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Genotyping of *Trichophyton mentagrophytes* infections in animals in Italy through sequencing of the ITS region

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Objectives

Trichophyton mentagrophytes is a zoophilic dermatophyte which recognizes lagomorphs and rodents as primary hosts. The fungus can also infect other animals, such as dogs and cats. While *T. mentagrophytes* is a polymorphic sexual species, *T. interdigitale* is recognized as its clonal offshoot. This delineation is meaningful from a clinical point of view in human patients. *T. interdigitale* is exclusively anthropophilic and mainly causes non-inflammatory chronic tinea pedis or onychomycosis. *T. mentagrophytes* is predominantly of animal origin and often leads to the development of inflammatory lesions. These two dermatophytes form a species complex and have several ribosomal internal transcribed spacer (ITS) region genotypes. Identifying the ITS type allows species attribution and simultaneously strain typing. Many studies have been dedicated to this argument concerning human infections, while scarce information is available regarding animals. This study aimed to gain insights into the current epidemiology of *T. mentagrophytes* genotypes in animals.

Methods

The fungal isolates included in the study regarded cases involving various animal species seen at multiple veterinary clinics in Italy (n=39) and France (n=1) between 2005 and 2021. DNA was extracted from isolates cultured on Sabouraud dextrose agar using a commercially available kit (NucleoSpin® Tissue, Macherey-Nagel, Düren, Germany). PCR was performed with the primer pair V9G and LR3. PCR products were sequenced using ITS5 and ITS4 primers through a commercial service (Macrogen Europe). Using MEGA11 software (<https://www.megasoftware.net/>), ITS sequences were aligned with the currently recognized genotypes (six and twenty-two for *T. interdigitale* and *T. mentagrophytes*, respectively).

Results

Figure 1 shows the ITS Type attribution for our isolates within a phylogenetic tree that includes the currently recognized genotypes. A new genotype (that, following the nomenclature, we called XXVII) was found in two isolates coming from a dog and a cat living in the same city. Figure 2 shows the distribution of the genotypes according to the animal host.

Twenty-three samples out of 40 (57.5%) belonged to the ITS Type III*. It was the lone found in rabbits and the most prevalent in cats. This finding agrees with past literature, which reported a wide distribution of this ITS type in European animals. Of note is the high number of isolates with ITS Type II* found in dogs. ITS Type II* differs only by one nucleotide substitution from *T. interdigitale* and is considered an “intermediate” entity between it and *T. mentagrophytes*. Clinical pictures, as well as molecular data, would suggest attributing this genotype to *T. interdigitale*. On the other hand, it has been detected from animal sources (chinchilla, guinea pig, and brown rat) which would justify its interpretation as *T. mentagrophytes*. Our data support the latter possibility.

Though we could not have a detailed description of all the dogs harbouring ITS Type II*, it is noteworthy that many showed the same clinical presentation, i.e. exfoliative chronic disseminated alopecia. Moreover, in most cases, despite the extensive lesions, the infection was not transmitted to the owners.

Conclusions

This study adds information on the molecular epidemiology of *T. mentagrophytes* infections in animals.

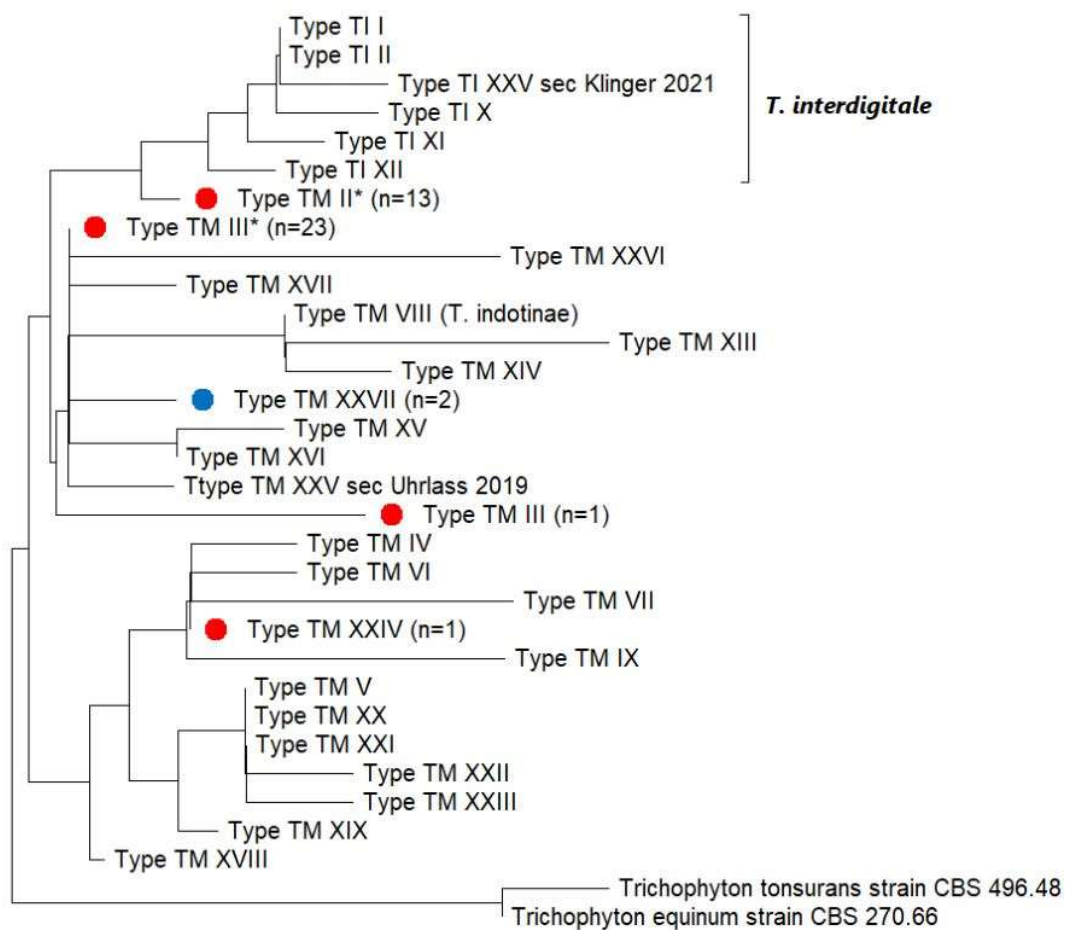


Fig. 1

Summary of *T. mentagrophytes* infection cases included in the study with the corresponding ITS Types

	ITS Type	Animals involved					tot
		II*	III*	III	XXIV	XXVII	
Dog		11 (61.1%)	6 (33.3%)	0	0	1 (5.6 %)	18
Cat		0	5 (71.4%)	1* (14.3%)	0	1 (14.3%)	7
Rabbit		0	10 (100%)	0	0	0	10
Guinea pig		2 (100%)	0	0	0	0	2
Chinchilla		0	0	0	1 (100%)	0	1
Other animals**		0	2 (100%)	0	0	0	2

* Isolate coming from France; all the other isolates came from Italy; ** one hunted chamois, two rhinoceros hosted in a Bio-Park

Fig.2