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The use of cytology as auxiliary diagnosis of canine demodectic mange - report of two cases.

O uso da citologia como diagnóstico auxiliar de sarna demodécica canina: relato de dois casos.

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Abstract

Demodicosis is a parasitic disease caused by the imbalance in the number of mites of the genus *Demodex sp.* The skin scraping is the gold standard method for diagnosis, but other methods can be used. Based on this, the aim of this work is to report cases in which cytology served as an auxiliary diagnosis for demodectic mange. Two dogs went to a private clinic with skin lesion of different aspects and in which both dogs the cytology was performed by skin imprint, being possible to observe the presence of *Demodex canis*. Thus, cytology could be used as an auxiliary diagnostic method this disease.

Keywords: Mite. *Demodex canis*. Auxiliary diagnosis.

Resumo

A demodicose é uma doença parasitária causada pelo desequilíbrio no número de ácaros do gênero *Demodex sp.* A raspagem de pele é o método padrão ouro para o diagnóstico, mas outros métodos podem ser utilizados. Com base nisso, o objetivo deste trabalho é relatar casos em que a citologia serviu como diagnóstico auxiliar da sarna demodécica. Dois cães foram a uma clínica privada com lesões cutâneas de diferentes aspectos e em ambos os cães a citologia foi realizada por imprint cutâneo, sendo possível observar a presença de *Demodex canis*. Assim, a citologia poderia ser utilizada como método auxiliar no diagnóstico dessa enfermidade.

Palavras-chaves: Ácaro. *Demodex canis*. Diagnóstico auxiliar.

Introduction

Demodicosis or demodectic mange is a parasitic disease caused by the mite of the genus *Demodex sp.*, occurring in dogs and cats (MILLWARD, 2018). This disease is characterized by the presence of the parasite in hair follicles and adnexa, generating alopecia, erythema, desquamation, folliculitis and furunculosis (GROSS et al., 2009; GASPARETTO et al., 2018). The clinical disease begins when there is an imbalance in the number of mites present in skin, so that they exceed the average tolerated by the immune system, culminating in varied clinical signs (RHODES; WERNER, 2014). The disease can be classified in localized or generalized demodicosis based on the locations and number of lesions (HORNE, 2010). Thus, animals generally have circular areas of alopecia, a diffuse area of fine hair, itching is often absent or occurs when there is a concomitant allergy or secondary infection. Furthermore, they can develop hyperpigmentation and lichenification, papules and pustules and the severity of symptoms may vary according to the extent of the disease and the presence and type of concomitant disease (HEINRICH et al., 2019).

Deep skin scrapes are the gold standard for diagnosing demodicosis, but trichograms, adhesive tapes, cytological and histopathological exams can assist in the identification of the mite, being useful according to the aspect and lesional location (MUELLER et al., 2020). Based on the above, the present study aims to report cases in which cytology served as an alternative diagnosis for canine demodectic mange.

Material and methods

In case 1, a two-year-old female poodle dog went to a clinic in Fortaleza, with an erythematous and pruritic lesion in the region of the left pelvic limb, with an evolution of 25 days. The deworming was up to date and so was the vaccination. The animal did not have leg licking and it used the Leevre[®] antiparasitic collar. At the general clinical examination, the following observations were made: active patient, hydrated, temperature of 38.7°C and non-palpable lymph nodes. Dermatological examination revealed a circular erythematous lesion in the region of the left pelvic limb with a granulomatous aspect. The dog did not use any Isoxazoline drug within the last year. Complementary exams were performed with Wood lamp, complete blood count, cytology by skin imprint in which the sample was stained using a pan-optic and the parasitological skin exam using the acetate tape impression.

In case 2, a 5-month-old mongrel female dog went to a clinic in Fortaleza, with dermatological lesions distributed throughout the body. The animal had previously been seen by another professional, presenting a lesion in the left periocular region, which was treated with anti-inflammatory and ophthalmic antibiotic ointment, but without an adequate therapeutic response. At the time of the current assessment, the presence of diffuse papules and pustules was observed throughout the body, including inside the ear. Clinical examination also revealed normal temperature (38.2°C), normal hydration and active patient. The deworming was up to date and no ectoparasites were seen. Vaccination protocol was incomplete and the animal did not use any Isoxazoline drug since birth. Complementary exams were performed using cytology by skin imprint and parasitological exam by acetate tape impression.

Results and discussion

In case 1, the hematological parameters were within the reference values and cytology by skin imprint revealed the presence of scaly and inflammatory cells (neutrophils, macrophages and eosinophils), moderate amount of coccoid bacteria and the adult evolutionary forms of *Demodex canis* were seen, which were also visualized in the cutaneous parasitological examination by the acetate tape impression, confirming the diagnosis of canine demodicosis (Fig. 01).

The treatment was based on the use of oral sarolaner in a single dose every 30 days and washing the lesion site with benzoyl peroxide every 4 days until the lesion clinical improvement.



Fig. 1 - Clinical and laboratorial aspects of canine with demodectic mange. **A.** Lesion in the pelvic limb region with an exudative, alopecic and erythematous aspect. **B.** Parasitological cutaneous examination with acetate tape with the presence of *D. canis* (arrow) at 400x amplification. **C.** Cytology performed by skin imprint, stained with pan-optic, with the presence of *D. canis* in 100x amplification.

In case 2, in cytological analysis by skin imprint (Fig. 02) was possible to observe the presence of scaling and inflammatory cells, similar to the first case, in addition to the *Demodex canis* mite in adult forms. The treatment had the same therapeutic choice as in case 1, showing similar clinical improvement. It should also be noted that the dogs were followed up for 3 consecutive months with monthly skin scrapings for parasitological assessment.

According to Patel and Forsythe (2011), the treatment can be followed up by serial skin scrapes to compare with previous exams. This monitoring is important because clinical cure precedes the elimination of parasites.

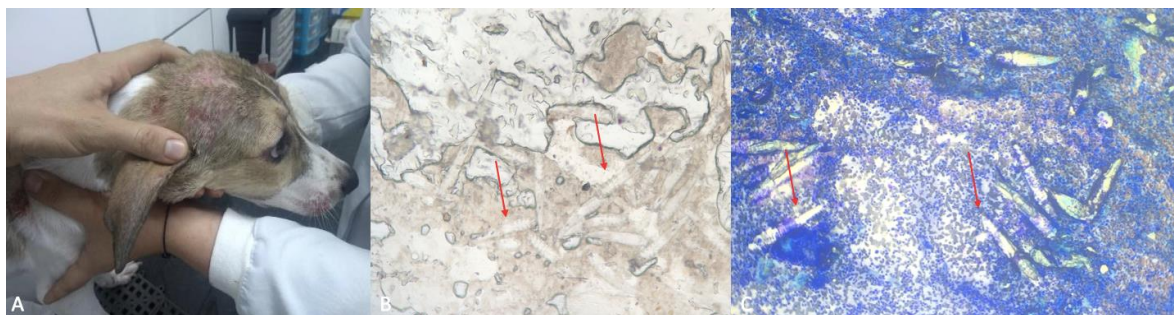


Fig. 2 - Clinical and laboratorial aspects of canine with demodectic mange. **A.** Generalized lesions with erythematous and alopecic aspect in canine with demodicosis. **B.** Parasitological examination with the presence of *D. canis* (arrow) by acetate tape impression at 100x amplification. **C.** Cytology performed by scarification and skin imprint revealing the presence of *D. canis* (arrow). Pan-optic stain in 100x amplification.

Demodectic mange or demodicosis is an inflammatory disease caused by the exacerbated increase in the number of mites of the genus *Demodex*, who lives on the skin of dogs (SIX et al., 2016). These mites live in the ducts of the sebaceous glands and in hair follicles present in normal skin. However, qualitative immunological changes and predisposing hereditary factors can cause cutaneous proliferation of the mite, culminating in the appearance of clinical signs associated with the disease (MILLWARD, 2018). Dogs with demodicosis contain a specific immunodeficiency of T cells, contributing to a proliferation of the mite, causing an immunosuppression, related to the development of the disease (PATEL; FORSYTHE, 2011). In addition, immunosuppression can be related to numerous factors such as malnutrition or even injuries by another endo or ectoparasites (NAGELSTEIN, 2010).

Demodicosis can be classified as localized or generalized, ranging from moderate to severe. The first phenotype is generally defined when there are four or less cutaneous lesions, with approximately 2.5 cm in diameter or smaller, containing multifocal, asymmetrical and well-circumscribed lesions with scaling, hyperpigmentation, alopecia and/or erythema. The affected sites are usually the face, head, neck, forelimbs and trunk (HEINRICH et al., 2019).

Generalized demodicosis is defined when there are more than four lesions that affect the entire region of the body or limbs (HEINRICH et al., 2019). Among the clinical signs, multifocal to generalized alopecia with desquamation, hyperpigmentation, follicular cylinders and crusts are cited. Secondary bacterial infections can occur and lesions can include papules, pustules, pyoderma, erosion, scabs, itching and pain (GASPARETTO et al., 2018). Thus, the clinical signs observed in the patients in question corroborated with the literature, with the first case being defined as localized demodicosis and the second generalized. In addition, the itching found in animal 1 is characteristic of the secondary infection.

The diagnosis is made from dermatological signs and microscopic examination to search for the mite, such as deep skin scraping, cytology and trichogram (GUERETZ, 2005; RHODES; WERNER, 2014). The use of cytology is important for the evaluation of the dog with demodicosis (MUELLER et al., 2012), and can also be used to identify other infectious agents, such as dermatophytic fungi, bacteria, inflammations and infections by *Malassezia* spp. (ROSENFELD; SHARON, 2010; DUNCAN, 2011), in addition to indicate the inflammatory pattern involved in the lesion (ALBANESE, 2017). Although these parasites live on the deep layers of the integument, they reproduce on the surface of the skin, and the female enters the follicular lumen to perform oviposition (ALBANESE, 2017). Thus, it is possible to search for positive samples by scarifying and imprinting the lesion area. However, according to Heinrich et al. (2019) to find *Demodex* spp. through cytology it is necessary to press the skin hardly and then apply the adhesive tape or the microscope slide.

When conducting research on mites, attention should be paid to the number of parasites present in the sample, as they live on the animal's skin normally. Known for this, the presence of more than one mite in the collected material is indicative of demodicosis (MUELLER et al., 2020). The correct diagnosis for demodicosis contributes to focus on more appropriate therapies that allow to eradicate infestation to the type of mites present in the skin, because knowing the species, it is possible to define a better therapeutic and prognostic protocol. Demodicosis can mimic other pruritic and non-pruritic skin diseases, in addition to being associated with other diseases. Thus, other conditions should be considered as possible aggravating or suppressing the skin response to

demodicosis, such as atopic dermatitis, seborrhea, endocrine diseases and neoplasms (HEINRICH et al., 2019).

The use of isoxazoline drugs such as sarolaner, which was used in the report, has shown a therapeutic efficacy in the treatment of demodectic mange (BEUGNET et al., 2016; SIX et al., 2016). Benzoyl peroxide has numerous qualities for supporting treatment of demodicosis, due to its ability to clean the hair follicle through its penetration, helping to control infections by its broad-spectrum antibacterial action, and by the dissolution of the corneal layer of the skin by its keratolytic action (SPINOSA et al., 2017). Due to such characteristics of the aforementioned products, they were chosen for the treatment of animals affected by the disease, resulting in improvement and clinical and parasitological control in subsequent skin evaluations in the period of three months.

Conclusion

From the above, it can be concluded that canine demodicosis is a dermatopathy present in the clinical routine of dogs, requiring from the Veterinarian the knowledge of the different diagnostic techniques that exist to identify the parasite. The possibility of using the cytological exam in such disease is also emphasized, especially in the suspicion of secondary infections and in places with difficult access to perform conventional exams, such as skin scraping.

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