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CASE REPORT
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Canine Ringworm Caused by *Trichophyton mentagrophytes* - Detection by SYBR-Green real-time PCR

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ABSTRACT

***Background*:** Dermatophytes, fungi of universal distribution, invade semi or fully keratinized structures, such as skin, fur/ hair and nails. The various species of dermatophytes are classified into three genera anamorphic: *Microsporum*, *Trichophyton* and *Epidermophyton*. The genus *Epidermophyton* includes only *E. floccosum*, that rarely affects animals. The main species responsible for the disease in dogs and cats are *Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophytes*, which were characterized through conventional mycological methodology (microscopic examination with KOH and culture). Molecular methodologies, such as real-time PCR, can contribute to a rapid laboratory diagnosis, helping clinicians to initiate an early antifungal treatment. This case report describes a case of canine dermatophytosis due to *Trichophyton mentagrophytes* detected from a clinical sample by SYBR-Green real-time PCR.

***Case*:** A 8-year-old dog, rescued from the street, was referred to a private veterinary clinic in the city of Canoas, RS, Brazil, presenting generalized lymphadenomegaly, crusted lesions all over the body, generalized alopecia, signs of excoriation and epistaxis. Initially, were administered prednisone [1 mg/kg every 48 h, BID] and cephalexin [30 mg/kg, BID]. Weekly baths with benzoyl peroxide were also given. The therapy was not clinically successful. Wood's Lamp Test was negative. As a differential diagnosis, PCR for detection of *Leishmania* was negative. Complete blood count and serum biochemical assay were also performed. For mycological diagnosis, hair specimen was clarified and examined microscopically using 10% potassium hydroxide (KOH) for the visualization of chains of arthroconidia (ectothrix invasion of hair). The infected hair was plated onto Mycosel™ Agar, incubated at 28°C for 15 days. Microscopy of hyphae/ conidia and macroscopic colony characteristics (colors and texture) were conducted for the differentiation of the species within the genus *Microsporum* and *Trichophyton*. In addition, real-time PCR was applied for direct analysis of the fungal DNA obtained from the hair sample. Microscopic examination was negative. The dermatophyte present in the hair sample was confirmed as *Trichophyton mentagrophytes* by culture and qPCR (melting-point analysis). The patient was treated with systemic itraconazole [10 mg/ kg SID - 90 days]. Twice-weekly application of 2.5 % miconazole and 2% chlorhexidine shampoo until complete cure.

***Discussion*:** Dermatophytosis is often listed as self-limiting infection; however, animal dermatophytosis can spread between pets, as well as a zoonotic transmission to humans. The literature on dermatophytosis indicates that *Microsporum canis* is the predominant etiological agent, followed by *M. gypseum*. *Trichophyon mentagrophytes* that appear in a lower percentage of isolation. The culture of hair, even with specific medium containing chloramphenicol and cyclohexamide, may present contaminating fungi, not related to dermatophytosis, which can inhibit or override the growth of dermatophytes. The use of real-time PCR provided a faster and specific diagnosis of dermatophytosis when compared to the conventional mycological methodology for detection and identification of *T. mentagrophytes*, which takes around 10 to 15 days for culture. It is possible to use this technique as an alternative diagnosis for dermatophytes associated to clinical hair samples of dogs.

Keywords: dermatophytosis, dog, pets, qPCR, hair samples, diagnosis, molecular methodology.

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INTRODUCTION

Dermatophytes, fungi of universal distribution, invade semi or fully keratinized structures, such as skin, fur/hair and nails. The various species of dermatophytes are classified into three genera anamorphic: *Microsporum*, *Trichophyton* and *Epidermophyton*. The genus *Epidermophyton* includes only the specie *E. floccosum*, which rarely affects animals. Three species of fungi (*Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophytes*) are responsible for more than 95% of all ringworm cases in pets [5,7].

The conventional diagnosis of dermatophytosis is based on clinical signs, direct examination (microscopic examination with KOH) and culture of hair specimens. The gold standard test for diagnosis of dermatophytosis is still the mycological culture [3], but its time consuming for accurate species identification is considered how a major handicap of the method. Molecular methodologies, such as real-time PCR [2,4], widely used in human and veterinary medicine, can contribute to rapid laboratory diagnosis, helping clinicians to initiate antifungal treatment [11,13]. This work report a case of canine dermatophytosis due to *Trichophyton mentagrophytes* detected from the clinical sample by SYBR-Green real-time PCR.

CASE

A 8-year-old dog, rescued from the street, was referred to a private veterinary clinic in the city of Canoas, RS, Brazil, presenting generalized lymphadenomegaly, crusted lesions all over the body, generalized alopecia (Figure 1A), signs of excoriation and epistaxis.

Initially, prednisone[1] [1 mg/kg every 48 h, BID] and cephalexin[2] [30 mg/kg, BID] were administered. Weekly baths with benzoyl peroxide manipulated shampoo were also given. The therapy was not clinically successful. A biopsy was performed 10 days after the 1st visit. Contacts without injury - there was no contagion to veterinarians who handled the dog. Wood's Lamp Test was negative. As a differential diagnosis, PCR for detection of *Leishmania* was negative. Complete blood count and serum biochemical assay were also performed. The hematological and biochemical changes observed were due to the corticosteroid treatment. After the end of the medication, the test results returned to normal.

For mycological diagnosis, hair specimen was clarified and examined microscopically using 10% potassium hydroxide (KOH) for the visualization of chains of arthroconidia (ectothrix invasion of hair). The culture was performed onto Mycosel™ Agar[3], incubated at 28°C for 15 days. Microscopic examination of conidia features and macroscopic colony characteristics (colors and texture) were conducted for the differentiation of the dermatophyte species.

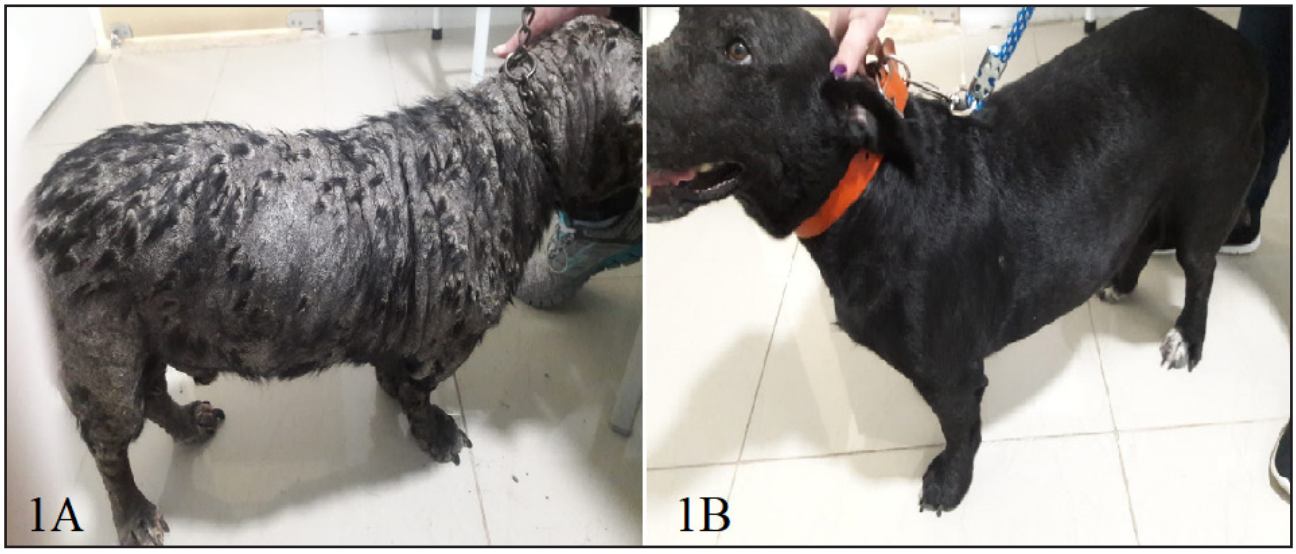

Figure 1. A- Clinical presentation of dermatophytosis in a dog caused by *Trichophyton mentagrophytes*. B- Dog After complete cure with systemic itraconazole.

In addition, DNA extraction and real-time PCR were applied according Spanamberg *et al*. [14]. Real-time PCR was performed using the pan-dermatophyte primers for detecting a DNA fragment encoding chitin synthase 1 (CHS1), panDerm1 (5'- GAA GAA GAT TGT CGT TTG CAT CGT CTC -3') and panDerm2 (5'- CTC GAG GTC AAA AGC ACG CCA GAG -3') using a StepOne™ Real-Time PCR System[4] (Applied Biosystems).

Microscopic examination of the hair sample was negative. The dermatophyte present in the hair sample was confirmed as *Trichophyton mentagrophytes* by culture and qPCR. Real-time PCR detected the *T. mentagrophytes*-specific PCR product, successfully identified by melting point analysis. The hair sample melted at 83.11°C (Figure 2), showing that the isolated clinical curve was distinct from the controls (*M. canis*).

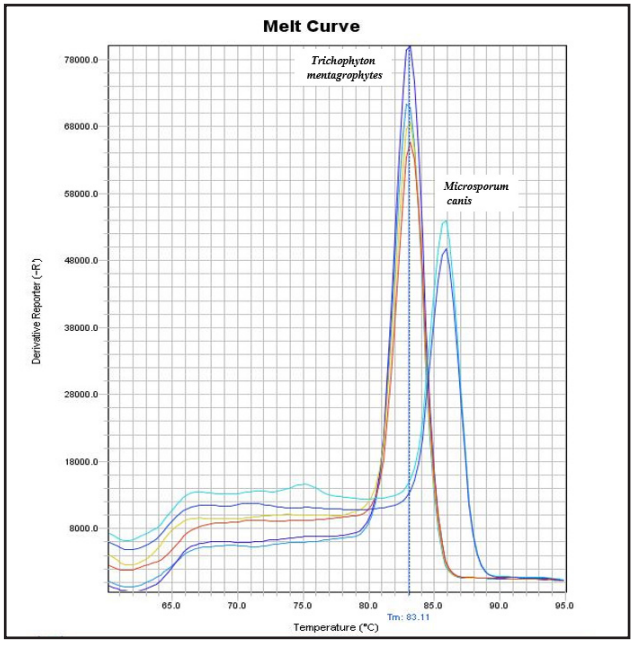

Figure 2. The different PCR products are separated by melting-point analysis: *Trichophyton mentagrophytes* (hair clinical sample) and *Microsporum canis* (positive control).

The patient was treated with systemic itraconazole manipulated [10 mg/kg SID - 90 days]. Twice-weekly application of 2.5 % miconazole and 2% chlorhexidine manipulated shampoo until complete cure (Figure 1B).

DISCUSSION

Animal dermatophytosis is often described as a frequently self-limiting infection, even though it might also spread to other animals, as well as, to humans. Extensive chronic dermatophytic infections can show severe inflammation, pruritus, and a total alopecia in most severe cases [3], as reported in this paper.

Trichophyton mentagrophytes is isolated from various hosts such as carnivores, horses, rabbits, and less frequently from ruminants and swine [7]. In pets, the literature indicates that *Microsporum canis* is the predominant isolate, followed by *M. gypseum* [2,8]. Often, *T. mentagrophytes* appears in a lower percentage of isolation [9,12]. In a retrospective study of dermatoses in Brazilian animals during a 1979-2009 period, 5,584 dog samples had dermatophytes represented by *M. canis* (78.4%), *M. gypseum* (16.2 %) and *T. mentagrophytes* (5.1%) [1]. In another Brazilian study, *M. canis* was the most common dermatophyte species in culture (93%), followed by *M. gypseum* and *T. mentagrophytes* (3.5% each one) [6].

Chronic and extensive dermatophytosis due to a mixed *Microsporum canis* and *Trichophyton* mentagrophytes infection in a dog can also be seen in some cases [3]. In our case report, the mycological culture did not show coinfection by different species of dermatophytes in the hair sample and, consequently, only one melting peak in qPCR was detected.

The culture of hair, even with specific medium containing chloramphenicol and cyclohexamide, may present contaminating fungi, not related to dermatophytosis, which can inhibit or override the growth of dermatophytes [5,10]. The use of real-time PCR provided a faster e specific diagnosis of dermatophytosis when compared to the conventional mycological methodology for detection and identification of *T. mentagrophytes*, which takes at least around 10 to 15 days for the final result. The isolate found in the clinical sample showed a Tm 83.11°C, similar to the Tm observed for positive controls in another study [14], when the melting curve analysis was performed to distinguish the main species causing animal dermatophytosis.

It is important to emphasize that this technique, using universal primers, is a fast and accurate method for the identification of the etiological agent of ringworm directly from a clinical sample. Besides that, it can be easily performed in a routine laboratory, with a major advantage of provide results in a remarkable short turnaround time.

MANUFACTURERS

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