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Status of canine demodicosis by using different diagnostic methods and its correlation with endoparasitic infections in dogs of Mumbai and thane region of Maharashtra state

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Abstract

The present study was undertaken to determine the prevalence, clinical manifestations, and haematological alterations associated with canine demodicosis, and to compare the diagnostic performance of deep skin scraping with the Superglue Slide Impression (SSI) technique in dogs from the Mumbai and Thane region of Maharashtra state. The overall prevalence of canine demodicosis was 34.96% (50/143). Among affected dogs, 18% exhibited localized lesions, while 82% presented with generalised demodicosis. Based on mite index, 39 dogs (78%) had a mite index >1, indicating progressive disease, whereas 11 dogs (22%) demonstrated features consistent with the recovery phase. A higher prevalence was recorded in adult dogs (82%), nondescript dogs (60%), and males (62%), compared with puppies (18%), pedigree breeds (40%), and females (38%). The predominant clinical signs included alopecia, pruritus, and pustular dermatitis, commonly distributed over the dorsum, head, and hindlimbs. When comparing diagnostic methods in demodectic dogs, SSI demonstrated a slightly higher detection rate (96%) compared with deep skin scraping (94%), highlighting its high diagnostic sensitivity; concurrent conditions were frequently observed. Ectoparasitic infestations included fleas (16%), ticks (12%), sarcoptic mange (6%), and maggot wounds (6%). Faecal examination revealed helminth infections in 28% of dogs, comprising hookworm (12%), *Dipylidium caninum* (8%), and mixed infections (8%). No haemoprotozoan parasites were detected upon blood smear evaluation. Hypothyroidism was identified in 6% of animals. Haematological analysis of the 50 demodectic dogs revealed normocytic, normochromic anemia, reduced packed cell volume (PCV), and leucocytosis characterised by neutrophilia, lymphocytopenia, eosinopenia, and monocytopenia, indicating inflammatory and immunosuppressive tendencies commonly associated with chronic demodicosis. Overall, the SSI technique proved to be as sensitive as deep skin scraping, and is rapid, inexpensive, minimally invasive, owner and pet-friendly, and does not induce adverse skin reactions. Additionally, viable mites can be collected using SSI, making it a valuable method for evaluating miticidal efficacy.

Keywords: Canine demodicosis, deep skin scraping, Superglue slide impressions (SSI)

1. Introduction

In today's veterinary field, small animal dermatology practice has become an important aspect. The skin, being the largest organ of the body, often provides information to the clinician that reflects both local and systemic changes, providing valuable clues for early diagnosis (Barlaskar *et al.*, 2023; Marchegiani *et al.*, 2020) [5, 15]. In canines, these changes are an important indicator as they reflect the general health of an animal. Over the past decade, veterinary clinics have seen a noticeable rise in dermatological consultations due to increased owner awareness and the growing prevalence of skin-related diseases. Dermatitis, defined as inflammation of the skin, may be produced by a myriad of agents, including parasites, bacteria, fungi, external irritants, burns, and allergens.

The contribution of ectoparasites viz. ticks, fleas, lice and mites causing dermatitis is conspicuous. One of the causes of parasitic dermatitis in dogs is the follicular mite, *Demodex canis* or 'red mange'. Canine demodicosis is a refractory skin disease caused by the excessive

Proliferation of demodectic mites in the animal's hair follicles and sebaceous glands (Kumari, 2017) ^[12]. Clinically, affected dogs may show lethargy, reduced appetite, anaemia, and secondary bacterial infections, especially when additional endoparasitic or ectoparasitic burdens are present (Abel *et al.*, 2023; Sousa *et al.*, 2020) ^[1, 39]. Severe or chronic cases may require months of therapy before full recovery is achieved. Although *Demodex* mites are considered to be commensals on dog skin, disease develops when the immune system fails to regulate their numbers, allowing unchecked multiplication. Prompt and accurate diagnosis is crucial because inappropriate treatment can worsen the condition (Silva *et al.*, 2021) ^[33]. Corticosteroids, for instance, may be useful in sarcoptic mange but can aggravate demodicosis by promoting mite proliferation (Almeida *et al.*, 2021; Swe *et al.*, 2014) ^[2, 42]. Tentative diagnosis is usually based on the presence of signs such as alopecia, erythema, crusting, pruritus, follicular pustules, furunculosis, ulceration, malodour, and, in cases of pododemodicosis, marked interdigital swelling (Sharma, *et al.*, 2018; Sousa *et al.*, 2020) ^[29, 31, 39]. Secondary bacterial infections are also common in advanced disease. Deep skin scraping remains the most widely used diagnostic technique and is regarded as the gold standard, as the finding of even a single mite confirms demodicosis (Mueller *et al.*, 2020) ^[16]. However, it is invasive, uncomfortable for the animal, may cause capillary bleeding, and is often poorly tolerated by both dogs and their owners (Singh, 2019) ^[35]. In addition, the procedure requires sample processing, which can delay diagnosis (Suartha *et al.*, 2018) ^[41]. Other methods such as acetate tape impressions and trichograms are available, but they generally offer lower sensitivity or are better suited for generalized lesions (Madureira and Brum, 2017; Mueller *et al.*, 2020) ^[13, 16]. Superglue Slide Impressions (SSI), or cyanoacrylate skin surface biopsies, offer a less invasive, quick, and economical alternative. Well-established in human dermatology for identifying *Demodex* spp. and *Sarcoptes scabiei*, this method has recently been adapted for use in dogs. In this technique, cyanoacrylate adhesive is applied to the skin to extract mites, which can subsequently be examined directly under a microscope without additional processing. SSI also allows collection of viable mites for monitoring the effectiveness of treatment (Singh, 2019) ^[35]. Given the clinical relevance of demodicosis and the limitations of existing diagnostic approaches, the present study was designed with the following objectives

- To determine the incidence of canine demodicosis using different diagnostic methods in dogs from the Mumbai and Thane regions of Maharashtra.
- To document the clinical manifestations and haematological changes in dogs with generalised demodicosis.
- To compare the diagnostic performance of currently available techniques for detecting *Demodex* spp. in dogs.

Methodology

Dogs exhibiting clinical signs suggestive of dermatitis such as erythema, alopecia, crusts, pruritus, papules, pustules, and comedones were included in the study (Fig. 1, 2). Samples were collected from the outpatient and inpatient departments of Bai Sakarbai Dinshaw Petit Hospital for Animals, Mumbai, as well as from various NGOs and private clinics in Mumbai and Thane. Information on breed, age, sex, and the type of demodicosis (localized or generalised) was recorded for analysis.

From all suspected cases, deep skin scrapings, Superglue Slide Impressions (SSI), faecal samples, and blood samples were collected. Deep skin scrapings were obtained by scraping the skin with a sterile blade dipped in liquid paraffin until capillary oozing of blood was observed. The samples were processed according to the method described by Soulsby (1982) ^[40]. The scraping was mixed with 5-10 ml of 10% KOH, then heated for 10-15 minutes. After discarding the supernatant, 2-3 drops of sediment were mounted on a slide with a coverslip and examined under low power for the presence of mites. SSI samples were collected according to the technique described by Singh (2019) ^[35]. A drop of cyanoacrylate adhesive was placed on the centre of a clean glass slide; the affected skin was gently squeezed between the thumb and index finger, and the slide was pressed to the area for approximately 30 seconds. The slide was then removed against the direction of hair growth. A drop of immersion oil was added to the impression and was covered with another slide and examined under low magnification for live mites (Fig. 3, 4). Mite Index for *Demodex* spp. was calculated by counting all life stages of *Demodex canis* (eggs, larvae, nymphs, and adults) in the ratio of immature to mature stages.

$\text{Mite Index} = \frac{\text{Number of immature mites}}{\text{Number of mature mites}}$

Faecal samples from suspected or confirmed demodicosis cases were examined for concurrent endoparasitic infection using the sedimentation method (Soulsby, 1982) ^[40]. Approximately 2 g of faeces was mixed with 10 ml of water and strained through cotton gauze. The filtrate was transferred into a sedimentation flask, topped with water, and left undisturbed for 30 minutes. After decanting the supernatant, a drop of the sediment was placed on a slide and examined under 10× magnification for parasite eggs or larvae.

Peripheral blood was collected from the cephalic vein of dogs confirmed positive for *Demodex* spp. for haematological analysis. Parameters evaluated included haemoglobin concentration, packed cell volume (PCV), erythrocyte indices, Total Leukocyte Count (TLC), and Differential Leukocyte Count (DLC). Thin blood smears were also prepared to screen for haemoprotozoan parasites, following the method described by Rathore and Sengar (2005) ^[21].

All data were analyzed statistically following the methods of Snedecor and Cochran (1994) ^[36].

Results

3.1 Prevalence of canine demodicosis

During the study period, 143 dogs with clinical signs of dermatitis such as alopecia, pruritus, and erythema were examined for demodicosis using deep skin scraping and Superglue Slide Impressions (SSI). Of these, 50 dogs were confirmed positive for canine demodicosis, resulting in an overall prevalence of 34.96%.

3.1.1 Clinical forms of canine demodicosis

Canine demodicosis was categorised into localised and generalised forms based on lesion distribution. Among the 50 affected dogs, 9 (18%) had localised demodicosis and 41 (82%) showed generalised involvement.

3.1.2 Age-wise prevalence

Based on the age of the dog, demodicosis can be classified as juvenile onset or adult onset demodicosis. Out of 50 dogs positive for canine demodicosis, nine dogs (18%) showed juvenile onset, while 41 dogs (82%) had adult onset of the

disease (Table 1). The difference between both groups was statistically highly significant ($p < 0.05$).

3.1.3 Breed-wise prevalence

Examined dogs were broadly classified as pedigree or non-descript. Among the 50 demodectic dogs, non-descript dogs accounted for 60% of cases, while pedigree dogs constituted 40%. Within pedigree dogs, Labradors (14%) were most affected, followed by Pomeranian (6%), American Bulldog, Pug, and Shih Tzu (4% each), and Doberman, Lhasa Apso, Pitbull, and Rottweiler (2% each). Statistical analysis showed no significant difference in prevalence between pedigree and non-descript dogs ($p > 0.05$).

3.1.4 Sex-wise prevalence

Of the 50 affected dogs, 31 (62%) were males and 19 (38%) were females. Although males were numerically more affected, the difference between sexes was statistically non-significant ($p > 0.05$).

3.2 Clinical findings

Dogs with demodicosis showed a variety of dermatological lesions and systemic signs. The most common lesions observed were alopecia (100%), followed by pruritus (76%), pustules (62%), crusting (56%), erythema (52%), scaling and lichenification (48% each), ulceration (26%), comedones (24%), pododemodocosis (24%), and foul odour (22%). (Table 2, Fig. 5-8)

3.2.1 Distribution of lesions

Lesions were either confined to specific regions or distributed over multiple body sites. Generalized demodicosis, defined as involvement of five or more body regions, was noted in 41 dogs (82%). The most frequently affected areas were the head and dorsum (68% each), followed by hindlimbs (62%), forelimbs (58%), neck (56%), abdomen (50%), and tail (24%) (Table 3).

3.3 Diagnosis of canine demodicosis

Diagnosis was based on deep skin scraping and Superglue Slide Impressions (SSI).

Out of the 50 affected dogs, skin scraping detected 47 positives (94% sensitivity), whereas SSI detected 48 positives (96% sensitivity)

3.3.1 Skin scraping

Deep skin scraping (DSS) detected *Demodex canis* in 47 of 50 dogs (94%). All developmental stages such as eggs, larvae, nymphs, and adults were observed (Fig. 9). Concurrent *Sarcoptes* spp. infection was detected in 3 dogs (6%), while no cases of *Otodectes cynotis* infestation were recorded. (Fig. 10)

3.3.2 Superglue slide impressions (SSI)

Using SSI, 48 of 50 dogs (96%) were found positive for *D. canis*. All life stages of the mite were observed, and live mites were frequently detected, indicating that SSI can also be used to collect viable mites for assessing miticidal efficacy (Fig. 11-12). No other mange mites were detected on SSI in the present study.

3.3.3 Comparison of mite counts

Total mite counts (adult and immature stages) from both tests are presented in Table 4. Although numerical differences were seen between methods, the differences were statistically

non-significant ($p > 0.05$), indicating comparable quantitative performance. Qualitatively, SSI was easier to perform, required less time, and was more acceptable to both dogs and owners, and no adverse reaction to cyanoacrylate adhesive was observed.

3.3.4 Mite index

The mite index was calculated as the ratio of immature to mature stages of *D. canis* in each case. Among the 50 dogs, 39 (78%) had a mite index greater than 1, indicating a predominance of immature stages and suggesting an active, progressive infection. The remaining 11 dogs (22%) had mite indices ≤ 1 , consistent with recovering or less active infections.

3.4 Concurrent infections

3.4.1 Concurrent ectoparasitic infestation

Out of 50 demodectic dogs, 8 (16%) had flea infestation, 6 (12%) had ticks, 3 (6%) had sarcoptic mange, and 3 (6%) had maggot-infested wounds (Table 5).

These concurrent infestations were associated with additional skin irritation, self-trauma, and hypersensitivity reactions.

3.4.2 Concurrent endoparasitic infection

Fecal examination of the 50 demodectic dogs revealed endoparasitic infection in 14 dogs (28%) (Table 6). Hookworm eggs were detected in 6 dogs (12%), *Dipylidium caninum* egg capsules in 4 dogs (8%), and mixed infections with both hookworm and *D. caninum* in 4 dogs (8%).

A higher proportion of endoparasitic infections was seen in non-descript dogs compared to pedigree dogs.

3.4.3 Blood smear examination

All 50 demodectic dogs were negative for haemoprotozoan parasites on blood smear examination.

3.4.4 Hormonal imbalance

Serum thyroxine evaluation revealed hypothyroidism in 6% of the demodectic dogs.

These findings suggest that thyroid dysfunction may be a contributing factor in a subset of cases.

3.5 Haematological findings

Haematological values of demodectic dogs and corresponding reference ranges are summarized in Table 7.

3.5.1 Haemoglobin and packed cell volume

Haemoglobin values ranged from 7.1 to 13.3 g%, with a mean of 10.27 ± 0.22 g/dL, indicating a mild to moderate reduction compared to the reference range. Packed cell volume (PCV) ranged from 20.1 to 38.76%, with a mean of 30.13 ± 0.63 %, also lower than normal reference values.

3.5.2 Erythrocyte indices

The mean MCV, MCH, and MCHC were 65.88 ± 1.86 fl, 22.52 ± 0.70 pg, and 34.2 ± 0.36 g/dl, respectively, which are compatible with normocytic, normochromic anaemia.

Total and differential leucocyte counts

Total leucocyte counts were elevated, ranging from 13 to $56.6 \times 10^3/\mu\text{L}$, with a mean of $24.73 \pm 1.12 \times 10^3/\mu\text{L}$. Differential counts showed neutrophilia ($84.2 \pm 0.70\%$), lymphocytopenia ($13.44 \pm 0.67\%$), eosinopenia ($1.26 \pm 0.22\%$), and monocytopenia ($1.2 \pm 0.06\%$).

Discussion

The overall prevalence of canine demodicosis (34.96%) recorded in this study aligns with earlier reports from Maharashtra and Jammu where similar levels of occurrence have been described (Pawar, 2021 and Berian *et al.*, 2018) [4, 18]. Conversely, much lower prevalence values ranging from 2.7% to 6% have been observed in other regions of India, including Eastern Maharashtra and Andhra Pradesh (Raut *et al.*, 2006 and Sowjanya, 2019) [14, 22], while Sarma *et al.* (2013) [27] reported a substantially higher prevalence of 51.92%. These discrepancies likely reflect differences in climate, seasonal conditions, owner awareness, management practices, and diagnostic sampling strategies. Generalised demodicosis was significantly more common than the localised form in the present study, a pattern consistent with findings from Assam, Tirupathi and Himachal Pradesh (Pradhan, 2016; Kumari, 2017; Sharma *et al.*, 2018) [12, 20, 29, 31]. Owners may overlook mild localised lesions or delay veterinary consultation, resulting in the presentation of more advanced cases. In contrast, studies from Gujarat and Kerala have reported more localised cases (Nair, 2019; Solanki, 2007) [17, 37] possibly due to spontaneous resolution of mild disease. Adult-onset demodicosis predominated in this investigation, a finding consistent with reports from Gujarat (Solanki, 2007) [37] and Himachal Pradesh (Sharma *et al.*, 2018) [29, 31]. Adult-onset disease may result from relapse of juvenile demodicosis or from underlying triggers such as chemotherapy, hormonal imbalance, endoparasitism, or compromised immunity (Kumar *et al.*, 2018) [11]. Conversely, Gunaseelan, 2011 [8] reported higher susceptibility in juveniles, likely reflecting immature immunity and close neonatal contact with carrier dams. Nondescript dogs were more frequently affected, probably due to poorer nutrition, inadequate housing, and delayed veterinary care consistent with observations in Palampur (Sharma *et al.*, 2018) [29, 31]. On the other hand, a higher prevalence among purebred dogs has also been documented (Nair, 2019) [17], suggesting breed predisposition and possible hereditary immune defects (Pradhan, 2016) [20]. Although more males were affected, the difference was not statistically significant. Testosterone-related immunomodulation has been suggested as a factor (Klein, 2004 and Roberts *et al.*, 2004) [10, 24], but many studies, including those from Gujarat and Assam, also reported no meaningful sex association (Pradhan, 2016; Solanki, 2007) [20, 37].

The clinical signs noted were alopecia, pruritus, pustules, crusting, erythema, scaling, lichenification, ulceration, comedones, pododemodicosis, and foul odour, which reflect classical presentations described across India (Chakraborty, 2012; Sivajothi *et al.*, 2015) [7, 34]. These manifestations result from folliculitis, inflammation and secondary bacterial infection caused by *Demodex* proliferation. The distribution of lesions on the head, dorsum, limbs and neck mirror trends (Sood *et al.*, 2012) [38]. Deep skin scraping remained highly sensitive (94%) and detected all life stages of the mite, reinforcing its role as a standard diagnostic tool. However, Superglue Slide Impressions (SSI) demonstrated slightly higher sensitivity (96%) and have several advantages like faster performance, less invasiveness, higher owner acceptability, and the ability to recover viable mites. Comparable findings were reported in humans using cyanoacrylate biopsies (Bunyaratavej *et al.*, 2016; Zorbozan *et al.*, 2020) [6, 44], and by Singh (2019) [35] in dogs. Mite counts between both methods did not differ significantly, confirming the quantitative reliability of SSI.

Concurrent flea infestation, tick infestation, sarcoptic mange and maggot wounds were relatively common, and similar co-infections have been noted in Nepal (Sharma and Pokharel, 2019) [30]. These additional skin irritants are likely to aggravate pruritus, self-trauma, and immune dysregulation, contributing to worsening disease. Endoparasitic infections were observed in 28% of demodectic dogs, mainly hookworms and *Dipylidium caninum*. Endoparasites are recognized contributors to immunosuppression and demodicosis (Shaw and Foster, 2000) [32], and juvenile demodicosis has been linked to hookworms and coccidia (Plant *et al.*, 2011) [19]. Higher prevalence in earlier studies from West Bengal (Chatterjee, 2007; Chakraborty, 2012) [7] may reflect differences in deworming practices. Hypothyroidism (6%) also contributed in some dogs, as low thyroxine is known to impair cell-mediated immunity and facilitate mite proliferation (Sakhare, 2017) [25]. Haematological changes showed mild to moderate anaemia, low PCV, and normocytic-normochromic indices supporting the presence of anaemia of chronic disease, which is commonly reported in demodicosis (Sakhare, 2017; Sharma *et al.*, 2018) [25, 29, 31]. Leucocytosis and pronounced neutrophilia indicate chronic antigenic stimulation and secondary bacterial infection, as previously documented (Reddy *et al.*, 2015; Boda, 2016) [23]. Lymphocytopenia aligns with earlier suggestions of T-cell suppression (Scott *et al.*, 1974; Sakina *et al.*, 2012) [26, 28]. Eosinopenia, although counterintuitive, has been reported internationally (Hillier & Desch, 2002) [9] and may reflect stress-mediated glucocorticoid release (Benjamin, 1978) [3]. Monocytopenia, although observed, is not clinically significant (Valenciano *et al.*, 2014) [43].

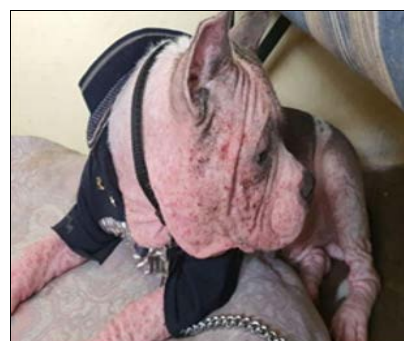


Fig 1: Dog showing erythema, alopecia with pustules



Fig 2: Dog showing alopecia with crust



Fig 3: SSI method by squeezing of skin while applying cyanoacrylate adhesive containing slide on skin lesion

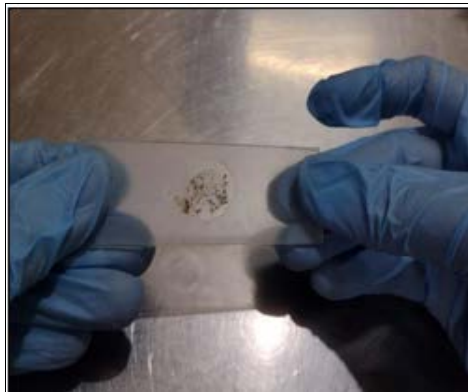


Fig 4: Placing a drop of immersion oil before applying a cover slide in SSI method



Fig 5: Generalized alopecia in dog



Fig 6: Lichenification of skin on face of dog



Fig 7: Pododemodicosis in dog (erythema with oedema of legs)



Fig 8: Ulceration with oozing of blood

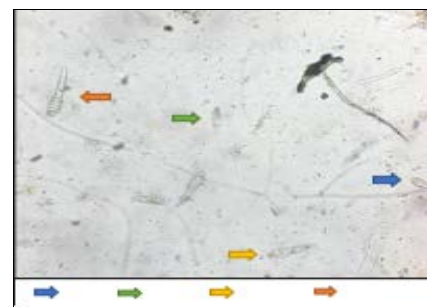


Fig. 9: Different life stages of *Demodex* in Skin scraping (10x)



Fig. 10: *Sarcoptes* spp. Of mite in Skin scraping (10x)

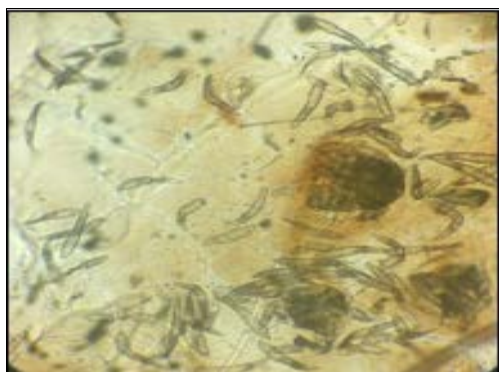


Fig. 11: Observation of Demodex mites in Superglue Slide Impression (10x)



Fig. 12: Observation of Demodex mites in Superglue Slide Impression (4x)

Table 7: Haematological values of demodectic dogs

Parameter	Demodectic dogs (mean±SE)	Reference range (Weiss & Wardrop, 2011)
Hb (g%)	10.27±0.22	12.0-18.0
PCV (%)	30.13±0.63	37-55
MCV (fl)	65.88±1.86	60-77
MCH (pg)	22.52±0.70	19.5-26
MCHC (g/dl)	34.2±0.36	32-36
TLC (×10 ³ /cmm)	24.73±1.12	6.0-17
Neutrophils (%)	84.2±0.70	50-67
Lymphocytes (%)	13.44±0.67	16-28.2
Eosinophils (%)	1.26±0.22	1.6-7.3
Monocytes (%)	1.2±0.06	2.5-7.9

Conclusion

Canine demodicosis is a common dermatological condition with higher prevalence in adult dogs and predominantly generalized presentation. Superglue slide impression (SSI) is a reliable, non-invasive alternative to skin scraping for diagnosis. The disease is strongly influenced by underlying factors such as concurrent infections and hormonal imbalances. Effective control requires both accurate diagnosis and management of these predisposing conditions.

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