Is Trichophyton simii endemic to the Indian subcontinent?

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Trichophyton simii is considered to be prevalent only in the Indian subcontinent where it was isolated from soil, as well as from infections of humans and animals. We have investigated a case of onychomycosis caused by this exotic dermatophyte, not traceable to endemic areas. This case, as in others due to this fungus in man or animals, that have been previously and sporadically reported worldwide, suggests infections caused by T. simii might be underestimated, especially outside its primary geographic areas. Indeed, there are isolates that do not show species-specific morphology, as in our case isolate, and as a result may be misidentified by classical methods. By checking the identity of some strains preserved in the collection BCCM/IHEM, we found several that proved to be T. simii, originating from non-endemic areas (Belgium, France and Ivory Coast). Therefore, the natural distribution of T. simii is probably not as restricted as has previously been proposed.

Keywords Trichophyton simii, Arthroderma simii, onychomycosis, ITS rDNA

Introduction

Whereas onychomycosis refers to any fungal infection of the nail, in tinea unguium the invasion of the nail plate is caused by dermatophytes. Tinea unguium is relatively common affecting about 3% of the population in most temperate countries [1]. Most lesions (up to 80%) occur on the feet and more specifically on the big toes [2]. Distal subungual infection is the most frequent invasion encountered. It first develops as a subungual hyperkeratosis at the distal border of the nail plate, which causes lifting of the free edge of the nail. The lesion may progress to the detachment of the nail plate from the nail bed (onycholysis), characterized by the discoloration of the nail plate. The most frequently identified dermatophyte species causing nail infections are T. rubrum and Trichophyton interdigitale. Trichophyton mentagrophytes and, to a lesser extent, Epidermophyton floccosum are also reported as isolated from nail specimens [1].

We have been confronted with a case of lateral onychomycosis with yellowish discoloration of the nail-plate of the big toe, caused by Trichophyton simii. This was rather surprising because this dermatophyte is considered as an exotic species as it supposedly is restricted to the Indian subcontinent where it causes lesions on monkeys and fowl [3]. Moreover, to our knowledge, this species was reported as an agent of nail infections only once before in North India [4].

Molecular identification is not always used in routine clinical laboratories where isolates are generally identified through the use of only phenotypic methods. In this case, the identification of T. simii was molecularly confirmed. Therefore, the identity of this isolate could not, in our estimation, be challenged, and we tried to find an explanation for the presence of this species in our region.

Materials and methods

Clinical history

A 21-year-old woman presented to her dermatologist in Brussels with an infection of the nail of the right big toe. The infection affected one third of the nail plate, with a yellowish discoloration in the lateral nail groove indicating a lateral onychomycosis. While at least half of the surface of the nail plate remained intact, at the distal part of the

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nail, the discoloration was spreading in the transverse direction from one lateral edge towards the other. The outer surface of this portion of the nail was crumbled and showed irregularities resulting from its progressive destruction (Fig. 1). Apart from the onychomycosis, the woman was in good health. She remembered that four years before she had a small spot located near the intersection between the right lateral and proximal nail edges. Over time, this spot gradually spread along the right edge of the nail. Later, someone stepped on her foot and her nail fell off in 2011. The new nail was quickly infected. She had no contact with animals, nor had she left Belgium except for a short stay in Spain two years before her visit to the dermatologist.

**Mycological investigation**

Scrapings of the nail were submitted to the Mycology & Aerobiology Service of the Scientific Institute of Public Health, Brussels. Microscopic examination of a nail scraping mounted in a 10% potassium hydroxide solution showed hyphal invasion (Fig. 2). A slant tube containing Sabouraud glucose agar supplemented with 0.5 mg/ml chloramphenicol, and another containing the same mixture supplemented with 2 mg/ml cycloheximide were inoculated with fragments of nail which resulted on both media with the development of identical fungal colonies. Colonial features and the microscopic morphology were examined from colonies on Sabouraud (SAB) and diluted SAB glucose agar. The case isolate was morphologically identified as *Trichophyton* spp., and sequencing of the ITS1-5.8S-ITS2 region was performed in order to obtain a correct identification at the species level.

**Therapy**

The patient was treated with terbinafine, 250 mg/day (Lami-sil, Novartis). The continuous 3-month oral treatment with terbinafine (250 mg/d) is considered the most effective method of treating toenail infections caused by dermatophytes [5]. The antifungal drug was well tolerated by the patient and no side-effects were observed. Fourteen weeks later, the coloured yellow surface had clearly regressed and the irregularities on its surface had disappeared. Cultures inoculated with new scrapings of the nail remained negative.

**Molecular identification**

Genomic DNA was extracted from the strain cultivated for at least 5 days in Sabouraud dextrose broth by using the Invisorb Spin Plant Mini Kit for DNA extraction (Invitek), according to the manufacturer’s instructions. Some adaptations were made to the protocol in that the fungal material was lyophilized prior to lysis and time of lysis was augmented to 2 hours. The ITS region was amplified and sequenced using the primers ITS5 and IT2 (5′-CCTCCGCTTATTGATATGCTTAGG-3′, modified from ITS4) [6]. PCR products were purified using the Wizard PCR Preps DNA Purification System (Promega). Sequencing was performed with the ABI 3130xl Genetic Analyser and ABI Prism BigDye Terminator 3.1 chemistry Cycle Sequencing Kit. To eliminate any undetermined sites, both forward and reverse sequences were analyzed and assembled using Lasergene SeqMan Pro v 8.1.3.418 (DNASTAR).
Results

On SAB at 25°C the case isolate produced flat colonies, cream-coloured, with a powdery surface and an unpigmented or slightly yellowish reverse. Microscopic examination of portions of colonies on diluted SAB glucose agar revealed abundant pyriform microconidia, 2.6–6.5 μm long by 1.6–2.7 μm wide, arranged along the hyphae, and in small groups. Numerous macroconidia, clavate to fusiform, regularly shaped, thin-walled and smooth, often produced in loose clusters, were present (Fig. 3). The macroconidia of this fungus were within the size range of and morphologically consistent with those of *T. mentagrophytes* sensu lato, i.e., 3–7 septate when mature (mostly 5) and measure 32–49 × 7–10 μm. No chlamydospores were observed. Subsequent subcultures tended not to produce macroconidia but still produced very large numbers of microconidia.

The sequence of the ITS region was used for a BLAST search in the GenBank database. Our sequence corresponded exactly (100% identity) to several *T. simii* sequences, including that of ex-type strain (IHEM 4420) previously deposited in GenBank (accession number is JQ407210). The new sequence was deposited at EMBL (HE962124), and the isolate is preserved in the BCCM/IHEM culture collection under accession number IHEM 25456.

Discussion and conclusion

The original description of *T. simii* dates back to 1912 when Pinoy described and named a fungus *Epidermophyton simii* that had been isolated from vesicular skin lesions of a monkey [7]. While it was subsequently reduced to synonymy with *T. mentagrophytes* [8], it was recognized 25 years later as the separate species *T. simii*. Indeed, it was shown by mating experiments that strains identified as *T. mentagrophytes* were in fact different species which are, morphologically very similar, but sexually incompatible [9,10]. The study that redescribed *T. simii* and perfect state, *Arthroderma simii* Stockdale, Mackenzie & Austwick, was published in 1965 [11]. In this report the fungus was isolated from ring-worm infections in monkeys, poultry, man and a dog, and, since all these infections originated in India, *T. simii* was regarded as being endemic to this region. According to authors, the strains of *T. simii* are characterized by scarce microconidia, that are predominantly clavate to pyriform, borne on simple hyphae and numerous macroconidia that differ from those of *T. mentagrophytes* by their tendency to degenerate rapidly with the formation of intercalary chlamydospores.

It is after the redescription of *T. simii* that surveys concerning its natural habitat were conducted. *Trichophyton simii* occurs sporadically in soil and most records are from India [12–15]. It can infect a broad variety of animals, and occasionally causes tinea corporis, tinea capitis, and tinea cruris in man. However, the incidence of this species in dermatophytoses in India is limited [16], with a prevalence of only 1% in various clinical samples [17]. A single case of an infection of fingernails (and hands) was reported in a worker in a poultry farm in North India [4]. The species was also reported from Sri Lanka where, over a period of 10 years, it was recovered from nine cases of tinea capitis. The explanation would be that the patients with *T. simii* infections lived in rural areas where they were likely to come into contact with infected animals [18]. It should be noted that during the same time period, 29 cases of tinea capitis caused by *T. mentagrophytes* were described in this same report. *T. simii* is thus a rather occasional infectious agent on this island off southern India (as is the situation in India).

Until 1972, no infections caused by this fungus had been reported outside India, except in a population of guinea baboons (*Papio papio*) of African origin which had been living for a short period of time in Paris [19]. Since then, other cases of non-endemic origin have been described and, when the infections could not be traced back to India, they remained unexplained. The review of the literature shows that over time there has been an increase in such cases. A number of infections caused by *T. simii* in humans and animals not traceable to the Indian subcontinent have indeed been diagnosed around the world, i.e., first in Brazil, [20], then in USA [21], again in France [22], in Saudi Arabia [23,24], in Argentina [25], in Iran [26], and now in Belgium. In addition to its involvement in clinical cases, this fungus was also once recovered from a beach in France [27].

![Fig. 3 Macro- and microconidia of *Trichophyton simii* on Sabouraud diluted 1/10 plus salts for 50 days (Nomarski optics). Scale bar: 20 μm.](https://academic.oup.com/mmy/article-abstract/51/4/444/1027652/678675)
With globalization, certain fungal infections occasionally emerge outside their region of endemicity, but they have always been considered as imported cases (unlike those mentioned above). The primary geographic distribution of *T. simii* is undoubtedly the Indian subcontinent because its prevalence there is higher, but the fact that it has been repeatedly observed worldwide, rather suggests that its distribution is less limited, contrary to what is assumed. This dermatophyte was indeed reduced to synonymy with *T. mentagrophytes* prior to 1965 and was thus invariably ignored before that date in case reports or soil surveys. In addition, not all isolates of *T. simii* have the species-specific morphological characteristics. Most isolates of the reports mentioned above were identified based on their microscopic examination, whereas atypical isolates of *T. simii* are likely to be misidentified as *T. mentagrophytes*. In our isolate, the macroconidia were extremely numerous and the macroconidia were not fragmented or transformed into intercalary chlamydospores, even after several months. Therefore, the formal identification of the fungus necessitated DNA sequencing. It is quite conceivable that isolates of *T. simii*, without striking characteristic morphology, may go unnoticed with routine identification methods, especially outside the Indian subcontinent, where this species is not expected to be encountered. Therefore, we cannot exclude that this species was involved in previous cases of dermatophytosis in Belgium.

*Trichophyton simii*, although infrequently recovered, have probably been underestimated, and its presence outside its geographical areas is probably more common than the number of case reports listed above. To test this assumption, we looked for possible isolates of this species in our collection. We found in the RV collection (Raymond Vanbreuseghem Collection – integrated in the BCCM/IHEM Collection) six other strains, from which the ITS rDNA sequences formally showed that they are representatives of *T. simii*. All these sequences were obtained in an identical way as described above and are deposited at EMBL. Accession numbers are given in Table 1. One of these strains is currently sterile, the other five produce large quantities of microconidia and only one produces macroconidia without chlamydospores. Years ago, these strains, presumptively identified, were sent by a clinical laboratory to the RV collection. Macroscopic and microscopic examinations of these strains had not allowed their identification, or had led to their misidentification. However, mating tests indicated that they were representatives of *T. simii*. We assume that, since these strains were not characteristic and not traceable to India, their identification remained putative. Now their identity could be confirmed with their DNA sequences. Two of these strains were isolated in 1984 from two Belgian patients with onychomycosis.

The case reported in this study was therefore not the first of onychomycosis caused by *T. simii* outside the Indian subcontinent, nor the first time that this species was involved in a human disease in Europe.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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<th>Strain no.</th>
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</table>

SA, single ascospore.

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