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Veterinary Dermatology

CASE REPORT

Cases of dermatophytosis caused by *Trichophyton benhamiae* var. *luteum* and *T. europaeum*, newly described dermatophytes within the *T. benhamiae* complex

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Abstract

Trichophyton benhamiae var. *luteum* and *T. europaeum* – recently described dermatophytes within the *T. benhamiae* complex – were identified in nine cases of dermatophytosis involving guinea pigs, chinchillas and dogs. The diagnosis was obtained through direct hair/scale examination, culture and sequencing of the internal transcribed spacer region of ribosomal DNA.

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 approach[2] 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 "Americano-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 study[3] 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.[3,4] 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).[3] The African race is more distantly related phylogenetically and is now classified as *T. africanum*. Its distribution, although less well-characterised as a consequence of the low number of available isolates, is mainly on the African continent.[3]

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).[6] 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.[6] 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.[2,3,5] DNA was extracted using a commercially available kit (NucleoSpin Tissue, Macherey-Nagel; Düren, Germany). PCR was performed with the primer pair V9G and LR3.[7] PCR products were sequenced using ITS5 and ITS4 primers[8] 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[3,9] 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.[3,9,10] 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,[3] and adds the chinchilla to the list of rodents that may harbour this fungus. Another recognised host is the common degu (*Octodon degus*).[3] 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.[3,10] Other proven infection cases have included rabbits and foxes.[3,11,12]

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.[3,5]*Trichophyton japonicum*, responsible for most rabbit and guinea pig dermatophyte infections in Japan, also occurs in Europe and with low prevalence.[3]*Trichophyton concentricum* is the lone anthropophilic species of the entire complex and is distributed in Oceania, Southeast Asia, and Central and South America.[3]*Trichophyton persicum* was identified in several human cases and a cat, mainly in southern and western Iran. *Trichophyton spiraliforme* is known only from a case in a Czech person who probably contracted the infection from a dog.[9]

The lesions in the guinea pigs described herein (alopecia with scaling and crusting, located principally on the head) correspond to what has been reported previously.[3] 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*[2]) *persicolor*.[1] Such diseases derive from digging activity in soils contaminated by fungal elements or direct contact with wildlife.[1] Lesions occur on the muzzle and extremities, as in the cases presented here. Contact with guinea pigs (the primary host of *T. europaeum*[3]) 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.[3,11,12]

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 (Figure 3b). For samples that include

TABLE 1 Overview of dermatophytosis cases: history, clinical aspects and laboratory results. The last column reports the molecular identification based on internal transcribed spacer (ITS) sequencing and the accession codes of representative sequences deposited in GenBank

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 infamiliar 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 infamiliar 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*).

FIGURE 1 Clinical presentation of dermatophytosis cases in guinea pigs and chinchillas (a–e) Guinea pig: highly inflammatory lesions revealed after plucking hair tufts lumped by keratinous debris; numerous scales entrapped in the hairs; exfoliative alopecic lesion on the nose. (f) Chinchilla: exfoliative lesion on the nose

FIGURE 2 Dog: areas of alopecia affecting the muzzle and the head, with erythema, erosions, and haemorrhagic crusts

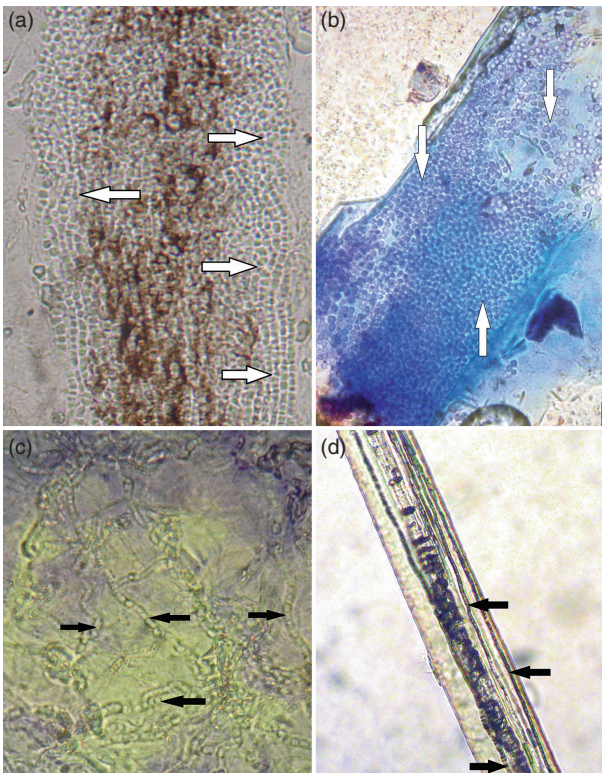

FIGURE 3 Direct microscopic examination showing fungal elements invading hair and scales (black arrows, hyphae; white arrows, arthroconidia in chains or clusters) (a) Hair cleared in 20% NaOH; (b,c) scales collected by the sticky tape technique and stained with the blue solution of the Hemacolor kit (Sigma-Aldrich); (d) hair observed in paraffin oil

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 visible within the hair shaft (Figure 3d).

All fungal isolates grew easily in culture, confirming what is known for these species.[3] A detailed mycological description of the *T. benhamiae* clade can be found elsewhere.[3,9] 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,

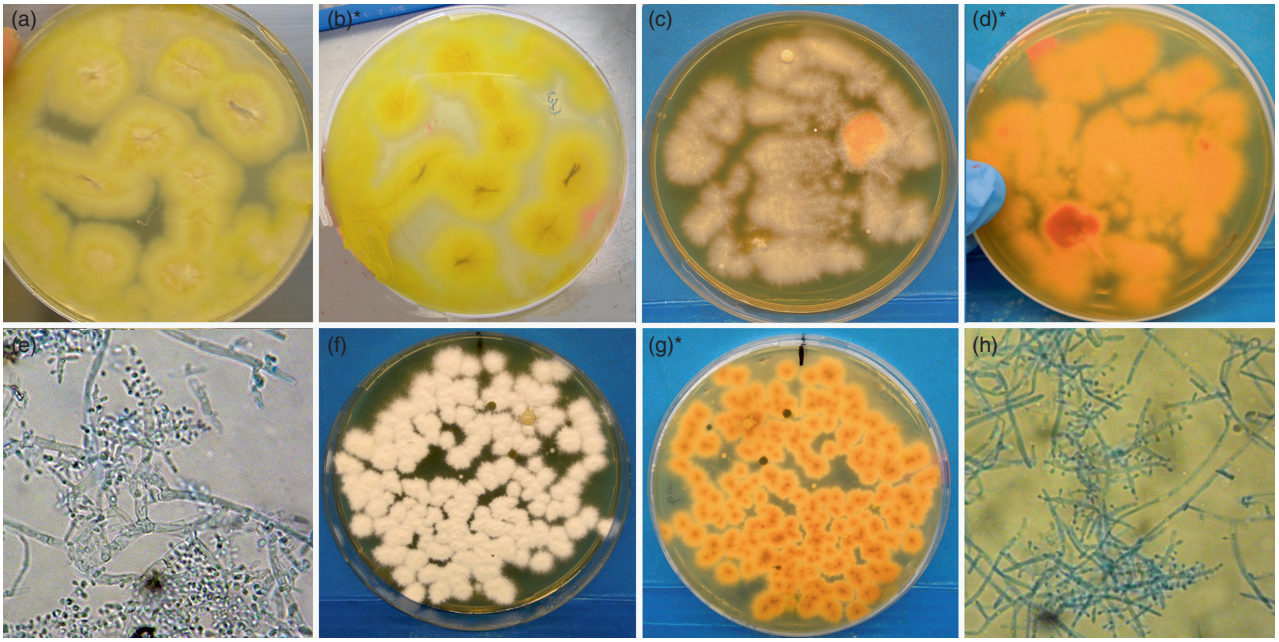

FIGURE 4 Macro- and micromorphology of some fungal isolates obtained from dermatophytosis cases. (a–e) *Trichophyton benhamiae* var. luteum; (f–h) *T. europaeum*. *Plate reverse

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.[3] 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.[3] 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.[3]

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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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Résumé

Trichophyton benhamiae var. *luteum* et *T. europaeum* – dermatophytes récemment décrits au sein du complexe *T. benhamiae* – ont été identifiés dans neuf cas de dermatophytose de cobayes, de chinchillas et de chiens. Le diagnostic a été obtenu par examen direct des poils/écailles, culture et séquençage de la région ITS de l'ADN ribosomique.

Resumen

Trichophyton benhamiae var. *luteum* y *T. europaeum*, dermatofitos recientemente descritos dentro del complejo *T. benhamiae*, se identificaron en nueve casos de dermatofitosis que involucraron a cobayas, chinchillas y perros. El diagnóstico se obtuvo a través del examen directo de pelo/escamas, cultivo y secuenciación de la región espaciadora transcrita interna del DNA ribosómico.

Zusammenfassung

Trichophyton benhamiae var. *luteum* und *T. europaeum* – zwei unlängst beschriebene Dermatophyten innerhalb des *T. benhamiae* Komplexes – wurden bei neun Fällen mit einer Dermatophytose, die Meerschweinchen, Chinchillas und Hunde betraf, identifiziert. Die Diagnose wurde mittels direkter Untersuchung von Haaren/Schuppen, Kultur und Sequenzierung der internen transkribierten Spacer Region der ribosomalen DNA, gestellt.

要約

モルモット、チンチラ、イヌの皮膚糸状菌症9例において、*Trichophyton benhamiae* var. *luteum* および*T. europaeum* (*T. benhamiae* complexに属する近年報告された皮膚糸状菌) が同定された。診断は、被毛・鱗屑の直接鏡顕、培養、リボソームDNAの内部転写スペーサー領域の塩基配列決定により行われた。

摘要

本哈米变种橙色毛癣菌和欧细辛毛癣菌——最近报道的本哈米毛癣菌复合体内的皮肤癣菌——在9例豚鼠、黄鼠和犬的皮肤癣菌病中被发现。通过直接毛发/皮屑检查、核糖体DNA内转录间隔区培养和测序获得诊断。

Resumo

Trichophyton benhamiae var. *luteum* e *T. europaeum* – dermatófitos recém descritos dentro do complexo *T. benhamiae* – foram identificados em nove casos de dermatofitoses envolvendo porquinhos da Índia, chichilas e cães. O diagnóstico foi obtido por exame direto de pelos e escamas, cultura e sequenciamento da região espaçadora transcrita interna do DNA ribossomal.