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**Epidemiology of *Trichophyton verrucosum* infection in Rennes University Hospital,
France: a 12-year retrospective study**

Short title: *Trichophyton verrucosum* infection in Brittany, France

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Abstract

Trichophyton verrucosum is a zoophilic dermatophyte, known as a causative agent of inflammatory mycoses of the skin and the scalp in humans. In this study, we reviewed all cases of *T.verrucosum* infection diagnosed in our laboratory over a 12-year period, to determine epidemiological and clinical characteristics. Among 18,340 samples analyzed, 5,186 cultured positive with dermatophytes (2674 patients), of which 64 samples (41 patients) were positive for *T.verrucosum*. Our data show that there was a strong influence of age on the type of lesion, with children and adults presenting more frequently with tinea capitis and skin infections, respectively ($p<0.0001$). Infection of children and adults resulted more frequently from indirect and direct exposure to cattle, respectively ($p<0.01$). We observed a marked increase of cases over the last four years, with a correlation of the number of cases and the mean annual rainfall ($p<0.05$), suggesting that increasing humidity favors cattle infection, and thereby, human infection. Whether this increase is the consequence of climate changes remains to be determined, but should be considered.

INTRODUCTION

Dermatophyte infections are worldwide superficial fungal infections, mainly due to *Trichophyton* and *Microsporum* genus. Both genera can be responsible for *tinea corporis* or *tinea capitis*, but the clinical type of dermatophytosis mostly depends on the species involved. It has been shown that the spectrum of dermatophyte infections has changed in Western Europe during the 20th century, with a dramatic increase of *T. rubrum* onychomycoses and a decrease of *tinea capitis*¹, probably in line with increased standard of living. Nowadays, the frequency of isolation of fungi responsible of *tinea capitis* depends on the exposure to animals (cats, rodents, cattle...), and on migration of population², thus it may differ according to countries or geographic areas. Knowledge of the local epidemiology of dermatophytes is important to guide the practitioner during consultation, as well as the mycologist during fungal identification. Few studies describe the recent epidemiology of dermatophytoses in France, in particular those due to animal dermatophytes.

Our laboratory of Parasitology and Mycology (University Hospital of Rennes) is a regional reference lab for parasitic and fungal infections and is located in a region of animal breeding, mainly of cattle and pig. Therefore, patients with recurrent or inflammatory skin infections or treatment failure are frequently referred to us, and we regularly diagnose *Trichophyton verrucosum* infections in patients with *tinea capitis profunda* or *tinea barbae*. This 12-year retrospective study aims at describing the epidemiology and the frequency of *T. verrucosum* infection, as well as the clinical characteristics of patients.

PATIENTS AND METHODS

Patients and samples

Patients were seen at the consultation unit of the Laboratory of Mycology and Parasitology of the University Hospital of Rennes. After clinical evaluation and questioning the patient,

samples were collected (scalp, skin scrapings, hair) and processed for mycological examination. Clinical information, including sex, age, site of lesions, type of lesion, occurrence of lesions in contact persons, profession, and contact with animals, was recorded on medical charts.

All patients with a mycological culture positive to *T. verrucosum* from January 2004 to March 2016, were retrospectively selected through the information system of the laboratory and included in the study. The study was approved by the ethics committee of the University Hospital of Rennes (approval No 16.96).

Mycological examination

For each sample, direct microscopic examination was performed using 30% KOH. Cultures were seeded on Sabouraud-chloramphenicol-gentamicine agar medium and Sabouraud-chloramphenicol-cycloheximide medium, and incubated at 25°C for at least 3 weeks. In addition, samples were cultured in Brain-Heart-Infusion (BHI) broth and incubated at 30°C. Identification of dermatophytes was based on macroscopic and microscopic examination of cultures. When identification was not possible, colonies were seeded on malt agar or other adequate medium to favor conidia formation. *T. verrucosum* could be identified in all cases without needing sequencing.

Statistical analysis

Quantitative data were compared using Mann-Whitney test. Qualitative data were analyzed using a Chi-square test or a Fisher's exact test. Association between variables was analyzed using Spearman's correlation test. Statistical analysis was made using GraphPad® Prism V5 (GraphPad software, USA). $P < 0.05$ was considered significant.

RESULTS

Over the 12-year period, a total of 18,340 samples were collected, of which 5,186 cultured positive for dermatophytes (2674 patients). Sixty-four samples from 41 patients were positive

to *Trichophyton verrucosum*. The frequency of *T. verrucosum* isolation was therefore 1.53% (41/2674). Regarding other zoophilic species, the frequency of isolation of *Microsporum canis* and *T. mentagrophytes* was 1.83% (49 patients) and 2.58% (69 patients), respectively. Besides, we observed two patients with *M. gypseum* over the period.

In *T. verrucosum* infected patients, males were predominant, with a sex ratio of 1.73 (26 men versus 15 women). About half of patients were below 16 year-old (19 patients, 34 samples), and 22 were over 17 years old (30 samples). There was no difference in the sex ratio according to age category (Table 1). Among these 64 positive samples, 25 were obtained from scalp, 6 from beard, and 33 from skin lesions (14 from lower limb, 6 from upper limb, 11 from face, 1 from thorax, 1 with no precision). Overall, 4, 16 and 21 patients were diagnosed with *tinea barbae*, *tinea capitis* and *tinea corporis*, respectively. Multiple sites of infection (beard and skin or hair and skin) were observed in 7 patients. Face lesions were more frequently observed in children than in adults ($p < 0.01$, Table 1). Multiple cutaneous lesions were observed in 14/28 patients, independently of age (Table 1). There was no influence of sex regarding the type and the number of lesions (Table 2). But there was a strong influence of age on the type of lesion, with children and adults presenting more frequently with *tinea capitis* and skin infections, respectively ($p < 0.0001$) (Table 1).

The lesion was described as a kerion in 13 cases, mostly observed on the scalp (10 cases), and there was no influence of age on kerion onset;(Tables 1&2).

The contamination resulted from professional exposure to cattle in 17 cases (breeding farmers) or from direct contact with a relative, simultaneously or previously diagnosed with a *T. verrucosum* infection, in 17 further cases. In the 7 remaining cases, no specific information was recorded on medical charts, but most of them lived in rural areas. Skin dermatophytosis was more frequently associated with direct contact with cattle ($p < 0.05$). Infection of children and adults resulted more frequently from indirect and direct exposure, respectively, as children most

often reported not to wander near animals ($p < 0.01$) (Table 1). Male patients were more often in direct contact with cattle and presented more frequently with a kerion than female patients (65% versus 40% and 46% versus 20%, respectively), although these differences were not statistically significant (Table 2).

As the survival and growth of fungal spores and mycelium are favored by humid environment, we searched for retrospective climatic data from Brittany during the study period (www.meteofrance.com). We observed a correlation of the number of cases and the mean annual rainfall ($p < 0.05$, Spearman correlation), but no correlation with the mean temperature (Figure 1).

DISCUSSION

The frequency of *T. verrucosum* isolation among other dermatophyte species is relatively high (1.53%), compared to some studies, as it was estimated at 0.04% in Italy³, 0.3% in the USA⁴, 0.01% in Czech Republic⁵, and 0% in a German study⁶. However, it was comparable to other European studies conducted in Poland (1%)⁷, Greece (1.8%)⁸ and Slovenia⁹ (0.9%). Breeding methods could account for these discrepancies of incidence in developed countries. In France, traditional outdoor breeding is still predominating and free wandering and gathering of animals could explain the acquisition and transmission of disease. A high rate of *T. verrucosum* infections has been reported in other geographical areas, such as Iran¹⁰ and Iraq¹¹, but the overall transmission rate of dermatophytes is higher than in Europe, and the contact with animals can be considered closer. As seen in tinea capitis infections¹², we observed more cases in male, than in female patients. This could be due either to hormonal factors or to the fact that women have less often occupational activities in close contact with cattle.

In the present study, a direct or indirect exposure to cattle was documented in 83% (34/41) of patients, confirming that contact with this animal reservoir is the main risk factor for *T.*

verrucosum infection. In about half of cases, the occurrence of lesions in animals was not noticed or acknowledged by the farmer. Although this fungal infection could be considered as a professional disease, there is no survey of human cases, neither official recommendation to vaccinate or to treat animals. Being considered as an old and rare disease to-date, this fungal infection becomes an ill-known disease for some practitioners from Western countries and can lead to diagnosis delay or misdiagnosis.

This strongly inflammatory mycosis, which is localized predominantly at the face in children, can lead to disfiguring lesions and to school eviction. Therefore, reliable diagnosis and treatment are needed. We use systematically BHI broth medium to improve and accelerate *T. verrucosum* growth. We do not know if this practice could explain a higher rate of positive cultures than in other regions. A recent study conducted in France in another rural area ¹³ showed a lower incidence of *T. verrucosum* infections (0.9%), but similar results for *M. canis* (1.8%). It should be noticed that molecular methods are still rarely used for the diagnosis of dermatophytosis, probably because they are considered as benign infections, which diagnosis is not urgent.

It is noteworthy that nearly half of patients acquired *T. verrucosum* infection through indirect exposure to cattle, as they lived in a farm or their relatives worked in a farm. This observation suggests that they acquired contamination through contact with spores present on the soil. The spores can persist up to 4 years in a viable and infectious form ¹⁴. As fungal growth and persistence is usually as better as humidity is higher, oceanic humid climate in Brittany could account for the higher rate of *T. verrucosum* isolation in our center. As we observed a trend in diagnosing more cases in latest years, we compared the annual number of cases to the annual rainfall observed in Brittany over the study period. Interestingly, there was a trend in observing more cases during rainy years, while fewer cases were diagnosed during dry years. Additionally, during the last four years, 24 cases were diagnosed, i.e. more than during the

previous 8 years (17 cases), suggesting an increasing incidence. Interestingly, we also observed an increase in *T. rubrum* isolation over the past 2 years, for which the meteorological records reported a 1 degree-increase of the mean annual temperature (data not shown). Whether this higher incidence is the consequence of climate changes remains to be determined, but should be considered.

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Disclosure of Conflict of Interest:

The authors have no conflicts of interest to declare

LEGENDS TO FIGURES

Figure 1: Correlation of the annual number of cases and the annual rainfall (mm) observed in Brittany, France, during the study period

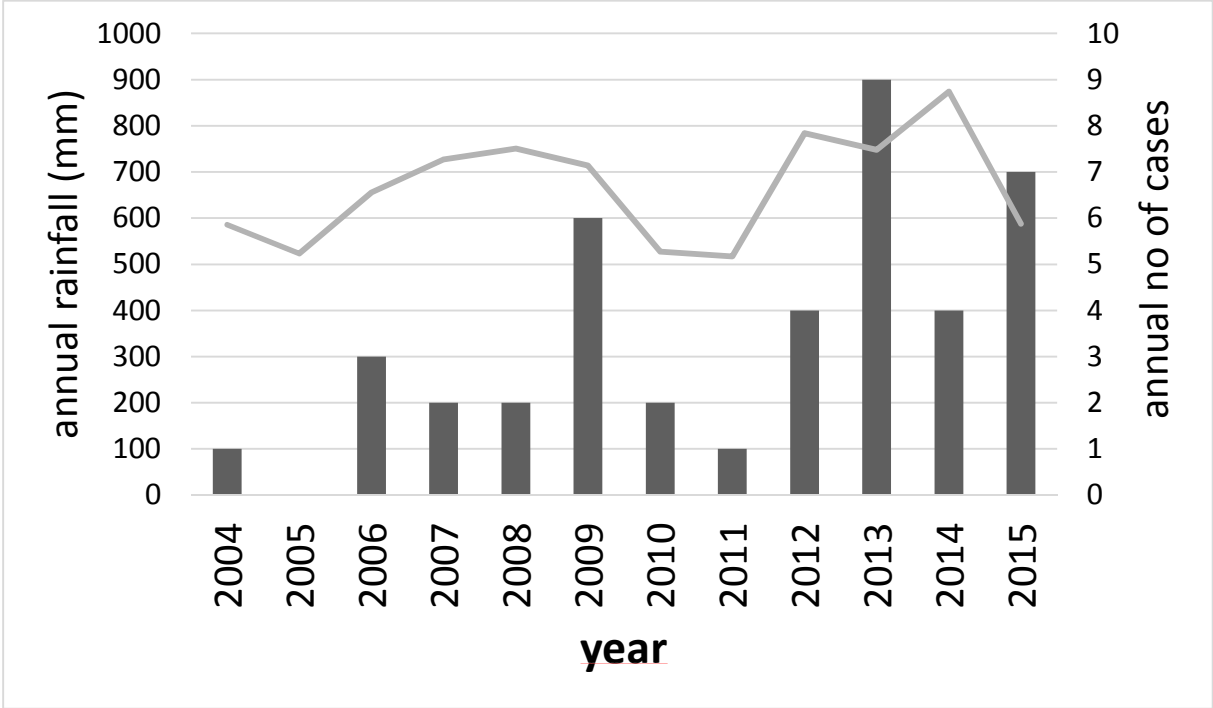


Table 1. Clinical and epidemiological characteristics according to age

Characteristics	Patient age (yrs)		p-value
	< 16 (n = 19)	≥ 17 (n = 22)	
Age, mean ± SEM	6.44 ± 4.41	40.96 ± 12.17	na
Sex ratio (M/F)	1.71	1.75	ns
Lesion site, n (%)			
Scalp ± skin	14 (74)	2 (9)	<0.0001
Skin only	5 (26)	16 (73)	
Beard	0 (0)	4 (18)	
Face lesion (skin, hair or beard)	17 (89)	11 (50)	<0.01
No of patients with multiple sites, n (%)	7 (37)	9 (41)	1, ns
No of patients with >1 skin lesion, n (%)	7 (37)	10 (45)	1, ns
Kerion, n (%)	9 (47)	5 (23)	0.114, ns
Mode of contamination, n (%)			
Direct contact with cattle	6 (32)	17 (77)	<0.01
Indirect	11 (58)	2 (9)	
Unknown	2 (11)	3 (14)	

SEM, standard error of the mean; yrs, years; na, not applicable; ns, not significant

Table 2. Clinical and epidemiological characteristics according to sex

Characteristics	Male (n = 26)	Female (n = 15)	p-value
Age, mean \pm SEM	23.96 \pm 19.71	25.51 \pm 19.7	
Lesion site, n (%)			0.261, ns
Scalp \pm skin	9 (35)	7 (47)	
Skin only	13 (50)	8 (53)	
Beard	4 (15)	0 (0)	
No of patients with multiple sites, n (%)	10 (56)	6 (40)	1, ns
No of patients with >1 skin lesion, n/N(%)	8/18 (44)	6/9 (67)	0.420, ns
Kerion, n (%)	12 (46)	3 (20)	0.177, ns
Mode of contamination, n (%)			0.248, ns
Direct contact with cattle	17 (65)	6 (40)	
Indirect	7 (27)	6 (40)	
Unknown	2 (8)	3 (20)	

SEM, standard error of the mean; yrs, years; na, not applicable; ns, not significant