

Evaluation of Therapeutic Approaches for Canine Demodicosis with Microscopic and Haemato-Biochemical Assessment

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ABSTRACT

Demodectic mange is a clinically significant parasitic dermatosis in dogs and represents one of the most common dermatological presentations in veterinary practice in India. The present randomized controlled clinical study was conducted to assess the diagnostic reliability of deep skin scraping and evaluate the therapeutic efficacy of ivermectin, amitraz, and their combination in 48 naturally infected dogs exhibiting alopecia, erythema, crusting, pruritus and pustular dermatitis. Diagnosis was confirmed by microscopic detection of *Demodex canis*, identifiable by its elongated cigar-shaped body and short legs. Dogs were randomly assigned to three treatment groups each of 16 dogs: ivermectin (0.4 mg/kg PO daily) monotherapy, amitraz (0.05% weekly dip) monotherapy, and combined ivermectin-amitraz therapy. Supportive care included benzoyl peroxide shampoo twice weekly and systemic antibiotics when pyoderma was detected. Clinical and parasitological response was monitored biweekly for 8 weeks, and haemato-biochemical analysis on 0 (before), 4 and 8 week of therapy. The combination group demonstrated a significantly higher cure rate (87.5%) with rapid reduction in mite load and lesion severity, compared to ivermectin alone (69%) and amitraz alone (50%) including reversal of haemato-biochemical alterations. Relapse was lowest in the combination group. Deep skin scraping proved an effective diagnostic and monitoring tool. The findings support multimodal therapy as the most effective clinical strategy for managing generalized demodicosis under field conditions.

Key words: Amitraz, Canine, *Demodex canis*, Haemato-biochemical profile, Ivermectin, Mange, Microscopy.

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INTRODUCTION

Canine demodicosis (demodectic mange) is a chronic parasitic dermatopathy caused by the excessive proliferation of *Demodex canis*, a normal inhabitant of canine hair follicles and sebaceous glands (Mueller *et al.*, 2020). Although small numbers of mites are commonly present on clinically healthy dogs, immunosuppression, genetic predisposition, nutritional deficiencies or concurrent systemic disease may disrupt host-parasite equilibrium, resulting in clinical disease (Paterson *et al.*, 2019). Young growing animals, poorly nourished dogs, and certain breeds such as German Shepherds, Pugs and Bulldogs show greater susceptibility, indicating a possible hereditary and immunological basis (Rosenberg *et al.*, 2016).

Clinically, demodicosis may present in localized or generalized forms. Localized lesions often manifest as patchy alopecia, scaling and erythema and may resolve spontaneously (Singh *et al.*, 2019a; Boyd and Reuter, 2012). In contrast, generalized demodicosis is characterized by widespread alopecia, crusting, comedones and involvement of the face, trunk and limbs. Secondary bacterial pyoderma and pododemodicosis are frequent complications that markedly worsen prognosis, clinical discomfort and overall therapeutic outcomes (Rosenkrantz and Griffin, 2017). Without appropriate early diagnosis and targeted therapy,

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the condition can become chronic and debilitating. Accurate and timely diagnosis is therefore critical and relies mainly on deep skin scrapings and microscopic identification of the mite in various developmental stages (Sharma *et al.*, 2023a). While alternative diagnostic approaches such as trichograms, biopsy, and dermoscopy may support the diagnosis, they are less practical in routine field practice in regions such as Gujarat, where veterinary clinics frequently rely on affordable, rapid methods (Kumar *et al.*, 2017).

Treatment options for demodicosis have evolved over the decades. Conventional drugs include macrocyclic lactones such as ivermectin and topical amitraz, while newer isoxazoline-based acaricides have demonstrated high efficacy and safety (Beugnet and Halos, 2020; Sahoo *et al.*, 2021). However, in India, ivermectin and amitraz are widely used due to availability, affordability and familiarity among clinicians. Considering increasing case incidence in Gujarat and limited structured therapeutic outcome data for this region, the present study was aimed to evaluate the diagnostic features, haemato-biochemistry and comparative therapeutic efficacy of ivermectin, amitraz and their combination in naturally infected dogs under field conditions. The study further assessed parasitological cure, clinical response and follow-up relapse rates to provide practical recommendations for regional veterinary practitioners.

MATERIALS AND METHODS

Study Population

The study was conducted from January 2021 to August 2023 in Veterinary clinical complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat. Forty-eight dogs of varying age, sex and breed presenting dermatological signs suggestive of demodicosis were screened. The inclusion criteria were dogs to exhibit visible dermatological signs such as alopecia, erythema (Fig.1), scaling, or pustules. Additionally, a positive deep skin scraping demonstrating the presence of five or more mites per low-power field (≥ 5 mites/LPF) was necessary for participation. Dogs that had received any form of acaricidal treatment within the preceding three months were excluded to ensure accurate evaluation of therapeutic outcomes.

Diagnostic Microscopy

Samples were collected using deep skin scraping from five active lesion areas until pinpoint bleeding was observed. Scrapings were mixed with 10% KOH and examined microscopically under 10 \times and 40 \times magnification. The

recorded parameters included detailed observations of mite morphology, specifically noting the characteristic adult "cigar-shaped" form (Fig. 2). The presence of different developmental stages, like eggs, larvae, and nymphs, was also documented. In addition, the average number of mites per low-power field (LPF) was calculated to assess the level of infestation.

Treatment Groups

Dogs were randomly assigned into three equal groups A, B and C, each of 16 dogs. Dogs in group A received Ivermectin (0.4 mg/kg PO daily) + Amitraz 0.05% weekly dip, those in group B Ivermectin monotherapy, and group C Amitraz monotherapy for up to 6-8 weeks. Supportive care included benzoyl peroxide shampoo twice weekly and systemic antibiotics when pyoderma was detected.

Clinical Evaluation

Clinical improvement was assessed based on percentage of hair regrowth, reduction in erythema and scaling, resolution of pustules and crusts and decrease in mite load on follow-up scraping. Follow-ups occurred at week 2, 4, 6 and 8. Cure was defined as $\geq 90\%$ clinical improvement and two consecutive negative skin scrapings.

Haemato-Biochemical Analysis

Blood samples were obtained from each dog from the cephalic or saphenous vein on week 0 (pre-treatment), week 4 (mid-treatment), and week 8 (end of treatment) in vacutainers with EDTA for haematology and in clot activator vials for serum biochemistry. Whole blood was used for haematological parameters like haemoglobin, packed cell volume, total leukocyte count, and neutrophil and eosinophil percentage using automated blood cell analyser (Abacus junior Vet-5).

Serum separated from clot activator vials was stored at -20°C in deep freeze for biochemical analysis of alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), and globulin (GLB) using assay kits and automated serum clinical chemistry analyser (CKK 300) at Veterinary Clinical Complex, Anand.



Fig. 1: Dog with severe alopecia and erythema

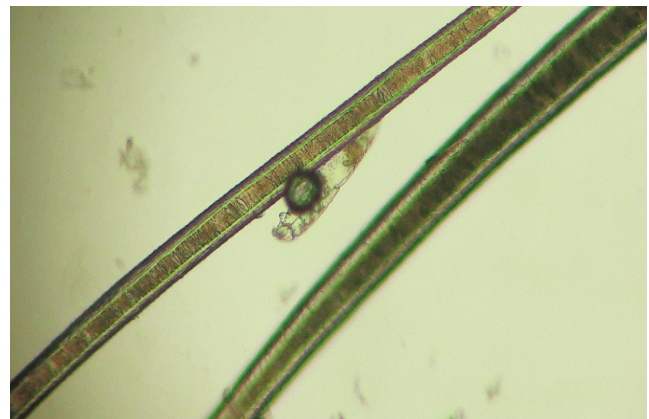


Fig. 2: *Demodex canis* under microscope

Statistical Analysis

The data generated on clinical and haemato-biochemical parameters was analysed using Repeated Measures ANOVA (RM-ANOVA), and *Post-hoc* analysis was performed using Bonferroni correction, and significance was accepted at $p < 0.05$. Highly significant results were noted at $p < 0.01$. Values are expressed as Mean \pm Standard error.

RESULTS AND DISCUSSION

All forty-eight dogs enrolled in the study were confirmed positive for demodicosis based on deep skin scraping, where microscopic examination revealed *Demodex canis* adults, larvae, nymphs, and eggs, confirming active mite replication. The mean baseline mite count was 40.3 ± 8.6 mites/LPF, indicating moderate to severe generalized demodicosis. The observed cigar-shaped morphology of adult mites with short legs (Fig. 2) was consistent with previous diagnostic descriptions by Mueller *et al.* (2020) and Sharma *et al.* (2023b), and reaffirming deep skin scraping as a sensitive and field-appropriate diagnostic tool.

Therapeutic Efficacy

Marked clinical improvement and reduction in mite burden were observed across all treatment groups over the 8-week period; however, the rate and magnitude of recovery varied significantly depending on treatment protocol (Table 1). The combination therapy group (Group A: ivermectin + amitraz) demonstrated the most rapid and significant clinical response. By week four, visible improvement including hair regrowth, reduced erythema, and healing of pustules was observed in most cases. By week eight, 87.5% (14/16) of dogs achieved complete clinical and parasitological cure. No relapse was recorded in the three-month follow-up period. Similar findings were reported by Unnikrishnan *et al.* (2022), and the therapeutic synergy was consistent with the enhanced follicular penetration mechanism proposed by Fourie *et al.* (2007).

The ivermectin monotherapy group-B demonstrated a moderate cure rate (69%, 11/16 dogs) with gradual improvement. Mite load reduction became significant after week four. Mild reversible neurological signs noted in two animals correspond with report of Paterson *et al.* (2019). While ivermectin remains effective, outcomes indicate monotherapy may be inadequate in advanced generalized cases, especially with concurrent pyoderma (Sahoo *et al.*, 2021). The amitraz monotherapy group-C showed the lowest cure rate (50%, 8/16 dogs). Improvement was slow and inconsistent, requiring extended therapy in several cases. Owner compliance issues including dip-associated odor, irritant reactions and handling difficulty were reported. Such limitations were also documented by Rosenberg *et al.* (2016) and Singh *et al.* (2019a).

Table 1: Therapeutic outcome of three treatment protocols used in naturally infected dogs with generalized demodicosis

Treatment Group	Total dogs	Cured	Relapse	Cure rate (%)
Ivermectin + Amitraz	16	14	0	87.50
Ivermectin alone	16	11	2	69.00
Amitraz alone	16	8	3	50.00

Statistical analysis confirmed significant differences among treatment responses ($p < 0.05$). Relapse occurred in Group B ($n=2$) and Group C ($n=3$), whereas none occurred in Group A, confirming superior long-term therapeutic stability. Under Gujarat field conditions, where drug availability, affordability and ease of use influence compliance, a combination therapy provided the most practical and effective outcome. The findings aligned with the consensus that generalized canine demodicosis requires multimodal therapy for optimal cure (Rosenkrantz and Griffin, 2017; Mueller *et al.*, 2020).

Haemato-Biochemical Alterations

Parallel to parasitological and clinical improvements, notable changes were observed in haematological and biochemical parameters from week 0 to week 8 (Table 2). At baseline, dogs displayed mild to moderate anaemia reflected by low haemoglobin and packed cell volume levels, alongside marked leucocytosis, neutrophilia, and eosinophilia. These alterations are characteristic of chronic parasitic dermatitis, prolonged inflammation, and associated pyoderma (Kumar *et al.*, 2017; Salem *et al.*, 2020). By week 4, all groups began showing partial haematological correction, but the most significant improvement was evident in Group A. By week 8, Group A demonstrated near normalization of haemoglobin and packed cell volume, accompanied by a marked reduction in total leukocyte count and eosinophils, reflecting effective suppression of inflammation and recovery from parasitic stress. Group B showed moderate improvement, while Group C showed only partial correction of haematological abnormalities. This progressive normalization is consistent with the biological expectation that effective mite removal results in gradual resolution of systemic inflammatory responses (Thrall *et al.*, 2012).

Hb and PCV increased significantly in Groups A and B, suggesting successful reversal of anaemia and chronic inflammation. Combination therapy (Group A) showed the strongest recovery ($p=0.000$). Similar improvements have been reported during recovery from demodicosis due to reduced cytokine-mediated bone marrow suppression and improved erythropoiesis (Kumar *et al.*, 2017; Singh *et al.*, 2019b). Group C exhibited slower, weaker improvement, which aligned with studies showing that amitraz monotherapy is less effective in severe or generalized cases (Rosenkrantz and Griffin, 2017; Beigh *et al.*, 2020). The pattern of erythrocyte improvement matches earlier observations that chronic



parasitic dermatitis leads to lowered RBC indices due to anorexia, stress, and prolonged inflammatory response (Sarasola *et al.*, 2021).

All groups showed a significant decrease in TLC, particularly Groups A and B, reflecting resolution of systemic inflammation. Elevated leukocyte levels in demodicosis are associated with secondary pyoderma and bacterial folliculitis (Miller *et al.*, 2013; Nuttall and Harvey, 2021). Neutrophils declined significantly in Groups A and B, consistent with rapid healing of pustules and follicular inflammation. Non-significant reduction in Group C suggests incomplete suppression of underlying infection, supporting earlier findings that amitraz alone is often inadequate in

highly inflamed cases (Mueller *et al.*, 2012; Paterson, 2020). Eosinophils decreased significantly in Groups A and B, typical of reduced parasitic irritation as mite burden fell (Saridomichelakis, 2006; Sharma *et al.*, 2023b). Group C showed mild, non-significant decline, further indicating slower therapeutic response.

Biochemical parameters also showed similar trends. At week 0, elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels reflected hepatic stress commonly associated with chronic inflammation and long-term dermatological disease (Boyd, 2017). Total protein and albumin levels were below normal limits, indicating protein loss through exudation, inadequate nutrition, and reduced

Table 2: Mean (\pm SE) values of haemato-biochemical parameters at different time intervals in dogs affected with demodicosis following various treatment protocols

Parameter	Treatment Group	Weeks of treatment			p-value
		Week 0	Week 4	Week 8	
Haemoglobin (g/dL)	Group A	9.10 \pm 0.4 ^a	11.45 \pm 0.5 ^b	13.80 \pm 0.5 ^c	0.000
	Group B	9.40 \pm 0.3 ^a	10.80 \pm 0.4 ^b	12.20 \pm 0.4 ^c	0.000
	Group C	9.20 \pm 0.5	10.15 \pm 0.4	11.10 \pm 0.3	0.255
PCV (%)	Group A	28.30 \pm 1.2 ^a	34.85 \pm 1.2 ^b	41.40 \pm 1.1 ^c	0.000
	Group B	29.10 \pm 1.0 ^a	33.15 \pm 1.2 ^b	37.20 \pm 1.3 ^c	0.000
	Group C	28.60 \pm 1.3 ^a	31.65 \pm 1.3 ^b	34.70 \pm 1.2 ^c	0.000
TLC ($\times 10^6/\mu\text{L}$)	Group A	19.70 \pm 1.0 ^a	15.55 \pm 0.9 ^b	11.40 \pm 0.7 ^c	0.000
	Group B	20.10 \pm 1.1 ^a	16.95 \pm 0.9 ^b	13.80 \pm 0.6 ^c	0.000
	Group C	19.50 \pm 1.2 ^a	17.35 \pm 1.0 ^b	15.20 \pm 0.7 ^c	0.007
Neutrophils (%)	Group A	82.30 \pm 1.8 ^a	76.40 \pm 1.9 ^b	70.50 \pm 2.0 ^c	0.000
	Group B	81.40 \pm 2.1 ^a	77.35 \pm 2.0 ^b	73.30 \pm 1.9 ^c	0.001
	Group C	82.00 \pm 1.9	78.70 \pm 1.9	75.40 \pm 1.8	0.605
Eosinophils (%)	Group A	11.50 \pm 0.6 ^a	8.35 \pm 0.5 ^b	5.20 \pm 0.4 ^c	0.000
	Group B	12.00 \pm 0.7 ^a	9.55 \pm 0.6 ^b	7.10 \pm 0.5 ^c	0.000
	Group C	11.70 \pm 0.6 ^a	10.55 \pm 0.6 ^b	9.40 \pm 0.6 ^c	0.085
ALT (U/L)	Group A	62.40 \pm 3.2 ^a	49.30 \pm 2.7 ^b	36.20 \pm 2.1 ^c	0.000
	Group B	59.10 \pm 2.9 ^a	50.30 \pm 2.6 ^b	41.50 \pm 2.3 ^c	0.000
	Group C	61.80 \pm 3.1 ^a	53.75 \pm 3.1 ^b	45.70 \pm 3.0 ^c	0.006
ALP (U/L)	Group A	198.30 \pm 5.4 ^a	159.95 \pm 5.1 ^b	121.60 \pm 4.8 ^c	0.000
	Group B	193.20 \pm 4.7 ^a	167.75 \pm 4.9 ^b	142.30 \pm 5.1 ^c	0.000
	Group C	196.50 \pm 5.6 ^a	177.00 \pm 5.3 ^b	157.50 \pm 5.0 ^c	0.000
Total Protein (g/dL)	Group A	4.80 \pm 0.2 ^a	5.85 \pm 0.25 ^b	6.90 \pm 0.3 ^c	0.000
	Group B	4.90 \pm 0.1 ^a	5.50 \pm 0.15 ^b	6.10 \pm 0.2 ^c	0.000
	Group C	4.70 \pm 0.2 ^a	5.15 \pm 0.25 ^b	5.60 \pm 0.3 ^c	0.030
Albumin (g/dL)	Group A	2.00 \pm 0.1 ^a	2.60 \pm 0.1 ^b	3.20 \pm 0.1 ^c	0.000
	Group B	2.10 \pm 0.1 ^a	2.45 \pm 0.1 ^b	2.80 \pm 0.1 ^c	0.000
	Group C	2.00 \pm 0.1 ^a	2.30 \pm 0.1 ^b	2.60 \pm 0.1 ^c	0.000
Globulin (g/dL)	Group A	2.80 \pm 0.1 ^a	3.25 \pm 0.15 ^b	3.70 \pm 0.2 ^c	0.000
	Group B	2.80 \pm 0.1	3.05 \pm 0.15	3.30 \pm 0.2	0.515
	Group C	2.70 \pm 0.1 ^a	2.85 \pm 0.15 ^b	3.00 \pm 0.2 ^c	0.050

Means with different superscripts (a,b,c) within the row differ significantly between periods within group ($p < 0.05$).

hepatic synthetic capacity. By week 8, Group A showed the most significant decline in ALT and ALP levels, suggesting improved hepatic function as inflammation subsided. Concurrently, total protein and albumin levels increased markedly, indicating restored metabolic balance and improved protein synthesis. Groups B and C demonstrated similar but less pronounced trends, with Group C showing only partial biochemical recovery by the end of the trial period.

Total protein and albumin increased significantly in all groups, demonstrating nutritional and hepatic recovery. Hypoalbuminemia is very common in chronic demodicosis due to protein-losing dermatitis, tissue exudation, and systemic inflammation (Plant *et al.*, 1992; Scott *et al.*, 2001). Group A showed the most robust recovery, which aligned with faster lesion healing and reduced inflammatory exudation. Globulin levels increased significantly in Groups A and C. Mild globulin increase during early recovery corresponds to antibody-mediated responses to bacterial and mite antigens (Koutinas *et al.*, 2001; Saridomichelakis and Koutinas, 2004). Later stabilization suggests restored immune homeostasis.

Overall, the week-based evaluation revealed a strong correlation between parasitological improvement and haematological as well as biochemical recovery. As mite load declined, markers of inflammation, hepatic stress, and protein metabolism progressively normalized. These findings confirm that haematological and biochemical indices are reliable indicators for monitoring therapeutic response in canine demodicosis and reflect the systemic recovery associated with effective mite control. The consistent superiority of the ivermectin–amitraz combination across clinical, parasitological, haematological, and biochemical parameters supports its use as the most effective protocol for generalized demodicosis under field conditions. This treatment not only accelerated clinical healing but also minimized relapse risk, a key factor influencing long-term disease management in endemic regions.

CONCLUSION

The present study demonstrated that generalized canine demodicosis responds most effectively to a combination regimen of ivermectin and amitraz. Dogs treated with the combined protocol exhibited the fastest reduction in mite burden, the most rapid clinical improvement, and the most consistent normalization of haematological and biochemical parameters compared to monotherapy groups. The week-based evaluation clearly showed that improvements in haemoglobin, leukocyte counts, serum proteins, and liver enzymes closely paralleled parasitological clearance, indicating that systemic recovery is strongly dependent on the speed and efficiency of mite elimination. In contrast, ivermectin alone provided moderate improvement, while amitraz monotherapy resulted in delayed and incomplete recovery in several animals. Overall, the findings confirm that multimodal therapy is superior to single-agent treatment for

managing generalized demodicosis under field conditions. The study highlights the diagnostic value of deep skin scraping, the importance of monitoring haemato-biochemical changes during treatment, and the necessity of combining systemic and topical therapy for successful long-term management. The ivermectin–amitraz combination can therefore be recommended as an effective, practical, and field-suitable therapeutic protocol for generalized canine demodicosis.

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