirlendi. Yaş, cinsiyet ve ırk özelliklerine göre farklılıklar istatistiki olarak önemsiz bulundu (P>0,05). Sonuç olarak, köpeklerden yüksek oranda *M. pachydermatis* izolasyonunun otitis eksterna infeksiyonlarında önemli role sahip olduğunu düşündürmektedir.

Introduction

Manifestations of otitis externa such as a skin rash (exanthema), flaking off, inflammation and stinky smell continuously leave the patient restless. One of the most important factors that cause otitis externa is the species of *Malassezia.* The members of *the Malassezia* genus are lypophilic and/or lipid dependent unipolar budding yeasts characterized by an oval-shaped and thick cell wall (Cafarchia et al., 2012).

It has been reported that species of *Malassezia* exist in the skin flora of healthy people and in the skins and mucosae of cats, dogs and birds, and the organisms lead to dermatological diseases in animals as well as being associated with otitis externa and in cats and dogs (Gemmer et al., 2002; Guillot and Bond, 1999; Turan et

al., 1997). Therefore, they are reported as a form a transmission phase between commensalism and pathogenicity. The diseases associated with species of *Malassezia* in humans were stated to be pityriasis versicolor, seborrhoeic dermatitis, folliculitis and systemic infections (Gemmer et al., 2002; Guillot and Bond, 1999).

M. pachydermatis was reported to have often been isolated from the toe skin of healthy dog paws, from their external auditory canal, anus and from around the mouth/lips (El-Hadidy et al., 2007).

Malassezia genus has been re-arranged in time according to the morphological, physiological, biochemical and molecular studies conducted on this matter, and genus of *Malassezia* currently includes 14 species. Thirteen of these species are identified as lipid-dependent (lipophilic) (*Malassezia furfur, Malassezia sympodialis*, *Malassezia globosa*, *Malassezia obtusa*, *Malassezia slooffiae*, *Malassezia restricta*, *Malassezia dermatis*, *Malassezia japonica*, *Malassezia yamatoensis, Malassezia nana*, *Malassezia caprae*, *Malassezia equina* and *Malassezia cuniculi*), and one of them is identified as non-lipid dependent (*M. pachydermatis*) (Cabañes et al., 2007).

It is considered that the agent in question exists within the normal cutaneous microflora of dogs, and these yeasts turn into a pathogenic state as they increase in number depending on several endogenic and exogenic factors, such as high temperature, high relative humidity, fatty skin, corticosteroid therapy and immunodeficiency (Ashbee and Evans, 2002; El-Hadidy et al., 2007; Swiecicka et al., 2015). Recently, it has also been reported that lipophilic species are associated with otitis externa (Crespo et al., 2000).

The researchers have reported that *M. pachydermatis* is transmitted by humans through to animals and vice versa, posing a great risk for humans (Morris et al., 2005; Velegraki et al., 2015).

In this study, it was aimed that determination of the ratio of *M. pachydermatis* in dubious otitis externa cases of mycotic origin.

Materials and Methods

Samples

Swab samples taken from a total of 100 dogs lived in İstanbul which were otitis externa suspected symptoms such as ear discharge, skin rash (exanthema), flaking off, inflammation and stinky smell. A total of 100 dogs were investigated, 46 of them were female and 54 of them were male. The ages of the dogs were between three months and 15 years old (mean value = 5.8). The samples were collected from 16 different dog breeds.

Isolation and identification

Swabs were inoculated onto Sabouraud Dextrose Agar (SDA) containing 0.05% chloramphenicol (Merc Darmstadt, Germany) and 0.05% cycloheximide (Sigma, St. Louis, MO, USA), and were incubated at 37 °C for 7 days. Gram staining (Figure 1), urease activity, catalase activity, esculin hydrolization and Tween assimilation (reproduction in SDA mediums into which 10% tween-20, 0.5% tween-40 and 0.1% tween-80 had been added) tests were performed on the cultures that had been purely obtained (Figure 2). The growth characteristics at 32 °C and 40 °C were examined (Ashbee and Evans, 2002; Khosravi et al., 2009; Petrov et al., 2013).

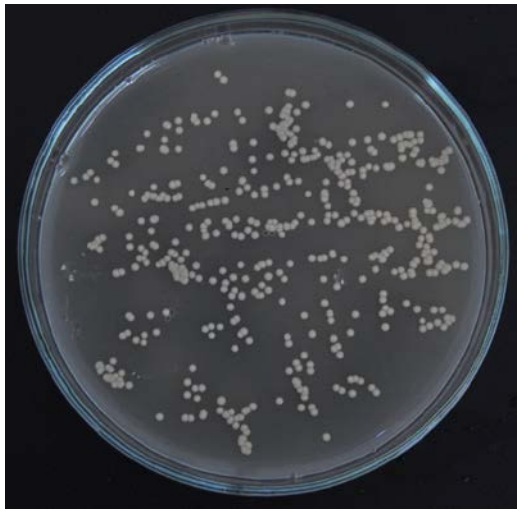

Figure 1. Colony morphology of *Malassezia pachydermatis* on SDA.

Şekil 1. *Malassezia pachydermatis*' in SDA' da koloni morfolojisi.

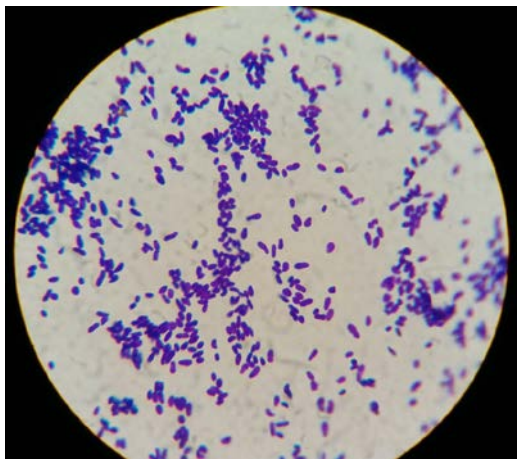

Figure 2. Microscopic morphology of *Malassezia pachydermatis* (Gram staining).

Şekil 2. *Malassezia pachydermatis*' in mikroskobik morfolojisi (Gram boyama).

Statistical analysis

In order to determine the significance of age, gender and breed characteristics of the dogs, a chi-square test was performed using SPSS 10.0 statistical package (SPSS, 1999). In the current study, ages were categorised as less than one year, one to three years old, four to six years old, seven to nine years old, ten to twelve years old and more than 12 years old.

Results

Thirty six *M. pachydermatis* strains were isolated while six of *Candida* spp., and two of *Aspergillus* spp. *M. pachydermatis* was isolated from 15 of 33 (45.4%) one to three years old, 10 of 30 (33.3%) four to six years old, 8 of 19 (42.1%) seven to ten years old, 1 of 8 (12.5%) ten to twelve years old and 2 of 9 (22.2%) older than twelve years old dogs. Differences in age were not statistically significant (P= 0.428). The agent was isolated 17 of 46 (36.9%) females, while 19 of 54 (35.1%) males. Differences in gender were not statistically significant (P=0.856).

M.pachydermatis isolation rates about within breeds and *M.pachydermatis* isolation rates from the breed verses all affected dogs were shown in Table 1. The differences in occurrence of the *M. pachydermatis* in relation to the breed of the dogs were not also statistically significant (P=0.780).

Table 1. *M. pachydermatis* isolation rates according to breeds.
Tablo 1. Irklara göre *M. pachydermatis* izolasyon oranları.

Breed	Number of dogs	Number of dogs with *M.pachydermatis* isolation	Percentage of *M.pachydermatis* isolation within a breed	Percentage of *M.pachydermatis* isolation from the breed vs. all affected dogs
Boxer	1	0	-	-
Bulldog	1	0	-	-
Crossbred	18	9	50	25
Cocker	20	6	30	16.7
Collie	1	0	-	-
Dalmatian	1	0	-	-
German Shepherd	7	4	57.1	11.1
Golden retriever	10	5	50	13.9
Kangal	4	2	50	5.6
Labrador retriever	1	0	-	-
Pekinese	2	0	-	-
Pitbull	1	0	-	-
Rottweiler	2	1	50	2.7
Setter	4	1	25	2.7
Terrier	27	8	37.3	22.2
Total	100	36	36,00	100,00

Discussion

Otitis externa is a disease which is quite commonly seen in dogs, most probably due to their anatomy and many other factors (Lyskova et al., 2007). One of the most important agents among the yeasts is the *Malassezia* species that are isolated as most cases of diseases. It was reported that the other yeast and fungi species isolated in the cases of otitis externa, *Candida* and *Aspergillus* species (Kumar et al., 2002).

Thirty six of the isolates were identified as *M. pachydermatis*, six of them as *Candida* spp., and two of them were identified as *Aspergillus* spp. The results of the current study were similar to findings reported by Kumar et al. (2002).

M. pachydermatis can be isolated from the external auditory canals and mucosae of animals. It was reported that the isolation rate was higher in the dogs than the other animals, which were reported to be 20% in cats and 42% in dogs (Guillot and Bond, 1999). Through various studies, *M. pachydermatis* was suggested to have had a higher incidence in clinical otitis externa, particularly in chronic cases. In addition, the isolation rate of *M. pachydermatis* had varied between 17.3% and 82.18% (Bardshiri et al., 2014; Borum et al., 2014; Kumar et al., 2002; Lyskova et al., 2007; Masuda et al., 2000; Oliveira et al., 2008).

In the current study, *M. pachydermatis* isolation was performed in 36 of the samples (33.3%), and this rate was rather lower than results of most studies; yet, it was determined to be quite higher than 17.3%. However, it is a debatable situation to the pathogenicity of *M. pachydermatis* and as for otitis externa, the agent in question is considered to allow an opportunist infection to develop as the result of the fact that the primary factors change the cutaneous micro-environment (the increase in humidity and surface lipids, the failure of the barrier function of stratum corneum, etc.) (Crespo et al., 2002). In the light of these findings, it is necessary to investigate in detail the high isolation rate of otitis externa cases associated with *Malassezia* species.

The gender predisposition on Malassezia otitis has been excluded in most of the studies. Bardshiri et al. (2014) and Girão et al. (2006) reported the prevalence of *M.pachydermatis* in female and male dogs was equal and the differences between *M.pachydermatis* occurrence and gender were not statistically significant. Kumar et al. (2002) also stated that gender had no influence on the frequency of Malassezia otitis. On the contrary, Cafarchia et al. (2005), reported Malassezia otitis was observed from males more than females and the researchers suggested the results had been indicating a possible gender predisposition. In the current study, it has been determined that the isolation rates of *M. pachydermatis* in female and male dogs were approximately same in each gender, which supports the results of the previous studies (Bardshiri et al., 2014; Girão et al., 2006; Kumar et al., 2002) had reported that gender was not a determinant in the occurrence of otitis externa caused by *M. pachydermatis*.

In the previous studies, a possible age predisposition about Malassezia otitis was reported (Bardshiri et al., 2014; Cafarchia et al., 2005; Girão et al., 2006; Kumar et al., 2002). However, most of the studies had not found any significant correlation between Malasezia and age. Cafarchia et al. (2005), detected higher occurrence in Malassezia population in dogs younger than one year old. Kumar et al. (2002) found highest occurrence of Malassezia otitis in 2-4 years old dogs. Bardshiri et al. (2014), reported *M.pachydermatis* was more prevalent in adult dogs (1-8 years old). However, Girão et al. (2006), determined 1-3 years old, dogs were the most representative age group linked to Malassezia otitis, also they found that *M.pachydermatis* occurrence and age was statistically significant. In the current study isolation from dogs of 1-3 years of age (42.2%) was found higher than other age groups. But, the differences in occurrence of the *M. pachydermatis* in relation to age of the dogs were not statistically significant.

Considering the breed characteristics, in the previous studies recognized that German shepherd, poodle, terrier and crossbred animals had a higher occurrence rate of Malassezia otitis. Girão et al. (2006), reported a higher occurrence of Malassezia otitis in poodles (39.2%) followed by crossbred dogs (12.5%). Kumar et al. (2002), found the highest percentage of otitis externa associated with *M. pachydermatis* in German shepherds (83.33%). Bardshiri et al. (2014), reported occurrence of *M. pachydermatis* was more prevalent in terriers (56.25%). In the current study, crossbred animals and the terriers have the highest occurrence rates 25% and 22.2% respectively, when the percentage of *M. pachydermatis* isolation from a breed verses all affected dogs was evaluated. Though, German Shepherds has the highest occurrence rate (57.1%) when evaluation assessed within the breeds. As a conclusion, a high level of *M. pachydermatis* isolation seems to have a major role in otitis externa in dogs.

REFERENCES

Ashbee, H.R., Evans E.G.V., 2002. Immunology of diseases associated with *Malassezia* species. Clinical Microbiology Reviews 15, 21-57.

Bardshiri, B., Tavana, M., Peighambarzadeh, S.Z., Abdolrahimi, M., 2014. Occurrence of *Malassezia pachydermatis* in the external ear canals of dogs with and without otitis externa. WALIA Journal 30, 24-26.

Borum, A.E., Çeçen, G., Demir G., Cetin, C., Şentürk, S., 2014. Determination and Antimicrobial Susceptibility of Microorganisms Isolated from Otitis Externa with Dogs. Kocatepe Veterinary Journal 7, 27-31.

Cabañes, F.J., Theelen, B., Castellá, G., Boekhout, T., 2007. Two new lipid-dependent *Malassezia* species from domestic animals. FEMS Yeast Research 7, 1064-1076.

Cafarchia, C., Gallo, S., Capelli, G., Otranto, D., 2005. Occurrence and population size of *Malassezia* spp. in the external ear canal of dogs and cats both healthy and with otitis. Mycopathologia 160, 143-149.

Cafarchia, C., Figueredo, L. A., Iatta, R., Montagna, M.T., Otranto, D., 2012. In vitro antifungal susceptibility of *Malassezia pachydermatis* from dogs with and without skin lesions. Veterinary Microbiology 155, 395-398.

Crespo, M.J., Abarca, M.L., Cabañes, F.J., 2000. Atypical lipid-dependent *Malassezia* species isolated from dogs with otitis externa. Journal of Clinical Microbiology 38, 2383-2385.

Crespo, M.J., Abarca, M.L., Cabañes, F.J., 2002. Occurrence of *Malassezia* spp. in the external ear canals of dogs and cats with and without otitis externa. Medical Mycology 40, 115-121.

El-Hadidy, G.S., Gomaa, N.I.M., Abo bakr, R.A.E., Metwally, L.A., 2007. Direct molecular identification of *Malassezia* species from skin scales of patients with seborrheic dermatitis by nested terminal fragment length polymorphism analysis. Egyptian Journal of Medical Microbiology 16, 437-444.

Gaitanis, G., Magiatis, P., Hantschke, M., Bassukas, I. D., Velegraki, A., 2012. The Malassezia genus in skin and systemic diseases. Clinical Microbiology Reviews 25, 106-141.

Gemmer, C.M., DeAngelis, Y.M., Theelen, B., Boekhout, T., Dawson, T.L., Jr., 2002. Fast, noninvasive method for molecular detection and differentiation of Malassezia yeast species on human skin and application of the method to dandruff microbiology. Journal of Clinical Microbiology 40, 3350-3357.

Girão, M.D., Prado, M.R., Brilhante, R.S.N., Cordeiro, R.A., Monteiro, A.J., Sidrim, J.J.C., Rocha, M.F.G., 2006. *Malassezia pachydermatis* isolated from normal and diseased external ear canals in dogs: a comparative analysis. The Veterinary Journal 172, 544-548.

Guillot, J. and Bond, R., 1999. *Malassezia pachydermatis*: a review. Medical Mycology 37, 295-306.

Khosravi, A.R., Eidi, S., Katiraee, F., Ziglari, T., Bayat, M., Nissiani, M., 2009. Identification of different *Malassezia* species isolated from patients with Malassezia infections. World Journal of Zoology 4, 85-89.

Kumar, A., Singh, K., Sharma, A., 2002. Prevalence of *Malassezia pachydermatis* and other organisms in healthy and infected dogs ears. The Israel Veterinary Medical Association 57, 145-148.

Lyskova, P., Vydrzalova, M., Mazurova, J., 2007. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. Journal of Veterinary Medicine Series A 54, 559-563.

Masuda, A., Sukegawa, T., Mizumoto, N., Tani, H., Miyamoto, T., Sasai, K., Baba, E., 2000. Study of lipid in the ear canal in canine otitis externa with *Malassezia pachydermatis*. The Journal of Veterinary Medical Science 62, 1177-1182.

Miranda, K.C., de Araujo, C.R., Costa, C.R., Passos, X.S., de Fátima Lisboa Fernandes, O., do Rosário Rodrigues Silva, M., 2007. Antifungal activities of azole agents against the *Malassezia* species. The International Journal of Antimicrobial Agents 29, 281-284.

Morris, D.O., O'Shea, K., Shofer, F.S., Rankin, S., 2005. *Malassezia pachydermatis* carriage in dog owners. Emerging Infectious Disease Journal 11, 83- 88.

Oliveira, L.C., Leite, C.A., Brilhante, R.S., Carvalho, C.B., 2008. Comparative study of the microbial profile from bilateral canine otitis externa. The Canadian Veterinary Journal 49, 785-788.

Petrov, V., Mihaylov, G., Tsachev, I., Zhelev, G., Marutsov, P., Koev, K., 2013. Otitis externa in dogs: microbiology and antimicrobial susceptibility. Revue De Medecine Veterinaire 164, 18-22.

SPSS, 1999. Statistical Package for the Social Sciences, Release 10.0. Chicago, IL, USA: SPSS Inc.

Święcicka, N., Bernacka, H. ,Fac, E., Zawiślak, J., 2015. Prevalence and commonest causes for otitis externa in dogs from two polish veterinary clinics. Bulgarian Journal of Veterinary Medicine 18, 65-73.

Turan, N., Bilal, T., Arıkan, N., Uysal, A. K., Yılmaz, H., 1997. Köpeklerde *Malassezia pachydermatis* infeksiyonları. İstanbul Üniversitesi Veteriner Fakültesi Dergisi 23, 119-130.

Velegraki, A., Cafarchia, C., Gaitanis, G., Iatta, R., Boekhout, T., 2015. Malassezia Infections in Humans and Animals: Pathophysiology, Detection, and Treatment. PLoS Pathogens 11, 1-6.