INTRODUCTION

Dermatophytes are fungal pathogens that invade the keratinised tissues of humans and animals.¹ The taxonomy of these organisms has been revised recently through a multilocus phylogenetic approach² and is still rapidly evolving. One fungus mainly involved in these changes is *Trichophyton benhamiae*, included in the homonymous species complex.² As summarised in Čmoková et al.,³ this species was first described in 1967 as *Arthroderma benhamiae* from human and canine infections in North America. Subsequently, two races were recognised based on biological compatibility experiments: an “Americana-European” and an “African” race. Furthermore, two phenotypic variants (a “yellow” and a “white” phenotype) were progressively identified among the Americano-European race. Human infections caused by yellow phenotype strains, contracted mostly from guinea pigs, have increased since 2010 in various European countries. The fungus is distributed widely in guinea pig breeding populations and pet shops, with animals typically showing scarce or no evidence of disease.⁴ The reason for the sudden increase of this variety is unclear, because the popularity of guinea pigs as pets began before the rise of human infections. Therefore, the cause may be the rise of a new, virulent and highly transmissible genotype.³ White phenotype strains probably have existed worldwide for many years. Indeed, sporadic human and animal infections were described before the diffusion of yellow phenotype strains in Europe.³ Notably, the white phenotype is very similar to *T. mentagrophytes*. Thus, the epidemiology of *T. benhamiae* infection has been elucidated only as a result of the increasing application of molecular techniques for fungal identification.²,⁵ Finally, a recent study³ showed that isolates previously designated as the Americano-European race of *A. benhamiae* harbour three species and two...
varieties. The yellow phenotype strains correspond to *T. benhamiae* var. *luteum*, which has a wide distribution in guinea pigs in Europe. 

The white phenotype corresponds to *T. benhamiae* var. *benhamiae* (found mainly in the USA), *T. japonicum* (distributed mainly in Asia) and *T. europaeum* (the second most common species affecting guinea pigs in Europe). 

The African race is more distinctly related phylogenetically and is now classified as *T. africanaum*. Its distribution, although less well-characterised as a consequence of the low number of available isolates, is mainly on the African continent.

In order to aid veterinary practitioners in becoming familiar with these newly described dermatophytes, we herein describe some cases of infection in various animal species (guinea pig, chinchilla, dog) due to *T. benhamiae* var. *luteum* and *T. europaeum*.

**CASE DESCRIPTIONS AND METHODOLOGY**

Nine cases (some involving multiple animals), examined at the Department of Veterinary Sciences (Turin University, Italy) between 2007 and 2021, showed alopecic patches with various degrees of inflammation (Table 1; Figures 1 and 2). The material collected for laboratory diagnosis included hairs plucked using sterile forceps, and hair and scales obtained by pressing a piece of transparent cellophane tape against the lesions (sticky tape technique). 

The procedure for microscopic examination (performed in five cases) varied according to the sample type. Hairs were suspended in mineral oil on a microscope slide. Samples including abundant keratinous material were cleared in 20% NaOH before microscopy. The tape pieces were placed on a microscope slide over a drop of stain (blue solution of the Hemacolor kit (Sigma-Aldrich; Waltham, MA, USA) or lactophenol blue). Cultures were performed on Mycobios Selective Agar (Biolife; Milan, Italy) at 25°C. Results of laboratory exams are reported in Table 1, with some examples shown in Figures 3 and 4. Human contagion was never reported. Available information on therapies is summarised in Table S1 in Supporting information.

Fungal identification was achieved by sequencing the internal transcribed spacer region (ITS), one of the molecular markers most employed in dermatophyte taxonomy studies. DNA was extracted 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 MEGA 11 software (https://www.megasoftware.net/), ITS sequences were aligned with those of the species belonging to the *T. benhamiae* complex deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and a phylogenetic tree was constructed by the neighbour-joining analysis. The robustness of the branches was assessed by bootstrap analysis with 1,000 replicates. The tree (shown in Fig. S1), allows subdivision of the complex into three clades/lineages (*T. benhamiae*, *T. erinacei* and *T. bullosum*) and eleven species. Our isolates were identified as *T. benhamiae* var. *luteum* (six cases) and *T. europaeum* (three cases). Representative sequences concerning our fungal isolates were deposited in GenBank (Table 1).

**DISCUSSION**

Our findings confirm that in Europe, *T. benhamiae* var. *luteum* is the most prevalent species from the *T. benhamiae* clade to cause guinea pig infections, and adds the chinchilla to the list of rodents that may harbour this fungus. Another recognised host is the common degu (*Octodon degus*). To our knowledge, the cases caused by *T. europaeum* are the first ever to be reported in dogs. This fungus has been isolated predominantly from guinea pigs in previous reports. Other proven infection cases have included rabbits and foxes.

Not unexpectedly, we did not identify other species of the clade. *Trichophyton benhamiae* var. *benhamiae* occurs mainly in the USA; it seems to have a broader range of primary host including dogs, foxes and the North American porcupine (*Erethizon dorsatum*), a close relative of the guinea pig. *Trichophyton japonicum*, responsible for most rabbit and guinea pig dermatophyte infections in Japan, also occurs in Europe and with low prevalence. *Trichophyton concentricum* is the lone anthropophilic species of the entire complex and is distributed in Oceania, Southeast Asia, and Central and South America. *Trichophyton persicorum* was identified in several human cases and a cat, mainly in southern and western Iran. *Trichophyton spiralisforme* is known only from a case in a Czech person who probably contracted the infection from a dog.

The lesions in the guinea pigs described herein (alopecia with scaling and crustng, located principally on the head) correspond to what has been reported previously. The presentation in chinchillas was very similar to that of the guinea pigs. The two dogs showed highly inflammatory forms that recall those reported during infections due to “sylvatic” dermatophytes, such as *T. erinacei*, *T. mentagrophytes* and *Nannizia* (formerly *Microsporum*) *persicolor*. Such diseases derive from digging activity in soils contaminated by fungal elements or direct contact with wildlife. Lesions occur on the muzzle and extremities, as in the cases presented here. Contact with guinea pigs (the primary host of *T. europaeum*) was not recorded in the case histories for the dogs in our report, yet it cannot be ruled out, especially for Case 3 that was free to roam in a rural context. Other sources of infection may have been foxes and rabbits.

Concerning direct microscopic examination results, the fungal elements were abundant and easily visualised in samples acquired from the guinea pigs and chinchillas. The sticky tape technique represented a practical and straightforward way of sampling scales and hairs. This technique makes it possible to stain the sample, which improves the visualisation of hyphae and arthroconidia. For samples that include...
| Case | Year | Animals | Environment of life | Clinical picture | Direct hair/scale examination | Colonies macro- and micromorphology | Molecular Identification (ITS) |
|------|------|---------|---------------------|------------------|-------------------------------|-------------------------------------|--------------------------------|
| 1    | 2007 | Three long-haired guinea pigs (3-year-old female; 4-month-old female; 1-year-old male) | Cage with unfamiliar breeding. The male had been introduced recently (possible source of infection) | Multifocal alopecia with abundant scaling. Plucking of hair tufts lumped by keratinous debris (follicular casts) allowed observation of highly inflammatory lesions (Figure 1a–d) | A massive number of arthroconidia in chains or clusters. The hairs appear to be nearly “substituted” by the fungal elements (Figure 3a) | Yellow, exceptionally bright on the plate reverse (Figure 4a). Sparse pyriform microconidia | T. benhamiae var. luteum* |
| 2    | 2010 | Guinea pig | Indoor (cage) | Alopecic patches on the nose and ears | Not performed | White, brown reverse. Sparse pyriform microconidia | T. europaeum |
| 3    | 2013 | Dog; 2-year-old neutered female; mixed-breed | Outdoor, free to roam (rural area with horse stables and rabbit farms) | Pruritic alopecic area on the hind left limb | Hairs invaded by rare, regular, long hyphae along the major shaft axis, without arthroconidia (Figure 3d) | Granular, white, deep orange to brown reverse. Abundant pyriform microconidia (Figure 4) | T. europaeum MZ960421 |
| 4    | 2018 | Dog; 3-year-old male; pit bull | Box in a courtyard, with three other dogs (healthy and negative on culture by the toothbrush technique) | Alopecic areas affecting the muzzle and the head, with erythema, erosions and haemorrhagic crusts (Figure 2) | Not performed | Granular, white, pale brown/orange reverse. Pyriform microconidia and spiral hyphae (Figure 4f,g) | T. europaeum MZ710118 |
| 5    | 2018 | Long-haired guinea pig; 4-month-old male | Indoor (cage) | Pruritic, alopecic exfoliative areas on the left flank and ear | Not performed | Pale yellow, more vivid on the plate reverse. Rare pyriform microconidia (Figure 4e) | T. benhamiae var. luteum* OK335270 |
| 6    | 2018 | Guinea pig; 2-month-old male | Indoor (cage) | Exfoliative and crusty lesions on the nose and ears | Not performed | Yellowish white, pale orange/brown reverse. Sparse microconidia | T. benhamiae var. luteum* |
| 7    | 2020 | Three guinea pigs | Abandoned and hosted in a rescue centre | Areas of exfoliative alopecia on the nose and flanks (Figure 1e) | Hair fragments and scales invaded by 2–3 μm round-to-oval arthroconidia (Figure 3b) | Yellowish white, pale orange reverse (Figure 4c,d). Sterile, only hyphae | T. benhamiae var. luteum* OK335263 |
| 8    | 2021 | Three long-haired guinea pigs | Cage within a household | Alopecic, crusty areas on the ears | Hair fragments and scales invaded by 2–3 μm round-to-oval arthroconidia | Yellowish white, yellow reverse. Sterile, only hyphae | T. benhamiae var. luteum* OK335264 |
| 9    | 2021 | Two chinchillas (one male, one pregnant female) | Cage with unfamiliar breeding | Exfoliative alopecic lesion on the nose (Figure 1f) | Numerous branching hyphae within the scales (Figure 3c). Hair fragments and scales invaded by 2–3 μm round arthroconidia | Pale yellow, more vivid on the plate reverse. Sterile, only hyphae | T. benhamiae var. luteum* OK335269 |

*Differentiation of T. benhamiae var. benhamiae based on colony reverse (yellow, var. luteum; brown or red-brown, var. benhamiae) and colony texture (submerged, velvety or cottony, var. luteum; granular/powdery, var. benhamiae).
abundant keratinous material (as in Case 1), use of a
clearing agent (NaOH) allows for easier interpretation
(Figure 3a). A direct diagnosis was more challenging in
Case 3 (a dog) owing to the absence of arthroconidia
and the position of the hyphae, which were hardly visi-
ble within the hair shaft (Figure 3d).

All fungal isolates grew easily in culture, confirm-
ing what is known for these species. A detailed
mycological description of the T. benhamiae clade
can be found elsewhere. To summarise, the more
widely distributed zoophilic species (T. benhamiae
var. benhamiae, T. europaeum and T. japonicum) have
a “T. mentagrophytes-like” appearance (Figure 4).
Colonies are white, velvety, powdery to granular,
with a brown, orange or brown/red reverse pigment. Microscopically they show sparse to abundant pyriform microconidia, and usually also cigar-shaped macroconidia and spiral hyphae, especially in older colonies. *Trichophyton benhamiae* var. *luteum* has a yellow pigmentation of the colony reverse. It typically exhibits poor sporulation and slower growth compared with the other species. It produces pyriform microconidia and no macroconidia, and spiral hyphae. Molecular differentiation of the two varieties of *T. benhamiae* can be achieved by the use of microsatellite markers. Notably, the yellow pigmentation may make it challenging to differentiate *T. benhamiae* var. *luteum* from *Microsporum canis*, also because the latter strains sometimes do not produce conidia in culture. A further complication in distinguishing between these species is that the morphological traits may vary as a function of culture conditions. For example, yellow pigmentation is not always evident in primary cultures (Figure 4c,d). Therefore, definitive identification is possible only through molecular analyses.

Despite the zoonotic potential of these pathogens, human involvement was not reported in our cases. One reason may be the clear evidence of lesions (Figure 1) that discouraged contact with the animals. Moreover, the guinea pigs and the chinchillas were confined to cages most of the time, while the dogs were housed outdoors. Although further studies are necessary for confirmation, dogs most likely represent “accidental” hosts for these fungi, which may be another explanation for the lack of human contagion.

**CONFLICT OF INTEREST**
None declared.

**AUTHOR CONTRIBUTIONS**
Andrea Peano: Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. Vit Hubka: Conceptualization; data curation; methodology; writing – review and editing. Paola Cavana: Investigation; writing – review and editing. Chiara Ottino: Investigation; writing – review and editing. Miriam Blandolino: Investigation; writing – review and editing. Anna Rita Molinar Min: Conceptualization; data curation; formal analysis; methodology; writing – review and editing. Mario Pasquetti: Data curation; formal analysis; methodology; writing – original draft; writing – review and editing.

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**REFERENCES**
1. Chermette R, Ferreiro L, Guillot J. Dermatophytoses in animals. Mycopathologia 2008;166:385–405.
2. de Hoog GS, Dukik K, Monod M, et al. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia 2017;182:5–31.
3. Čmoková A, Kolafik M, Dobiáš R, et al. Resolving the taxonomy of emerging zoonotic pathogens in the *Trichophyton benhamiae* complex. Fungal Divers 2020;104:333–387.
4. Berlin M, Kupsch C, Ritter L, et al. German-wide analysis of the prevalence and the propagation factors of the zoonotic dermatophyte *Trichophyton benhamiae*. J Fungi. 2020;6:161.
5. Sieklucki U, Oh SH, Hoyer LL. Frequent isolation of *Arthroderma benhamiae* from dogs with dermatophytosis. Vet Dermatol. 2014;25:39–e14.
6. Moriello KA, Coyer K, Paterson S, et al. Diagnosis and treatment of dermatophytosis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2017;28:266–e68.
7. Alastruey-Izquierdo A, Hoffmann K, de Hoog GS, et al. Species recognition and clinical relevance of the zygomycetous genus *Lichtheimia* (syn. Absidia pro parte, Mycocladus). J Clin Microbiol. 2010;48:2154–2170.
8. White TJ, Bruns T, Lee S et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis
SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

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