RESEARCH ARTICLE

Efficacy of Different Treatment Protocols in the Management of Malassezia Infected Dogs

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Abstract

Dogs presented to outpatient medicine unit, Veterinary College, Bengaluru, with clinical signs suggestive of Malassezia dermatitis (such as rancid odor, erythema, greasiness, hyperkeratinisation, hyperpigmentation and lichenification) were screened by cytological examination and these dogs comprised the study group. Upon cytological examination, 24 dogs that were found positive for *Malassezia* spp. were divided into three groups of eight dogs each. Skin swabs from these 24 dogs were cultured on Modified Dixon's Agar (MDA) for the isolation of *Malassezia* spp. Treatment was initiated with Itraconazole @ 5 mg/kg b. wt. orally once daily for 21 days for group I and itraconazole @ 5 mg/kg once orally, two days in a week for three consecutive weeks for group II and twice daily application of topical imidazole antifungal luliconazole, for 21 days for group III. All the 24 dogs were treated with systemic broad spectrum antibacterial cefadroxil @ 22 mg/kg b. wt. twice daily for 14 days and antihistamine, hydroxyzine @ 0.5 mg/kg twice orally for 5 days with supportive care. The study proved that topical luliconazole and itraconazole pulse therapy are more effective clinically than conventional itraconazole on 21 day treatment regimen.

Key words: Dogs, Itraconazole conventional, Itraconazole pulse, Luliconazole topical, Malassezia.

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Introduction

Fungal infections are a major health problem and an important cause of morbidity. In recent days superficial fungal infection due to Malassezia is considered an extremely common cause of skin disease in dogs. The genus Malassezia belongs to the phyllum Basidiomycota and consists at present of 17 species (Theelen *et al.,* 2018) of which all are lipophilic, except *Malassezia pachydermatis* (Glatz *et al*., 2015). *M. pachydermatis* has a high intraspecies diversity, and certain genetic subtypes may have host specificity (Puig *et al*., 2016). *Malassezia pachydermatis*, a normal commensal on canine skin can multiply and become pathogenic when cutaneous factors such as an excessive production or modification of sebum and/or cerumen, cutaneous folds, excess of moisture or a breach in the epidermal barrier prevails. These changes may occur due to underlying common causes such as cutaneous hypersensitivity including atopic dermatitis, pyoderma, ectoparasitic skin disease (demodicosis), endocrine disorders (hypothyroidism), keratinization disorders, epidermal dysplasia of the West Highland white terrier, idiopathic seborrhoea and previous treatment with glucocorticoids or antibiotics.

Malassezia dermatitis is a seasonal dermatitis with no age or sex predilection, but some breeds are more predisposed such as Shih Tzus, West Highland white terrier, Dachshund, Basset Hound, English Setters, Cocker Spaniel, American Cocker Spaniel, Poodle and German shepherd. There is no indication that *Malassezia* dermatitis is contagious. The density of skin colonization depends on age, body site and other comorbid conditions. Malassezia dermatitis is more common in warm and humid climates (Thayikkannu *et al*., 2015). The diagnostic methods that are available for the confirmation of Malassezia yeasts include direct microcopy, culture based methods (often a combination of morphological features of the isolate, microscopy and biochemical tests), and molecular based methods such as PCR techniques, and Matrix Assisted Laser

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Desorption / Ionization-Time of flight mass spectrometry. Skin diseases caused by Malassezia are usually treated with antifungal therapy and if there are associated bacterial load and inflammatory skin mechanisms, this is often supplemented by antibiotic and anti-inflammatory therapy, respectively (Saunte *et al*., 2020). This paper highlights the diagnosis and therapeutic efficacy of different treatment protocols in the management of Malassezia dermatitis in dogs.

Materials and Methods

Sample Collection, Processing and Culture

Twenty-four client owned dogs of different breed, gender, and aged between 1 and 10 years, were included in the study. They were all tentatively diagnosed as Malassezia dermatitis based on history of moderate to severe pruritic dermatitis affecting the neck, ears, feet and/or ventrum which did not completely resolve following antimicrobial and antiparasitic therapy.

Skin was sampled from each animal using a sterile cotton swab from different affected sites. Two cotton swabs rubbed on the affected sites were used for cytological and cultural studies, respectively. Smears prepared from the swabs collected from affected skin surface were then rolled onto a glass slide, air dried and heat fixed. The heat fixed impression smears were stained with Loeffler's alkaline methylene blue for 2-4 min (Selvi *et al*., 2015). As there is no standard accepted number of organisms needed to diagnose Malassezia dermatitits, more than one yeast cell per oil immersion field that correlated with clinical signs is considered positive for Malassezia dermatitis (Pinchbeck *et al*., 2002; Nardoni *et al*., 2007; Ganguly *et al*., 2013; Haimbach, 2019).).

Twenty four dogs that were positive on direct microscopic/ cytological examination with associated clinical signs were divided into three groups of eight dogs each and subjected to treatment with oral itraconazole @ 5 mg/kg b.wt. orally once daily for 21 days (conventional, Group I), itraconazole @ 5 mg/kg b. wt., once orally for two days in a week for three weeks (pulse, Group II) and topical application with Oint. Luliconazole (1 %) twice daily (Group III).

Blood (5 mL) was collected from cephalic or saphenous vein for determination of various haematological and biochemical parameters. Numbers of budding yeasts per high power oil immersion field were counted for 15 fields and mean yeast count was recorded pre-treatment and post-treatment. Based on the history, each dog in the study group was assigned a value from 1-5 for pruritus, where higher number represented more intense pruritus and lower number represented a milder pruritus (Marsella *et al*., 2000). Therapeutic evaluation, for the three treatment groups pre- and post-treatment was based on cytological evaluation (mean yeast count) and visual analog scale for pruritus (history) generated on day 0 and day 22 and the same was tested for statistical significance by paired 't' test.

Samples from the sterile swabs were cultured onto Modified Dixon's agar supplemented with chloramphenicol (0.5%) and cycloheximide (0.5%). All the plates were incubated at 37°C for about 7 days (Rathnapriya *et al*., 2016; Daniel *et al*., 2022) and inspected daily for Malassezia growth (Nardoni *et al*., 2007). Confirmation was based on identification of yeasts both on macroscopic appearance of colonies and microscopic cell morphology.

Results and Discussion

Dogs with dermatological problems suggestive of Malassezia dermatitis were screened and those which were found positive for Malassezia by direct microscopy formed the study group. Amongst 24 dogs, the highest and equal occurrence of Malassezia dermatitis was observed in non-descript, Labrador, Pug and Shih Tzu (n=5 each), followed by Dachshunds (3) and Beagle (1). In this study, Malassezia dermatitis was observed significantly higher in dogs aged above 6 years (n=9), followed by 1-3 year age group (8), 3-6 years (3) and less than 1 year (2). However, the sex-wise predilection was the same (n=12 each male and female). Neck, ventrum and median thighs were the commonly affected sites in the diseased dogs (Fig. 1).

Predominant clinical signs observed in the affected dogs were pruritus, erythema, malodour, lichenification, hyperpigmentation and hyperkeratinisation. Haematological and serum biochemical values revealed no statistical significance between pre-treatment (0 day) and post-treatment (22 day) group (Table 1). The impression smear stained using methylene blue produced a monochrome image of Malassezia organisms, which microscopically appeared as small, oval to peanut or footprint shaped (Fig. 2) (Haimbach, 2019)

Out of 24 clinical samples 20 isolates suggestive of *Malassezia* spp. were obtained on Modified Dixon's agar. The colonies obtained were smooth, round, convex, friable and cream in color (Fig. 3). Microscopic examination of colonies revealed dark blue coloured oval, footprint shaped organisms (Fig. 4) on staining with methylene blue for a minute (Bhaswanth *et al*., 2019).

Clinical response was monitored in all the three groups on day 22 after 3 weeks of treatment in terms of cytological evaluation of mean yeast count and pruritus score. The response to therapy on day 22 as compared to day 0 (prior to treatment) was statistically highly significant with considerable reduction in the mean yeast count and pruritus score post-treatment in group II and III, which received itraconazole pulse therapy and topical luliconazole, respectively. In the group, which received itraconazole conventional therapy (Group I) no significant reduction was noticed in yeast count, but there was significant reduction in the pruritus score (Table 1, Figs. 5, 6, 7).

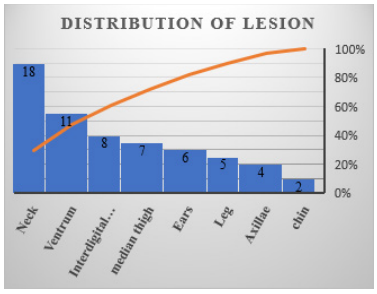

Fig. 1: Distribution of lesion of Malassezia dermatitis in dogs

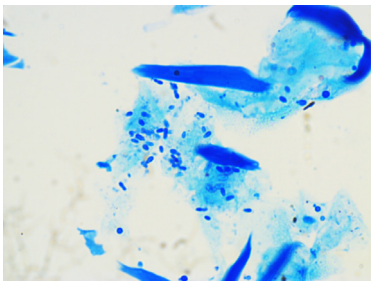

Fig. 2: Direct impression smear – Loefflers's alkaline methylene blue (100x): Small, oval to peanut or footprint shaped Malassezia organisms

Fig. 3: Macroscopic colony morphology (Modified Dixon's agar)

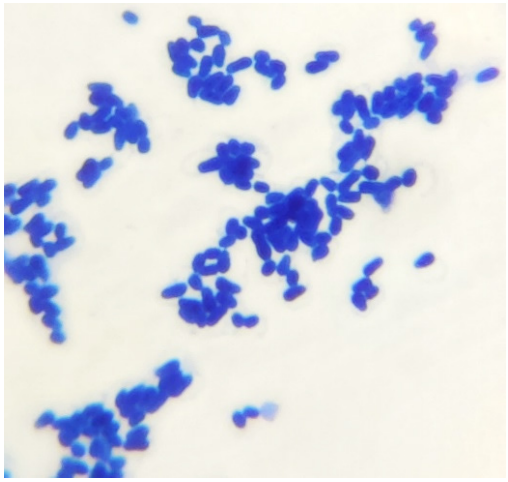

Fig. 4: Microscopic culture morphology (100x), stained with Loffler's alkaline meyhylene blue

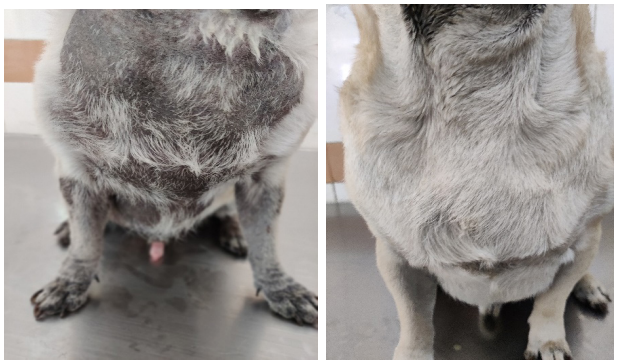

Fig. 5: Gr-I: Itraconazole conventional therapy, Lesions before and after treatment

Table 1: Comparison of Visual Analog Scale (VAS) for pruritus, mean yeast count, haematological and serum biochemical parameters pre- and post- treatment (Pre-T, Post-T) between groups in Malassezia affected dogs

Mean ± SE	Itraconazole -Conventional		P value	Itraconazole - Pulse		P value	Topical		P value
	Pre-T	Post-T		Pre-T	Post-T		Pre-T	Post-T	
Yeast count	4.25± 0.861	2.75± 1.114	0.1114^{NS}	3.63± 0.498	1.38 ± 0.263	0.0066**	3.88± 0.398	1.13± 0.125	0.0003**
VAS for Pruritus	4.00± 0.378	1.13± 0.125	0.0003**	3.38 0.420	1.13 ± 0.125	0.0005**	3.13± 0.295	1.13± 0.125	0.0001**
TLC ($x10^3$/µL)	17.15± 1.902	15.13± 1.883	0.2592^{NS}	14.59± 2.256	14.78 ± 2 .864	0.8940^{NS}	14.43± 1.320	13.56± 1.379	0.5846^{NS}
Hb (g/dL)	13.24± 0.497	13.26± 0.623	0.9694^{NS}	14.39± 0.754	14.00 ±0.635	0.4236^{NS}	15.24± 0.716	15.80± 0.627	0.3070^{NS}
Platelets ($x10^3$/µL)	374.63± 38.005	396.00± 32.777	0.4684^{NS}	318.36± 48.917	329.13± 29.125	0.6825^{NS}	298.13± 7.603	293.00± 17.348	0.7416^{NS}
PCV (%)	40.21± 1.610	41.10± 1.844	0.6560^{NS}	41.69± 3.006	43.03 ± 1.877	0.6387^{NS}	46.53± 2.241	48.38± 1.405	0.1415^{NS}
ALT (IU/L)	25.11± 3.175	32.30± 4.087	0.1227^{NS}	29.08± 4.274	29.05 ± 3.571	0.9958^{NS}	37.73± 4.495	33.70± 2.930	0.3199^{NS}
Creatinine (mg/dL)	0.99± 0.069	0.90± 0.027	0.2625^{NS}	1.14± 0.092	1.05 ± 0.080	0.2470^{NS}	1.10± 0.102	1.00± 0.098	0.3064^{NS}

** Significant at 0.01 level (P<0.01)

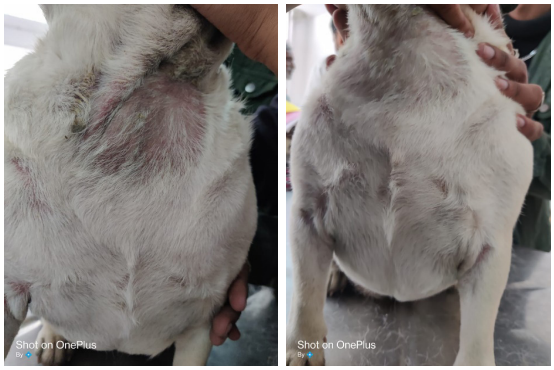

Fig. 6: Gr-II: Itraconazole pulse therapy, Lesions before and after treatment

Fig. 7: Gr-III: Topical Luliconazole therapy, Lesions before and after treastment

In clinical cases of Malassezia dermatitis in dogs, bacterial infections often co-exist. Therefore, broad spectrum antibiotic, cefadroxil @ 22 mg/kg b. wt. orally twice daily for 14 days was advised. Shampoo containing a combination of 2% miconazole with 2% chlorhexidine was recommended twice weekly for topical application in dogs with *Malassezia* dermatitis (Mueller *et al*., 2012). Ketoconazole though well tolerated, adverse effects and drug interactions are more common as compared to itraconazole. Further, itraconazole has better tissue penetration, has a longer elimination half-life, and is less toxic, compared to ketoconazole. The pharmacokinetics of itraconazole, along with sustained high concentrations in the skin that develop following oral administration, suggests that pulse administration (*i.e.,* intermittent drug administration at the recommended dose with a longer interval between doses than is commonly accepted) could be as efficacious as once daily administration (conventional) for the treatment of *M. pachydermatis* infection in dogs (Pinchbeck *et al*., 2002).

Itraconazole displayed the best *in vitro* activity and can be used as optimal antifungal agent in the management of Malassezia dermatitis in veterinary clinical practice (Rincon *et al*., 2006; Cafarchia *et al*., 2015). Luliconazole has a unique chemical structure, which is augmented by introduction of a ketene dithioacetate structure in the imidazole moiety (Koga *et al.,* 2009). It has high potency inhibitory action against Malassezia and filamentous fungi, dermatophytes (Hassan and Ranjan, 2020). Topical therapy may be useful and considered in patients as a prevention measure or in patients unfit for systemic therapy (Khanna and Bharti, 2014). Recurrence is common after treatment is completed, in such instances maintenance therapies such as weekly topical or monthly oral antifungal treatment is recommended as preventive measures (Rubenstein and Malerich, 2014).

Conclusions

A positive treatment response to antifungal, backed by reduction or temporary elimination of the organisms is highly suggestive and confirmatory, of a Malassezia etiology, but there are other variables such as the host's general condition and the species involved to be focussed upon. The importance of recheck examinations at regular intervals must be stressed for recurrent cases of Malassezia dermatitis. Cytology can confirm resolution of infection or can show residual yeast organisms that might indicate the need for maintenance antifungal therapy. This present study proves that topical luliconazole and itraconazole pulse therapy are more effective clinically than conventional itraconazole on 21 day treatment regimen. These results underscore the clinical utility of luliconazole as a potent, broadspectrum antimycotic agent.

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