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Assessment of the Therapeutic Efficacy of Doramectin with and Without Levamisole in Managing Canine Demodicosis

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ABSTRACT

Background: Canine demodicosis, a parasitic skin disease, is known to T-cell suppression causing immunosuppression. The use of immunomodulators in the treatment of demodicosis along with a miticidal agent needs to be assessed.

Methods: 12 dogs positive for demodicosis, on the basis of skin scraping examination, were divided into 2 groups of 6 dogs each and were treated with weekly inj. Doramectin @ 0.6 mg/kg body weight s/c alone and in combination with tab. Levamisole @ 2.5 mg/kg body weight weekly. Clinical progress was assessed based on skin scraping and haemato-biochemical examination. Immunomodulation was assessed using ANAE staining technique.

Result: All of the demodectic dogs showed a borderline reduction in Hb, PCV and TEC levels which showed a non-significant yet apparent rise after treatment. Also, there was leucocytosis, accompanied by neutrophilia, eosinophilia and lymphopenia that resolved significantly with treatment. Demodectic dogs had normal ALT and AST levels, normal serum total protein, hypoalbuminemia, hyperglobulinemia and reduced A:G ratio. The ANAE-positive cells (T lymphocytes) were reduced in demodectic dogs on day 0 and increased highly significantly in both groups on day 42. Both groups showed 83.33% recovery and 98.04% reduction in mite count on day 42. Hence, combination of doramectin and levamisole therapy can be used for efficient management of canine demodicosis.

Key words: ANAE staining, Demodicosis, Doramectin, Immunomodulation, Levamisole.

INTRODUCTION

Canine demodicosis, also known as follicular mange or red mange, is a parasitic skin disease characterised by the presence of larger-than-normal numbers of demodectic mites *viz. Demodex canis*, *Demodex injai* and *Demodex cornei* (Miller *et al.*, 2012). Malik *et al.*, (2023) recorded 28.16% overall prevalence of canine demodicosis with higher prevalence of generalized form than localized form of demodicosis (Thakur *et al.*, 2022). Canine demodicosis is notoriously known for its prolonged and laborious treatment regimens and its relapsing nature. Mueller *et al.* (2012) suggest that immunosuppression or a defective skin immune response contributes towards the development of clinical demodicosis. Dogs with generalised demodicosis suffer from an immune dysfunction called T-cell exhaustion, an antigen-specific effector T-cell dysfunction, characterised by stepwise progressive loss of T-cell functions. One or several combination of genotypes increases the probability of developing the disease phenotype which is a possible explanation of the hereditary basis of canine demodicosis Ferrer *et al.* (2014).

Generally, the treatment of canine demodicosis includes miticidal therapy to check the over-proliferating mites; however, it only seems logical that an immunomodulatory agent may be beneficial for treating dogs with demodicosis (Mueller *et al.*, 2020). Levamisole, an anti-nematodal parasiticide, has also been used as an immunostimulant because of its role in increasing T-cell activation. Levamisole enhances the function of T and B

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lymphocytes, monocytes and neutrophils, possibly via its effects on the metabolism of cyclic nucleotides (c-AMP, c-GMP) (Day, 2011). Mojžišová *et al.*, (2004) recommended levamisole as a suitable immunomodulator for improvement of the immune reactivity in immuno-compromised dogs and it also enhances the efficacy of protective vaccines. With this vantage point views, the following study was conducted to evaluate the therapeutic efficacy of doramectin, alone and in combination with levamisole in treating canine demodicosis.

MATERIALS AND METHODS

The present research work was conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence; College of Veterinary and Animal Science, MAFSU, Parbhani; during the period from May, 2022 to December, 2022 (five months). A total of 12 dogs with canine demodicosis, irrespective of age, sex and breed, presented to the Veterinary Clinical Complex, Parbhani, were divided randomly into 2 treatment groups, each comprising 6 dogs having extensive localized lesions (Fig 1). The experiment was conducted after approval from the ethical committee of college and research was conducted as per the standard ethical guidelines. Dogs from group I were treated with inj. Doramectin @ 0.6 mg/kg BW SC once a week. Dogs from group II were treated with inj. Doramectin @ 0.6 mg/kg BW SC once a week and tab. Levamisole @ 2.5 mg/kg BW PO once a week. All dogs from both groups received tab. Hydroxyzine @ 2-3 mg/kg BW PO BID and omega fatty acids supplement skin tonic as a supportive therapy.

The dogs were treated until recovery and clinical progress was assessed based on skin scraping, haematological and biochemical examinations. Skin scraping examination was performed every 2 weeks to establish a mite count and treatment was continued until two consecutive negative skin scraping (zero mite count) were obtained. Haematological parameters *viz*. Hb, PCV, TEC, TLC, Absolute counts of Neutrophil, Eosinophil, Lymphocyte and Monocytes; and biochemical parameters *viz*. ALT, AST, total protein, albumin, globulin and A:G ratio were examined on day 0 (before treatment) and on the 14th, 28th and 42nd days post-treatment. The reference range of these parameters as described by Brar *et al.* (2000) was used as a normal reference range for dogs. Skin scraping samples were collected aseptically from an area of at least 4 cm^2 of the skin and the scrapped material, after treatment with KOH (10%), was observed under the microscope.

Fig 1: Demodicosis positive dog with lesions.

For assessment of immunomodulation a cytochemical staining technique *viz.* the acid alpha naphthyl acetate esterase (ANAE) staining technique was used as described by Gadge, (1994). Six healthy dogs of either sex were selected and grouped as the healthy control group for ANAE staining technique. Heparinised blood was collected on day 0 and day 42 and the smears were prepared immediately after the blood collection and processed for ANAE staining. Lymphocytes containing one to four bright red cytoplasmic globules or large granules were counted as ANAE-positive lymphocytes. A total of 300 lymphocytes from the blood smear were counted under an oil immersion lens and the number of ANAE-positive lymphocytes was expressed as percentages. The therapeutic efficacy of both treatment regimens was assessed based on the rate of recover, recovery percentage and percent reduction in mite count on day 42 post-treatment with respect to day 0 values.

RESULTS AND DISCUSSION

The haematological and biochemical parameters of both groups are presented in Table 1 and Table 2, respectively. Dogs from both groups showed a borderline reduction in the Hb, PCV and TEC levels and followed a non-significant yet apparently increasing trend in their values post-treatment. The borderline levels of Hb, PCV and TEC on day 0 observed in this study may be due to loss of blood during scratching and loss of protein through the skin (Chakraborty and Pradhan, 2015) or poor nutrition as dogs are distracted from eating because of scratching. The apparent increase in the Hb, PCV and TEC in this study can be due to an improvement in skin lesions as a result of a reduction in mite count due to the miticidal action of doramectin. The amelioration in the Hb, PCV and TEC after treatment on subsequent days observed in this study is in accordance with the findings of Chakraborty and Pradhan, (2015); Chander *et al.* (2020) and Patowary *et al.* (2022); and can be attributed to improved skin lesions, appetite and general health.

All the demodectic dogs showed leucocytosis accompanied by neutrophilia, eosinophilia and lymphopenia. Post-treatment the leukogram improves back to the normal range. The increase in TLC and absolute neutrophil count was an inflammatory response to the over-proliferating mites and their levels recuperated to normal as the mite burden reduces (Chander *et al.*, 2020). The eosinophilia was due to the irritation of the skin tissues because of *Demodex spp.* mite infestation which stimulated the mast cells to release more histamine and since the histamine is chemotactic for eosinophils, eosinophilia developed (Chakraborty and Pradhan, 2015). Also, lymphopenia observed in demodicosis can be ascribed to a phenomenon known as T-cell suppression resulting from certain blastogenesis-suppressing factors *i.e.*, various

cytokines altered in demodectic dogs (Ferrer *et al.*, 2014 and Chakraborty and Pradhan, 2015). A highly significant reduction in TLC, absolute neutrophil count and absolute eosinophil count on day 0, day 14, day 28 and day 42 was observed in both groups and can be due to an improvement in skin lesions and general health of the dogs as a result of a reduction in mite count due to doramectin. Also, hydroxyzine, an antihistamine, given as supportive therapy in this study may have played a role in reducing the eosinophilia as it competes with histamine for the site on H1-receptors on effector cells and antagonizes the effect of histamines (Plumb, 2011). The highly significant increase in the absolute lymphocyte count observed in this study can be due to an improvement in skin lesions and the general health of the dogs as a result of a reduction in mite count due to doramectin. Also, levamisole played a role in restoring lymphocyte levels as it restores cell-mediated immune function in peripheral T-lymphocytes and stimulates phagocytosis by monocytes (Plumb, 2011). On day 14, day 28 and day 42 the TLC of group I differed highly significantly from group II such that later had a comparably lower TLC count. Similarly, the absolute neutrophil count of group I differ highly significantly from group II on day 14 and day 28 and on day 42 it differs significantly such that the absolute neutrophil count of group II was comparably lower than group I. With respect to the absolute lymphocyte count, group II had significantly higher levels on day 14 than group I. However, a non-significant difference was observed between the absolute lymphocyte count of group I and group II on day 28 and day 42.

Table 1: Pre and Post-treatment haematological parameters of demodectic dogs.

Parameters		Day 0	Day 14	Day 28	Day 42
Haemoglobin (g/dl)	Group I	11.10±0.78	11.85±0.84	12.28±0.82	12.75±0.81
	Group II	12.35±0.40	12.87±0.40	13.25±0.34	13.23±0.35
PCV (%)	Group I	34.63±2.43	35.92±2.55	37.00±2.55	38.53±2.41
	Group II	36.50±1.12	38.08±1.00	39.43±1.13	39.97±1.08
TEC ($\times 10^6$/mm^3)	Group I	5.50±0.23	5.87±0.34	5.88±0.27	6.20±0.32
	Group II	6.03±0.19	6.22±0.16	6.30±0.12	6.32±0.12
TLC ($\times 10^3$/mm^3)	Group I**	16.58±0.21^{a}	15.72±0.05^{b†}	14.70±0.12^{c†}	13.65±0.04^{d†}
	Group II**	16.70±0.10^{a}	14.13±0.11^{b}	13.37±0.16^{c}	13.08±0.10^{c}
Absolute neutrophil count (/mm^3)	Group I**	14037.67±180.02^{a}	12889.17±165.41^{b†}	11415.50±91.24^{c†}	10123.50±95.85^{d†}
	Group II**	14024.33±79.78^{a}	10999.83±221.41^{b}	10123.50±131.46^{c}	9611.33±195.13^{d}
Absolute eosinophil count (/mm^3)	Group I**	1466.83±88.84^{a}	1309.50±77.22^{a}	1054.17±61.66^{b}	910.17±46.30^{b}
	Group II**	1533.67±08.77^{a}	1200.67±105.46^{b}	1028.33±130.92^{b}	896.00±69.39^{b}
Absolute lymphocyte count (/mm^3)	Group I**	856.67±78.01^{d}	1203.67±83.64^{c‡}	1984.33±64.67^{b}	2411.67±77.68^{a}
	Group II**	835.83±46.63^{c}	1649.67±124.15^{b}	2013.00±197.59^{ab}	2314.00±172.33^{a}
Absolute monocyte count (/mm^3)	Group I	222.17±36.67	314.33±40.81	246.00±32.28	204.67±30.42
	Group II	306.17±51.56	283.17±37.89	201.83±31.75	262.00±47.69

Means with different superscripts ^{a,b,c} in a row differ significantly P<0.05; Means with superscripts † differ highly significantly from group II at P<0.01 ; Means with superscripts ‡ differ significantly from group II at P<0.05; * indicate P<0.05; ** indicate P<0.01

Table 2: Pre and Post-treatment Biochemical parameters of demodectic dogs.

Parameters		Day 0	Day 14	Day 28	Day 42
ALT (U/L)	Group I	46.21± 7.77	47.18± 7.69	49.14± 9.07	45.02± 7.01
	Group II	47.18± 5.46	48.03± 5.71	47.98± 6.01	47.96± 5.77
AST (U/L)	Group I	36.93± 5.32	36.66±5.33	35.06±3.65	34.24± 3.12
	Group II	37.50± 2.77	39.04± 2.93	38.62± 2.76	36.98± 2.37
Total Protein (g/dl)	Group I	5.76± 0.20	5.98± 0.12	5.75± 0.13	5.97± 0.20
	Group II	5.72± 0.07	5.83± 0.12	5.67± 0.18	5.91± 0.18
Albumin (g/dl)	Group I**	1.80± 0.12^{c}	2.36± 0.13^{b}	2.39± 0.13^{b}	3.07± 0.28^{a}
	Group II**	1.88± 0.07^{c}	2.16±0.10^{bc}	2.43± 0.19^{b}	3.05± 0.18^{a}
Globulin (g/dl)	Group I**	3.96± 0.15^{a}	3.62± 0.14^{ab}	3.36± 0.15^{b}	2.89± 0.11^{c}
	Group II**	3.84± 0.07^{a}	3.67± 0.12^{a}	3.24± 0.15^{b}	2.86± 0.06^{c}
A:G ratio	Group I**	0.46± 0.03^{c}	0.66± 0.06^{bc}	0.72± 0.06^{b}	1.08± 0.14^{a}
	Group II**	0.49± 0.03^{c}	0.59± 0.04^{bc}	0.76± 0.09^{b}	1.07± 0.08^{a}

Means with different superscripts ^{a,b,c} in a row differ significantly P<0.05; Means with superscripts † differ highly significantly from group II at P<0.01; Means with superscripts ‡ differ significantly from group II at P<0.05; * indicate P<0.05; ** indicate P<0.01.

Table 3: Pre and Post-treatment ANAE-positive cell levels of demodectic dogs.

	ANAE-positive cells (%)		
	Group I	Group II**	Healthy control
Day 0**	58.67+0.76^{b†}	56.00+1.18^{c†}	81.17+0.40^{a}
Day 42**	67.50+1.61^{b}	78.00+0.37^{a}	80.67+0.42^{a}

Means with different superscripts ^{a,b,c} in a row differ significantly P<0.05; Means with superscripts † differ highly significantly from group II at P<0.01; Means with superscripts ‡ differ significantly from group II at P<0.05; * indicate P<0.05; ** indicate P<0.01.

Absolute monocyte count, ALT, AST and total protein did not show a significant variation and were in the normal range. Demodectic dogs showed hypoalbuminemia, hyperglobulinemia and a reduced A:G ratio before treatment and a highly significant improvement was seen on day 42. Chander *et al.* (2020) ascribed the decreased serum albumin levels to the excessive breakdown of proteins due to skin trauma and mites' proliferation. The improvement in Albumin, Globulin and A:G ratio after treatment can be attributed to the resolution of infection.

The pre and post-treatment values of ANAE-positive cells of both groups is presented in Table 3. As implied by Bayraktaroğlu *et al.* (2015) ANAE-positive cells represent T lymphocytes. The lower levels of ANAE-positive cells are thus indicative of reduced T lymphocyte levels, a feature also documented by Ferrer *et al.* (2014) in demodectic dogs. On day 0, ANAE-positive cells *i.e.*, T lymphocyte levels were highly significantly lower in group I and group II than in the healthy control group. Post-treatment on day 42, dogs from group II showed comparable ANAE-positive cell values *i.e.*, T lymphocyte levels to that of the healthy control group and dogs from group I, though exhibited a rise in ANAE-positive cells *i.e.*, T lymphocytes in comparison to their day 0 values, showed ANAE-positive cells *i.e.*, T lymphocyte levels lower than that of dogs from the group II and healthy control group. A highly significant rise in ANAE-positive cells *i.e.*, T lymphocytes in group I can be because of the reduction in mite burden due to the miticidal action of doramectin (Ferrer *et al.*, 2014). A highly significant rise in ANAE-positive cells *i.e.*, T lymphocytes in group II can be because of the combined effect of the reduction in mite burden due to the miticidal action of doramectin and the immunostimulatory action of levamisole (Plumb, 2011 and Ferrer *et al.*, 2014).

Dogs from group I and group II showed a highly significant reduction in mean mite count from 8.67±1.26 and 8.67±0.42 on day 0 to 0.17±0.17 and 0.17±0.17 on day 42, respectively. This can be attributed to the potent miticidal action of doramectin as documented by Hutt *et al.* (2015); Cordero *et al.* (2018) and Parwari *et al.* (2022). The average number of days required to achieve 1st negative skin scraping was 44.33±2.33 days and 35.00±4.78 days for group I and group II respectively. This was comparable with the findings of Parwari *et al.* (2022) and Hutt *et al.* (2015) who reported the mean rate of recovery to be 5.75±0.37 weeks and 7.1 weeks, respectively. However, statistical analysis indicated a non-significant difference in the rate of recovery between group I and group II. Out of 6 dogs from Group I and 6 dogs from Group II, 5 dogs from each group achieved negative skin scraping on/by day 42. In other words, both groups showed 88.33 % recovery on/by day 42. These findings resonate with the findings of Hutt *et al.* (2015) and Cordero *et al.* (2018). Hutt *et al.* (2015) who recorded a 94.8% remission rate with weekly subcutaneous injections of doramectin. Similarly, Cordero *et al.* (2018) recorded an 81% success rate for weekly subcutaneous injections of doramectin and a 9% success rate for weekly oral administration of doramectin. The per cent reduction in mite count, on day 14, day 28 and day 42 with respect to the baseline mite count of day 0 was 44.29%, 80.74% and 98.04% in group I and 50.06%, 92.27% and 98.04% in group II, respectively.

Dogs treated with doramectin alone and in combination with levamisole group both showed significant improvement in comparison to their day 0. However, a non-significant difference was observed between the rates of recovery for both groups and also the per cent recovery and per cent reduction in mite count were comparable to each other. The ANAE-positive cell *i.e.*, T-lymphocyte levels improved highly significantly in both groups in comparison to their initial levels and post-treatment ANAE-positive cells *i.e.*, T-lymphocytes levels were comparably higher in group II than group I. Post-treatment the haematological and biochemical parameters showed improvement in group I and group II both, however, did not differ significantly from each other. From this study, we can say that the use of levamisole does improve T-lymphocyte levels in demodectic dogs but doesn't contribute to the parasitological recovery of the dog.

CONCLUSION

In conclusion, the combination of doramectin and levamisole therapy has better efficacy than doramectin alone for management of canine demodicosis. Levamisole can improve T cell suppression with slight effect on the clinical recovery of the demodectic dog.

Conflict of interest

The authors declared that there is no conflict of interest for the manuscript.

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