Ringworm by *Trichophyton erinacei* in calves: description of two Italian outbreaks

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SUMMARY

Trichophyton erinacei is a zoonotic dermatophyte mainly isolated from hedgehogs. In other hosts (human or animals) it can cause skin infections, with typical clinical features of dermatophytosis. It has never been reported in cattle in which *T. verrucosum* is frequently isolated, particularly in calves. The present case report describes two outbreaks (case A and case B) of cattle ringworm caused by *T. erinacei* in Northern Italy; the first case involved a group of 5 month old Bruna Alpina calves, while the second one concerned a group of 24 months (approximate) Italian Frisona cows. Herd A was subjected to a regular vaccination protocol against *T. verrucosum*, but the calves involved in the outbreak had not yet been vaccinated. Crusts, alopecic and pruritic skin lesions, appeared about 4 months before the clinical visit, were mainly distributed on the head, trunk and flanks in all the animals involved. The veterinarian of farm B detected abdomen skin lesions compatible with dermatophytosis about 2 weeks after the appearance of the disease in the animals. Animal and human hairs/crusts samples were collected for mycological investigations by cultural and molecular methods (PCR and sequencing). The colonies observed showed macro- and microscopical features attributable to *T. mentagrophytes*, however the strain has been confirmed as *T. erinacei* by molecular analysis. The described cases showed that *T. erinacei* can cause bovine ringworm, with clinical features completely comparable to those expressed by *T. verrucosum*. The use of vaccination in some animals of herd A appears to have contributed to their protection against the mycosis, as demonstrated by the fact that only non-vaccinated animals showed *T. erinacei* skin lesions. The use of different laboratory diagnostic methods was essential to reach a correct fungal identification. Furthermore, it was possible to confirm that this is the first Italian isolation of *T. erinacei* from calves.

KEY WORDS

Trichophyton erinacei; ringworm; calves; outbreak.

INTRODUCTION

T. erinacei is a zoonotic dermatophyte mainly isolated from the hedgehogs, which are often in a role of asymptomatic carriers[1]; in other hosts (human or animals) it can cause skin infections, with typical clinical features of dermatophytosis[2]. Although it has commonly been considered belonging to the *T. mentagrophytes* complex, recent studies allow classification as a different species[3]. This report describes two outbreaks of dermatophytosis due to *T. erinacei* occurred in two Italian cattle farms.

MATERIALS AND METHODS

The outbreaks involved a group of 14 Bruna Alpina calves (farm A) and a group of 40 Italian Frisona cows (farm B). Animals of farm A were represented by 5-month old calves, bred in a mountain farm in North-Western Italy and composed of about 60 animals. These calves were housed in outdoor cages. Animals of farm B were about 24 months old and were bred in a farm composed of 200 specimens, located in North-Eastern Italy. Cows of this farm were housed in typical external fences with sheds for night shelters.

In both two cases, multifocal circular, crusted and mild thickened areas of alopecia, highly pruritic, 1-4 cm in diameter were noticed which appeared about 4 months before the clinical visit. The lesions were widely distributed to the head, trunk, and flanks (Figure 1, 2).

Herd A, according to the breeder, was normally subjected to a regular vaccination protocol against *T. verrucosum*, but the calves involved in the outbreak had not yet been vaccinated. On the contrary, herd B was never vaccinated for *T. verrucosum*. The latter case also involved a veterinarian, who developed two inflamed and ellipsoidal skin lesions on the abdomen, compatible with dermatophytosis, after about two weeks the onset of the disease in animals (Figure 3).

Hair and crusts samples from animals of farm A and B and from the vet involved were collected for mycological investigations, through cultural and molecular methods. The sampling areas were previously decontaminated with alcohol 70% and sterile pliers were used for the collection. Samples were inoculated into Dermasel Agar and into Sabouraud with thiamin and inositol plates (specific for the growth of *T. verrucosum*), then incubated at 25°±1°C for 3-4 weeks and daily observed. At the same time, samples were investigated

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Figure 1 - Skin lesions in a calf of the farm A: multifocal circular, crusting and mild thickened areas of alopecia, distributed to the head, trunk, and flanks.

Figure 2 - Skin lesions in a cow of the farm B, similar in appearance to those of the animal in Figure 1, but more distributed to the trunk and flanks.

through molecular techniques (PCR and sequence analysis), following the protocol of Cafarchia *et al.*[4]. To this purpose, after an initial step of lysis with lysozyme, DNA was extracted using the QIAamp DNA mini kit® (Qiagen), according to manufacturer's instructions. DNA was then subjected to heminested-PCR. The primers used were DMTF18SF1 (5'-CCAGGGAGGTTGGAAACGACCG-3') and DMTF28SR1 (5'-CTACAAATTACAACTCGGACCC-3') for the one-step PCR and DMTF18SF1 and DMTFITS1R (5'-CCGGAACCAAGAGATCCGTTGTTG-3') for the second step. They were designed to the region ITS+ of nuclear ribosomal DNA. PCRs and the following DNA amplifications (first and second step) were performed according to Cafarchia *et al.*[4]. PCR products were analysed by 2% agarose gel electrophoresis with Midori Green Advance DNA stain® (NIPPON Genetics) and purified using the QIAquick® PCR Purification Kit (Qiagen), following the manufacturer's instructions. The identity of dermatophites were revealed by sequence analysis with primers DMTF18SF1 and DMTFITS1R, using BigDye® Teminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Consensus sequences were created by BioEdit Sequence Alignment Editor software v 7.0.9.0 and then aligned against Genbank database.

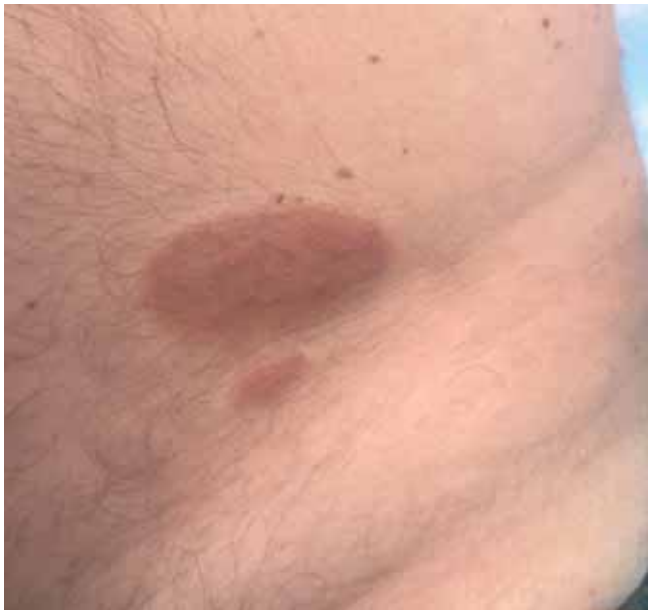

Figure 3 - Abdomen skin lesions of the veterinarian involved in the farm B outbreak.

From all the samples examined, growth of white, flat, powdery, with lemon-yellow reverse colonies has been observed on both culture media, within 7-10 days (Figure 4A). At microscopy, smooth-walled, two to six-celled, clavate, variable in size macroconidia and hyaline, smooth-walled, and predominantly spherical in shape microconidia were observed. (Figure 4B).

Sequence analysis showed a degree of similarity of 99% with *T. erinacei*, with an e-value: 0.0 (accession number MF153407.1). Therefore, the final fungal identification was found to be *T. erinacei*.

A spontaneous regression of the disease in both herds started approximately one month after the clinical visit. The human lesions began to regress after about two weeks of treatment with azole compounds.

DISCUSSION

Dermatophytosis by *T. erinacei* is mainly related to contact with quills and underbelly of hedgehogs[1]. Although isolation of *T. erinacei* has been reported in other animals - hunting dogs, wild rodents and brown hares[5,6,7], there is no prior evidence, as far as the authors are aware, of this fungal infection being detected in calves. Cattle are subject to skin fungal in-

Figure 4 - Macroscopical (**A**) and microscopical (**B**) features of *T. erinacei* (light microscope, 40X).

fections from *T. verrucosum*, the main ringworm agent in ruminants, also described in other species[8,9]. Therefore, it is possible to say that the outbreaks described here represent the first *T. erinacei* infection in calves in Italy.

In keeping with other animal fungal diseases, the potential for transmission to humans should not be underestimated. Breeders, veterinarians and/or livestock workers are the occupational categories most at risk of contracting the infection and of disseminating it to other people not directly in contact with animals. This potential was recognized when the veterinarian demonstrated abdominal skin lesions compatible with dermatophytosis approximately 2 weeks after the appearance of the disease in the animals. Laboratory examinations confirmed the role of *T. erinacei* as a cause of the human disease, in accordance with similar studies conducted in other countries[10,11].

In the outbreaks described, it was not possible to establish for certain a direct transmission of the fungus from hedgehogs to cattle; although the farms are located near to wooded areas, according to the breeders, there should be no hedgehogs or, at least, not close to the fences. However, once can hypothesize the presence of other animals that play the role of intermediate hosts between the hedgehogs and the calves, developing the mycotic disease and then remaining potential spore carriers. These alternative hosts to the hedgehog could play the simple role of asymptomatic spore carriers with no disease effect. Wild ungulates, red foxes, wild boars or rodents could be animals acting as a suitable reservoir of the fungus in the areas where the farms are located. Therefore, it would be interesting to carry out further mycological investigations on hair samples from these species, in order to isolate any strain of *T. erinacei*.

Other important factors which could be considered in the transmission of *T. erinacei* into the investigated farms could be breeding management errors such as the lack of sanitation in routine procedures or factors related to the immunity status of the animals. Generally, the use of preventive measures i.e. vaccination, plays an important role in the prevalence of the *T. verrucosum* infection in bovine. On the contrary, no specific immunity derived from natural exposure to *T. erinacei* was demonstrated, like in other animal species[12].

From the laboratory perspective, the use of different diagnostic methods was essential: molecular investigations allowed the correct fungal identification surpassing the culture method, proving to be a useful adjunct tool in the mycological diagnostics, thus enhancing the epidemiological knowledge of the organism.

CONCLUSIONS

In conclusion, the described outbreaks have demonstrated the disease potential of the dermatophyte *T. erinacei* in animal species other than hedgehog and allow the authors to state that they are the first cases of cattle dermatophytosis by *T. erinacei* in Italy.

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