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ORIGINAL ARTICLE

Dermatophytoses Due to Anthropophilic Fungi in Cadiz, Spain, Between 1997 and 2008

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Abstract
Background and objectives: Cutaneous fungal infections are a major public health problem. The distribution of the dermatophytoses varies between countries and geographical areas. The aim of this study was to determine the incidence, epidemiology, etiology, and clinical course of the dermatophytoses caused by anthropophilic fungi in Cadiz, Spain, over the past 12 years.
Material and methods: The study, conducted between 1997 and 2008, included 2235 samples from lesions of the skin, hair, and nails of 2220 patients with a clinical suspicion of mycosis. Samples were examined by microscopy using potassium hydroxide and were cultured on mycological media. The dermatophytes were identified by their macroscopic and microscopic characteristics.
Results: Cultures were positive in 283 cases (12.7%). Anthropophilic dermatophytes (53.3%) were more common than zoophilic (41.3%) and geophilic (5.3%) dermatophytes. *Trichophyton rubrum* (38.2%) was the predominant pathogen isolated, followed by *Microsporum canis* (22.3%) and *Trichophyton mentagrophytes* (15.5%). Five other species of anthropophilic fungi were identified: *Trichophyton tonsurans* (5.6%), *Trichophyton violaceum* (4.9%), *Epidermophyton floccosum* (2.8%), *Trichophyton soudanense* (1.0%), and *Trichophyton schoenleinii* (0.7%). Infections caused by the anthropophilic fungi included tinea unguium (29.1%), tinea corporis (25.8%), tinea pedis (19.2%), tinea cruris (11.9%), tinea capitis (5.3%), and tinea faciei (3.3%).
Conclusions: The principal fungus responsible for dermatomycosis in Cadiz was *T. rubrum*, and its incidence has been rising since 2000. The prevalence of other anthropophilic fungi, such as *T. tonsurans* and *T. violaceum*, has increased, though this is not directly related to immigration. *E. floccosum*, *T. soudanense*, and *T. schoenleinii* are isolated occasionally.

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PALABRAS CLAVE
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Trichophyton tonsurans

Dermatofitosis por hongos antropofílicos en Cádiz (1997-2008)

Resumen

Introducción y objetivos: Las infecciones cutáneas producidas por hongos constituyen un importante problema de salud pública. La distribución de las dermatofitosis varía en diferentes países y áreas geográficas. El objetivo de este estudio ha sido determinar la epidemiología, etiología y evolución de las dermatofitosis por hongos antropofílicos en Cádiz durante los últimos 12 años.

Material y métodos: El estudio se realizó de 1997-2008 sobre 2.235 muestras de lesiones de piel, pelo y uñas de 2.220 pacientes con sospecha clínica de micosis. Las muestras fueron analizadas mediante examen microscópico con hidróxido potásico y cultivo en medios micológicos. Los dermatofitos se identificaron de acuerdo con las características macroscópicas y microscópicas.

Resultados: El cultivo fue positivo en 283 muestras (12,7%). Los dermatofitos antropofílicos (53,3%) predominaron sobre los zoofílicos (41,3%) y geofílicos (5,3%). *Trichophyton rubrum* (38,2%) fue el patógeno más frecuente, seguido de *Microsporum canis* (22,3%) y *Trichophyton mentagrophytes* (15,5%). Se identificaron otras cinco especies de hongos antropofílicos: *T. tonsurans* (5,6%), *T. violaceum* (4,9%), *Epidermophyton floccosum* (2,8%), *T. soudanense* (1,0%) y *T. schoenleinii* (0,7%). Las infecciones por hongos antropofílicos fueron onicomicosis (29,1%), tiña corporal (25,8%), tiña del pie (19,2%), tiña crural (11,9%), tiña del cuero cabelludo (5,3%) y tiña facial (3,3%).

Conclusiones: El principal responsable de dermatofitosis en Cádiz es *Trichophyton rubrum*. Su incidencia es ascendente desde el año 2000. Otros hongos antropofílicos como *T. tonsurans* y *T. violaceum* son cada vez más prevalentes, aunque no están directamente relacionados con la inmigración. *Epidermophyton floccosum*, *T. soudanense* y *T. schoenleinii* se aíslan ocasionalmente.

Introduction

Dermatophytoses are superficial infections caused by keratinophilic fungi that invade the stratum corneum and other keratinized tissues. The clinical and epidemiological relevance of this mycosis is widely recognized as it constitutes a major public health problem. To prevent the disease from spreading, it is important to identify the cause and control the source of infection.

Dermatophytic fungi are extremely common and can be found all over the world. The majority of dermatophytoses are caused by 5 species. In less-developed countries, the main species is the anthropophilic *Trichophyton rubrum*, which causes tinea pedis, onychomycosis, and tinea cruris.[1,2] In Western Europe, most cases of dermatophytosis in humans, until recently, were caused by zoophilic species from dogs, cats, and rabbits. In Spain, for example, the most common species reported before 1990 was *Trichophyton mentagrophytes* but more recent publications have reported an increasing incidence of infections due to *Microsporum canis*.[3-13] The types of species isolated changes with time and varies considerably between geographical regions depending on factors such as climate, economic development, cultural habits, the availability of antifungal agents, and above all, demographic changes. In recent years, increased population flows as the result of tourism, war, and migration have resulted in the dissemination of certain species and variations in clinical forms of dermatophytosis. Tinea pedis and onychomycosis, for example, predominate in developed countries, whereas tinea capitis and tinea crural are more common in developing countries.[1,5-8,14-16]

Numerous studies conducted in different parts of Spain have demonstrated the changing nature of dermatophytosis over the years, with reports of variations in clinical presentation, prevalence and etiology, and the frequency with which different species are isolated.[8,17-19] It is therefore advisable to perform systematic studies that analyze the incidence of dermatophytosis in particular regions. To this end, we investigated the epidemiology, etiology, and variations in infections caused by anthropophilic dermatophytes in Cádiz, southern Spain, over the last 12 years.

Materials and Methods

We analyzed 2235 samples from primary care and dermatology patients with a clinical suspicion of mycosis from the health care district of Cádiz-San Fernando, which serves a population of approximately 250 000 mostly urban inhabitants. The samples were processed by the Mycology Unit at Hospital Universitario Puerta del Mar in Cádiz between January 1997 and December 2008. There were 1279 skin samples, 730 nail samples, and 226 hair samples, all collected following cleaning of the corresponding sites with 70% ethanol. Skin scrapings were taken from the active

border of lesions using a slide or scalpel, nail specimens were collected by scraping the surface of the nail or using clippers, and hair samples were collected by scraping scalp lesions or removing hairs with tweezers.

The samples were processed by direct microscopic examination with lactophenol cotton blue or KOH to detect fungal structures such as hyphae and conidia, followed by inoculation onto Sabouraud glucose agar containing chloramphenicol and cycloheximide (Difco, USA) and incubation for 4 weeks at between 28ºC and 30ºC, with twice-weekly observation. Colonies were identified by assessing growth rate, macroscopic features (topography, texture, color, and colony surface and reverse), and microscopic characteristics (hyphae, conidia, and other fungal structures) either directly in the primary culture media or following subculture on potato dextrose agar and cornmeal agar (Difco, USA) to favor the growth of conidia. Urease production was also used for identification purposes. A single sample was analyzed per patient (ie, samples taken for follow-up studies of previously diagnosed patients were excluded).

Results

Of the 2235 samples processed, 283 (12.7%) were positive, of which 195 were skin samples (68.9%), 52 were nail samples (18.4%), and 36 were hair samples (12.7%). The correlation between a positive microscopic examination and detection of species by culture was 79.5% for skin samples, 100% for hair samples, and 30.8% for nail samples.

The species isolated and the clinical forms of dermatophytosis are shown in Table 1. There were 151 samples corresponding to anthropophilic species (*Trichophyton rubrum*, *Trichophyton violaceum*, *Trichophyton tonsurans*, *Epidermophyton floccosum*, *Trichophyton schoenleinii*, and *Trichophyton soudanense*), accounting for 53% of all species isolated; 117 (41.3%) corresponding to zoophilic species (*Trichophyton mentagrophytes*, *Microsporum canis*, and *Trichophyton verrucosum*); and just 15 (5.3%) corresponding to geophilic species (*Microsporum gypseum*, *Microsporum nanum*, and *Microsporum racemosum*) (Figure 1). When analyzed by 4-year periods, the prevalence of infection by anthropophilic species increased from 28.8% between 1997 and 2000 to 57.1% between 2001 and 2004 and to 66.7% between 2005 and 2008. The most common species isolated in these cases were *T rubrum* (38.2%) (mostly in association with onychomycosis, tinea pedis, and tinea corporis); *T tonsurans* (5.6%) (tinea corporis, tinea faciei, and tinea capitis); *T violaceum* (4.9%) (tinea corporis, tinea pedis, and tinea capitis); *E floccosum* (2.8%) (tinea pedis and tinea cruris); and *T soudanense* (1.0%) and *T schoenleinii* (0.7%) (both in association with tinea corporis, onychomycosis, and tinea capitis).

The most common clinical form of dermatophytosis observed over the 12 years of the study was tinea corporis (35.3%), followed by onychomycosis (19.1%), tinea pedis (13.1%), and tinea capitis (12.4%). There were 4 cases in which 2 clinical forms were observed in the same patient: tinea pedis with onychomycosis in 2 patients and tinea

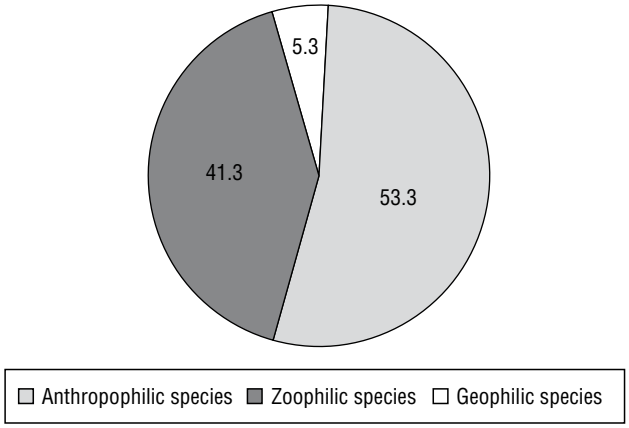

Figure 1 Distribution of dermatophytes in Cádiz, Spain (1997-2008).

Table 1 Clinical Forms of Dermatophytosis and Distribution of Species in Cádiz (1997-2008)

Dermatophytes	Tinea Corporis	Onycho-mycosis	Tinea Pedis	Tinea Capitis	Tinea Corporis	Tinea Faciei	Tinea Manuum	Total	Percentage
Tinea rubrum	25	36	25	1	14	0	7	108	38.2
Microsporum canis	35	0	0	17	1	9	1	63	22.3
Trichophyton mentagrophytes	16	8	6	4	3	2	5	44	15.5
Trichophyton tonsurans	6	1	1	3	1	4	0	16	5.6
Trichophyton violaceum	5	5	0	2	0	1	1	14	4.9
Microsporum gypseum	6	1	0	3	0	3	0	13	4.6
Trichophyton verrucosum	3	0	2	3	1	0	1	10	3.5
Epidermophyton floccosum	1	1	3	0	3	0	0	8	2.8
Trichophyton soudanense	1	1	0	1	0	0	0	3	1.0
Trichophyton schoenleinii	1	0	0	1	0	0	0	2	0.7
Microsporum nanum	1	0	0	0	0	0	0	1	0.3
Microsporum racemosum	0	1	0	0	0	0	0	1	0.3
Total	100	54	37	35	23	19	15	283	100
Percentage	35.3	19.1	13.1	12.4	8.1	6.7	5.3	100	100

Table 2 Annual Distribution of Dermatophyte Species Isolated in Cádiz, Spain (1997-2008)

Dermatophytes	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Tinea rubrum	3	4	1	2	4	4	11	27	12	18	11	11
Microsporum canis	2	5	3	3	9	5	5	7	6	5	6	7
Trichophyton mentagrophytes	7	9	3	2	1	6	5	2	2	3	1	3
Trichophyton tonsurans	0	0	0	0	4	2	1	1	3	1	3	1
Trichophyton violaceum	0	1	2	2	0	0	1	3	2	0	2	1
Microsporum gypseum	0	1	0	1	0	1	5	2	1	0	2	0
Trichophyton verrucosum	5	0	2	2	0	0	0	0	0	0	1	0
Epidermophyton floccosum	1	0	0	1	1	2	0	2	0	1	0	0
Trichophyton soudanense	0	1	0	0	0	0	0	0	0	1	1	0
Trichophyton schoenleinii	0	0	1	0	0	0	0	1	0	0	0	0
Microsporum nanum	1	0	0	0	0	0	0	0	0	0	0	0
Microsporum racemosum	1	0	0	0	0	0	0	0	0	0	0	0
Total	20	21	12	13	19	20	28	45	26	29	27	23

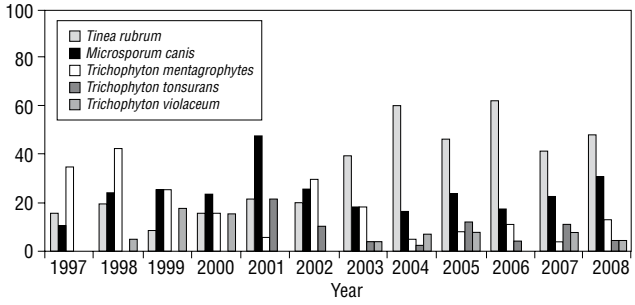

Figure 2 Changes in main species of dermatophytes in Cádiz, Spain (1997-2008).

pedis with tinea cruris, also in 2 patients. The most common infections caused by anthropophilic dermatophytes were onychomycosis (29.1%) and tinea corporis (25.8%), followed by tinea pedis (19.2%), tinea cruris (11.9%), tinea capitis (5.3%), and tinea faciei (3.3%). There were only 12 cases of anthropophilic infection detected in immigrants (7.9%): 5 due to *T rubrum* (4.6% of all cases of *T rubrum* infection), 1 due to *T tonsurans* (6.2%), 4 due to *T violaceum* (36.6%), 1 due to *T soudanense* (33.3%), and 1 due to *T schoenleinii* (50%).

Patient age ranged from 5 months to 87 years. Infections were most common in those aged between 20 and 30 years (27.2%), those aged over 50 years (23.3%), and those aged under 10 years (21.2%). Frequency of infection did not differ significantly between men (53%) and women (47%). Tinea corporis was mainly observed in children and young patients (64%), tinea capitis in children under 10 years (91.4%), tinea cruris in young patients (78.3%), and onychomycosis in patients over 50 years (85.2%).

Table 2 shows the species detected each year and Figure 2 shows the changes observed over the 12 years. There was a marked increase in the incidence of *T rubrum* from 2000 onwards; indeed this dermatophyte has become the main cause of dermatophytosis in our area in recent years. The incidence of *M canis* varied while that of *T mentagrophytes* decreased. Finally, there were outbreaks of infections due to *T tonsurans*, *T violaceum*, and *M gypseum*. In all years, there was a peak in the number of cases detected between February and June and between September and December.

Discussion

We observed a gradual but marked increase in the incidence of infection due to anthropophilic dermatophytes in the health care area of Cádiz between 1997 and 2008. In the 1990s, the 2 predominant dermatophytes in Cádiz and most other Spanish regions were the zoophilic species *T mentagrophytes* and *M canis* followed by the anthropophilic dermatophyte *T rubrum*.[8,17,18,20-27] In more recent years, however, *T rubrum* has become the predominant species in certain parts of Spain[3-8,13] and other parts of the developed world,[2,28] and we also found this to be the case for Cádiz. The species is still relatively uncommon, however, in many developing countries.[14-16] In our study, there was a marked increase in the incidence of *T rubrum*, particularly from 2003 onwards, and a decrease in that of zoophilic dermatophytes and *T mentagrophytes* in particular. This increase in anthropophilic infections, predominantly due to *T rubrum*, was accompanied by an increase in the number of patients consulting for onychomycosis and tinea pedis. According to our findings, *T rubrum* is currently the main pathogen responsible for all clinical forms of dermatophytosis in Cádiz.

The appearance and spread of infections caused by unusual anthropophilic dermatophytes in Spain is linked to increased tourism and immigration.[29] While few such cases are detected in Cádiz, which has low immigration levels, occasional infections due to *T violaceum*, *T soudanense*, and *T schoenleinii* have been detected in immigrants. Although there have been outbreaks of dermatophytosis due to *T tonsurans* since 2001, these have not been directly related to immigration. Prior to this date, *T tonsurans* was unknown in Cádiz and had always been relatively uncommon in the rest of Spain. *T violaceum* is becoming

increasingly common and occasional cases of infections due to *E floccosum*, *T soudanense*, and *T schoenleinii* are detected.

We also observed an increase in the geophilic dermatophyte *M gypseum*, which was the sixth most common cause of dermatophytosis in our study. The high incidence observed in 2003, which was unusual given the relative infrequency of this pathogen in humans, was related to a local outbreak caused by the infection of skin wounds and erosions through contact with soil or animals.[30] There were only a few other cases of dermatophytosis caused by soil-inhabiting dermatophytes. The single case of onychomycosis due to *M racemosum* was a highly unusual finding and indeed the first report of such a case in humans in Europe.[31]

The most common clinical form of dermatophytosis in our area was tinea corporis, followed by onychomycosis and tinea pedis, coinciding with the majority of reports published to date.[4,8,13,19,23,26] Although once common in Spain, the incidence of tinea capitis has decreased over the years with improved hygiene, although a recent surge in cases has been linked to immigration.[29]

Unlike our study, which showed no difference between men and women in terms of the frequency of dermatophytosis, the majority of Spanish studies have reported a predominance of men.[17,19] In agreement with previous reports,[4,8,21] all the clinical forms of dermatophytosis except tinea capitis and onychomycosis were more common in middle-aged patients, with a peak in cases in spring and autumn.

The low percentage of positive microbiological results observed is also consistent with reports from other studies[4,8,19] and is possibly due to the inconsistent quality of the samples. Proper collection of samples, thus, is key to improving diagnostic yield. It is important to perform mycological cultures to confirm clinical suspicion of dermatophytosis and identify the causal agent in all cases as this has important clinical and epidemiological implications and will help to tackle the source of contamination, prevent the spread of disease, and take appropriate treatment decisions.

The pattern of dermatophytosis in the health care area of Cádiz has changed with an increase in the incidence of cases caused by anthropophilic species. Epidemiological changes in different areas are closely related to improved diagnosis and the implementation of appropriate disease control measures.

Conflicts of Interest

The authors declare no conflicts of interest.

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