Case Report

Canine dermatophytosis caused by an anthropophilic species: molecular and phenotypical characterization of *Trichophyton tonsurans*R. S. N. Brilhante,^{1,4} R. A. Cordeiro,^{1,3,4} J. M. F. Gomes,² J. J. C. Sidrim¹ and M. F. G. Rocha^{1,2}

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Microsporum canis is the most common species isolated from canine and feline dermatophytosis in the world. However, this study reports a rare case of canine dermatophytosis caused by the anthropophilic dermatophyte *Trichophyton tonsurans* in the city of Fortaleza, Ceará, Brazil. The fungal characterization was performed by classical mycological examination and by genotypical analysis using the restriction enzymes *Sau3A*, *RsaI*, *DdeI* and *EcoRI*. The phenotypical characteristics were compatible with *T. tonsurans*. The results obtained in the genotypical analysis were similar to the digestion pattern of the ITS sequences for *T. tonsurans* strains. In addition, an antifungal susceptibility test was performed with griseofulvin, ketoconazole and itraconazole. The MICs were 0.5 µg ml⁻¹ for griseofulvin, 0.25 µg ml⁻¹ for ketoconazole and 1 µg ml⁻¹ for itraconazole. This study emphasizes the adaptability of anthropophilic fungi such as *T. tonsurans* to animal conditions.

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Introduction

Dermatophytoses are the most common fungal infections in dogs and cats (Khosravi & Mahmoudi, 2003; Simpanya & Baxter, 1996). The dermatophytes have a high affinity for keratin, an important component of fur, skin and nails, which are the primary sites of fungal infection (Borgers *et al.*, 2005). Clinical presentations of dermatophytic lesions include multifocal alopecia, mild or intense pruritus and round scaly lesions with erythematous and scaly borders (Cafarchia *et al.*, 2004). According to their natural reservoir, dermatophytes are classified as anthropophilic, zoophilic or geophilic (De Hoog *et al.*, 2000). Several reports have stated that *Microsporum canis*, a typical zoophilic species, is the most common dermatophyte isolated from dogs and cats worldwide (Brilhante *et al.*, 2003; Cafarchia *et al.*, 2004; Khosravi & Mahmoudi, 2003; Segundo *et al.*, 2004). On the other hand, *Trichophyton tonsurans* is a classic anthropophilic species usually isolated from human dermatophytosis in northeast Brazil (Brilhante *et al.*, 2004).

This study describes a rare case of canine dermatophytosis caused by *T. tonsurans*. The fungal characterization was done by morphological and molecular analysis. In addition, an *in vitro* antifungal susceptibility test was performed.

Case report

A 2-year-old female Doberman Pinscher with suspected dermatophytosis was examined in a veterinary clinic located in Fortaleza, Ceará (northeast Brazil). The animal showed a rounded lesion of 3 cm in diameter, patches of scalp hair loss and scaling. The lesion was not inflamed, and it was in the distal portion of the right femoral region of the leg (Fig. 1a).

Clinical specimens were obtained from the animal skin by scraping epidermal scales from the lesion with a surgical blade, and then transported to the Specialized Medical Mycology Center (Federal University of Ceará, Brazil). Direct microscopic examinations of the epidermal scales, using 30% KOH, were negative for mites, but showed hyaline-septated arthroconidiate hyphae suggesting dermatophyte infection. Ectothrix or endothrix parasitism was not observed in the hair. Cultures of the clinical specimens, placed on blood agar, Sabouraud dextrose agar, Sabouraud with chloramphenicol and Mycosel agar, showed a colony which suggested *T. tonsurans*.

Complementary laboratory tests showed that the animal had no blood abnormalities as there was no other evidence of disease.

Fig. 1. (a) Round scaly lesion of 3 cm in diameter with scalp hair loss situated in the distal portion of the right femoral region of the leg. (b) Granular to cottony colonies of *T. tonsurans*, apiculated, with bright yellow to brownish pigment. (c) Numerous microconidia of variable sizes.

Methods

Classical mycological examination. The fungal macro- and micromorphology were analysed on potato dextrose agar (Difco) and Sabouraud dextrose agar (Sanofi) after 10 days of incubation at 28 °C. The following macromorphological characteristics were analysed: texture, surface of the colony, and the presence of pigmentation. Slide cultures in agar potato block were used for the micromorphological study, according to De Hoog *et al.* (2000). In addition, fungal identification was confirmed by the *in vitro* hair perforation test, urease production in Christensen's medium and analysis of vitamin requirements in *Trichophyton* agar media (Difco).

Genotypical analysis. The fungal DNA was extracted with CTAB buffer according to the procedure described by Talbot (2001). PCR amplification of the ITS 1 and ITS 2 ribosomal regions was achieved with the universal primers ITS-4 and ITS-5 (Brilhante *et al.*, 2005a). The amplified products were analysed by endonuclease digestion assays with *Sau3A*, *RsaI*, *DdeI* and *EcoRI* (Gibco-BRL) and compared with the products from one strain of *T. tonsurans* isolated from a human and with the products from one strain of *Trichophyton mentagrophytes* isolated from a dog. The digested products were separated by electrophoresis on 6% polyacrylamide gels in 0.5 × TBE buffer and silver stained as described in the literature (Brilhante *et al.*, 2005a; Sanguinetti *et al.*, 1994).

Antifungal susceptibility test. The microdilution assay was performed in 96-well microdilution plates with RPMI 1640 medium (Sigma), with L-glutamine and without sodium bicarbonate, and buffered to pH 7.0 with 0.165 M MOPS (Sigma). A standardized inoculum of 0.5×10^4 – 5×10^4 c.f.u. ml⁻¹ was challenged against the following antifungal drugs: griseofulvin (0.0312–8 µg ml⁻¹) and ketoconazole and itraconazole (0.0156–16 µg ml⁻¹). The microdilution plates were incubated at 35 °C and the results were read visually after 4 days (Brilhante *et al.*, 2005b).

Results

The mycological analysis confirmed the identification of *T. tonsurans*. Granular to cottony colonies, apiculated, with bright yellow to brownish pigment were detected (Fig. 1b). Clavate to cigar-shaped macroconidia were rare; numerous microconidia of variable sizes, often with an almost cylindrical shape, were seen (Fig. 1c). Physiological tests proved negative for both hair perforation and urease production. Enhanced growth in medium supplemented with thiamine was observed.

The PCR assay detected an amplicon of approximately 720 bp. The *Sau3A*-digested product consisted of four fragments of 45, 60, 280 and 335 bp. *DdeI*, *RsaI* and *EcoRI* cut the amplicon regions into two fragments with the

following lengths: 120 and 600 bp for *RsaI*; 280 and 440 bp for *DdeI*; 380 and 330 bp for *EcoRI* (Fig. 2). The results obtained from the animal strain and from the human strain were similar to the expected digestion pattern of the ITS sequences for *T. tonsurans* strains registered in GenBank. The results obtained from the PCR assay with *T. mentagrophytes* and with *T. tonsurans* strains were similar.

By using standardized conditions, the obtained MICs were as follows: 0.5 µg ml⁻¹ for griseofulvin, 0.25 µg ml⁻¹ for ketoconazole and 1 µg ml⁻¹ for itraconazole.

Discussion

Many members of the anamorph genus *Trichophyton* are anthropophilic and have co-evolved with the human host (Graser *et al.*, 1999). In dogs, the distribution of these fungi is less frequent, *T. mentagrophytes* being found most frequently in such cases (Brilhante *et al.*, 2003).

According to Graser *et al.* (1999), some members of the genus *Trichophyton*, such as *T. tonsurans*, *Trichophyton interdigitale*, *T. mentagrophytes*, *Trichophyton simii* and *Trichophyton erinacei*, must be reclassified as synonymous.

Fig. 2. PCR-enzyme restriction patterns in PAGE (6% gel) of a human control strain of *T. tonsurans* (lanes 4, 6 and 9) and the *T. tonsurans* strain obtained from a dog (lanes 3, 5 and 8). A 100 bp ladder was used to estimate the product sizes (lanes 1 and 7). Lane 2 is a negative (no template) control.

This suggestion has been based on the similarities observed through molecular biology techniques such as analyses of the ITS regions.

In the present study, genetic similarities were detected in the strains of *T. tonsurans* and *T. mentagrophytes* isolated from dogs with dermatophytosis. However, according to De Hoog *et al.* (2000), these fungi can be phenotypically identified through different tests, where, for instance, *T. mentagrophytes* is always urease-positive and *T. tonsurans* shows variations in this test. In addition, *T. tonsurans* does not perforate hair and *T. mentagrophytes* does or does not perforate hair *in vitro*. Differences in the micromorphological characteristics are well described: *T. mentagrophytes* presents spherical microconidia, grape-like clusters and spiral hyphae, and *T. tonsurans* presents clavate to nearly cylindrical microconidia, sometimes inflating to balloon-shaped, without spiral hyphae (De Hoog *et al.*, 2000).

Strains of *Trichophyton rubrum* are primarily adapted for parasitism in humans, but occasionally may cause infection in susceptible animals (Cabañes, 2000). *T. tonsurans* has been the cause of 90–95 % of *Tinea capitis* infections in adults and children throughout the world (Hainer, 2003). It is considered a cosmopolitan anthropophilic fungus found in various geographic regions. Brilhante *et al.* (2004), evaluating the incidence of *Tinea capitis* in Fortaleza, Ceará, Brazil, noted that *T. tonsurans* was the main (62.8 %) dermatophyte isolated. This species has been recognized as a causative agent of Majocchi's granuloma (Rajpara *et al.*, 2005) and has been cited in cases with clinical features that mimicked the concentric rings of *Tinea imbricata* caused by *Trichophyton concentricum* (Ouchi *et al.*, 2005).

The rarity of dermatophytosis caused by *T. tonsurans* in small animals can be explained by the fact that this fungus is an anthropophilic species. The first case of animal dermatophytosis caused by an anthropophilic species was described by Kushida & Watanabe (1975). The authors reported the isolation of *T. rubrum* in a dog whose owner had *Tinea pedis*, probably caused by the same fungal species. More recently, Cabañes (2000) and Kano *et al.* (2002) described *T. rubrum* infections in dogs. These animals may have acquired dermatophytosis after direct or indirect contact with an infected human, as molecular typing suggested that isolates of *T. rubrum* from both sources were genetically identical (Kano *et al.*, 2002). In the present study, we guess that the source of infection was the asymptomatic owner.

The dog was treated with 10 mg ketoconazole kg⁻¹ once a day for 30 days, followed by 10 mg itraconazole kg⁻¹ once a day for 28 days, and finally pulse therapy maintaining the same dosage every other week for 2 months. Complete resolution was achieved after 3 months of itraconazole treatment, corroborating the *in vitro* results with itraconazole. The resistance of dermatophytes to drugs *in vitro* is considered rare, having been reported only once in strains of *T. rubrum* (Osborne *et al.*, 2005).

Dermatophytes possess unique characteristics in the way they adapt to the environment. The anthropophilic fungi probably reached different stages in their phylogenetic scale until they adapted to humans. In this way, the study emphasizes the adaptability of anthropophilic fungi, uncommon micro-organisms involved in canine dermatophytosis.

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