



Prevalence of Dermatophytes in Red Deer (*Cervus elaphus*) in The Stelvio National Park, Italy.

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CORRESPONDING AUTHOR

Roberta Perego,

Dipartimento di Scienze
Veterinarie per la Salute, la
Produzione Animale e la
Sicurezza Alimentare (VESPA) -
Università degli Studi di Milano

Via G. Celoria 10, 20133, Milano,
Italy

e-mail: roberta.perego@unimi.it

phone: +39 02 50318188

fax: +39 02 50318171

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Roberta Perego¹, Daniela Proverbio¹, Eva Spada¹, Giada Bagnagatti
De Giorgi¹, Emanuela Valena¹ and Elisabetta Ferro¹

¹ Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale
e la Sicurezza Alimentare (VESPA), Università degli Studi di Milano,
Milano, Italy.

ABSTRACT.

Dermatophytosis has been described in wildlife, but the literature reporting dermatophyte prevalence in deer is incomplete. To determine the prevalence of dermatophytes and to evaluate the hypothetical role of asymptomatic carriers hair samples were collected from 30 legally hunted wild red deer (Cervus elaphus) in the Stelvio National Park, Italy. All deer were visually examined for dermatologic lesions and the coat was brushed using a modified Mackenzie collection method. A small sample of hair was used for direct microscopical examination and subsequently fungal culture was performed on the hair samples. Macroscopic and microscopic examinations were used to identify dermatophytes, saprophytic fungi and yeasts. None of the deer had visible cutaneous lesions. No dermatophyte spores or hyphae were found on direct microscopical examination and, when hair samples were cultured, dermatophytes were not demonstrated in any sample. Only saprophytic fungi were grown, predominantly Alternaria spp., Mucor spp., Cladosporium spp. These results did not reveal the presence of asymptomatic carriers of dermatophytes in the deer sample population of Stelvio National Park and suggest that it is unlikely that, at least in the investigated geographical area, the red deer act a reservoir for transmission of dermatophytes to other wild animals, livestock or people living locally.

1 Introduction

Dermatophytoses, also called ringworm, are common contagious infections of keratinized tissues caused by keratinophilic/keratinolytic fungi named dermatophytes which affect a wide range of mammals, including man (Chermette *et al.* 2008). Many species of dermatophyte have been reported as animal pathogens, but these may also be carried as normal flora on the haircoat of pets (Cabanes *et al.* 1997; Cafarchia *et al.* 2006; Boyanowsky *et al.* 2000). Dermatophyte fungi can also be found in farm animals and rodents (Chermette *et al.* 2008, Cabanes *et al.* 1997; Sargison *et al.* 2002; Mahmoud 1995; Moretti *et al.* 1998; Papini *et al.* 2009). There have been previous reports of isolation of dermatophytes from the haircoat of healthy carriers (Alteras *et al.* 1966; Chabasse *et al.* 1987, Marcianti *et al.* 1993) and cases of clinical dermatophytosis (Alteras *et al.* 1968, Knudtson *et al.* 1980, Peano *et al.* 2008) also in wild animals. Knudston and Robertstad (1970) demonstrated a positive specimens prevalence of 26.8% for keratinophilic fungi in 224 animals tested from 30 wildlife species in South Dakota. In a hair sample study in 2005 by Gallo *et al.* (2005), the alpine marmot (*Marmota marmota*) was identified as a reservoir for many dermatophytes, including *Microporum canis*; and dermatophytosis has previously been reported in water buffalo (*Bubalus bubalis*) (Pal *et al.* 1995). Clinical dermatophytosis has been reported in mule deer (*Odocoileus hemionus*) (Wobeser *et al.* 1983), barking deer (*Muntiacus muntjak*) (Pal and Thapa 1993) and Rusa deer (*Cervus timorensis*) (Le Bec and Beugnet 1994). Conversely, in a recent survey of fungal flora in white-tailed deer (*Odocoileus virginianus*) in Virginia (USA) no dermatophytes were identified (Hall *et al.* 2011). Rare studies of prevalence of dermatophytosis and ringworm episodes have also been signalled in wild animals in captivity (Kuntze *et al.* 1967, Janovitz and Long 1984). However, the literature reporting the prevalence of dermatophyte carriers in the majority of wild species is still incomplete, and the role of these species as reservoir for these pathogens is unclear.

The red deer (*Cervus elaphus*) is one of the most represented animal throughout the Alps. Measures have been taken to preserve and manage these deer populations. Currently, nearly 10,000 red deer are present in the in the Stelvio National Park (PNS) and adjacent areas, of which around 60% live in a more or less stable community in the protected area that covers about 485 sq km. In 2000, the Consortium of PNS started a project for the management of deer populations in the Park allowing, in some protected areas, controlled culling (reduction in numbers of populations by controlled hunting) to reduce the density of the deer population, since high densities have significant impacts on ecosystems and cause economic damage to agricultural activities. The project, however, has also provided a mechanism for investigation of the health status of the deer and their relationship with the ecosystem and with man. In this regard wildlife surveillance (to assess the presence of potentially zoonotic pathogens on the skin of wild deer) is an important area for which data is lacking. To our knowledge no studies report dermatophytes prevalence from red deer populations.

The aims of this study were to determine the prevalence, and identify species, of dermatophyte fungi in a population of wild alpine red deer in Italy and to evaluate whether asymptomatic deer may carry dermatophyte fungi as part of the normal haircoat flora. Given the importance of the deer population in the Stelvio National Park this knowledge is important in identifying the risk of human and livestock exposure to this zoonotic pathogen.

2 Materials and methods

Under a scientific project pursuant to art. 11, paragraph 4 of Law 6 December 1991 n. 394, and with permission of the Italian Ministry of Environment and Protection of Land and Sea (DPN 2010 – 13760, 17 June 2011) and of the National Institute for Protection and Environmental Research (protocol n. 15387/T-A25, 6 May 2010), hair samples were collected from legally hunted red deer in Stelvio National Park, Italy. The culling programme provided a reduction in the density of the red deer population by allowing hunting within a small wintering area of about 1400 hectares, comprising the unit of management referred to as "Valfurva-Sondalo", belonging to the Forestry Station of Valfurva in the Lombard sector of PNS. This forestry station of about 24,600 hectares, the largest of the park, is of sufficient size to provide all the needs of the red deer (neighbourhoods for summering, wintering areas and breeding areas).

Hair samples were collected between November and December 2012. All deer tested had been killed no more than 12 hours before sampling. All deer sampled were visually examined for dermatologic lesions.

Samples obtained from 30 red deer consisted of 11 fawns (5 females and 6 males) and 19 adult red deer (16 females, including 10 pregnant females, and 3 males) ranging in age from yearling deer to mature deer (20 years).

Although several methods are available for dermatophyte testing, including Wood's lamp examination, direct microscopic examination of hairs and fungal culture is considered the gold standard (Moriello 2003) and was the method used in this study.

The deer coat was brushed using a modified Mackenzie collection method (MacKenzie 1963; Moriello 2001). Briefly, a new sterilized toothbrush was used to collect hair samples by brushing each deer for a minimum of 2 minutes (at least 30 strokes) over the face, neck, abdomen, thorax and limbs (Hall et al. 2011) and immediately after brushing, all accumulated material was transferred to a sterile pouch until it could be examined in the laboratory. In the laboratory, a small sample of hair was collected from the toothbrush with sterile, rubber-covered, hemostats and examined under a microscope (direct hair examination). The hairs were positioned in the same orientation on a microscope slide, suspended in mineral oil, and examined, with the low-power objective of the microscope, for dermatophyte hyphae and arthrospores. 20% potassium hydroxide diaphanisation for 30 minutes of hair sampled was finally performed to microscopically detect ectotrix spores of *Trichophyton verrucosum*.

The coat samples were then subjected to fungal culture: sample toothbrushes were gently imprinted onto the surface of 9 cm Petri dishes containing Sabouraud dextrose agar (with chloramphenicol 0.5% and actidione 0.4% added) on one half and Dermatophytes Test Medium (DTM) agar on the other half. An aliquot of samples was cultured on Sabouraud dextrose agar supplemented with inositol and thiamine to obtain more easily the possible growth of *Trichophyton verrucosum*, a dermatophyte difficult to cultivate in normal dermatophyte media (Peano et al. 2008). The Petri dishes were incubated upside down in two laboratory ovens (MICRA 9T, I.S.Co Srl, Pieve Emanuele, Italy) in the dark, both at a constant temperature of 25 °C and 37 °C, and examined daily for 3 weeks. After 3 weeks (4 weeks for dishes at 37 °C), the dermatophyte colonies on the medium were macroscopically and microscopically examined and identified to species level. Macroscopic and microscopic examinations were also used to identify saprophytic fungi and yeasts, but only to the genus level. For each sample with

saprophytic growth, the three predominant colony types were selected for saprophyte identification.

3 Results

No deer included in this study presented with cutaneous lesions other than those inflicted by gunshot. No dermatophyte spores or hyphae were identified in any sample using direct hair examination and no abnormalities (at the level of the bulb, shaft or tip) were identified in any hair samples.

DTM colour change did not occur with any sample of cultured hair and no dermatophyte colonies were grown. Only saprophytic fungi were grown - predominantly *Alternaria* spp., *Mucor* spp., and *Cladosporium* spp. (Table I)

Samples from three subjects (10%) showed no fungal colony growth, while two or more colonies were grown in samples from 15 subjects (50%).

Table 1 Saprophytic fungi isolated alone or together on the same subject in the study deer population. The isolation could be pure or a deer could have more of a saprophyte.

Genus	Number of isolation	%
Penicillum spp.	4	9.1
Alternaria spp.	13	29.5
Mucor spp.	10	22.7
Cladosporium spp.	7	16.0
Fusarium spp.	6	13.6
Candida spp.	4	9.1
Total	44	100

4 Discussion

This is the first study of the presence and prevalence of fungi on the haircoat of wild red deer and one of the first on the fungal flora carried out on wild deer. The results of our study did not reveal the presence of asymptomatic carriers of dermatophytes in the red deer sampled population of Stelvio National Park; in the investigated geographical area seems therefore unlikely that the red deer act as a source of dermatophyte transmission to other wild animals, livestock or people with whom they might come into contact. Due to the absence of previous studies it is difficult to compare the results of this study with published literature. In a study published in 2013 by Nemeth *et al.* on the incidence, clinical manifestations and demography of bacterial and parasitic dermatological diseases in white-tailed deer in the southeastern United States an incidence of 5.7% of fungi (n.5 subjects on 88 deer with positive

isolation) was reported. However, the study did not specify whether the fungal isolates were dermatophytes, or whether isolations came from asymptomatic animals or those with dermatologic lesions. The only study of prevalence previously reported by Hall *et al.* in 2011 studied 60 adult white-tailed deer in Virginia (USA) but failed to isolate any dermatophytes. .

In our study fawns were well represented and there have been anecdotal reports of outbreaks of dermatophytosis caused by fawns (Hall *et al.* 2011). Young animals are much more susceptible to infection, probably due to their naive immune system (Stannard and White 2002). Nevertheless, all the fawns examined in our study, were negative for dermatophytes by culture.

The results of our study may be influenced by the sample group, geographic limitations and season. The sample group consisted of only 30 animals. This low number of animals cannot be truly representative of the entire deer population in the park, (nearly 10,000 red deer) notwithstanding the fact that there should have been a random selection of animals killed by hunters and the study group had a good representation of both sexes and the various age groups. All red deers were from a limited geographic region and therefore represent a small subset of the Italian red deer population. Finally the deer were sampled in the late fall/early winter, during cooler weather, whereas dermatophyte flora is likely to be more prevalent in hot, humid weather (Stannard and White 2002). Deer culling in the Stelvio National Park is always done in the fall/ winter season because at this time the deer population is closer to the more accessible areas of the park where the rangers place supplementary sources of food. In calves of domestic cattle, dermatophytosis is usually a self-limiting disease with a maximum duration of 4 months (Stannard and White 2002). It is possible that in the red deer population outbreaks occur in fawns during spring and summer and resolve by the fall.

The saprophytic fungi isolated from the haircoat of 90% of the red deer in this study were similar to those reported in the USA on white-tailed deer (Hall *et al.* 2011), showing that the different geographic area does not affect the commensal flora of the wild deer.

5 Conclusion

In conclusion, studies on a larger sample of red deer, or with hair samples taken in other seasons, would be needed to confirm that red deer population from the Stelvio National Park doesn't act as a reservoir for dermatophytes in this area. It would be also interesting to evaluate the prevalence of zoophilic dermatophytes and dermatophytosis in other wildlife, livestock and people living in the same geographic area.

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