



# Diagnosis and therapeutic management of malasseziosis in dogs



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## Abstract

*Malassezia spp.* are commensals of the normal cutaneous microbiota of humans and animals. These yeasts may become opportunistic pathogens under certain conditions and cause dermatitis and otitis externa in dogs. *Malassezia pachydermatis* is the most common cause of malasseziosis in dogs. In this study skin and ear swabs from suspected cases were cultured on Modified Dixon's Agar (MDA). The isolates obtained were initially characterized on the basis of colony characteristics, result of Gram staining and microscopic morphology. Total DNA was extracted from the pure cultures of the isolates and subjected to confirmation by polymerase chain reaction (PCR) targeting large subunit ribosomal RNA gene. Positive cases were treated with oral itraconazole at 5 mg/kg bodyweight, orally once daily for 28 days.

**Keywords:** *Malassezia pachydermatis*, MDA, PCR

Members of the genus *Malassezia* are lipophilic basidiomycetous yeasts, which are part of the normal cutaneous microbiota of humans and other warm blooded animals. Malasseziosis is recognized as a secondary complication to other conditions like keratinization defects, hypersensitivity disorders, staphylococcal pyoderma and endocrinopathies. Itraconazole is a keratinophilic and lipophilic triazole antifungal agent used to treat malassezia infection (Pinchbeck *et al.*, 2002). The paper reports the isolation of *Malassezia* species from clinical cases in dogs and its successful management.

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## Materials and methods

Ten dogs of different breed, age and gender that were presented to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, with clinical signs of alopecia, pruritus, erythema, hyperpigmentation, lichenification, foul odour and otitis externa suggestive of malasseziosis were subjected to detailed clinical examination. Cytological examination of skin and ear swabs were carried out by Methylene blue staining. On cytology, at least one body site having a mean of  $\geq 1$  yeast organism per oil immersion field (OIF) during microscopic examination of 10 consecutive OIF with corneocytes considered positive for malasseziosis (Pinchbeck, 2002). Cultural examination was done by inoculating the samples onto Modified Dixon's Agar and incubating at 37°C for up to seven days (Rathnapriya *et al.*, 2016). Total DNA was extracted from the pure cultures of the isolates using conventional phenol – chloroform method and were subjected to polymerase chain reaction targeting large subunit ribosomal RNA gene of *Malassezia* spp. (Guillotet *et al.*, 2000). Dogs with malasseziosis were treated with oral itraconazole at 5 mg/kg bodyweight, orally once daily for 28 days along with supportive therapy, which included essential amino acids and herbal immuno-stimulants. Six apparently healthy dogs were selected for the control group. Haematological and serum biochemical

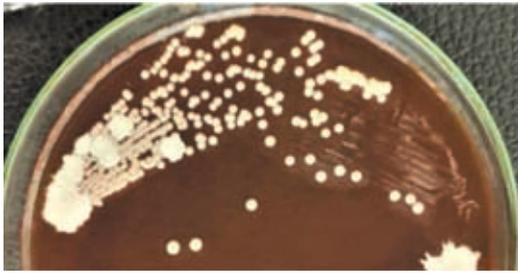
studies were performed. Comparison between study and control animals was done by using independent t-test.

## Results and discussion

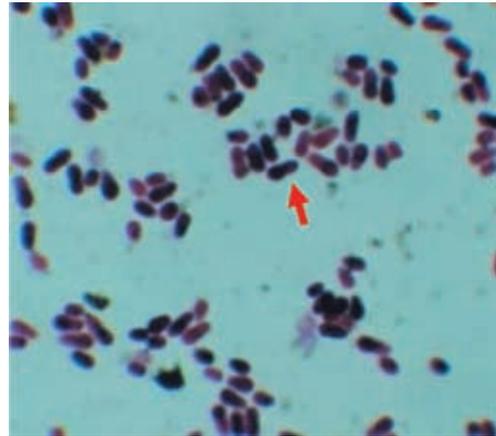
Clinical parameters were found to be within the normal range upon general clinical examination with mean respiratory rate of  $27.22 \pm 1.02$ / min, pulse rate of  $92 \pm 0.6$ / min and temperature of  $102.14 \pm 0.15$  °F. Haematological and serum biochemical study revealed leucocytosis and mild non regenerative anaemia in affected dogs. Significantly less Haemoglobin (Hb), Volume of Packed Red Cells (VPRC) and Mean Corpuscular Haemoglobin (MCH) ( $p < 0.05$ ) values were obtained in dogs with malasseziosis when compared to control group (Table 1). Takahira (2009) reported that mild to moderate non regenerative anaemia is the most common anaemia in dogs with fungal, bacterial, viral and protozoal diseases. Further, it was stated that reduced erythrocyte production and erythrocyte removal by immunologic mechanisms or oxidative damage could lead to development of anaemia in the above conditions. Significantly high values of TLC ( $p < 0.01$ ) were observed in affected dogs when compared to control group (Table 1). Hromada *et al.* (2005) also observed a significant increase in total leukocyte count in dogs infected with malassezia yeasts and, which might be due to inflammatory changes in the skin. There is



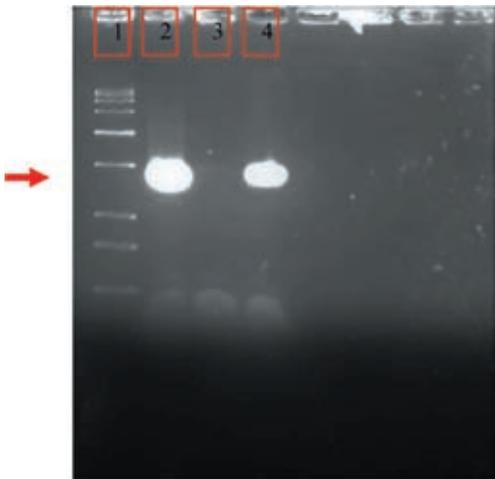
**Fig. 1.** Clinical manifestations of malasseziosis in dogs



**Fig. 2.** Colonies of *Malassezia* spp. on MDA



**Fig. 3.** Microscopic examination of colonies of *Malassezia* spp. using Gram's staining



**Fig. 4.** Molecular detection of *Malassezia* spp. (Lane 1 – 100 bp ladder; Lane 2, 4 – Isolates of *Malassezia* spp.; Lane 3 – Negative control; Amplicon size between 541 bp and 579 bp)



**Fig. 5a.** 1st day



**Fig. 5b.** 14th day



**Fig. 5c.** 28th day

**Fig. 5.** Clinical response to treatment with Itraconazole

no statistically significant difference in ALT, ALP, total protein, albumin and globulin values in dogs with malasseziosis in comparison with control group (Table 2). Detailed examination of the skin revealed alopecia, pruritus, erythema, hyperpigmentation, lichenification, epidermal collarettes, pustules, vesicles and nodules (Fig. 1). These findings were in accordance with Bond *et al.* (2020) who reported that pruritus, erythema, hyperpigmentation, malodour and traumatic alopecia were the major clinical signs in dogs with malasseziosis. Microscopic examination of acetate tape impression smears revealed the presence of Gram positive budding yeast cells. Jatavath and Kumar (2014) identified malassezia yeasts by cytology using the tape-strip technique. Impression and swab smear examinations of ten diseased dogs revealed the presence of cocci in six dogs suggestive of bacterial pyoderma. Dogs in association with

concurrent bacterial infections were treated with antimicrobial therapy. Ten isolates suggestive of *Malassezia* spp. were obtained on MDA. The colonies were cream to buff coloured with smooth, convex surface (Fig. 2). Of these, seven were isolated from skin lesions of dogs and the rest were obtained from cases of otitis externa.

**Table 1.** Comparison of haematology of dogs with malasseziosis and control animals

Sl. No.	Parameter	Mean $\pm$ SE		t-value	P-value
		Study Group	Control Group		
1	Hb(g/dL)	13.63 $\pm$ 0.73	16.03 $\pm$ 0.70	2.2*	0.046
2	PCV (%)	40.12 $\pm$ 2.36	48.18 $\pm$ 1.89	2.36*	0.033
3	TEC ( $\times 10^6$ /cmm)	6.40 $\pm$ 0.3	6.87 $\pm$ 0.26	1.09 <sup>ns</sup>	0.292
4	TLC ( $\times 10^3$ /cmm)	23.9 $\pm$ 2.9	11.85 $\pm$ 0.66	3.06**	0.009
5	Platlet count ( $\times 10^3$ / $\mu$ L)	339.1 $\pm$ 28.9	274.67 $\pm$ 25.28	1.52 <sup>ns</sup>	0.151
6	MCV (fL)	63.05 $\pm$ 2.6	70.2 $\pm$ 0.6	2.08 <sup>ns</sup>	0.056
7	MCH (g/dL)	21.3 $\pm$ 0.59	23.23 $\pm$ 0.22	2.48*	0.026
8	MCHC (pg)	34.1 $\pm$ 0.76	33.2 $\pm$ 0.2	0.873 <sup>ns</sup>	0.398
9	N (%)	79.63 $\pm$ 2.63	76.75 $\pm$ 1.66	0.79 <sup>ns</sup>	0.445
10	L (%)	15.1 $\pm$ 2.42	16.97 $\pm$ 1.79	0.54 <sup>ns</sup>	0.595
11	M (%)	3.2 $\pm$ 0.32	4.55 $\pm$ 0.44	2.54 <sup>ns</sup>	0.23
12	E (%)	2.1 $\pm$ 0.3	1.73 $\pm$ 0.38	0.78 <sup>ns</sup>	0.446

\*\* Significant at 0.01 level ( $P < 0.01$ ); \* Significant at 0.05 level ( $P < 0.05$ ); ns Non-significant ( $P > 0.05$ )

**Table 2.** Comparison of serum biochemical values of dogs with malasseziosis and control animals

Sl. No.	Parameters	Mean $\pm$ SE		t-value	P-value
		Diseased Group	Control Group		
1	ALT (U/L)	30.6 $\pm$ 8.22	25.16 $\pm$ 2.13	0.5 <sup>ns</sup>	0.626
2	ALP (U/L)	106.8 $\pm$ 14.21	77.16 $\pm$ 7.19	1.53 <sup>ns</sup>	0.148
3	TP (g/dL)	5.5 $\pm$ 0.24	6.11 $\pm$ 0.12	1.85 <sup>ns</sup>	0.085
4	Albumin (g/dL)	2.4 $\pm$ 0.17	2.88 $\pm$ 0.08	2.15 <sup>ns</sup>	0.05
5	Globulin (g/dL)	3.12 $\pm$ 0.25	3.23 $\pm$ 0.05	0.34 <sup>ns</sup>	0.738

ns Non-significant ( $P > 0.05$ )

Rathnapriya *et al.* (2016) and Marin *et al.* (2018) also successfully isolated malassezia yeasts on Modified Dixon's Agar at 37 °C for five days with a higher isolation rate compared to Sabouraud Dextrose Agar with olive oil overlay. Microscopic examination of colonies revealed dark blue coloured footprint shaped organism on Gram's staining. Microscopically, budding yeast cells with buds attached to the mother cell by a broad base were observed (Fig. 3). All the ten isolates were positive in the PCR targeting LSU region of *Malassezia* spp. (Fig. 4). Affeset *et al.* (2009) reported that the use of PCR method provides a sensitive and rapid identification system for *Malassezia* species, which can be applied in epidemiological surveys and routine practice. Clinical response to itraconazole therapy was

monitored at 14<sup>th</sup> and 28<sup>th</sup> day of treatment. An excellent clinical response along with quick recovery (by 14<sup>th</sup> day itself) was noticed in all the ten dogs treated with oral itraconazole. By 28<sup>th</sup> day of treatment, all the animals showed complete recovery from all the clinical signs. Clinical response to treatment with itraconazole were shown in Fig. 5a, 5b and 5c. Haimbach (2019) reported that itraconazole given at 5 mg/kg bodyweight daily for three weeks had good efficacy against malassezia infection in dogs.

### Conclusion

The present study concluded that cytological, cultural and molecular examination of clinical samples can be effectively used for diagnosis of *Malassezia* species. Orally

itraconazole was found to be effective for the treatment of canine malasseziosis with excellent clinical response and quick recovery.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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