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CASE REPORT
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Equine Dermatophytosis caused by *Nannizzia gypsea* - Molecular Diagnosis by qPCR

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ABSTRACT

***Background*:** Dermatophytes, fungi of universal distribution, invade semi or fully keratinized structures, such as skin, fur/hair and nails. In companion animals (cats, dogs, or small mammals like rabbits, guinea pigs, and chinchillas) as well as in large animals (mainly in horses and cattle). Frequently are responsible for skin diseases including alopecia and crusts. This work reported a case of equine ringworm due to *Nannizzia gypsea* (*Microsporum gypseum*) detected from the clinical sample by SYBR-Green real-time PCR. The strategy was based on the DNA extraction directly from the infected hair followed by real-time PCR and melting-point analysis.

***Case*:** A 2-year-old horse was referred to the Veterinary Clinic Hospital, Federal University of Rio Grande do Sul (HCV-UFRGS), Porto Alegre, Brazil, presenting circular areas of alopecia and lesions with dry aspect and thin powdery scales and hairs broken at their base mainly on head and neck. No previous antifungal treatment was carried out. The sample was obtained by plucking the hair with forceps and scales from the peripheral area of the lesions. For mycological diagnosis, hair specimen was clarified and examined microscopically using 10% potassium hydroxide (KOH) for the visualization of arthroconidia (ectothrix type). The infected hair was plated onto Mycosel™ Agar and Mycosel Agar with nicotinic acid requirement, incubated at 25-30°C for 10-15 days. Microscopic features (macroconidia) and colony characteristics (colors and texture) were conducted for the differentiation of the species within the genus *Microsporum*. In addition, real-time PCR was applied for direct analysis of the fungal DNA obtained from the hair sample. Qiagen DNeasy® plant mini DNA extraction kit protocol was used to extract DNA from the hair sample according to the manufacturer's instructions. A real-time PCR was performed using the pan-dermatophyte primers for detecting a DNA fragment encoding chitin synthase 1 using SYBR Green PCR Mix. The melting curve data were obtained by continuous fluorescence acquisition from 60 to 95°C with a ramp rate of 0.3C. Microscopic examination of hair sample was negative. The culture was positive and dermatophyte present in the hair sample was confirmed as *Nannizzia gypsea* (*M. gypseum*) following the amplification of CHS1 gene. The hair sample melted at 83.78°C, showing that the isolated clinical curve was distinct from the control (*M. canis*) melted at 85.3°C.

***Discussion*:** Animals can be infected by a variety of dermatophytes. *Nannizzia gypsea* (*Microsporum gypseum*) is a geophilic keratinophilic fungus with a worldwide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion. The dermatophytic infection in horses is generally follicular and the most common clinical sign is one or many circular areas of alopecia with variable erythema, scaling and crusting. Is extremely important the culture of samples from skin lesions, because many agents may be involved and, frequently KHO test is negative. Conventional methods (direct exam and fungal culture) lacks the ability to make an early and specific diagnosis. The qPCR assay introduced in this study allows the specific detection of relevant dermatophytes in veterinary medicine in a short time. In the case reported here, dermatophytosis due *Nannizzia gypsea* (*Microsporum gypseum*) in a horse was confirmed based on mycological diagnosis and SYBR-Green real-time PCR.

Keywords: horse, ringworm, zoonosis, *Microsporum gypseum*, geophilic, real-time PCR.

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INTRODUCTION

In companion animals (cats, dogs, or small mammals like rabbits, guinea pigs, and chinchillas) as well as in large animals (horses and cattle), dermatophytes are frequently responsible for skin diseases including alopecia and crusts [2,7]. Fungal culture is still considered as the reference standard for diagnosis and samples of hairs, crusts, scales, or even cutaneous tissue (in the specific case of pseudomycetoma) should be collected for culture [4].

PCR-based tests that are available for the direct diagnosis of dermatophytosis from clinical samples proved to be effective, including the identification of the species involved. Furthermore, another advantage is related to the diagnosis time, which is reduced to a few days [1,10].

The aim of this work was to report one case of equine dermatophytosis caused by *Nannizzia gypsea* which was confirmed by SYBR-Green real-time PCR using clinical sample directly.

CASE

A 2-year-old horse was referred to the Veterinary Clinic Hospital, Federal University of Rio Grande do Sul (HCV-UFRGS), Porto Alegre, Brazil, presenting circular areas of alopecia and lesions with dry aspect and thin powdery scales and hairs broken at their base mainly on head and neck (Figure 1). No previous antifungal treatment was carried out. The sample was obtained by plucking the hair with forceps and scraping scales from the peripheral area of the lesions. Hair specimen was clarified and examined microscopically using 10% potassium hydroxide (KOH) for the visualization of chains of arthroconidia (ectothrix type invasion of hair). In addition, samples were cultured on Mycosel Agar[1] and Mycosel Agar[1] with nicotinic acid requirement at 25-30°C for 10-15 days.

The Qiagen DNeasy® plant mini DNA extraction kit[2] protocol was used to extract DNA from hair sample according to manufacturer instructions. A real-time PCR was performed using the pan-dermatophyte primers for detecting a DNA fragment encoding chitin synthase 1 (CHS1). The PCR assay was performed as previously described in 2023 by Spanamberg *et al*. [10].

Microscopic examination of hair sample was negative. Mycosel Agar with nicotinic acid requirement was negative and only in Mycosel there was growth of *Nannizzia gypsea* (*Microsporum gypseum*). Real-time PCR detected the *N. gypsea*-specific PCR product, successfully identified by melting point analysis. The hair sample melted at 83.68°C (Figure 2), showing that the isolated clinical curve was distinct from the control isolate (*M. canis*) melted at 85.3°C. Negative control strains did not show any amplification.

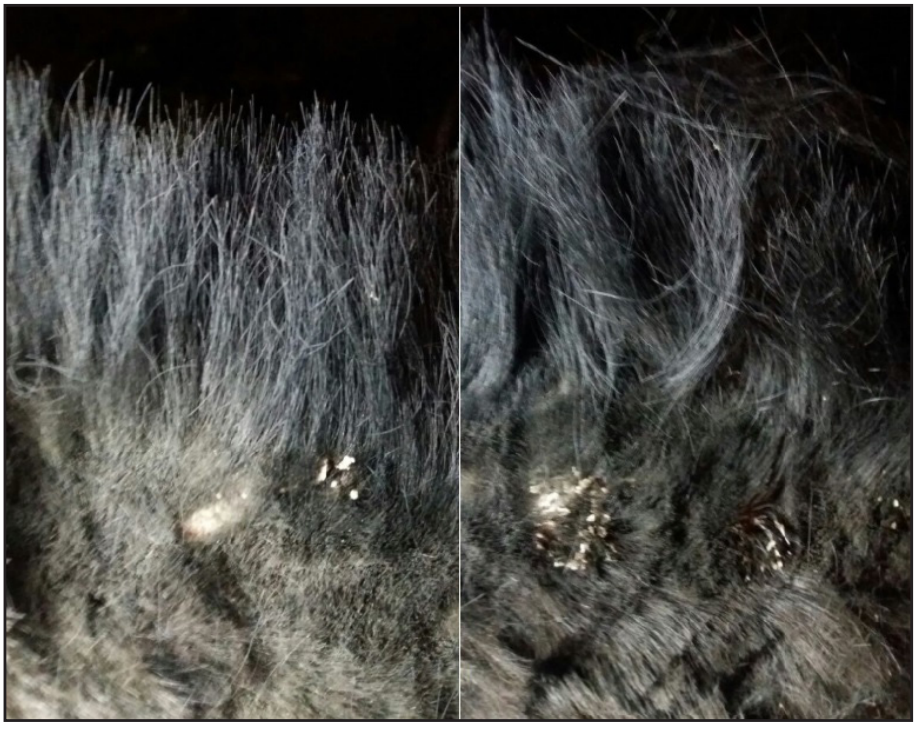

Figure 1. Clinical presentation of dermatophytosis in a horse caused by *Nannizzia gypsea*.

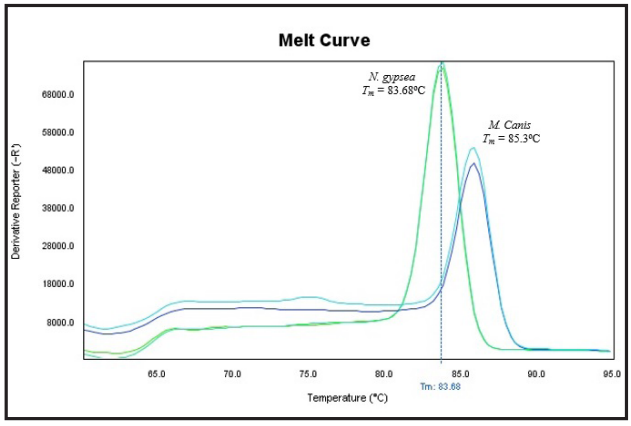

Figure 2. The different PCR products are separated by melting-point analysis.

DISCUSSION

Animals can be infected by a variety of dermatophytes. The most frequently isolated dermatophyte species from animals are *Microsporum canis*, *Nannizzia gypsea* and *Trichophyton mentagrophytes* [8-10]. Anothers species, like *Microsporum nanum*, *Trichophyton verrucosum* and *T. equinum* [2] are reported in the literature; however, occurrences have been rare [7].

Nannizzia gypsea is a geophilic keratinophilic fungus with a worldwide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a single inflammatory skin or scalp lesion. Invaded hairs show an ectothrix infection but do not fluoresce under Woods ultra-violet light [5]. For geophilic dermatophytes, the soil is the reservoir in which the fungi multiply. Thus, the risk of contamination is higher for animals with outdoor contacts, as described in this case report.

The dermatophytic infection in horses is generally follicular and the most common clinical sign is one or many circular areas of alopecia with variable erythema, scaling and crusting. Non-dermatophyte dermatoses mimicking dermatophytoses in the horse can occur and the primary differential diagnoses are follicular infections, such as bacterial folliculitis, demodicosis and eosinophilic folliculitis. In horses, the differential diagnosis also includes other skin diseases like *Pemphigus foliaceus*, systemic or chronic cutaneous lupus erythematosus, insect allergies, sarcoidosis, cutaneous lymphoma and multisystemic eosinophilic epitheliotropic disease [3]. Especially in the absence of pruritus and for horses living outdoor, the occurrence of dermatophilosis which is caused by the bacterium *Dermatophilus congolensis*, must be systematically explored [6].

The reference standard for the diagnosis of dermatophytosis is the isolation in culture of the etiologic agent from the lesion [2]. Is extremely important the culture of samples from animals with skin lesions, because many agents may be involved, even when potassium hydroxide (KOH) microscopy of hair evaluation is inconclusive. Conventional methods (direct exam and fungal culture) lacks the ability to make an early and specific diagnosis. The qPCR assay introduced in this study allows the specific detection of relevant dermatophytes in veterinary medicine in a short time [8-10]. In the case reported here, dermatophytosis due *Nannizzia gypsea* in a horse was confirmed based on mycological diagnosis and SYBR-Green real-time PCR.

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Declaration of interest. The authors report no conflict of interest. They are entirely responsible for the content and writing of the paper.

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