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Research Article

THERAPEUTIC MANAGEMENT OF *MALASSEZIA* DERMATITIS IN DOGS

RUCHA BORKAR, KABITA ROY*, P.C. SHUKLA and DEVENDRA GUPTA
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry
The Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, India

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ABSTRACT

Thirty six dogs affected with *Malassezia* dermatitis were divided into six groups (T_1 to T_6). Synthetic anti-fungals such as ketoconazole@5 mg/kg b. wt. (T_1) and 10 mg/kg b. wt. (T_2) and fluconazole@5 mg/ kg b. wt. (T_3) and 10 mg/kg b. wt. (T_4) were given orally on two successive days with a gap of 5 days/week for 4 weeks (total 8 doses). Dried neem leaf extract (powder) was given orally @25 mg/kg b. wt. (T_5) and 50 mg/kg b. wt. (T_6) daily for 4 weeks. In all six therapeutic regimens (T_1-T_6), broad spectrum anti-bacterial cephalexin @20 mg/kg b. wt. twice daily for 5 consecutive days and silymarin @5 ml twice daily for 4 weeks were administered orally. Under the concurrent topical therapy, herbal neem seed oil (NSO) was applied daily on the skin lesions of dogs in all the groups. Shampoo (2% miconazole plus 2% chlorhexidine), followed by body wash was recommended once a week for dogs in all groups. Six *Malassezia spp*. free healthy dogs served as the control group (T_0). Based on alanine transaminase activity, clinical intensity scores and lymphocyte count, fluconazole@5 mg/kg b. wt. and herbal Neem powder @50 mg/kg b. wt., each in synergistic combination with the anti-bacterial, sylimarin, concurrent with topical application of Neem seed oil and shampoo were found to be the most effective.

Key words: Azoles, dogs, herbals, *Malassezia* dermatitis, neem, sylimarin,

Malassezia pachydermatis is a yeast that is commensal of the dog skin (Morris, 1999; Brito *et al*., 2009) and assumes pathogenic role when the cutaneous micro-environment in immune-compromised states becomes propitious for its rapid proliferation. A wide range of cellulolytic enzymes including phospholipases and proteases appear to participate in accentuated cutaneous inflammation (Carlotti, 2001; Cafarchia and Otranto, 2004). Multiplication of the yeast cell is promoted by higher pH values in the specific cutaneous target sites (Mason *et al*., 1996; Matousek *et al*., 2003). We report therapeutic management of dogs affected with *Malassezia* dermatitis.

MATERIALS AND METHODS

A total of 60 client-owned dogs, irrespective of breed, sex, age and the reproductive status with a history of persistent dermatitis having foul odour, were included in this clinical trial. The cases that were brought for treatment at the Institute's Teaching Veterinary Clinical Complex from November, 2013 to April, 2014 were included in this study. Physical examination of the individual dog, alterations in the posture/aberrations in the behavioral profile and routine clinical evaluation (Kelly, 1973) were conducted. *Malassezia* dermatitis was diagnosed based on the case history, clinical findings and surface cytological examination of representative samples collected from the affected areas. Dogs with history of receiving any systemic antibiotic/anti-fungal medication within the last 30 days were excluded from this study.

Impression smear from the skin lesions was stained with modified Wright's stain (Diff Quick) and was then examined under oil immersion to identify *Malassezia spp*. yeast cells (3-5 µm in diameter) with unipolar budding, imparting the typical peanut or boot-shaped appearance (Guilott and Bond, 1999). Confirmation of *Malassezia spp*. was done by culturing a parallel sample from the skin lesion on Sabouraud's dextrose agar medium (32°C, 3-5 days). Blood (5 ml) was collected for determination of various haematological and biochemical parameters. Uniformly thin blood films were stained with modified Wright's stain for differential leucocyte count. Plasma total protein, albumin and globulin fractions and alanine transaminase activity (ALT) were estimated with an autoanalyzer (Erba-CHEM-5 Plus V2) using commercial kits. Experimental data were analyzed with randomized design.

Malassezia dermatitis dogs (n=36) were randomly divided into six equal groups, each comprising of 6 animals. Under systemic therapy, synthetic anti-fungals such as ketoconazole @5 mg/kg b. wt. (T_1) and 10 mg/ kg b. wt. (T_2) and fluconazole @5 mg/ kg b. wt. (T_3)

*Corresponding author: kabitaroy_59@yahoo.in

and 10 mg/kg b. wt. (T_4) were given orally on two successive days with a gap of 5 days/week for 4 weeks (total 8 doses). Herbal anti-fungal, dried neem leaf extract (powder) was given orally @25 mg/kg b. wt. (T_5) and 50 mg/kg b. wt. (T_6) daily for 4 weeks. Broad spectrum anti-bacterial cephalexin @20 mg/kg b. wt. twice daily for 5 consecutive days and silymarin @5 ml twice daily for 4 weeks were administered orally. Neem seed oil (NSO) was applied daily on the skin lesions of dogs in all the groups. Shampoo (2% miconazole plus 2% chlorhexidine), followed by body wash was recommended once a week for dogs in all groups. Six *Malassezia spp*. yeast infection free healthy dogs served as the control group (T_0).

Assessment of the comparative efficacy of the different combination therapeutic regimens, based on skin cytological as well as semi-quantitative clinical scores was made in the affected dogs (Aspres and Anderson, 2004). In surface cytology, localization of more than five *Malassezia spp*. yeast cells/100x microscopic field was considered clinically significant. Presence of 5-10 yeast organisms in 15 consecutive fields in 1.25 cm^2 area on the five most susceptible body areas, namely the face, neck, axillae, perianal area and the inter-digital spaces was assigned 1+ (2 points), 15-20 organisms 2+ (4 points), and more than 20 organisms 3+ (8 points, maximum). Five body areas, sampled for the yeast count were also evaluated at the initial and the follow-up visits to record the pre- and post-treatment clinical scores on a system adapted from Rosales *et al*. (2005). Thus, each of the specified body areas was assigned a numerical value ranging from 0 to 3+ (0=absent, 1+=mild, 2+=moderate, 3+=severe) for eight clinical parameters, characteristic of *Malassezia* dermatitis in dogs such as erythema, hyperpigmentation, greasy exudate, offensive odour, scales and crusts, pruritus, alopecia and lichenification in chronic cases. Subtotal clinical score was maximum containing 24 (3x8) points. In the combination therapeutic regimens T_1-T_6, at each pre-and post-treatment interval, total scores under the skin surface cytological (8) and clinical parameters (24) were tallied for each dog, and subsequently average value under each treatment group was computed out of maximum (total 32 points) on a Clinical Intensity Score Card (CISC; Table 1). Calculated percent drop in the CISC was used as an index of relative therapeutic efficacy of different combination regimens.

RESULTS AND DISCUSSION

Of the 60 dogs with dermatitis, 36 dogs were found positive for *Malassezia* dermatitis with lymphocytosis being the main alteration. Thus, lymphocytosis to the extent of 36% in *Malassezia* dermatitis-affected dogs (Table 2) in this study corroborated with the findings of Saranya *et al*. (2011) and may be related to chronic cutaneous inflammatory process. However, all six combination treatments resulted in bringing back the percent lymphocytes within the normal range by 28th day post-treatment.

While treating the Malassezia dermatitis dog maintenance of the structural and functional patency of the liver remained a matter of high priority in this study. Thus, herbal hepatoprotective agent, silymarin was given in all the six treatments. Further, hepatic health status was regularly monitored by ALT assay. The ALT activity after administration of neem leaf powder combination at both dose levels (T_5 and T_6) was comparable to group T_0 at all intervals. Orally administered fluconazole at lower dose level of 5 mg/kg b.wt. did not elicit any perceptible increase in the ALT titre that is in sharp contrast to the significant increase recorded with ketoconazole at both doses (T_1 and T_2) on day 14 post-treatment, and the values remained elevated even on day 28 post-treatment (Table 2). These findings suggested that in therapeutic efficacy, fluconazole showed similar results as shown by ketoconazole. Similar findings have also been reported by Sickafoose *et al*. (2010). Rudayana (2010) reported wide safety margin of fluconazole. Thus, it is reiterated that combination regimens in groups T_3 and T_6 concurrent with the anti-bacterial, supportive and topical therapies were the best in light of the overall clinical findings of this study that is significant reduction in the number of cutaneous *Malassezia spp*. yeast organisms concurrent with abatement of fungal/ bacterial

Table 1
Comparative evaluation of the different combination therapies in *Malassezia* dermatitis in dogs with Clinical Intensity Score Card*

Groups	Day 0	Day 14	Day 28
T_1	16.3±0.79	14.5±0.71	12.3±0.57
T_2	17.3±0.82	13.3±0.56	10.3±0.49
T_3	16.3±0.73	14.0±0.63	10.8±0.47
T_4	14.1±0.72	11.5±0.55	9.0±0.40
T_5	15.0±0.78	13.3±0.68	12.7±0.67
T_6	15.1±0.77	12.3±0.85	9.1±0.47

*Maximum points=32; Six animals in each groups

Table 2
Lymphocyte (%) and plasma ALT titre in different combination therapies in *Malassezia* dermatitis in dogs at varying intervals (Mean±SE)

	Groups	Day 0	Day 14	Day 28
Lymphocyte (%)	T_0	26.67±0.21^{c}	27.67±0.49^{c}	26.67±0.42^{c}
	T_1	36.50±0.43^{a}	34.67±0.76^{a}	28.00±0.58^{c}
	T_2	36.83±0.48^{a}	31.17±1.08^{b}	28.83±0.60^{c}
	T_3	37.50±0.43^{a}	34.83±0.70^{a}	27.83±0.60^{c}
	T_4	37.67±0.42^{a}	36.83±0.48^{a}	27.83±0.60^{c}
	T_5	37.83±0.48^{a}	36.50±0.43^{a}	27.50±0.76^{c}
	T_6	37.50±0.48^{a}	36.33±0.42^{a}	28.33±0.88^{c}
Plasma ALT	T_0	50.57±2.32^{d}	49.92±2.29^{d}	51.24±1.49^{d}
	T_1	51.75±2.21^{cd}	62.37±1.08^{b}	62.78±0.91^{b}
	T_2	47.86±2.03^{d}	72.51±1.61^{a}	75.41±0.57^{a}
	T_3	49.26±1.81^{d}	49.26±1.81^{d}	52.20±2.06^{cd}
	T_4	50.74±1.72^{d}	56.83±1.10^{c}	62.74±0.88^{b}
	T_5	50.18±2.06^{d}	51.55±1.91^{d}	48.82±1.50^{d}
	T_6	49.55±1.93^{d}	49.66±2.12^{d}	50.97±2.27^{d}

Means in the same row/ column with different superscript vary significantly ($P<0.05$)

dermatitis as evidenced by progressive resolution of pruritus and other patho-clinical parameters (Figs. 1 and 2), evaluated with the help of the clinical intensity score card (Table 1).

In this study, the maximum therapeutic efficacy in terms of percentage reduction in the clinical score (40%) at the end of the 28-day clinical trial was exhibited by orally administered ketoconazole @10 mg/ kg b.wt. (T_2) vs 33.7% and 39.1%, respectively by combination of fluconazole @5 mg/kg b.wt. (T_3) and Neem powder @50 mg/ kg b.wt. twice daily for 4 weeks (T_6), it is not rated by us as the drug of choice in the light of increased ALT activity. Perusal of the pertinent pharmacokinetic data on fluconazole will corroborate our contention. Given that plasma levels of fluconazole in dogs peaked to nearly 10 µg/ml following a single 10 mg/kg b.wt. oral dose (Humphrey *et al*., 1985), it is plausible that the drug concentration in the actual site of action, stratum corneum exceeds the reported minimum inhibitory concentration (MIC) values for *M. pachydermatis* yeast, ranging from 4 to 16 µg/ ml of plasma (Brito *et al*., 2009). A lower dose of fluconazole is, therefore, adequate to sustain the effective therapeutic level in the cutaneous target tissue. Thus, combination of fluconazole @5 mg/kg b.wt. in pulse therapy, was effective in the present study. On the contrary, ketoconazole is not concentrated in the stratum corneum (Faegermann and Laufen, 1993), and highly protein bound lipophilic drug molecules tend to accumulate in liver and adrenal glands (Plumb, 2002) to potentially toxic levels in susceptible dog patients (Mayer *et al*., 2008).

In clinical cases of dermatitis in dogs, secondary yeast and bacterial infections often co-exist. Therefore, broad spectrum antibiotic, cephalexin was employed along with anti-fungal drugs in line with an earlier communication (Sickafoose *et al*., 2010). Miconazole acts by inhibiting biosynthesis of ergosterol which is an integral component of the fungal cell membranes (Chen and Hill, 2005) and also exhibits marked anti-bacterial activity against Gram positive organisms such as *Staphylococcus spp*. Negre *et al*. (2009) recommended a combination of 2% miconazole with 2% chlorhexidine for topical application in dogs with *Malassezia* dermatitis. In our study, in addition to once weekly miconazole-chlorhexidine shampoo, twice daily topical application of

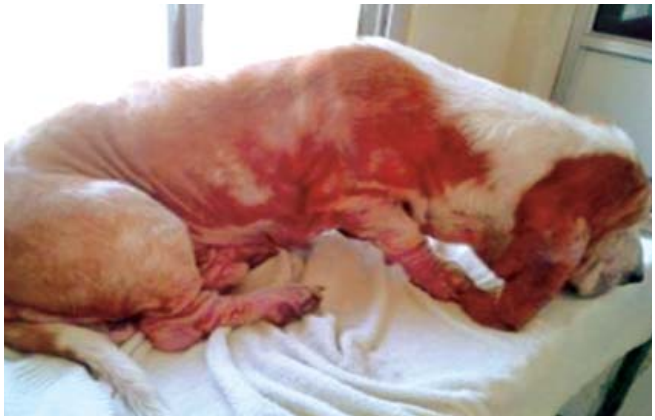

Fig 1. Prominent *Malessezia* dermatitis surface lesions in dog on day 0 pre-treatment

Fig 2. The same dog showing remission of skin lesions of Malessezia dermatitis on day 14 post-treatment (fluconazole combination, T_3)

organic shampoo was found to be very effective. Concurrent with orally administered holistic antimicrobial and supportive regimens, judicious use of this combination topical therapy is expected to pre-empt recurrent tenacious bouts of *Malassezia* dermatitis in the companion dogs. Depending on clinical judgment, the recommended combination of oral anti-microbial and supportive therapy along with proper anti-allergic dietary and other precautionary measures may need to be continued beyond the usual 4 week time schedule in some refractory individual dogs, till recovery is achieved.

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