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Clinical and histopathology studies on demodicosis in dogs

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Abstract

The present study was conducted over a period of 6 months at Veterinary College Hospital, Hebbal, Bengaluru and other animal facilities in and around Bengaluru. Out of 90 cases of dogs considered in the present study, Demodicosis was recorded in thirty-four dogs with an incidence of 35.55 per cent (32/90). Clinically, the lesions showed papules and pustular eruptions with erythematous patchy alopecic areas and also showed crusted lesions or plaques and hyperpigmentation. Histologically, four principal patterns of inflammation were observed in canine demodicosis which included folliculitis, furunculosis, mural folliculitis and parafollicular granulomas.

Keywords: Dogs, *Demodex canis* mites, skin histopathological changes

Introduction

Glossy coat and a healthy skin are the hallmarks in the cosmetic appearance of a dog. A skin ailment may be a cause of distress not only to the dog but also to the owner because of its unkempt appearance. Among different diseases affecting dogs, skin diseases pose a serious problem to the veterinarian. Negligence and ignorance of the owner or unawareness and improper diagnosis of skin diseases may augment the condition where maximum damage will be noticed to the pet, since routine and general treatment will not completely eradicate the condition always.

Skin being the largest organ in the body is likely to be influenced by a plethora of infectious and non-infectious, environmental agents. In several veterinary facility 20 per cent to 75 per cent of dogs presented at the small animal clinics are known to be with a dermatological complaint ^[1]. In dogs, the skin diseases could be due to parasitism, bacterial, fungal, allergies, immunological diseases, nutritional related dermatosis, hormonal disorders and cancers ^[2].

Demodicosis is relatively common disease among dogs, caused by common commensal present on the skin surface. The majority cases of canine demodicosis are caused by *Demodex canis* mites, although other species of demodicid mites also causes infection.

The main factors which influence extreme colonization of demodex mites could be due to immunological and ecological favourability, a genetically pre-programmed immunological defect and immunosuppression also responsible for the demodectic infection in dogs. However, immunological studies revealed evidence of suppressed T-cell function, especially T-cytotoxic cells activated by alteration in T helper cells function leads to specific defect in immunity which flares up the infection ^[1]. The mange-mite infestation is influenced by a number of factors such as age, body condition, breed, and climate conditions ^[3].

The demodicosis infection is divided into forms such as juvenile and adult onset which can be both localized and generalized form. Usually, juvenile onset is seen in puppies which are less than 1 year of age. Canine demodicosis (*D. canis*) is also one of the important mange mite infestations which occurs either in a localized or a generalized form with the clinical manifestation of pustular and squamous type skin lesions. Common clinical signs of mange mite infestations include irritation, rubbing, ear twitching, scratching and head shaking and produced intense irritation with a thick reddish-brown crust ^[4].

Clinical signs of mite proliferation depend on the degree of mite proliferation. Initially, develop as a noninflammatory hypotrichosis or alopecia. In later which leads to an inflammatory dermatitis with mild erythema, comedone formation, scaling and

associated hypotrichosis or alopecia. The lesions evolve as focal or multifocal to coalescing into larger areas [5].

The dermatologic essential baseline tests, such as skin scrapings, skin cytology for identifying parasitic and infectious causes of skin illness, are always the first step in the diagnostic evaluation of a patient with skin disease. The standard method for diagnosis and therapeutic monitoring of dogs with demodicosis is microscopic examination of deep skin scrapings [6]. The commonly employed diagnostic test in dermatology is procurement of superficial or deep skin scrapings that comprise the epidermal outer layers or the entire epidermis and superficial dermis. This technique principally used for the detection of ectoparasites such as follicular mites [7]. The mites are most commonly found in the pilosebaceous follicles, hence cutaneous scraping must be done from several spots before excluding a positive demodicosis diagnosis [8]. Deep skin scraping is performed by firmly squeezing the selected lesion between the thumb and index finger to expel mites from the hair and samples are analyzed for the number of eggs, larvae, nymphs and adult forms of *D. canis* [9]. Multiple scrapings of approximately 1 cm of affected skin should be performed in the direction of the hair growth and importantly the skin should be squeezed constantly or intermittently during scraping to extrude the mites from the depth of the follicles to the surface [10]. The areas chosen for deep scraping, are the areas with primary lesions associated with demodicosis (erythema, follicular papules and pustules, and comedones) [6, 8].

Skin biopsy samples can give the doctor vital information. Ironically, the pathologist is often better at helping the physician to rule out certain differential diagnoses such as an allergy, endocrine, or parasitic one. Punch biopsy is the simplest and fastest method; it uses skin "punches," which come in a range of sizes; however, pathologists prefer specimens with a minimum diameter of 6 mm⁷. Skin biopsies are required, in order to diagnose a demodex case. Punch biopsy would be advised if there is a high index of suspicion for demodicosis and skin scrapings are negative. Those with thick skin, chronic pododermatitis, or patients whose lesions have become fibrotic [10].

Materials and Methods

The present study was carried out over a period of 6 months in the Department of Veterinary Pathology, Veterinary College, Bengaluru, to study "clinical and histopathological changes in the demodicosis infected dogs." Clinical data such as age, sex, breed and previous history were noted. Clinical signs with reference to inflammatory changes, pruritus, erythema, pustules, vesicles, scales, crusts, lichenification, hyperpigmentation and odour were recorded. Presence of fleas, ticks and alopecia were recorded. For all affected skin lesions, following samples were collected and processed.

Skin scraping

The affected skin lesions showing gross changes was selected for scraping. Microscopic slides were labelled with site to be scraped and mineral oil/ glycerine was applied to the slide. It was important to either dip the scraping instrument (22 gauze BP blade) in mineral oil or to be applied mineral oil directly onto the area being scraped to ensure the material collected to be stuck to the instruments. Squeezing the site was necessary to extrude the

mites that were deep in hair follicles up onto the skin surface.

Scraping was done deep enough to get capillary bleeding, transferred scraped material to the mineral oil on the glass slide, coverslip was placed and examined microscopically, under 4X or 10X. Recorded the results from each material such as the number of mites found and their life stage (adult or larva). In some cases, materials were collected in test tube to which 10 per cent potassium hydroxide was added and gently heated to dissolve the cell debris. It was then cooled, centrifuged and sediment was examined under the microscope for the mites.

Punch biopsy

The dogs showing dermatological lesions with demodex mites in direct microscopic examination of skin scrapings were selected to perform skin punch biopsy. The hair over and around the site to be biopsied was clipped and swabbed with 70% alcohol taken care not to disturb the surface lesions. Mostly primary skin lesions and transition lesions were selected for biopsy. Representative tissue samples were obtained by using 6 mm punch biopsy under local anaesthesia using 2% lidocaine 2mg/kg body weight, the punch biopsy was inserted using combination of forward pressure and unidirectional rotation motions and was advanced to the depth of the subcutaneous fat layer. The detached piece of skin was then gently lifted at one edge using the point of a sterile needle or fine rat toothed forceps and any adherent tags of fat were excised and placed in container with at least 10 times their volume of 10% phosphate buffer formalin.

Histopathology

The biopsied samples were fixed in 10% neutral buffered formalin immediately and processed by routine paraffin embedding technique. Sections of 4-5 microns were cut using Leitz microtome with disposable blades and stained with Haematoxylin and Eosin (Luna, 1968) for the classification of different patterns of demodicosis based on its microscopic features.

Results and Discussion

Out of 90 cases of dogs considered in the present study, demodicosis was recorded in thirty-two dogs with an incidence of 35.55 per cent (32/90), these results were consistent with the reports of Kalyan *et al.* [11] who reported 35.7 per cent of demodectic lesions, whereas Solanki and Hasnani [12] reported 25.4 per cent. Jeong- Hyun *et al.* [13] reported lower incidence of demodicosis about 4.9 per cent in his study. In these cases, both general and localized forms were observed and the same was recorded in juvenile (5/32) and as well as adults (27/32) (Table 1 and Figure 1). Localized forms were characterised by developing of one to six small patches (Figure 3 a and b), whereas generalized forms showed severe changes with extensive skin lesions (Figure 3c and d). Juvenile onset was developed in dogs less than one year of age, these juvenile onsets were generalized forms and usually hereditary in nature [10, 14]. The current study showed increased occurrence of mites in male dogs (21/32) (Table 2 and Figure 2) which was in agreement with the findings of Mahato *et al.* [15] and Ali *et al* [16]. The probable reason could be due to the current study population was over presented with male dogs, could have been associated with greater number of owners preferring males

over females, preference and purpose of dog keeping, difference in market values, perceived notions on health issues in females etc.

Some authors has opined that, higher prevalence of mites in male dogs could be due to some hormonal influences. However, Roberts *et al.* [17] reported that elevated plasma testosterone levels were generally associated with increased parasitism. Klein [18] opined that testosterone could be the reason for increased susceptibility to infestation via suppression of the immune system.

Clinically, the lesions showed papules and pustular eruptions with erythematous patchy alopecic areas (Figure 3 e) and also showed crusted corrugated lesions or plaques (Figure 3 f) and hyperpigmentation. In severe chronic cases fistulous tracts and pus oozing lesions was observed (Figure 3 g). Exudative lesions (Figure 3 h) were seen in some dogs with moderate to severe pruritus, which were also reported by many researchers [10, 12, 19, 20].

The commonly affected areas included legs, paws, trunk, lumbo-sacral region, tail, face- periorbital region, nasal planum, forehead and commissure of lips (Figure 3 i). The commonly affected areas from the current study included legs, paws, trunk, lumbo-sacral ventrum region (Figure 3 j) with tail which was also observed by earlier workers [12, 16, 20]. Facial regions included mainly peri- orbital region, nasal planum, forehead and commissure of lips which was commonly affected as observed by Pin *et al.* [19], Chen [20] and Tamura *et al.* [21].

Whereas, Ali *et al.* [16] observed rough, dry and leathery condition, alopecia, thickening of skin, in some cases corrugation and presence of dandruff, erythema, pastules, crusts and pruritus. Pathological changes of mite infestation in dogs were described by other authors including Pin *et al.* [19] who found erythema, papules, lichenification, scales, crusts and alopecia in the feet, the face and/or the pinnae, the abdominal skin, the flank and the lumbar area. Solanki and Hasnani [12] found pruritus, encrustation, complete alopecia and wrinkling of the skin of the leg, head and trunk. Chen [20] found alopecia and scaling on the ventral aspects of the chest, all four limbs, ventral aspect of the neck and around the eyes. Tamura *et al.* [21] found scaly dermatitis with patches of pustules around the ears, eyes, lips, chin and legs and Horne [10] in his findings reported patients with demodectic pododermatitis can be especially pruritic and painful. In other findings, peripheral lymphadenopathy may be noted and systemic signs such as fever, depression and anorexia can occur if secondary bacterial infections are severe as reported by Gross *et al.* [22]. Demodicosis was confirmed in 32/90 cases in the present study, where confirmed using skin scraping in 20/90 which revealed for the presence of live adult mites (Figure 3 k to l) and the remaining cases (12/90) were confirmed through histopathology. Histopathologically, four principal patterns of inflammation were observed in canine demodicosis which included folliculitis (Figure 1m), furunculosis (Figure 3 n), mural folliculitis (Figure 3o) and para-follicular granulomas (Figure 3p). In few cases, demodicosis with panniculitis (Figure 3q) and malasseziasis were also observed. Mural folliculitis was consistently observed in all the cases. These findings were as per the observation made by Gross *et al.* [22]. Although some degree of mural folliculitis was present in almost all cases due to the presence of mites in the follicle which are usually deeply

burrowed as reported by Gross *et al.* [22] and Casewell *et al.* [23].

Microscopically, epidermis showed for orthokeratotic hyperkeratosis, acanthotic changes, apoptotic cells with chromatic condensation. Demodex being a follicular mite, the follicles along with pilosebaceous glands (Figure 3 r) were distended severe to moderate with the presence of elongated mite and mite particles, associated with follicular casts with or without mural folliculitis. Furunculosis was recorded with mite particles were extruded out of follicular ostia into dermis (Figure 3 t) and even subcutaneous layer with fibrous tissue proliferation around it along with chronic lymphocytic inflammatory infiltrates (panniculitis). Dermal haemorrhages were noticed along with severe sebaceous adenitis and hidradenitis (Figure 3 s). In the dermis extruded mite particles were surrounded by neutrophils and lymphocytes (Figure 3 u). In few cases neutrophilic micro-abscess was seen in subepidermal layers as clusters, pustular lesions have been noticed [24]. Sub corneal abscess with bacterial colonies were seen (Figure 3 v). The probable reason could be due to bacterial pyoderma often accompanies generalized demodicosis, which were in acceptance with Horne [10].

In the severe occult forms, mites could not be demonstrated in skin scrapping, showed para-follicular granulomatous forms. Histologically, in severe forms multiple giant cells (Langhan type) with lymphocytes were predominantly noticed in the current study, which was also reported by Ali *et al.* [16] and Sood *et al.* [25].

Whereas Rojko *et al.* (1978) [24] found ulceration, acanthosis, hyperkeratosis, parakeratosis, intraepidermal pustule formation and epidermal degeneration associated with neutrophilic infiltration. Mederle [8] observed hyperkeratosis with *Demodex canis* mite in the pilous follicle. Casewell *et al.* [23] found mural folliculitis in canine demodicosis. Baker [26] reported hyperpigmentation with melanocyte activity in the epidermis and pustular reaction follows follicular rupture. Ali *et al.* [16] observed acanthosis, slight hyperkeratosis, formation of hyperplastic rete-pegs and destruction of dermis and epidermis. Hyperplastic changes in sweat glands, sebaceous glands and hair follicular cells. In addition, pyogranuloma in papillary layer and in few hair follicles were observed with infiltration of neutrophils, eosinophils, lymphocytes and macrophages.

Conclusion

This higher incidence of demodicosis (35.55 %) noted in the present study could be due to the refractory nature of demodex mites to irrational and inadequate treatment which is also reported by Sridhar (2010). Dermatological diseases make an important part of daily clinical practice, require specific diagnosis for suitable therapeutic interventions. Skin punch biopsy serve as a gold standard technique for diagnosis of refractory skin diseases. Pet practices constitute a major portion of skin ailments with dermatological diseases.

Approval

The study protocol was reviewed and approved by Institutional Animal Ethical committee (IAEC), Veterinary college, Hebbal, Bangalore and the procedure was designed to avoid or minimize discomfort, distress and pain with IAEC approval No. VCH/IAEC/2022/39 dated: 10.08.2022.

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Table 1: Occurrence of Juvenile and Adult onset of Demodicosis in dogs.

Onset of demodicosis	No. of cases	Occurrence percentage
Juvenile onset	5	15.62 %
Adult onset	27	84.37 %

Table 2: Gender wise Occurrence of Demodicosis in dogs.

Occurrence of demodicosis	No. of cases	Occurrence percentage
Male	21	65.62 %
Female	11	34.37 %

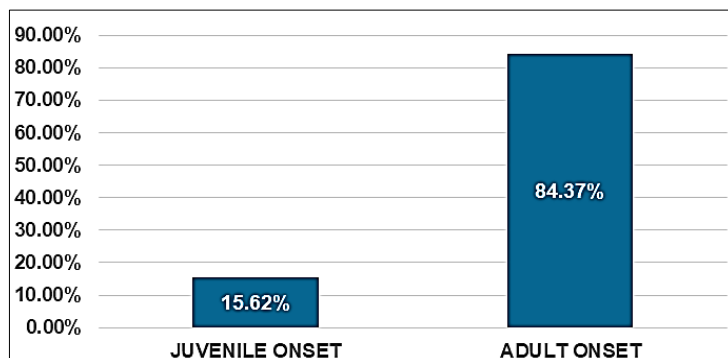
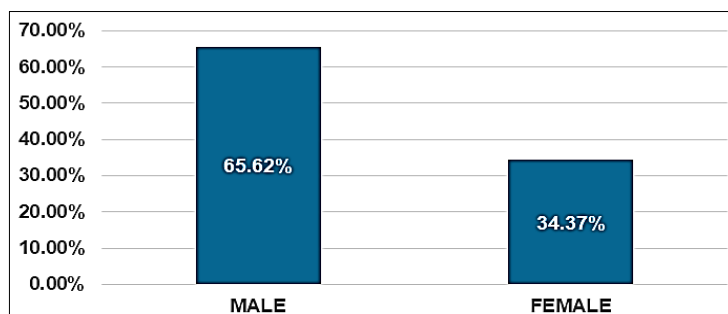
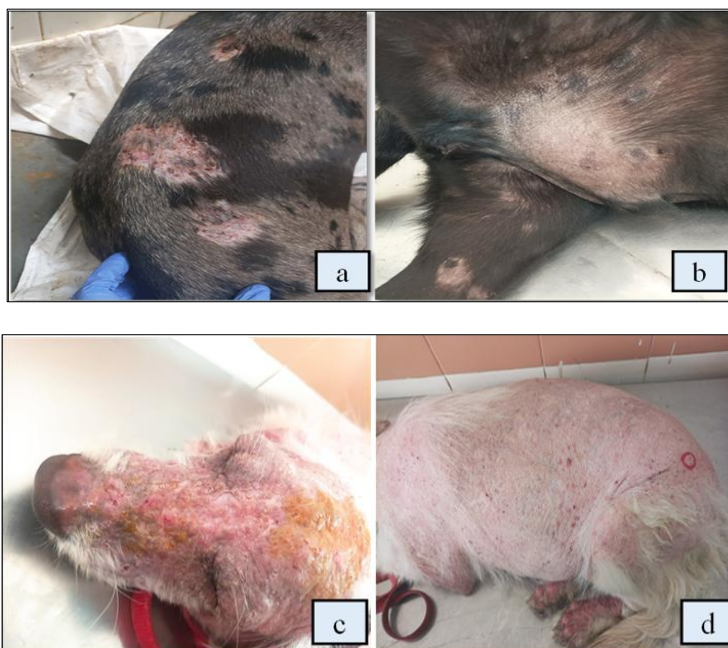
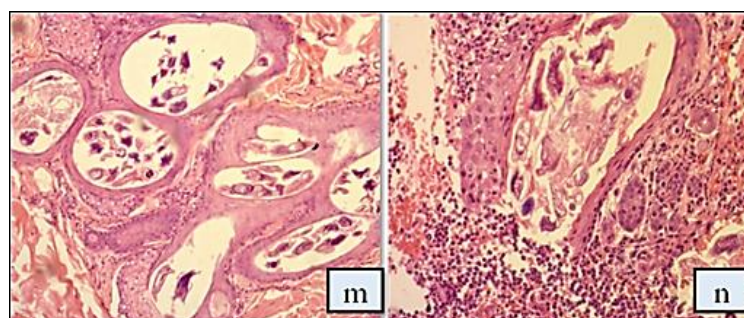
**Fig 1:** Onset of Demodicosis in Dogs**Fig 2:** Gender-wise occurrence of demodicosis



Fig 3 a: Photograph of demodex affected dog showing localized form of demodex with developing 3 patches of alopecia in Grate Dane dog. **B:** Photograph of demodex affected dog showing localized form of demodex with developing 6 patches of alopecia in German shepherd dog. **C:** Photograph of demodex affected dog showing generalized form of demodex with alopecia in Golden Retriever dog. **D:** Photograph of generalized demodicosis affected dog showing alopecia regions with hyper pigmentation **e:** Photograph of the demodex lesions showing papules and pustular eruptions with patchy alopecic areas. **F:** Photograph of the demodex lesions showing crusted corrugated lesions or plaques.



Fig 3 G: Photograph of podo demodex affected Shih tzu showing fistulous tracts and pus oozing lesions. **H:** Photograph of podo demodex affected Shih tzu showing exudative lesions were seen in some dogs with moderate to severe pruritus. **I:** Photograph of demodex affected dog showing facial lesions on periorbital region, nasal planum, forehead and commissure of lips. **J:** Photograph of demodex affected dog showing lesions on lumbo-sacral ventrum region. **K:** Photomicrograph of skin scraping cytology of demodex infected dogs showing egg, nymph, larva, adult of *Demodex canis* mites. **L:** Photomicrograph of skin scraping cytology of demodex infected dogs showing adult of *Demodex canis* mites within keratin casts



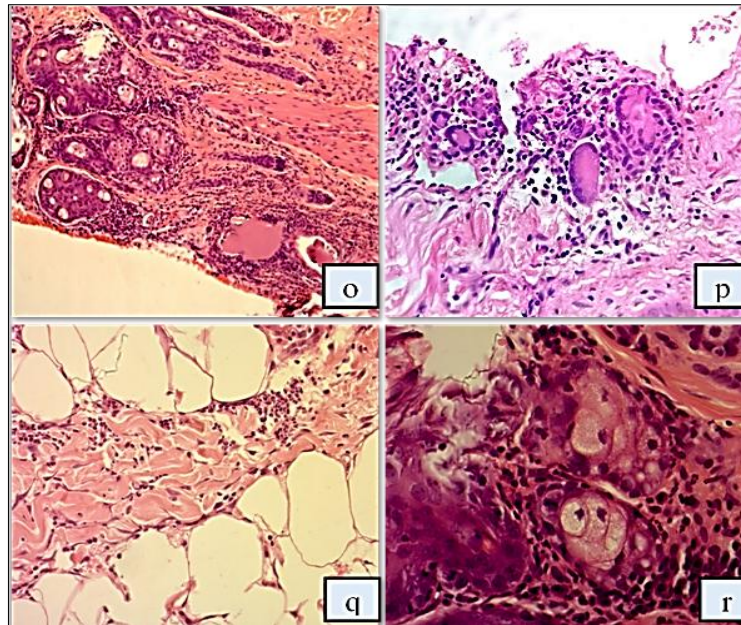


Fig 3 M: Photomicrograph of demodex skin lesions showing elongated mite and mite particle in the hair follicles with folliculitis. **N:** Photomicrograph of demodex skin lesions showing furunculosis with the inflammatory infiltrates around ruptured follicle **O:** Photomicrograph of demodex skin lesions showing chronic mural folliculitis with lympho-plasmocytic inflammatory infiltrates. **P:** Photomicrograph of demodex skin lesions showing chronic parafollicular granuloma with multi nuclear giant cell (Langhan type). **Q:** Photomicrograph of demodex showing panniculitis (chronic lymphocyte inflammatory infiltrates in the interstitium) **R:** Photomicrograph of demodex skin lesions showing sebaceous adenitis.

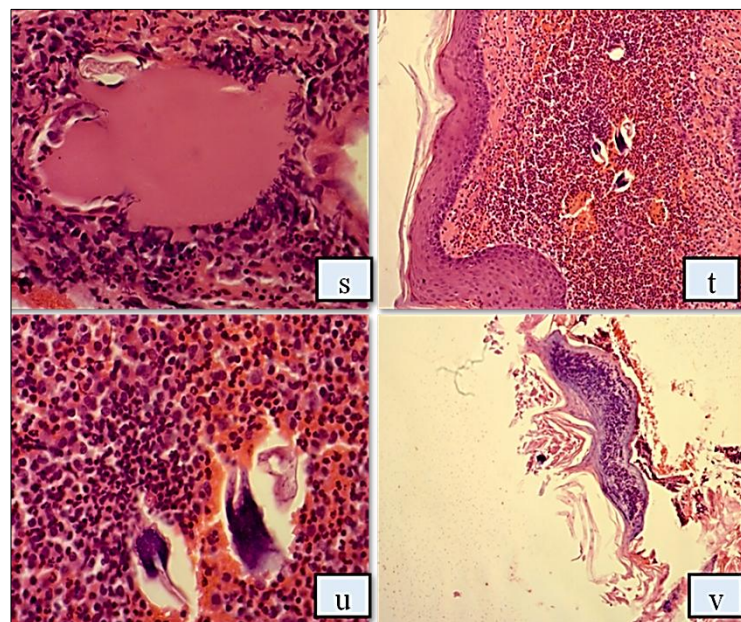


Fig 3 S: Photomicrograph of demodex skin lesions showing hidradenitis with mite particle. **T:** Photomicrograph of demodex skin lesions showing follicular mites extruded out of follicular ostia and present within the dermis with severe neutrophil and lymphocyte inflammatory infiltrates. **U:** Photomicrograph of demodex skin lesions showing dermal haemorrhages. **V:** Photomicrograph of demodex with secondary bacterial complication showing sub corneal abscess with neutrophils and bacterial colonies.

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