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Captive Wild Rabbits As Carriers Of Dermatophytes In Portugal

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ABSTRACT
The wild rabbit (*Oryctolagus cuniculus*) is one of the most important small game species in the Iberian Peninsula, but there is no information about the presence of dermatophytes in this species. Dermatophytosis or ringworm infection is a superficial skin infection with veterinary and public health importance. Data on dermatophyte carriage are crucial for epidemiological assessment and also for the design of potential control strategies in game farms, farmers and hunters. An epidemiological dermatophytosis survey was carried out in captive wild rabbits without clinical signs in Northern of Portugal, between August and December 2019. Specimen sampling was performed in 71 hair samples collected using the brush technique (MacKenzie). In this study, dermatophytes were identified in samples from five wild rabbits. The overall occurrence of dermatophyte positive animals was 7.0% (95% confidence interval: 2.3–15.6%). *Trichophyton mentagrophytes* was the only dermatophyte species isolated. This study suggests that wild rabbits may be carriers of *T. mentagrophytes* and are a potential source of infection for the farmer, hunters and other animals. Considering the scarcity of epidemiological reports in rabbits and the absence of information in this small game animal, these results can be a useful contribution towards the diagnosis and prevention in captive wild rabbit and farmers.

Keywords: Carrier; Dermatophytes; Occurrence; Subclinical; *Trichophyton mentagrophytes*; Wild rabbits

Introduction
Dermatophytosis is one of the most important zoonotic skin diseases, with dermatophytes having the ability to invade keratinized tissue to produce superficial mycoses in both animals and humans (Drouot et al., 2009). Three ecological groups of dermatophyte species are recognized, namely anthropophilic, zoophilic and geophilic. According to the new classification of dermatophytes by De Hoog et al. (2017), dermatophyte species have been grouped into seven genera: *Arthroderma*, *Epidermophyton*, *Trichophyton*, *Lophophyton*, *Microsporum*, *Nannizzia* and, *Paraphyton*. In general, zoophilic and geophilic species, as those in the *Trichophyton mentagrophytes* complex, cause lesions in humans that are more inflammatory than those induced by anthropophilic species (Drouot et al., 2009; Thakur and Kalsi, 2019). Infection occurs by direct or indirect contact with infected hair, scales or other materials (Chermette et al., 2008; Cruz et al., 2014).

Captive wild rabbits (*Oryctolagus cuniculus*) are very important among the managed small game species in the Mediterranean region (Machado et al., 2017), considering their relevant role in food chains, as those animals are an important prey for more than 30 predators (Delibes-Mateos et al., 2008). Decline in the wild rabbit population led to the development of rearing of wild rabbits in captivity systems for their subsequent release. Producers can breed and keep wild rabbits in a variety of conditions, ranging from a virtually absolute wild regime (rabbits living in colonies in enclosures) to very artificial ones (rabbits raised in cages) (González-Redondo & Sánchez-Martínez, 2014). In game farms, animals are subject to stress induced by capture, handling, captivity and transportation (Teixeira et al., 2007).

In rabbits, dermatophytosis most often occurs in kits, and the most common fungus identified in rabbits with dermatophytosis is *T. mentagrophytes* (Van Rooij et al., 2006). The *T. mentagrophytes* complex has in recent years received considerable attention due to increasing infections in humans (Tekin et al., 2019). Clinical signs of infection in rabbits vary from alopecia scaling, crusting or coalescing lesions with raised erythematous borders on the head, ears, back and limbs. The disease is usually self-limiting (Coelho et al., 2011; Moreira et al., 2012). In the Iberian Peninsula, few studies have been done to investigate ringworm occurrence in domestic rabbits (Torres-Rodríguez et al., 1992; Moreira et al., 2012; Mesquita et al., 2016), but the occurrence of dermatophytosis in captive wild rabbits has not been investigated, to the best of our knowledge.

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In Mediterranean countries, hunting wild rabbits is an activity of remarkable economic importance. The present study was done in response to concerns about the suspected occurrence of dermatophytosis in captive wild rabbits in the North of Portugal, in order to clarify potential sources of zoophilic infections, understand its economic impact and prioritize the allocation of resources to the control of this disease.

Methods

The study was conducted on 71 wild rabbits (*O. cuniculus*) without clinical signs from three privately-owned farms located in the North of Portugal. The animals were from captivity farms and were raised in semi-natural conditions. Samples were taken from the skin of the rabbits in a period between August and December 2019. For sample collection the Mackenzie toothbrushes technique was used (Moriello, 2001). Data as sex and age were recorded for each animal (Table 1).

Cultures were performed from samples collected from each animal. Individually sterilized human toothbrushes were used for each rabbit. The brush technique (MacKenzie) collected debris from the surface of the hair and from the surface of the skin. To ensure sufficient material for culturing, each brush was longitudinally combed for 1 min over the hair coat of each animal, starting from the head, followed by the neck, dorsum, trunk, ventrum, limbs and tail. After specimen collection, the toothbrushes were placed individually in new paper bags and submitted to the Medical Mycology Laboratory of the University of Trás-os-Montes and Alto Douro (UTAD). In the laboratory, each brush was pressed onto the surface of the agar for 1 min. Cultures were performed on Dermatophyte Test Medium (DTM, Liofilchem™) (formula per litre: 11 g soy peptone; 10 g glucose; 0.5 g cycloheximide; 0.1 g gentamicin; 15 g agar; phenol red 0.2%; pH 5.5). Plates were covered with plastic to prevent dehydration, incubated at 28°C and examined daily for 14-21 days. Suspected *T. mentagrophytes* colonies were identified by colony characteristics (Hoog et al., 2020). If no fungal growth was observed within 3 weeks, the culture was considered as negative. The number of morphologically suspicious colonies per plate was also counted. Fungal colonies were identified to the species level based on their macroscopic and microscopic morphological features. Monocultures were made from morphologically suspicious colonies on Potato Dextrose Agar (PDA) and incubated in the dark at 28˚C for 1.5–3 weeks. Dermatophytes were evaluated macroscopically and microscopically after 14–21 days for species identification. Morphology was examined through light microscopic examination. From the PDA colonies, microscopic preparations were made by staining with one drop of lactophenol cotton blue and further examined based on the description(s) by De Hoog et al. (2020). In this study the ability of *T. mentagrophytes* to hydrolyse urea was tested. Christensen's urea agar (Oxoid™) was inoculated with positive cultures. After the urea media were inoculated, the cultures were incubated at 28ºC for up to 2 weeks. The colour change of the media from orange or pale pink to purple-red indicated positive results, i.e. the presence of urease. Isolates were identified as species of the *T. mentagrophytes* based on the production of numerous pyriform or round microconidia (Drouot et al., 2009) and biochemical tests.
The UTAD Committee of Ethics waived ethical approval (Doc32/CE/2015) for this study.

Results

Out of 71 wild rabbits sampled (Table 1), 32.4% were female (25.4% young and 7.0% adult) and 67.6% males (50.7% young and 16.9% adult).

All the studied isolates formed colonies in DTM™ and PDA with a velvety surface and yellowish pigmentation on the reverse. Isolates were identified as *T. mentagrophytes* based on the production of numerous spherical to pyriform microconidia and multiseptated macroconidia cigar- to club-shaped (Figure 1). Based on culture colony morphology, dermatophytes were identified from samples of five wild rabbits (Table 2). None of the animals showed lesions and clinical signs of dermatophytosis such as scaling or crusting during visual control. The number of colonies counted was seven. The overall occurrence of dermatophyte positive animals was 7.0% (95% confidence interval: 2.3–15.6%). The isolates were identified as *T. mentagrophytes* on phenotypic examination. Dermatophytes were isolated from three males and two females, three juveniles and two adults.

Table 1. Samples distribution by sex and age.

Sex	Age	Sample size
Male	Adult	12 (16.9%)
	Juvenile	36 (50.7%)
Female	Adult	5 (7.0%)
	Juvenile	18 (25.4%)
Total		71 (100%)

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Figure 1. *Trichophyton mentagrophytes*: A – macroscopic view of *T. mentagrophytes* cultured for 8-15 days on DTM™ plates at 28ºC; 400x. B – microscopic structures with numerous spherical to pyriform microconidia and macroconidia with cigar to club shaped, lactophenol blue staining; 400×.

Table 2. Characterization of rabbits with positive cultures.

Positive Sample number	Sex	Age	Days to obtain visible colonies	Number of colonies
1	Male	Adult	12	2
2	Male	Young	12	1
3	Female	Young	8	2
4	Male	Adult	12	1
5	Female	Young	8	1

Discussion

Dermatophytosis or ringworm infection is a superficial skin infection, with veterinary and public health importance. Data on carriers of dermatophytes are ultimately important for their control in game farms, farmers and hunters. An epidemiological survey on dermatophytosis was carried out in captive wild rabbits without clinical signs in North of Portugal. To the best of our knowledge, this is the first report on the occurrence of dermatophytes in wild rabbit. Dermatophytes were identified from samples of five (7.0%) wild rabbits, with none of the positive animals showing clinical signs of dermatophytosis, such as broken hairs or skin scales. This study also shows the diagnostic usefulness of fungal culture using the toothbrush technique. The isolates were identified as *T. mentagrophytes* on phenotypic examination.

Specific studies of the prevalence of dermatophytosis in rabbits are scarce. To the best of our knowledge, this is the first asymptomatic dermatophyte carriage in wild rabbits. In pet rabbits some investigations have been conducted in apparently healthy animals (Kraemer et al., 2012; Overgaauw et al., 2017; Tekin et al., 2019). Previous studies showed that the zoophilic dermatophyte *T. mentagrophytes* was the most frequent species isolated from domestic rabbits with clinical signs (Van Rooij et al., 2006; Moreira et al., 2012).

Reported prevalences in domestic rabbits vary between 0% and 82.7% (Kraemer et al., 2012; Moreira et al., 2012). In pet rabbits a few investigations have been conducted in apparently healthy animals (Kraemer et al., 2012; Overgaauw et al., 2017; Tekin et al., 2019). In a study in Germany, none of 140 pet rabbits screened were found to be carriers (Kraemer et al., 2012). In a study in the Netherlands, dermatophytes were found in 4% of healthy rabbits (Overgaauw et al., 2017). Differences to the present study may be explained by the diverse prevalence levels of dermatophytosis in other countries, as well as differences in the sampling and mycological methods.

The risk of human infection with *T. mentagrophytes* spread by rodents and lagomorphs has been proved (reference?). An understanding of dermatophytosis epidemiology in captive wild rabbits is of public health concerns to reduce the spread of zoophilic fungal infections to humans and to other animals (Mesquita et al., 2016). Wild rabbits infected with dermatophytes in game farms are undesired. Not only because the zoonotic exposure for staff and customers, but also for the risk of spreading the infection to other animals when they are released in the nature. For this reason, preventive measures, including hygienic ones, should be put into practice. Farmers need to be informed about the zoonotic risks of dermatophytosis in wild rabbits. Their hands should be washed and disinfected after working with the animals and clothes should be changed. *Trichophyton mentagrophytes* is one of the most common zoophilic dermatophyte infecting humans (Drouot et al., 2009; Chadeganipour et al., 2016). When handling animals, specific preventive measures are recommended in addition to regular hygiene.

Conclusion

Despite the small sample size, the present study suggests that wild rabbits are carriers of the dermatophyte *T. mentagrophytes* and represent a potential source of infection to rabbit farmers and hunters. The results confirm the presence of dermatophytes in apparently healthy wild rabbits and support the need for new strategies and approaches used in the control of dermatophytosis in captive wild rabbits. Farmers and hunters should be encouraged to adopt prophylactic measures such as disinfection of equipment and tools, and to place animals in quarantine upon arrival until dermatophyte carriage has been ruled out. Additional studies are required to evaluate the risk of dermatophyte transmission from wild rabbits to farmers and hunters.

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