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Occurrence of *Malassezia* Otitis Externa in Dogs Presented at a Veterinary Clinical Complex, Jabalpur

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Abstract

The present study was conducted to determine the occurrence of *Malassezia* otitis externa in dogs presented to the Veterinary Clinical Complex (VCC), Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. A total of 2,479 dogs were examined during the study period from January to May 2026, of which 140 dogs presented with dermatological lesions. Ear swab samples were collected from clinically suspected cases and subjected to cytological examination and molecular confirmation using PCR targeting the 26S rDNA gene. The overall occurrence of *Malassezia* otitis externa was 1.85% (46/2,479), while the occurrence among dogs with dermatological disorders was 32.86% (46/140). The highest occurrence was observed in dogs aged 2-3 years (50.00%), followed by 3-4 years (35.00%), although the association with age was not statistically significant ($\chi^2 = 9.006$, $p > 0.05$). Breed-wise occurrence was highest in Beagle dogs (50.00%), followed by Spitz (42.85%) and Labrador Retrievers (40.00%), with no significant breed association ($\chi^2 = 10.055$, $p > 0.05$). Male dogs (37.18%) were more frequently affected than females (27.42%), but the difference was also non-significant ($\chi^2 = 1.492$, $p > 0.05$). PCR analysis confirmed *Malassezia pachydermatis* in 18 (39.13%) of the 46 cytologically positive samples. The study demonstrates that *Malassezia* otitis externa is a common fungal ear disorder among dogs with dermatological conditions and highlights the importance of cytology and molecular diagnostics for accurate epidemiological assessment.

Keywords: *Malassezia pachydermatis*, otitis externa, dogs, occurrence, PCR

Introduction

Otitis externa is one of the most frequently encountered disorders in small animal clinical practice and is characterized by inflammation of the external ear canal and its associated structures. The condition is considered multifactorial, involving primary, predisposing, perpetuating, and secondary factors, and is often associated with chronic pathological changes of the ear canal in affected dogs (Crespo *et al.*, 2002) [3]. Among fungal pathogens, *Malassezia* species, particularly *Malassezia pachydermatis*, are recognized as important opportunistic yeasts associated with canine otitis externa (Crespo *et al.*, 2002) [3].

Malassezia yeasts are common commensals of the skin and ear canal of warm-blooded vertebrates and may become pathogenic when changes occur in the host or the local ear canal environment. Increased humidity, excessive sebum production, allergic skin disease, endocrine disorders, and impaired host immunity can promote yeast proliferation and lead to clinical disease (Theelen *et al.*, 2018) [14]. Members of the genus *Malassezia* are lipid-dependent yeasts belonging to the family *Malasseziaceae*, order *Malasseziales*, and phylum *Basidiomycota*. At present, 18 species have been identified based on molecular and physiological characteristics (Laniri, 2022; Diaz *et al.*, 2024) [4, 10]. Unlike most *Malassezia* species, *M. pachydermatis* can grow on Sabouraud Dextrose Agar due to its ability to utilize lipid fractions present in the peptone component of the medium (Cabanès *et al.*, 2014; Ilahi *et al.*, 2017; Hobi *et al.*, 2022) [1, 7, 8].

Epidemiological studies have reported considerable variation in the occurrence of *Malassezia* otitis in dogs depending on geographical location, climate, breed composition, and diagnostic methods employed. Understanding the occurrence pattern of *Malassezia* otitis in relation to age, breed, and sex is essential for identifying susceptible populations and improving clinical management strategies. Molecular confirmation of *M. pachydermatis* also provides reliable species identification and contributes to epidemiological surveillance (Diaz *et al.*, 2024) [4].

The present investigation was therefore undertaken to determine the occurrence of *Malassezia* otitis externa in dogs presented to a veterinary clinical complex in central India and to evaluate its distribution according to age, breed, and sex, along with molecular confirmation of *M. pachydermatis* isolates.

Materials and Methods

Study location and duration

The study was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. The investigation was carried out over a period of six months from December 2025 to May 2026.

Selection of animals

Dogs presented to the Veterinary Clinical Complex (VCC), Jabalpur during the study period were screened for otitis externa irrespective of age, breed, or sex. Animals were classified as suspected cases based on history, clinical signs, and otoscopic examination. Information regarding age, breed, sex, and duration of illness was recorded for each dog using a structured questionnaire.

Video-otoscopic examination

The external ear canal was examined using a video otoscope (Global Technology Solutions LLC., Janakpuri, New Delhi) to assess erythema, cerumen accumulation, discharge, inflammation, and other lesions associated with otitis externa.

Sample collection and cytological examination

Aural exudate was collected from the junction of the vertical and horizontal ear canals using sterile cotton-tipped swabs. The swab material was rolled onto clean glass slides, stained with a modified stain, and examined under oil immersion (100×). Slides were assessed for the presence of yeast cells, bacteria, and inflammatory cells using a semi-quantitative scoring method.

Acetate tape impression smears were prepared from affected skin lesions by gently pressing transparent acetate tape onto the lesion and mounting it on a glass slide. The stained preparations were examined microscopically for the characteristic footprint-shaped budding yeast cells of *Malassezia* spp.

DNA extraction

Representative cytologically positive samples were subjected to molecular confirmation. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Catalog No. 51304) according to the manufacturer's protocol. Ear swab samples were lysed with Proteinase K and extraction buffers, followed by ethanol precipitation, spin-column purification, washing, and elution of DNA in Buffer AE.

PCR amplification

Molecular identification of *Malassezia pachydermatis* was performed using primers targeting the 26S rDNA region as described by Taha *et al.* (2011) [13].

Table 1: Primer sequences used for molecular identification of *Malassezia* spp

S. No.	Primer	Sequence (5'-3')	Product size	Reference
1	26S rDNA	F: TAACAAGGATTCCCCTAGTA R: ATTACGCCAGCATCCTAAG	580 bp	Taha <i>et al.</i> (2020) [13]

PCR was carried out in a final reaction volume of 25 µL.

Table 2: Composition of PCR reaction mixture

Component	Quantity (µL)
PCR master mix	12.5
Forward primer (20 pmol/µL)	1.0
Reverse primer (20 pmol/µL)	1.0
Template DNA	2.0
Nuclease-free water	8.5
Total volume	25.0

Amplification was performed in a thermal cycler under standardized conditions for the 26S rDNA gene.

Table 3: PCR cycling conditions

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	30 s	35
Annealing	58 °C	40 s	35
Extension	72 °C	45 s	35
Final extension	72 °C	10 min	1

Agarose gel electrophoresis

PCR products (10 µL) were resolved on 1% agarose gel containing ethidium bromide in 0.5× TAE buffer. Electrophoresis was performed at 70 V for 90 min alongside a 100 bp DNA ladder, and amplified products were visualized under a UV transilluminator.

Statistical analysis

Occurrence data were analyzed using the Chi-square (χ^2) test to determine the association of *Malassezia* otitis externa with age, breed, and sex. Statistical analysis was performed

using R software (R Core Team, 2013), SPSS, or equivalent statistical software, and differences were considered significant at $p < 0.05$.

Result and Discussion Overall occurrence of *Malassezia* induced otitis externa in dogs

The total number of dogs examined and screened at VCC Jabalpur were 2479 from January to May 2026. Among these the overall occurrence of dermatological lesions was 5.64% (140/2479). The results are shown in table 04.

The overall occurrence of *Malassezia* otitis externa was 1.85 % (46/2479). Among these the overall occurrence of

Malassezia otitis externa from dermatological lesions was 32.86%. (46/140). These findings are illustrated in table 05.

Table 4: Overall occurrence of dermatological lesions in dogs at VCC Jabalpur

Total no. of dogs	No. of dogs screened for dermatological lesions	Occurrence (%)
2479	140	5.64%

Table 5: Overall occurrence of *Malassezia* otitis externa in dogs at VCC Jabalpur

Particulars	No. of dogs screened	No. of dogs affected with <i>Malassezia</i>	Occurrence (%)
Total number of dogs	2479	46	01.85%
Dogs with dermatological disorder	140	46	32.86%

These observations are in close similarity with those of Walia *et al.* (2025) ^[15] who reported an overall incidence of otitis in dogs as 3.67% (89/2420) in contrast with Petrov *et al.* (2013) ^[11] who reported the 25% prevalence of *Malassezia* *Pachydermatis*.

The observed variations in the results may be assigned to several factors, including differences in sample size, geographical location and management practices adopted by dog owners across different studies. Variations in these factors may contribute to variance in the findings, as differences in environmental conditions and study populations can influence the revealed outcomes.

The age-wise occurrence of *Malassezia* otitis externa was screened in a total of 140 dogs having dermatological lesions. Among the 46 confirmed cases of *Malassezia* otitis, the occurrence across different age groups was as follows: 24.14% (07/29) in dogs under 1 year, 18.75% (03/16) in dogs aged 1-2 years, 50% (18/36) in dogs aged 2-3 years, 35% (7/20) in dogs aged 3-4 years, 20.83% (05/24) in dogs aged 4-5 years and 30% (6/20) in dogs older than 7 years. A non-significant variation in the occurrence of *Malassezia* otitis was observed across the age groups, with the highest occurrence noted in the 2-3 years age group and lowest in the age group of 1-2 years. These findings are illustrated in Table 06.

Age wise occurrence of *Malassezia* otitis externa in dogs

Table 6: Age wise occurrence of *Malassezia* otitis externa in dogs

Age group (years)	No. of dogs screened (n=140)	No. of dogs affected (n=46)	Occurrence (%)
0-1	29	07	24.14
1-2	16	03	18.75
2-3	36	18	50.00
3-4	20	07	35.00
4-5	19	05	20.83
>5	20	06	30.00
$\chi^2 = 9.006^S$ df=5		P<0.005 non-significant	

Similar observations were reported by Girao *et al.* (2006) ^[6] and Seetha *et al.* (2018) ^[12] reported highest prevalence of *Malassezia* 2-3 years of age. In contrast, Rakesh *et al.* (2023) reported highest percentage of *Malassezia* infection in 6-9 years group of age in dogs.

The highest percentage of *Malassezia* infection in 2-3 years age group could be due to various factors associated with proliferation of *Malassezia* otitis such as poor nutrition, higher production of sebum, difference in temperature and humidity.

Breed wise occurrence of *Malassezia* dermatitis in dogs

The occurrence of *Malassezia* otitis externa was found highest in Beagle dogs i.e. 50% (08/04) followed by Spitz i.e. 42.85% (03/07), Labrador i.e.40% (14/35), German Shepherd i.e. 30.00% (9/30), non-descript i.e. 28.57% (10/35), others (Great Dane, Lhasa Apso, Saint Bernard, Dachshund and Dalmatian etc.) i.e. 26.86% (04/15), Golden Retriever i.e. 20% (01/05) and Pug i.e. 20% (1/4). A non-significant difference ($p > 0.05$) was observed in breed wise distribution of *Malassezia* otitis externa in dogs. The details are summarized in Table 07.

Table 7: Breed wise occurrence of *Malassezia* otitis externa in dogs

Breed	No. of dogs screened (n=140)	No. of dogs affected (n=46)	Occurrence (%)
Labrador	35	14	40.00
Non-descript	35	10	28.57
German Shepherd	30	09	30.00
Beagle	08	04	50.00
Spitz	07	03	42.85
Golden Retriever	05	01	20.00
Pug	05	01	20.00
Others*	15	04	26.66
$\chi^2 = 10.055^{NS}$ non-significant ($p>0.05$)			

These finding are supported by Hedge *et.al* (2021) and Kumar *et al.* (2011) ^[9] who reported higher incidence of *Malassezia* dermatitis in Beagle in contrast with Giaro *et al.*

(2006) reported that Poodles were the most commonly breed affected by *M. pachydermatis* associated with otitis.

However, during present investigation Beagle were found to suffer more due to pendulous or pendant structure of ear pinna and hang down rather than standing erect, so there is inadequate air flow which leads to accumulation of wax and moisture in the ear canal which act as predisposing factor for *Malassezia* otitis externa in dogs.

Gender wise occurrence of *Malassezia* otitis externa in dogs

Gender-wise occurrence study of *Malassezia* otitis revealed 37.18% (29 out of 78) affection in males and 27.42% (17 out of 62) in female dogs. The details are shown in Table 08.

Table 8: Gender wise occurrence of *Malassezia* otitis externa in dogs

Gender	No. of dogs (n=140)	No. of dogs affected (n=46)	Occurrence (%)
Male	78	29	37.18
Female	62	17	27.42
	$\chi^2=1.492$	$p>0.005$	Non-significant

The *Malassezia* otitis infection was more common in males (37.83%) as compared to females 27.42%. These findings are in close agreement with Conkova *et al.* (2011) [2] and Seetha *et al.* (2018) [12] in contrast with Makaeel *et al.* (2025) observed a higher incidence in female dogs (60.71%) In the present study, sex wise prevalence revealed significant difference between male and female and males were more affected that could be due to assumption that androgen cause higher production of sebum (Conkova *et al.*, 2011) [2], which is one of the predisposing factors for development of latent infection. The change in the host immunity alteration in skin micro-climate disruption of epithelial barrier may also predispose the dogs for development of *Malassezia*.

Molecular identification of *Malassezia pachydermatis* in dogs

Published gene-based primers successfully amplified the target part of 26S rDNA from all *Malassezia* strains being tested and thus provides a single PCR product of the expected size (approximately 580 bp) (Taha *et al.*, 2011) [13] In the present study, all the samples collected from the lesions in dogs were positive for *Malassezia* spp. The representative forty-six samples of *Malassezia* yeasts obtained from the lesions were subjected to PCR based on 26S rDNA gene. Out of which 18 samples found to be positive for *Malassezia pachydermatis* spp. (Table 09 and Plate 01).

Table 9: No of species identified as *Malassezia pachydermatis*

No. of affected dogs with <i>Malassezia</i> otitis externa	No. of dogs identified as <i>Malassezia pachydermatis</i>	Percentage (%)
46	18	39.13



Plate 1: PCR based detection of *Malassezia* using 26S rDNA gene- based primers

- Lane 1- Negative control
- Lane 2- 100 bp marker (580bp)
- Lane 3-7 - Test samples
- Lane 8 - Positive control

In the present study identified *Malassezia pachydermatis* in 18 (39.13%) of the 46 cytologically positive canine otitis samples using PCR targeting the 26S rDNA gene. The successful amplification of the expected 580 bp fragment confirmed the reliability of the molecular method described

by Taha *et al.* (2011) [13]. Similar observations were reported by Eidi *et al.* (2011) [5], who identified *M. pachydermatis* as the predominant *Malassezia* species isolated from both healthy and diseased dogs. The findings of the present study

support the use of PCR as a specific and reliable method for confirming *M. pachydermatis* in dogs with otitis externa.

Conclusion

Malassezia otitis externa was detected in 1.85% of all dogs presented to the Veterinary Clinical Complex and in 32.86% of dogs with dermatological disorders. Young adult dogs (2-3 years), Beagle dogs, and male dogs showed higher occurrence, although age, breed, and sex were not significantly associated with disease occurrence. PCR confirmed *Malassezia pachydermatis* in 39.13% of cytologically positive cases. The findings indicate that *Malassezia* otitis externa is an important fungal ear disorder among canine dermatological patients in central India and support the combined use of cytology and molecular diagnostics for epidemiological investigations.

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Disclaimer (Artificial Intelligence)

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing interests

Authors have declared that no competing interests exist

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