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Clinical and Immuno-Pathology Aspects of Canine Demodicosis

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Abstract

Canine demodicosis is a common and often severe dermatopathy of dogs. It is caused mainly by *Demodex canis*, a parasitic mite of the skin of dogs of the genus *Demodex*, of the order *Acarina* and family *Demodecidae*. This study is aimed to review the clinical-pathological presentation of canine demodicosis and the cytokine-mediated immune response to the cutaneous density of the mite. Only dogs with a defective immune response will present the disease, whether localised or generalised. Microscopically, the dermal inflammatory response is similar among dogs. Localised and generalised demodicosis and pyoderma associated with a high cutaneous density of mites are factors associated with aggravation of lesions in both forms of disease presentation. In addition, the participation of cytokines has been investigated in the induction of the immune response in the different forms of the disease. Although different research groups have invested in studies aimed at elucidating the canine demodicosis pathogenesis, there is still insufficient data to understand the important role of the host immune system in triggering clinical signs and the reproductive management is still an effective preventive method for disease perpetuation.

Keywords: *Demodex canis*, parasite-host interaction, generalised demodicosis, localised demodicosis, dog

1. Introduction

Canine demodicosis is an inflammatory disease caused by a species of the genus *Demodex* frequently diagnosed in veterinary clinical routine [1–3] and is considered the most prevalent parasitic dermatopathy [4]. The genus *Demodex* belongs to the order *Acarina*, family *Demodecidae*, and *Demodex canis* is the species of greatest occurrence in dogs [5]. This relationship is considered commensal. The mites embed themselves in hair follicles, sebaceous ducts, and sebaceous glands, where they feed on cells, sebaceous material and epidermal debris [4, 6].

The clinical presentation of demodicosis occurs according to the extent of the affected area and may manifest in localised or generalised forms. These forms also differ among themselves in terms of disease progression, prognosis and therapeutic measures adopted [7].

Peri-folliculitis, mural folliculitis and furunculosis are histopathological findings observed, with demodicosis in both clinical forms of the disease due to the action of the mite inside the hair follicles [8]. However, the severity of the lesions may vary depending on the presence and extent of secondary bacterial infection, characterised by pyoderma [9, 10].

Until now, it has not been fully understood why *D. canis*, a mite that is proven to be present in the canine skin [6], triggers demodicosis. In addition, the fact that some dogs develop the most severe form of the disease while others limit themselves to localised lesions only is still being elucidated.

Several factors such as genetic, structural and biochemical alterations of the skin, immunological disorders, hormonal status, race, age, fur length, endoparasitism, and debilitating disease have been considered as predisposing to the disease [11]. In addition, it is possible for mites to induce local immunosuppression, stimulating the onset of their proliferation [12]. Despite the multifactorial nature, studies suggest the dysfunctions of patients with clinical disease may be directly associated with the pathogenesis of demodicosis [7, 13–15].

The number of parasites in dogs seems to be lower in relation to humans [16]. This is likely because they are distributed throughout the fur and not concentrated in certain areas, as in the human face [6, 17]. Regarding the clinical manifestations of canine demodicosis, the number of mites on the skin of dogs determines the occurrence of clinical signs, but does not define the severity of the lesions [16].

A number of studies involving the immunopathogenic mechanisms of demodicosis have been performed and although there is no evidence of any abnormalities related to nonspecific or humoral immunity, functional immunodeficiency was observed in T lymphocytes [7, 18]. Furthermore, the role of proinflammatory and immunosuppressive cytokines in modulating the immune response of demodicosis has been investigated and the results demonstrate the active participation of these proteins in recruitment and activation, as well as the suppression, of host immune system cells [11, 19–26].

This study reviews the morphophylogenetic characterisation of the *Demodex canis* mite, discusses the clinical and pathological features that appear in dogs with demodicosis in order to understand the effects of the action of *D. canis* on the skin of dogs with localised and generalised demodicosis, as well as discusses the participation of the immune system, especially cytokine activity, in the development of clinical disease.

2. Methodological procedure

For understanding the main hypotheses related to the development of canine demodicosis, classical and modern data on the pathogenesis of the disease were gathered through systematic review. The articles were obtained from bibliographic databases. We were preferred to search for free terms, without the use of controlled vocabulary, to guarantee the recovery of most published works within the area of interest. Original articles related to mite Epidemiology, Morphology, Physiology and Pathogenesis; and Immunology, Clinical, Pathology and Genetics of sick dog were used to support this approach. Separate terms have been disregarded because they are not the purpose of the review. In addition, book chapters related to parasitological dermatopathies were used.

3. Morphophylogenetic characterisation of *Demodex canis*

Demodex canis [27], genus *Demodex*, order *Acarina*, family *Demodecidae*, is a mite described as inhabiting commensal in hair follicles, sebaceous ducts and

sebaceous glands of dogs, found in small amounts in healthy animals [28, 29]. According to Scott et al. [7], the transmission of this mite occurs by direct contact of the mother with the neonates during the first 3 days of breast-feeding.

In its life cycle, the mite *D. canis* presents as an egg, larvae, protonymph, nymph, and adult (male and female), where all stages of the life cycle can be found in microscopic analysis of skin scalings [7, 28, 30]. The eggs in fusiform (length $81.5 \pm 3.5 \mu\text{m}$) hatch into small larvae (length $91 \pm 5.9 \mu\text{m}$) with three pairs of paws, next protonymphs (length of $130.7 \pm 10.7 \mu\text{m}$), then nymphs (length of $201.2 \pm 21.9 \mu\text{m}$) [30] and finally evolve into adult mites with four pairs of legs, which commonly measure from 40 to 300 μm [7].

In general, *Demodex* mites are described as small, with elongated bodies, having four pairs of legs. The body is separated into three distinct tagma: the gnathosoma, the small anterior segment with a trapezoidal or rectangular shape, containing mouth parts; the podosoma, which contains reduced and slightly projected legs beyond the podosoma line; and the opisthosoma, the posterior segment, elongated and formed by cuticular striae [31] (**Figure 1**). The morpho-biological characteristics of the adult mite *D. canis* are similar in several studies.

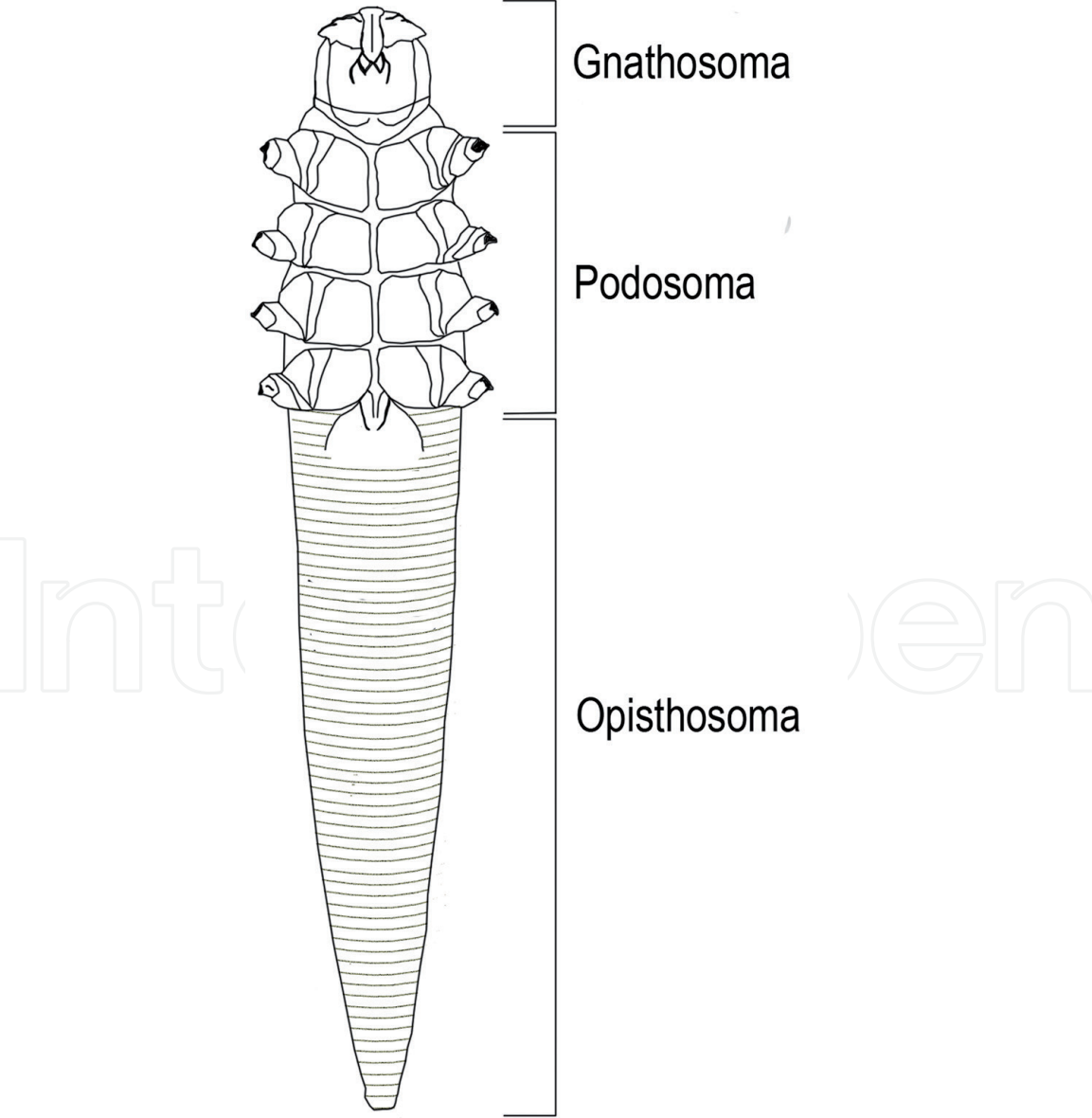


Figure 1.
Morphology of Demodex canis.

Tagmas	Nutting e Desch [30]	Tamuri et al. [32]	Izdebska e Fryderyk [31]	Rojas et al. [33]	Sivajothi et al. [34]
Gnathosoma (µm)	24	18.9	26.9	26.27	18.89
Podosoma (µm)	63.9	94.11	63.8	64.69	60.98
Opisthosoma (µm)	135.9	136.6	116.5	134.31	129.68
Total body lenght (µm)	224.3	229.7	195.2	224.80	214.32

Table 1.
Biometric means Demodex canis found in the literature.

Table 1 describes the biometric measurements of *D. canis* mite segments as described in the literature [30–34].

Although the *D. canis* mite is the most common species [7, 31, 35], two new species, *D. injai* [36] and *D. sp. cornei* [37–40], have also been documented causing dermatological alterations in dogs.

Rojas et al. [33], comparing the three species described in dogs, revealed inter-related but distinct populations in which *D. canis* presented with elongated opisthosoma (ratio opisthosoma length/total length 0.59), and an absence of a band-like segmental plate between the fourth coxisternal plate and opisthosoma. *D. injai* presented opisthosoma comprising 70% of the total length (ratio 0.70) and *D. sp. cornei* presented with a segmental plate, nearly rectangular (ratio 0.47), between the fourth coxisternal plate and opisthosoma.

In addition to the morphobiometric characteristics, Rojas et al. [33], using molecular markers of mitochondrial DNA, 16S rDNA, and cytochrome oxidase I genes, suggested that these three species could be polymorphisms of the same species. However, Sastre et al. [41] in the sequencing analysis of 16S rDNA demonstrated that *D. canis* and *D. injai* present a genetic distance of 23.3%, therefore are different species, while *D. sp. cornei* is likely a variant of *D. canis*.

Although *D. canis* is a common commensal mite, Fondati et al. [29] in a microscopic analysis of the presence of *D. canis* in healthy dogs, emphasised that the presence of *D. canis* in the skin should not be considered as normal. However, Ravera et al. [6] using real time PCR demonstrated that mite DNA was present in all examined dogs, regardless of age, sex, breed, coat or clinical status, albeit in smaller numbers in healthy dogs. Regardless, the positivity increased when a greater number of areas were analysed. A similar result was observed by Gasparetto et al. [16], detecting a higher number of mites in dogs with clinical demodicosis (6.2×10^4 copies/µl of the parasite in the generalised form and 1.2×10^4 copies/µl in the localised form) compared to healthy dogs, (8.7×10^2 copies/µl of the parasite) using the same technique.

4. Pathological clinical aspects of canine demodicosis

Clinical changes in demodicosis may be induced by the excessive proliferation of mites associated with weakness in the immune system, or induced by the mites themselves [14, 17, 42]. Variables such as breed, age, nutrition, oestrus, pregnancy, stress, endoparasitism and debilitating diseases are predisposing factors for the disease. Purebred dogs appear to be more predisposed. Based on the autosomal recessive inheritance hypothesis, this would lead to immune dysfunction [15, 43].

Bowden et al. [44] found that dogs of the American pit bull and West Highland White Terrier breeds and those with allergic diseases were more predisposed to demodicosis. Likewise, Gasparetto et al. [8] verified that dogs with a defined breed were the most affected.

Regarding classification, demodicosis can be divided according to age of onset of clinical signs (juvenile or adult), or the extent of lesions (localised or generalised), though there is no consensus on the criteria [15]. Kumari et al. [26] suggest classifying as generalised demodicosis when there are lesions on more than 50% of the body surface with the involvement of two or more limbs, and classifying as localised demodicosis when there are alopecia, erythematous and desquamative lesions with hyperpigmentation on the face and one thoracic limb. Other authors have suggested that cases in which there are four or fewer lesions (with a diameter less than 2.5 cm), including a maximum of one focal lesion on any limb, be classified as localised demodicosis and cases with extensive multiple limb lesions, be classified as generalised demodicosis [44–46].

In a retrospective study investigating demodicosis in an US region, dogs with juvenile onset of lesions had a mean age of 7.6 months, having a predominance of the generalised form (74.2%). Dogs with adult onset (over 48 months) of demodicosis were also more likely classified as generalised, with 87.1% of the cases [44]. In Brazil, a study involving 46 dogs, 24 males and 22 females showed generalised demodicosis (60.9%) was more common than localised (39.1%) with a mean age of onset of 23 months [8].

Dogs that develop lesions such as alopecia or erythema as juveniles, are not usually pruritic, have spontaneous remission of clinical signs, and progression to the generalised form is rare. Only in cases of external earwax associated with localised demodicosis, a rare form of the disease, will dogs require therapy [15].

Unlike the localised disease, the generalised form of demodicosis can reach serious proportions and clinical signs such as alopecia, desquamation and erythema (**Figure 2**) are particularly intense [8]. Secondary bacterial infection is often due to the proliferation of opportunistic microorganisms, mainly *Staphylococcus pseudintermedius* and *Pseudomonas* [47, 48], which progress from superficial folliculitis to severe cases of furunculosis and cellulitis [7, 10, 49]. Gasparetto et al. [8] observed pyoderma in 95.5% of dogs with generalised demodicosis and half presented with pruritus, indicating bacterial pyoderma and an immunological reaction against *Demodex* [9, 50]. In more severe cases, lymphadenopathy, fever, anorexia and lethargy associated with secondary bacterial infection may occur [51, 52]. Pododemodicosis, which affects the interdigital, palmar and/or plantar regions, has a poor prognosis. It manifests with severe erythema, oedema and fistulous tracts that cause intense localised pain, requiring prolonged periods of treatment [10, 15, 49].

In histopathological examination, mites are frequently observed in hair follicles that induce folliculitis, peri-folliculitis and furunculosis, as well as sebaceous gland hyperplasia [53]. According to Gasparetto et al. [8], hyperkeratosis was the most frequent epidermal alteration with either form of demodicosis. Mild to moderate interstitial and perivascular exudate containing lymphocytes, plasma cells and macrophages. Dogs with generalised demodicosis and pyoderma had lymphocytes, macrophages and plasma cells associated with the neutrophilic exudate. In chronic cases of generalised demodicosis, follicular hyperkeratosis predominates, and mononuclear inflammation of sudoriferous glands and sebaceous glands is present [9, 10].

Peri-folliculitis occurs in the early stage of the inflammatory process evidenced by the presence of macrophages and lymphocytes around the hair [7]. This finding is apparent both in dogs with the localised disease and in those with more severe

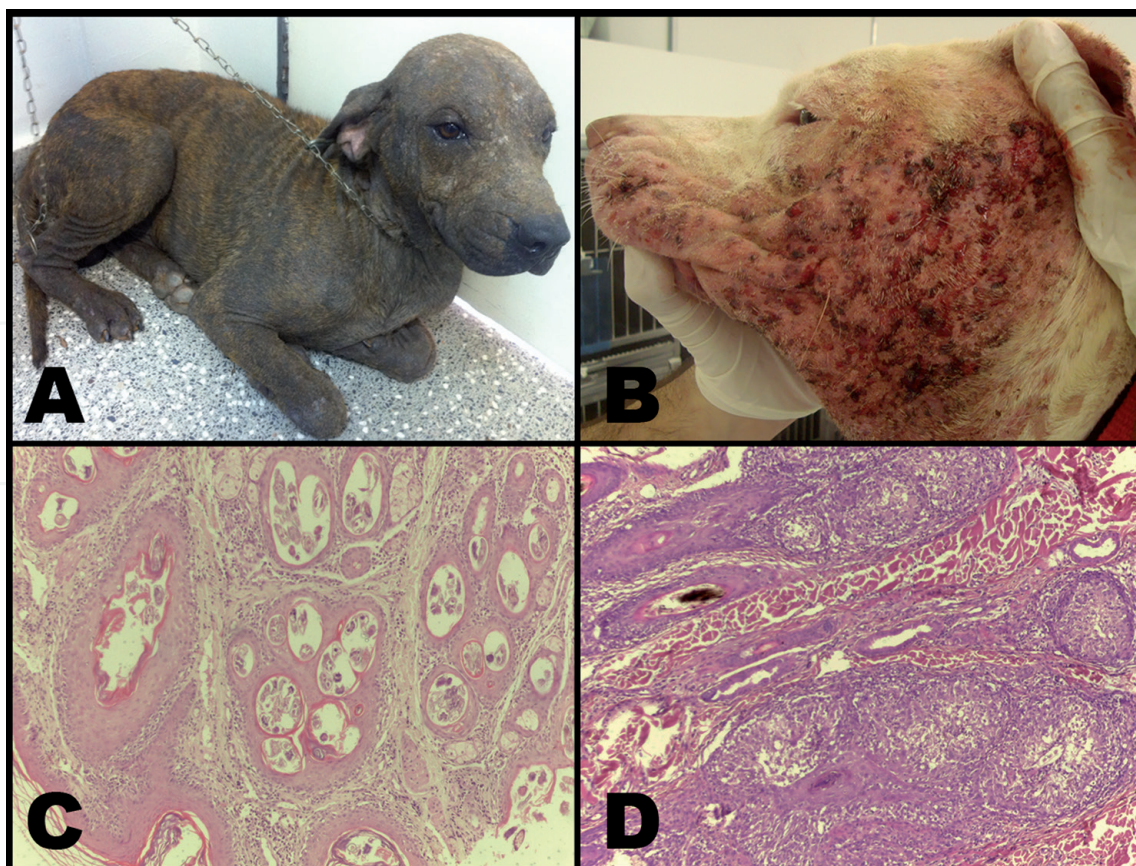


Figure 2.

(A) Generalised demodicosis in dog with cutaneous hyperpigmentation, alopecia and desquamation. (B) Pyoderma and generalised demodicosis in facial region of dog. (C) *Demodex* in the interior of the hair follicles and folliculitis. H&E, 10 \times . (D) Furunculosis. H&E, 10 \times .

clinical lesions [8]. As the disease progresses, mural folliculitis occurs due to the infiltration of lymphocytes and histiocytes into the follicular wall, causing injury to follicular keratinocytes. Hydropic degeneration, follicular keratinocyte apoptosis and follicular exocytosis occurs [9, 50]. Mural folliculitis, which has been reported most frequently in dogs with the localised disease [8], is observed to be a consistent and an important lesion pattern of active demodicosis. The histological lesion generated is often associated with diseases in which immune response is recognised as important in its pathogenesis [10, 50, 54, 55].

Finally, multiplication of *Demodex* in the interior of the hair follicles induces follicle dilation causing rupture and releasing mites into the dermal interstitium [10]. The observation of mural folliculitis and multifocal pyogranulomatous furunculosis more frequently in dogs with localised demodicosis indicates that the histological stages of follicular inflammation may have similar severity in the different clinical forms of the disease [8].

5. Host-Parasite Interaction, *Demodex canis* versus dog

Because they are natural inhabitants of the skin of mammals, mites of the genus *Demodex* usually do not generate adverse reactions to the host due to the capacity of the animal's immune system [6, 11, 17, 26, 56]. This is due to the recognition of mite chitin by host keratinocytes through their toll-like receptors (TLR), specifically TLR2, triggering an innate immune response. In addition, studies report that the immune systems of healthy dogs are especially effective at detecting the lipases and proteases secreted by *Demodex* mite, possibly stimulating the adaptive immune response, which is more specific and effective for the control of the *Demodex* mite [17, 57].

The reason for the progressive evolution of the disease in some dogs has not been completely elucidated. The most accepted hypothesis is that immune system dysfunctions play an important role in the manifestation of clinical signs of the disease in its different forms [7, 11, 13–15]. The proposition that the host immune system is the main mediator in the overpopulation of *Demodex* is sustained by the occurrence of the disease in patients who have undergone prolonged treatments with immunosuppressive drugs, in addition to clinical signs in immunodeficient mice, as well as in people and animals with chronic degenerative diseases [17, 56, 57]. However, studies in dogs indicate that immunosuppression occurs at various times in the course of the disease and may be induced by the action of the mite itself on the hair follicles and/or sebaceous glands and not as a primary trigger for parasitic proliferation [14, 17, 32, 42, 57]. This explains why not all immunosuppressed dogs develop clinical demodicosis and indicates that the manifestation of the disease may involve more than one factor.

Unlike humans, there is little evidence of humoral immune response being involved in canine demodicosis and although Ravera et al. [58] have shown the existence of immunoglobulin (Ig) G against *D. canis* with generalised juvenile demodicosis, the real meaning of this response remains unclear. On the other hand, dogs with generalised demodicosis tend to present functional immunodeficiency in T lymphocytes [7, 18]. Many of the studies indicate that the main mechanism of *Demodex* population control is cell mediated. When mite proliferation occurs, it is probable that there is impaired cellular immunity [7, 57].

This immune dysfunction is defined by the exhaustion of T cells. This type of depletion is not uniform and is generally characterised by high levels of suppressor cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)- β , low production of stimulatory interleukins, such as IL-2 and IL-21 and a reduction in circulating CD4+ [17].

Higher serum levels of IL-10 were observed in dogs with relapsing demodicosis, compared to healthy dogs and those with first manifestation. This change culminates in T cell suppression and antigen presentation ability by inhibiting the synthesis of cytokines and helper 1 T cells (Th1) [22].

Lemarié et al. [59] observed a reduction in the expression and in vitro production of IL-2 resulting from a decrease in Th1 cell response and pointed to a functional irregularity of this class of lymphocytes, directly affecting the balance between Th1 and Th2 responses during the course of the disease. The establishment and perpetuation of demodicosis was attributed to suppression of the Th1 response to Th2, resulting in an inflammatory process capable of inducing tissue damage but not eliminating or containing the proliferation of the mite.

The decrease in transcription of cytokines TNF- α and IFN- γ , and the unprecedented increase in IL-5, as evidenced by Tani et al. [20], appears to be due to Th2 lymphocyte overexpression in the presence of *Demodex* [59]. In addition, Yarim et al. [23] and Tani et al. [20] demonstrated an increase in circulating TGF- β concentrations in dogs with generalised disease compared to healthy animals. Elevated TGF- β levels may compromise the regulation of various biological processes, such as tissue homeostasis, angiogenesis, and cell differentiation, especially in cases of chronic disease, allowing the evolution of localised to generalised demodicosis [56].

Considering that most of these previously described changes were observed in dogs with generalised demodicosis, a recent study investigated the serum levels of a selection of proinflammatory cytokines in dogs with localised and generalised demodicosis in order to observe the levels of certain proteins. There was no difference in serum cytokine levels between groups of diseased animals, but IL-6 was significantly higher in dogs with localised disease than in healthy animals. Thus,

characterising the nonspecific inflammatory reaction that occurs shortly after tissue injury precedes the acquired immune response in the acute phase of the disease [16].

Moreover, a modern approach supports the involvement of the cholinergic pathway in the immunopathogenesis of canine demodicosis. In addition to acting as a neurotransmitter, acetylcholine (ACh) plays an important role as a mediator in the inflammatory process by inhibiting the release of certain proinflammatory cytokines, without affecting the production of inhibitory cytokines such as IL-10. The increased activity of its indirect biomarker, acetylcholinesterase, in the serum of dogs with demodicosis, has established the overproduction of ACh in diseased dogs, resulting in immunosuppression [26, 56].

Finally, it is known that TLR receptors play an important role in the identification and control of *Demodex* proliferation in the skin of healthy dogs [17]. However, in a recent study involving animals with demodicosis, important changes in the function of these receptors were detected. Kumari et al. [60] showed elevated expression of mononuclear type 2 TLRs (lymphocytes and monocytes), as well as a decrease in the expression of TLR types 4 and 6. These effects were directly attributed to the action of the mites, but it is not yet known how the mite stimulates or decreases the production of TLR receptors in the disease process [12, 60].

6. Conclusion

Although the *D. canis* mite is considered a commensal inhabitant of dog's skin, demodicosis is one of the most frequent parasitic diseases in this species. Clinical signs such as alopecia, desquamation, erythema and crusting are common in dogs with localised and generalised demodicosis and may be aggravated by secondary bacterial infection. Pyoderma produces severe dermal microscopic inflammation; however, the histopathological findings of dogs with localised and generalised disease tend to be similar. In addition, the increase in the parasitic load of mites in the canine tegument induces the clinical disease, but does not define the severity of the lesions, indicating that the predisposing factor for the mite proliferation likely relates to the immunocompetence of the host.

Low production of stimulating cytokines and high levels of suppressor cytokines coupled with reduced numbers of CD4⁺ lymphocytes are invariably observed in dogs that develop clinical signs of demodicosis, indicating T-cell depletion. However, due to the multifactorial nature of the disease, immunological mechanisms that allow the excessive growth of the parasites in the dog skin is still misunderstood and this limitation in the understanding of the host-mite interaction makes that the impediment of diseased animals reproduction prevail as the main strategy of control until now.

Currently, research groups from different countries have suggested several mechanisms to understand the immunopathogenesis of demodicosis and although the various hypotheses raised are not yet enough to establish the determining cause of clinical disease development, observed together they allow for new hypotheses that may serve as starting points for subsequent studies in the area.

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