



Research Article

DERMATOPHYTES FROM A ZOONOTIC POINT OF VIEW

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ABSTRACT

Dermatophytosis or ringworm (tinea) are superficial mycoses caused by a highly specialized group of fungi, which are manifested in a disease characterized by infection of the keratinized tissues, such as the epidermis (skin cornea), hair and nails. These play an important role because of their zoonotic potential, but most of the time this infecting power is minimized, for two reasons: sub-registries in the diagnosis of the disease, and because they do not cause direct mortality in the humans. It is worth mentioning that in recent years there has been an increase in zoophilic types, this due to having animals inside the home. Transmission of dermatophytes can occur by direct contact with infected animals and humans or by indirect contact with contaminated fomites. A specific analysis is required regarding the relevance of this disease, as it is a zoonosis of great clinical importance but totally ignored.

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INTRODUCTION

Dermatophytosis or ringworms (tinea) are an infection caused by a group of micro-organisms closely related to filamentous fungi, commonly known as dermatophytes, and can infect animals and people (Moretti *et al.*, 2013; Behzadi *et al.*, 2014). Dermatophytes appeared on earth in the Cenozoic era, that is to say, approximately 65 million years ago, when the continents had their current position. At this time mammals with skin similar to the animals of the present day appeared equipped with various types of keratins, so that when man domesticated animals and learnt to use products from them, the first infections from dermatophytes probably occurred (Méndez, 2015). Fungi are micro-organisms that are ubiquitous in the environment; the vast majority are soil micro-organisms or are present in vegetables. However, more than 300 species act as pathogens in animals (López *et al.*, 2008), with more than 20 different species of dermatophytes having been isolated from pet hair and skin.

Most taxonomists divide dermatophytes into three genera with their respective species: 21 species for the genus *Trichophyton*, 15 for *Microsporium* and 1 for *Epidermophyton* (García *et al.*, 2008), which are the most pathogenic genera in the canine and feline species. They are classified, according to their natural habitat, as: anthropophilic –primarily affect man, but can also infect animals; zoophilic– are pathogens typically of animal species although occasionally they affect man; geophilic –are found in the soil and are mainly associated with keratin, only occasionally infect man and

other animal species (López *et al.*, 2008; Ocaña *et al.*, 2011; Baldo *et al.*, 2012).

Animals carrying zoophilic fungi could be the source of infection for humans. One study in Barcelona shows that the most frequently isolated dermatophyte, both in humans and dogs, has been *M. canis* (36.1 and 36.0%), followed in both cases by *Trichophyton mentagrophytes* variety of *Mentagrophytes* (22.2% and 24.8%) (Bohada *et al.*, 1994). In another study performed at the Gregorio Marañón hospital in Madrid Spain it was reported that the most common species are *T. rubrum* (60%), *T. mentagrophytes* (21%) and *M. canis* (10%), in tinea unguium were *T. rubrum* (80%) and *T. mentagrophytes* (15%) [Hernández *et al.*, 2013], they are often asymptomatic carriers but can be important sources of infection (Mayorga *et al.*, 2016). In recent decades in humans it is estimated that cutaneous mycosis affects more than 20–25% of the world population; on the other hand we found a predisposition to dermatophytosis in domestic pets, such as Persian cats, Yorkshire and Jack Russell terrier dogs (Rivas, 2011). The cat is considered the main reservoir of *Microsporium canis*, an agent involved in dermato-zoonoses of urban populations: some studies have found 6.5% and more of 88% of the cats examined to be infected (Acha & Szyfres 2008). The third most common dermatophyte in human infections is this fungus. In Siena, Italy, an investigation showed that the majority of cases of human dermatophytosis was caused by this species of dermatophyte (*Microsporium canis*) as well as *Trichophyton mentagrophytes* through contact with apparently healthy cats. These (173 cats) were captured in different parts of Siena and examined for

dermatophytes, *Microsporium canis* was isolated in 82 cats, *Trichophyton mentagrophytes* in three cats and *Microsporium gypseum* in one cat (Romano *et al.*, 1997). *Microsporium canis* is responsible for 97–100% of cutaneous mycosis in Italy (Mancianti *et al.*, 2003). These zoonoses related to pet ownership would fundamentally affect children (Ilkit *et al.*, 2007; López *et al.*, 2012). Around 50% of people exposed to *M. canis* develop disease when their cats are carriers or are infected. The a etiological agents of this pathology in humans belong to anthropophilic and zoophilic species: fundamentally the most important dermatophytes that cause skin infections are *M. canis* with 70% of the cases, *M. gypseum* with 20% and 10% caused by *T. mentagrophytes* (Stojanov *et al.*, 2009; Samad, 2011). However these are not the only ones causing dermatophytosis in humans, Mendoza (Mendoza, 1986) sampled four human cases of tinea caused by *Trichophyton verrucosum*, var. *Autotrophicum*, by contact with fomites contaminated by animal carriers; Amor *et al.*, reported a case of tinea faciae caused by *Trichophyton equinum* affecting a five-year-old child after having ridden a pony a month earlier (Amor *et al.*, 2001). In Germany the first report of a human infection caused by *Trichophyton gallinae* was of a 67-year-old woman, with diabetes mellitus type 1 (Poblete *et al.*, 2006), but the first serious case caused by this dermatophyte was reported in a man in Spain. The patient, 25 years old, was HIV-positive with generalized dermatophyte infection (Del Palacio *et al.*, 1992) Sitterle and colleagues [2012] presented the first case of human dermatophytosis caused by *Trichophyton bulbosum* in a 21-year-old man who had been in contact through riding donkeys. Also, in the area of Dhamar, Yemen, six dermatophytes were identified in the domestic camel: *T. verrucosum*, *T. mentagrophytes*, *T. tonsurans*, *M. audouinii*, *M. canis* and *T. schoenleii*. The latter has been widely confirmed to produce a severe form of tinea capitis in humans called ‘favus’, which usually affects scalp hair (Baghza *et al.*, 2016). Paškevičius and Švedienė reported that in 2013, *M. canis* was the main cause of tinea capitis in Lithuania. In their study they found that *M. canis* mainly affects children aged 6 to 14 and only rarely affects adults [25]. In Mexico, Dr Arenas, who has been investigating dermatophytosis for many years, comments that it is very frequent in the country and constitutes 80% of all mycoses and which occurs at a frequency of 5% in dermatological consultations (Arenas, 2002). The National Autonomous University of Mexico (UNAM) made isolates of 108 floors (8 spas, 38 shoemakers, 34 bedrooms, 28 clothing testers), 200 pets (100 dogs and 100 cats), and 1146 healthy people. Dermatophytes were isolated in 18% of the floors, *T. mentagrophytes* being the most frequent, and *M. canis* was isolated in 4% of the dogs and 26% of the cats. A sample of the scalp was taken from the group of healthy people and dermatophytes were isolated in 10%, mainly *T. tonsurans* (López, 1986). It should be mentioned that the diagnosis of ringworm or dermatophytosis does not appear among the 20 main causes of national disease (SINAVE, 2015). In addition, the direct or indirect risk factor of zoonosis by dermatophytes is not considered in the Guide of Clinical Practice of Diagnosis and Treatment of Ringworm and Onychomycosis in the First Level of Care (GPC, 2008). This could be due to the few studies related to this epidemiological association or on the other hand, to the sub-registry of the disease and the non-direct cause of mortality.

### Pathogeny

The first stage of dermatophyte infection involves the contact and deposition of arthrospores or hyphae, which adhere to the surface of the keratinized tissue to reach the epidermis. Through the germination of arthroconidia the growing hyphae enter the stratum corneum in multiple directions; the adherence of spores to host tissues is time-dependent.

The response of the immune system depends mainly on T lymphocytes, in a delayed-effect hypersensitivity reaction, the antibodies do not generate protection and the body's defence against these organisms depends on immunological factors that are not immunological (Tainwala & Sharma, 2011).

In a model carried out in 2007 by Kaufman *et al.*, *T. mentagrophytes* was inoculated into extracts of human skin, and the authors observed how the arthroconidia deploy fibrils that are responsible for joining the fungal structures to the surface of the skin [31]. These fibrils are long when they are in the most superficial part of the stratum corneum and short in the deeper layers. Apparently these structures are responsible for the correct anchorage of the dermatophyte to host cells and prevent them from being easily disconnected by external aggressions such as scratching, these fibrils have also been called fibrillar adhesins (Kaufman *et al.*, 2007; Uribe & Cardona, 2013).

During penetration, the dermatophytes secrete a variety of virulent enzymes, which have different substrate specificities, such as protease, lipase and cellulase. Once the dermatophyte is attached to the cells, the hyphae begin to grow and are anchored to the host, projecting longitudinally and transversely across the entire surface. However, the invasion process cannot be started without first reducing the disulphide bridges found in the compact network of proteins that make up keratinized tissues (Uribe & Cardona, 2013; Chinnapun, 2015). Subtilisins and fungalisins digest proteins into long-chain peptides, which are then converted into amino acids and short-chain peptides by the synergistic action of leucine aminopeptidases (Lap 2), and dipeptidylpeptidases (DppIV) (Monod *et al.*, 2002). When the keratin proteins are degraded, they result in amino acids, dipeptides and tripeptides; these are a nutritional source for the survival of the dermatophytes (Kunert, 1972). Once on the skin, they can be removed by detaching themselves mechanically, remaining in the same site without producing symptoms (asymptomatic carriers), or if the conditions are right, germinate by adhered to the keratinocytes and penetrate the stratum corneum invading the hair follicles (Ocaña *et al.*, 2011). In immunocompetent hosts, they cannot penetrate deeper than the granular stratum, however in patients with compromised immune systems there may be infection in the dermis and subdermis, fungi can enter the bloodstream and spread among the main distant organs, including lymph nodes, liver, brain and bone marrow (Si-Hyun *et al.*, 2016).

### Clinical Manifestations

In animals, lesions are mostly found on the face, ears, tail and claws; in cats there may be blackheads may form. The most important dermatological aspect is the follicular localization of the lesions, consequently the most characteristic are one or more erythematous alopecia circular spots in dogs, with follicular papules; general or localized folliculitis can be observed with or without boils. In most cases the lesions are scaly. Other clinical signs include dry seborrhoea, focal or

multifocal dermatitis, crust with erythematous margins, kerion, onychomycosis and / or paronychia. In dogs this can be confused with autoimmune disease since crusts are usually present and in some cases in a symmetrical and bilateral form. In dogs, *M. canis* usually shows more marked inflammation than in cats. *M. gypseum* or *T. mentagrophytes* often cause kerion, which presents an inflammation, with pus and an ulcerated infiltrate, which is associated with a secondary bacterial infection (Escobedo, 2011; Moretti *et al.*, 2013).

The clinical picture produced by dermatophytes in humans is called ringworm and is classified depending on the body region where it develops. These infections lead to a variety of clinical manifestations in humans, such as tinea pedis, tinea corporis, tinea cruris, Majocchi granuloma, tinea capitis and tinea unguium (Ocaña *et al.*, 2011; Goldstein & Goldstein, 2016).

The main clinical subtypes of dermatophyte infections are: tiña corporis (infection of the body surfaces other than the feet, groin, face, scalp or beard hair); tinea pedis (infection of the foot); tinea cruris (infection of the groin); tinea capitis (infection of the scalp); tinea unguium (infection of the nails) (Goldstein & Goldstein, 2016).

**Diagnosis**

In the case of any lesion suggested by a dermatophyte, it is essential to verify the presence of the micro-organism in order to confirm the diagnosis and guide the epidemiological survey and treatment (Viguié & Paugam, 2009).

factor is that there are additional factors that can inhibit fluorescence such as the topical application of iodine. The presence of bacteria such as *Corynebacterium minutissimum* and *Pseudomona aeruginosa*, can fluoresce even if the colour is slightly lighter. Keratin, soap and synthetic fibers or topical ointments can give a false positive. Taking this information in to account, Wood’s lamp is an auxiliary tool in the diagnosis of dermatophytosis but definitely not be used as the only diagnostic method. Isolated in culture media, it is the only method that allows the identification of the species, which is of great use in deciding on appropriate treatment of the infection, thus obtaining better results. In veterinary patients, we recommend taking the hair for the laboratory incubation. When fluorescence is obtained it can be used as an auxiliary in the selection of the sample of which is the collection with clamps or hemostats, it is possible to used brushing technique in which a sterile tooth brush is applied directly to the culture medium after brushing the affected areas. In humans, scrapings of the skin, nails and hair are the samples used. Unfortunately, often the samples are insufficient or inadequate; the clinician must be sure to take sufficient material for microscopic observation and culture. After sampling, if topical medication has been applied it is necessary to gently clean the sample with 70% alcohol. The observations of this material under the microscope using Potassium hydroxide (KOH) can identify the presence of a dermatophyte but it is necessary to cultivate the samples in order to differentiate them.

**Table 1** Principal dermatophytes and species affected

Species	Species affected	Author and year
<i>M. canis</i>	Dogs, cats, rabbits, hamsters, horses and humans	Viguié <i>et al.</i> , 2009; Cabañes, 2000.
<i>M. gypseum</i>	Dogs, pigs, rabbits, horses and humans	Viguié <i>et al.</i> , 2009; Ocaña <i>et al.</i> , 2011
<i>M. andouini</i>	Children	Acha <i>et al.</i> , 2001
<i>M. nanum</i>	Pigs and occasionally humans	Molina de Diego, 2011
<i>M. distortum</i>	Dogs, humans and primates	
<i>T. mentagrophytes</i>	Cattle, pigs, birds, rabbits, sheep, goats, felines, rodents, horses and humans	Paugam, 2009; Cabañes, 2000.
<i>T. equinum</i>	Horses	López <i>et al.</i> , 2008
<i>T. verrucosum</i>	Cattle, goats, sheep and occasionally other species	Molina, 2011
<i>T. gallinae</i>	Birds, especially chickens and rarely human	Acha <i>et al.</i> , 2001
<i>T. simii</i>	Wild animals, hens, dogs and human	Viguié., Paugman, 2009
<i>T. rubrum</i>	Human, dogs (very rare)	Prieto <i>et al.</i> , 2011

The clinic diagnosis can be made by the following methods: microscopic observation of the hair and lesion scales; a generic diagnosis can be established with this method. Using Wood’s light (filtered ultraviolet light) the skin normally shows a blue colouration and zones infected with dermatophytes show a bright green fluorescence. The fungus issues fluorescence even when it is not viable. Only some dermatophytes able invading the hair produced fluorescence: *M. canis* and *M. audouinii* always produce green fluorescence. It is possible to detect fluorescence in 80% of the cases of *M. canis*. This fluorescence is due to the active metabolism of tryptophan by the fungus to the infected hair that is in active growth, cannot replicate fluorescence *in vitro*. Other dermatophytes that can fluoresce are *M. Ferrugineum*, *M. distortum*, *M. audouinii* while *M. gypseum* and *M. nanum* only do so occasionally *T. shoeneleini* produces pale green fluorescence. Fluorescence is negative in *T. tonsurans*, *T. violaceum* and other species the *Trichophyton*, as well as in *Epidermophyton* (Molina de Diego, 2011). Another relevant

Cultivate media are used, such as: Saboraund glucose Agar (2%), DTM (Dermatophytes Test Medium) with antibodies, brain infusion and heart with blood agar (Acha & Szyffres, 2001; Antúnez *et al.*, 2014; Cabrera, 2014). The cultivate media with indicator of pH phenol red can be used, the dermatophytes utilize first the protein in the culture media causing alkaline metabolites change the colour to yellow to red, when the culture media proteins are depleted the dermatophytes use the carbohydrates generating acid metabolites and causing an inverse colour change again.

Most other types of fungi do not use nutrients this way and can cause colour changes in the culture gel after a very long time, so it is advisable to check the crops daily.

The traditional determination of species is mainly based on the morphological macroscopic characteristics (appearance, size and colour of the colony, and pigment production), such as microscopic (shape, size and arrangement of conidia, presence of chlamydoconidia and modality of hyphae: spiral,

racket and peridial). These characteristics are easily observed in conventional media, such as Sabouraud's dextrose agar, however the culture is negative in 40% of the positive cases by microscopy and also takes up too much time due to slow growth, sporulation and the need for more physiological examinations. The time required for species identification can vary from 1 week to 3–4 weeks, thus a quick and simple diagnostic method would, without a doubt, prove to be a very important improvement. The alternative method of detection of dermatophytes used is based on a specially developed multiplex PCR for the detection of onychomycosis, through a two-step DNA extraction of a multiplex PCR and an electrophoresis. The method allows the diagnosis of infection caused by any dermatophyte and in the case of *T. rubrum* includes species and gender identification. Unfortunately so far the use of PCR in the case of dermatophytes in veterinary medicine has been so sensitive that the test becomes unreliable as it gives a positive result to many indications that may have been contaminated or contain a tiny presence of a dermatophyte.

You can also use to perform a biopsy a histopathological study of the lesions. In routine stains occasionally you can see hyphae but special stains are usually required for fungi.

#### Prevention

It is very important to remember the potential for contagion and dissemination of dermatophytes. The most susceptible individuals are children, the elderly, immunosuppressed persons or cancer patients. Approximately 50% of humans exposed to dermatophytes are infected. Kittens are the biggest spreaders of spores in the environment.

#### General recommendations

Wash the hands of children who are in contact with young kittens

Wear gloves if you must handle an animal with dermatophytosis

Aspirate carefully – and daily – the areas of the house where there is an infected cat

Disinfect areas where the pet spends the most time

Restrict the area where the sick animal is housed

Avoid the pet coming into contact with immunocompromised persons

#### Treatment

The therapeutic management of dermatophytosis depends on the extent of infection as well as the location and affected tissues and is based on topical and oral medications. In the case of localized infections it is advisable to use topical products, but these are not recommended for the use of extensive, subcutaneous or hair infections. The drugs used topically are azoles; these alter the fluidity and permeability of the membrane and produce inhibition of growth and cellular replication. Inhibition of cytochrome P-450 is responsible for the adverse effects of azole in humans. Allylamines are the other group of topical drugs used and these cause membrane rupture and cell death, but their spectrum of action is more limited. The topical product should be administered into the lesion and spread 2cm outwards in healthy tissues. The newest topical products already available

are sertaconazole, which, in addition to fungostatic, has a prolonged antipruritic effect. Luliconazole is the other new topical product for use in humans approved by the FDA (Food and Drug Administration) in 2013.

The use of topical steroids may be necessary to give relief to the symptoms associated with these infections.

The use of systemic drugs is indicated in cases of extensive infections, immunosuppressed patients, as well as when topical products fail. When applying systemic treatments it is necessary to monitor the patients and consider possible drug interactions. Griseofulvin remains the antifungal of choice in children Itraconazole is considered safer than ketoconazole, being effective in doses of 100mg per day for two weeks; it is necessary to take into account the drug interactions that may occur. Ketoconazole is effective in many infections but carries an increased risk of liver problems. In the case of dogs, ketoconazole is very effective and well tolerated at doses of 5mg per kilogramme for short periods of time. Itraconazole is safer in dogs and can be used in cats but the cost is usually a limiting factor. For the prevention and control of infection, establish procedures of cleaning and disinfection of the habitat of the animals, as well as the timely treatment of infected patients (Cabañes, 2000; Brilhante *et al.*, 2003; Pacheco, 2003; Segundo *et al.*, 2004).

Oral terbinafine can be used at a dose of 250mg daily for two weeks, but may also cause drug interactions. The combination of topical and systemic therapy is recommended for a faster resolution and more effective control. In a study conducted by Manzano *et al* 2015., the antifungal that showed the best activity in humans was terbinafine, and therefore could be recommended as an option for the treatment of dermatophytosis, although it is important to correlate the minimum inhibitory concentration of the drug with the patient's response to treatment (Prieto *et al.*, 2011; Behzadi *et al.*, 2014).

In cats, immersion baths twice a week with dilute sulphur solutions are a great way to limit the amount of spores in the environment and avoid zoonosis and reinfection. It is important to note that the immunological state of animals and people often determines the presence of infection.

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