Diagnostic and epidemiological analysis of Trichophyton benhamiae *infection on an alpaca (*Vicugna pacos*) farm in Poland*

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Summary
Alpacas (*Vicugna pacos*) are growing in popularity and are increasingly being presented for veterinary care. Literature reports indicate that dermatophytosis occurring in alpacas accounted for about 3% of dermatological diagnoses. However, there are no reports regarding species of dermatophytes associated with alpacas and reservoirs of infection. In this study, we investigate the diagnosis and epidemiological origin procedure and the virulence enzymes activities of *Trichophyton benhamiae* isolates obtained from alpacas from a breeding farm. Identification was carried out traditionally by correlating clinical manifestations with micro- and macroscopic examination, and molecular differentiation methods based on Internal Transcribed Spacer (ITS) sequences. Epidemiological analysis was carried out on the basis of Melting Point PCR (MP-PCR) and Amplified Fragment Lenght Polymorphism (AFLP) genotyping. The production of virulence factors was evaluated phenotypically using specific test media. The results obtained from diagnostic tests indicated that the etiological factor of dermatophytosis is *T. benhamiae*. The same species was also isolated from cowsheds and insects. The MP-PCR and AFLP analyses indicated high invariability of the genomes of the strains isolated from the animals, cowsheds, and insects. In conclusion, animal husbandry outside the natural ecological niche may increase predisposition to dermatophytosis. The treatment of animals alone is insufficient, one should be aware that only elimination of all fungal sources is a long-term success and the key point of therapy.

Introduction

Alpacas (*Vicugna pacos*, formerly *Lama pacos*) are growing in popularity and are increasingly being presented for veterinary care (Scott *et al.* 2011, Halsby *et al.* 2017). These animals often present skin disorders that constitute diagnostic, therapeutic, and epidemiology challenges for practicing veterinarians (Foster *et al.* 2007). Additionally, alpacas may have regular and close human contact (Halsby *et al.* 2017). Understanding infectious diseases associated with these animals and the possible risks to human health is important for alpaca keepers and breeders, veterinary professionals, and others involved in recreational and therapeutically activities (Halsby *et al.* 2017, Scott *et al.* 2011).

Alpacas together with llamas (*Lama glama*) are domesticated species of South American camelids (SACs), whereas guanaco (*Lama guanicoe*) and vicuna (*Vicugna vicugna*) are wild species of SACs (Halsby *et al.* 2017). Camelids are regarded as rather exotic animals in Europe (Twomey *et al.* 2014, Halsby *et al.* 2017, Foster *et al.* 2007). As a rule, they live in zoological gardens, although in some countries, e.g. in Great Britain or Poland, alpacas are already farmed and raised due to the quality of their wool. Some are kept as pets, for trekking, guarding livestock, and in open farms (Halsby *et al.* 2017). In England and Wales, there is also a small but developing market for alpaca meat products (Twomey *et al.* 2014).

Literature reports indicate occurrence of dermatophytosis in alpacas (Foster *et al.* 2007,

D'Alterio *et al.* 2006, Halsby *et al.* 2017). This fungal infection accounts for about 3% of dermatological diagnoses (D'Alterio *et al.* 2006); nevertheless, in the Animal Health and Veterinary Laboratories Agency, only five cases of dermatophytosis in alpacas were reported between 2000 and 2015 (Halsby *et al.* 2017). Normally, symptoms of ringworm in alpacas are similar to these in other farm animals and include mild skin alopecia, with skin rarely covered by crust or hyperkeratotic areas (Scott *et al.* 2011). However, there are no reports regarding species of dermatophytes associated with alpacas and reservoirs of infection.

The dermatophyte *Trichophyton benhamiae* is an important zoonotic pathogen, and the infection rates have been increasing worldwide over the last 15 years (Sabou *et al.* 2018, Drouot *et al.* 2009). A study performed in Germany between March 2010 and March 2013 showed that *T. benhamiae* had already become the most frequent zoophilic dermatophyte responsible of human infections, with a prevalence of 2.9% (Jochen Brasch *et al.* 2015). The histories of many zoonotic infections suggest that guinea pigs are the main source of transmission. However, other small rodents (Jochen Brasch *et al.* 2015), rabbits (Nakamura *et al.* 2002), dogs (Sieklucki *et al.* 2014), or even porcupines (Takahashi *et al.* 2008) have also been associated with *T. benhamiae* infection, but no comparative investigations of different small animals have been published yet. So far, no *T. benhamiae* infections in South American camelids and other farm animals have been described.

T. benhamiae was formerly known as *Trichophyton* species of *Arthroderma benhamiae*; this species was considered part of the *T. mentagrophytes* species complex (Gnat *et al.* 2019c). In the past, *T. benhamiae* was often misdiagnosed as *Microsporum canis* (Mayser *et al.* 2013, Nenoff *et al.* 2014) or *T. mentagrophytes* var. *porcellae* (Sabou *et al.* 2018) because of a similar colony color or as *T. interdigitale* and microscopic similarities (Nenoff *et al.* 2014). Two phenotypes have been described for *T. benhamiae*: yellow and white (Brasch *et al.* 2015, Nenoff *et al.* 2014, Sabou *et al.* 2018). The first is characterized by strains with downy, pleated mycelium with a slow growth rate and poor sporulation on Sabouraud medium with rare microconidia or absence of macroconidia (Hiruma *et al.* 2015, Sabou *et al.* 2018). The white phenotype strains are powdery to floccose with a rapid growth rate (Sabou *et al.* 2018). Numerous spherical to clavate microconidia and sparse, thin-walled, cigar shaped macroconidia are present in microscopic preparations (Hiruma *et al.* 2015).

Due to its more than three different names, two different phenotypes, and several different possible hosts, the identification of *T. benhamiae* is difficult (Sabou *et al.* 2018). In the present study, we identified and analysed *T. benhamiae* infection in alpacas from a breeding farm. The aim of this study was to investigate the epidemiological origin of *T. benhamiae* isolates using morphological traits in combination with molecular analysis and to assess their capability to produce different enzymes.

Materials and methods

Strains and sampling methods

Strains were isolated in the Department of Veterinary Microbiology, University of Life Sciences in Lublin, Poland from clinical samples sent to our diagnostic laboratory for identification by an alpaca breeder and veterinarian in sterile Petri dishes. Forty-one samples were obtained from alpacas, including 11 with symptomatic dermatophytosis (each isolate from another animal), 10 collected from the cowshed (from mulch), and twenty from insects that were identified as the housefly (*Musca domestica*; insects collected from catching tapes suspended in the cowsheds).

The farm was located in south-eastern Poland and comprised 41 animals aged 1-5 years kept in an outdoor breeding system (standard maintenance and nutrition conditions). The alpacas were imported 6 years earlier from Chile and no new animals were introduced into the farm ever since. No case of dermatophytosis was diagnosed on the farm earlier. In eleven alpacas, in September 2018, round alopecia sites or ca. 3 cm excoriations covered with thickened scaling epidermis were noticed at the border of the head and neck with a distinct tendency to hair loss (from three to five in each of the suspected animals). The clinical changes were diagnosed as dermatophytosis. Hair from the margins of the clinical changes was collected for mycological diagnostics. At the same time, a routine veterinary assessment for superficial fungal infections of the other alpacas was made on the farm, and material from the neck and trunk taken with the brush technique was sent to the laboratory. Before the cowshed was disinfected, material from the bedding was collected for epidemiological purposes. During the cleaning works, rodent droppings were noticed in the cowshed, but no rats or mice were found. Mycological studies were also made in material taken from a dog and two cats living on the farm as well as from the nail of the breeder and veterinarian. At a later stage of the studies, a flytrap that was suspended in the cowshed with 20 insects stuck to it was subjected to mycological analysis.

Laboratory diagnostic procedure

After the material was delivered to the laboratory, the first stage of the diagnostics consisted in direct examination of unstained slides in a 10% KOH solution, which was then viewed at a magnification of 40x10 (Nikon Coolpix YS100). Simultaneously, cultures were inoculated with the bilayer tile method onto Sabouraud glucose agar (Becton Dickinson, New Jersey, USA), incubated at 37 °C for 14-21 days, and examined twice a week. Dermatophytes were identified based on colony texture and production of typical spores (Hoog *et al.* 2000). Confirmation of the initial identification was performed retrospectively by sequencing the Internal Transcribed Spacer (ITS) region (including parts of 18S and 28S rDNA, as well as the whole of ITS1, 5.8S rDNA, and ITS2) of the ribosomal DNA as previously described (Gnat *et al.* 2018a). Briefly, DNA was isolated from the dermatophytes with the phenol-chloroform method (Gnat *et al.* 2017). The ITS amplification reaction was carried out in a T Personal thermal cycler (Biometra GmbH, Goettingen, Germany) using Qiagen Taq PCR Master Mix (Qiagen, Hilden, Germany), 10 pmol of each primer: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.* 1990) (Genomed S.A, Warsaw, Poland), and 50 ng of DNA template. Electrophoretic separation of PCR products was carried out in 1% agarose gels. The gels were documented and analysed in GelDoc 2000 (Bio-Rad, Hercules, California, USA). The ITS sequencing reaction was carried out using a BigDye Terminator Cycle Sequencing Kit (Life Technologies, Carlsbad, California, USA) and primers ITS1 and ITS4. Two separate reactions were carried out for each primer. PCR was performed in a T-Personal thermal cycler (Biometra GmbH). The amplicon was purified using an ExTerminator kit (A&A Biotechnology, Gdynia, Poland) and then the DNA sequence was read in the 3500 Genetic Analyzer (Life Technologies, Carlsbad, California, USA). The identification was made using the BLAST (Basic Local Alignment Search Tool) in the GenBank database.

Epidemiological analysis

Epidemiological analysis was carried out on the basis of MP-PCR (Melting-Point-PCR) and AFLP (Amplified fragment Length Polymorphism) genotyping of whole genomes of the clinical isolates. The MP-PCR procedure was optimized for dermatophyte differentiation by Leibner-Ciszak and colleagues (Leibner-Ciszak *et al.* 2010). In this study, approximately 100 ng of DNA, endonuclease *Hind*III (Thermo Fisher, USA) and a mixture of two oligonucleotides Helper and Ligated (15 pmol; Helper: AGCTGTCGACGTTGG, Ligated: CTCACTCTCACCAACAACGTCGAC) were used. PCR was carried out in a 25-µl reaction mixture with primer PowaAGCT (25 pmol, 5′-CTCACTCTCACCAACGTCGACAGCTT-3′; Genomed, Warsaw, Poland). The AFLP analysis were performed with few modification as previously described by Graser and colleagues (Gräser *et al.* 2000). Briefly, one restriction enzyme *Eco*RI (Thermo Fisher, USA) and one primer with three selective nucleotides EcoRI-ATG (5′-GAC TGC GTA CCA ATT CAT G; Genomed, Warsaw, Poland) were used. The PCRs were carried out according to the source description in a TGradient thermal cycler (Analytik Jena AG, Jena, Germany). Electrophoresis of all PCR products was carried out in 3% agarose gels. All analyses were made at least in triplicate.

Evaluation of production of virulence factors

The production of virulence factors was evaluated using specific test media. Firstly, the dermatophytes were cultured onto Sabouraud glucose agar (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37 °C for seven days. Next, an inoculum with a 5 mm diameter from the edge of each culture was transferred onto plates containing the test medium (second passage). The following tests were performed: production of keratinase, phospholipase, lipase, elastase, protease, and gelatinase and detection of haemolytic activity as previously described (Gnat *et al.* 2018c). Each test was performed in triplicate and each strain was tested in duplicate in each experiment. The enzymatic activities were expressed as a difference between the total diameter of the colony plus the zone of precipitation and the colony diameters. In the case of detection of haemolytic activity, a transparent clearance zone around the colony indicated complete haemolysis.

The enzymatic activity of dermatophyte isolates was statistically analysed with the R program (Free Software Foundation, Boston, USA). Statistical inference was performed based on the results of the Student T test.

Results

In this study, 77 samples were investigated: 41 samples were collected from alpacas with and without clinical symptoms of trichophytosis, 10 from the cowshed where the alpacas were kept, 20 from a fly-stick hanging in the cowshed, one from a dog, two from cats, two from the breeders, and one from the veterinarian. Sixteen of these samples (21%) were *T. benhamiae* positive, as demonstrated by morphology and ITS sequencing (Table I).

The first group of strains included isolates from animals with dermatophytosis. On average, 27% of

the alpacas on the farm were affected with typical clinical symptoms. Furthermore, no dermatophytes were isolated from the asymptomatic animals (alpacas, dog, cats) and from three farm employees. Direct analysis of the material sampled from the clinical lesions in the symptomatic alpacas revealed the presence of arthrospores (Figure 1). The positive result of the examination of the culture confirmed the initial diagnosis. The macromorphological image of the isolated *T. benhamiae* showed two types of colonies: four isolates with a fluffy texture, a beige obverse, and a yellow reverse and the other type with a friable, powdery, white obverse and a brown reverse (Figure 2). The size of the colony was in the range from 5 to 8 mm. The edges of the colony were softly corrugated with prominent protrusions extending from the centre. The micromorphological image on the microscope slides revealed visible circular to clavate microconidia, which were much less numerous in the colonies with the beige phenotype (Figure 2). There were no macroconidia or spiral hyphae.

The second group of strains comprised isolates from the cowshed litter. *T. benhamiae* was isolated in 2 of the 10 collected samples (20%). Both strains represented the beige phenotype. The sporulation rate in these isolates was definitely lower than that observed in the samples collected from the animals. The emerging microconidia were not sufficiently abundant to dominate the preparation and the hyphal form was profusely visible.

The third group were strains isolated from flies collected on the adhesive tape of the flytrap. Three strains were isolated from the 20 collected flies (15%). They were two strains with a beige obverse and one with a white phenotype. The analyses of the strains showed distinct transition from the dominant hyphal form on day 4 to the sporous form dominating from day 14, especially in the case of the white-phenotype strain.

A comparative analysis of the ITS sequences of the isolated and reference strains available in the NCBI (Nucleotide Centre of Biotechnology Information) database was employed for a correct identification of the dermatophyte species. The ITS sequences obtained for all 16 isolates (accession number MK922472-MK922478, MK922512-MK922519, MK940328) using the BLAST (Basic Local Alignment Search Tool) software available in the NCBI (National Center for Biotechnology Information) database exhibited approximately 99% similarity to the *T. benhamiae* CBS623.66 sequences.

The MP-PCR and AFLP methods were used to determine the genomic differentiation of the *T. benhamiae* isolates. The agarose gel electrophoregrams indicated high invariability of their genomes (Figures 3 and 4). Although the dermatophytes were isolated from the different animals, samples taken from cowsheds, and insects, no different genomic profiles were obtained.

The virulence activities of the dermatophyte isolates

Table I. *Isolates of dermatophytes obtained from symptomatic animals, cowshed and insects with description of isolation source, location of changes and accession numbers of ITS sequences.*

Isolates	Host	Isolation source	Location of changes	Phenotypic type	Accession numbers of ITS sequences	Identification consistent with the NCBI database
TBA1	alpaca	clinical lession	neck	beige	MK922475	*Trichophyton benhamiae* CBS623.66 (accession number AB088677)
TBA2	alpaca	clinical lession	head	beige	MK922476	
TBA3	alpaca	clinical lession	head, neck	beige	MK922477	
TBA4	alpaca	clinical lession	head, neck	beige	MK922478	
TBA5	alpaca	clinical lession	head, neck	white	MK922514	
TBA6	alpaca	clinical lession	head, neck	white	MK922515	
TBA7	alpaca	clinical lession	head	white	MK922516	
TBA8	alpaca	clinical lession	head, neck	white	MK922517	
TBA9	alpaca	clinical lession	neck	white	MK922518	
TBA10	alpaca	clinical lession	head, neck	white	MK922519	
TBA11	alpaca	clinical lession	head	beige	MK940328	
TBB1	cowshed	litter	-	white	MK922512	
TBB2	cowshed	litter	-	beige	MK922472	
TBC1	insect	outer shell	-	beige	MK922473	
TBC2	insect	outer shell	-	beige	MK922474	
TBC3	insect	outer shell	-	white	MK922513	

NCBI = National Center for Biotechnology Information.

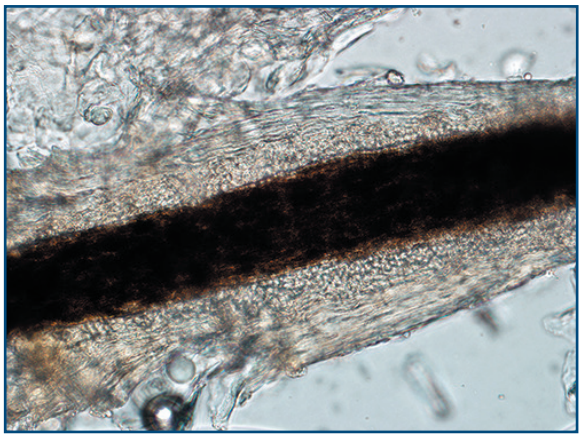

Figure 1. *Microscope slides from direct analysis of material sampled from clinical lesions in the alpaca (pictures taken with light microscopy at 400x, Nikon Coolpix YS100).*

were screened in a phenotypic assay, *i.e.* production of enzymes and determination of the haemolytic activity of the strains (Table II). All the clinical isolates of *T. benhamiae* showed keratinase, phospholipase, lipase, gelatinase, and protease activity. There were no statistically significant differences in the keratinase, gelatinase, and lipase activities between all the isolates. No elastase activity was detected in any of the isolates. The statistically highest phospholipase and protease activities, *i.e.* with a diameter of 9.1 mm and 9.9 mm, respectively, were noted for the *T. benhamiae* isolates derived from the animals. In addition, regardless of the source of the isolate, all strains caused type β haemolysis. Small differences between the distribution zones around the colonies were noted for the haemolytic activity of isolates obtained from the alpacas and the cowshed. Definitely weaker haemolytic properties were demonstrated by the *T. benhamiae* isolates derived from the insects.

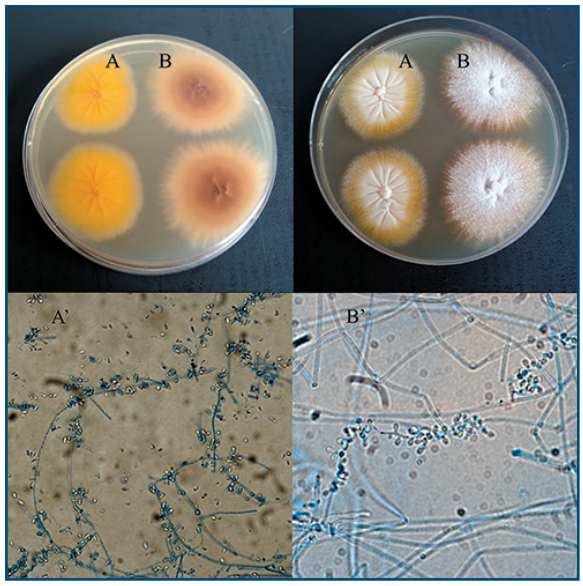

Figure 2. *Micro- and macroscopic morphology of dermatophytes isolated after 14 days of incubation, A - the beige phenotype, B - the white phenotype (pictures taken with light microscopy at 400x, Nikon Coolpix YS100).* A, B. macromorphology (obverse and reverse), A', B'. micromorphology (stained with lactophenol blue).

Discussion

Dermatophytes are generally cosmopolite, but in recent years, there has been a notable tendency towards limitation of the geographical range of some species and the strict connection with a sensitive animal host (Sabou *et al.* 2018, Gnat *et al.* 2019a). Nonetheless, population migration, including the import of animals that do not naturally occur in a given climate, improvement of the hygiene of animal breeding, changes in the human lifestyle, and the increase in physical activity in the company of animals have an impact on the geographical distribution and reservoirs of dermatophytes (Hiruma *et al.* 2015, Sabou *et al.* 2018).

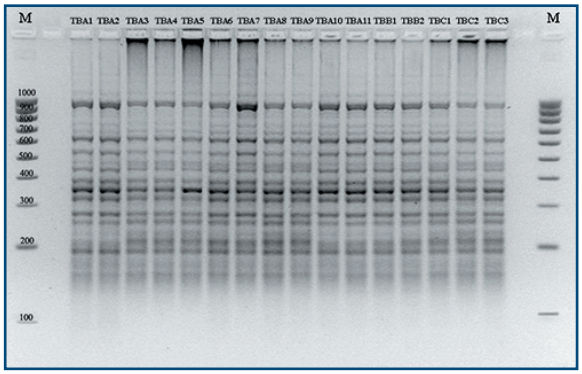

Figure 3. *Electrophoretic profile obtained with the MP-PCR fingerprinting method in 3% agarose gel.* M = Molecular weight marker GeneRuler™ (1000bp; Thermo Fisher, USA); TBA1-TBA11 = Strains isolated from alpacas; TBB1-TBB2 = Strains obtained from litter; TBC1-TBC3 = Strains isolated from insects.

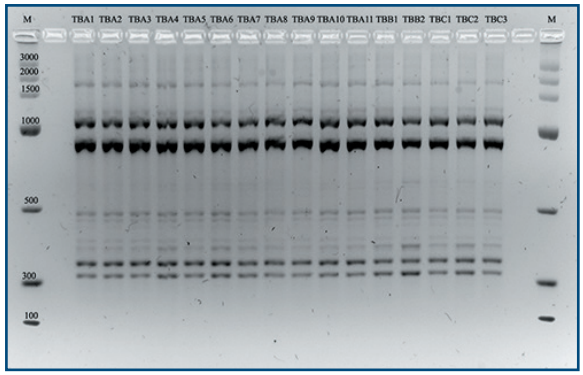

Figure 4. *Electrophoretic profile obtained with the AFLP method in 3% agarose gel.* M = Molecular weight DNA marker - Marker 3 (3000bp; A&A Biotechnology, Gdynia, Poland); TBA1-TBA11 = Strains isolated from alpacas; TBB1-TBB2 = Strains obtained from litter; TBC1-TBC3 = Strains isolated from insects.

Table II. *Enzymatic activity* in vitro *of isolates obtained from alpacas, cowshed, and insects expressed by the mean (in mm) of the diameter of the clear zone around the colonies after 21 days of incubation.*

Origin of isolates	Isolates	Keratinase	Phospholipase	Lipase	Elastase	Gelatinase	Protease	Haemolysis
Alpacas	TBA	8.4 (0.6)	9.1 (1.1)$^{+}$	8.8 (0.9)	0	*1.1 (0.3)*	9.9 (0.5)$^{+}$	11.2 (1.9)
Cowshed	TBB	7.5 (1.0)	7.4 (0.8)	7.9 (0.9)	0	1.0 (0.5)	8.4 (1.2)	11.8 (0.8)
Insects	TBC	5.9 (0.8)	3.1 (0.5)	4.0 (1.1)	0	0.7 (0.5)	5.2 (0.9)	6.7 (1.0)

Standard deviation in brackets; $^{+}$Statistically significance in column.

An important factor influencing the epidemiology of dermatophytosis is the knowledge of its possible transmission source. It is thought that transmission can take place after direct contact with an infected individual, even if asymptomatic, or indirectly via fomites, scales, and animal hairs (Moriello *et al.* 2017). Particularly dangerous in terms of epidemiology is the fact that infectivity may persist as long as two years (Contet-Audonneau *et al.* 2010). To date, only few reports on other potential sources of indirect infection, i.e. soil, water, plants, rodents, insects, etc., are available (Kornikłowicz-Kowalska *et al.* 2013). This contributes to the spread of infection, since they are neither identified as a potential source of infection for the animal milieu nor screened or removed from animal surroundings. Furthermore, trichophytia infections in animals are often misdiagnosed at the initial presentation and therefore wrongly treated, for example with steroids, modifying the clinical findings and resulting in difficulty in further diagnosis (Brillowska-Dabrowska *et al.* 2010). Improperly diagnosed treated or recurrent superficial mycoses are a potential source of infection via direct and indirect transmission to other animals and humans (Gnat *et al.* 2018b). For this reason, epidemiological studies on new potential sources of dermatophyte infections are an important element of knowledge for veterinarians.

Based on the increasing role of *T. benhamiae* in the development of dermatophytosis in various breeding and companion animals as well as humans, we believe that it is important to carry out epidemiological analysis of this pathogen from an outbreak on an alpaca farm. Morphological analysis failed to discover the source of dermatophyte infection; nevertheless, it resulted in highly probable species identification for all 16 *T. benhamiae* isolates investigated in this study and confirmed the species through ITS sequencing. Therefore, we used the MP-PCR and AFLP genotyping methods. Currently, PCR-fingerprinting techniques are believed to have sufficient suitability for epidemiological investigation of the origin of dermatophyte infection. Many investigators show that these methods can be a useful tool for analysis of the diversification of dermatophyte genomes in full agreement with identification based on both culture- and ITS-techniques (Leibner-Ciszak *et al.* 2010, Brillowska-Dabrowska *et al.* 2007, Gnat *et al.* 2018a, Shehata *et al.* 2008). Our study also revealed that the PCR-based band profiles obtained by MP-PCR and AFLP were identical in all the clinical isolates studied. The homology of the genomes in the *T. benhamiae* isolates obtained in this analysis indicates a high probability of the same origin of the etiologic agent of the dermatophytosis in the alpacas and its source location in the cowshed or originating from insects. Interestingly, this pathogen was not detected in the two cats and the dog kept on the same farm. This may be related to the greater mobility of these animals and the possibility of chasing away flies trying to sit on their fur, or the impossibility to enter the cowshed.

Bartosch and colleagues (Bartosch *et al.* 2019) showed that the high infection rate of 58% confirms that guinea pigs are a main reservoir for *T. benhamiae*, which was in agreement with previous studies (Nenoff *et al.* 2014). Furthermore, it is suggested that although rats, mice, and even rabbits are less susceptible to *T. benhamiae* infection than guinea pigs, the spread of these pathogens is much easier due to the lack of biosecurity in this animal stock (Bartosch *et al.* 2019, Nenoff *et al.* 2014). Because the animals are rodents or are kept only as feed animals, most often no skin infection therapy is applied. Moreover, Brasch and colleagues (Brasch *et al.* 2016) noted that the spread of *T. benhamiae* might be difficult to control, as infected guinea pigs are often free of clinical signs of dermatophytosis. Although there are no similar data for rodents, it is important in this case that they are usually unnoticed by breeders on the farm. In our study, *T. benhamiae* was isolated from the litter in the cowshed where the alpacas were kept. Although no rodents were noticed on the farm, the presence of probably rats' droppings was noted during the cleaning work in the cowshed. Therefore, guinea pigs and rodents, at least in breeding stocks and pet shops, should be screened regularly for dermatophytes and adequate biosecurity measures should be implemented to prevent this ignored zoonosis (Brasch *et al.* 2016).

The housefly has the potential for dissemination of microorganisms associated with animal faeces, skin, fomites, and natural environment (soil, water, seeds, and nuts) (Kornikłowicz-Kowalska *et al.* 2013, Zarrin

et al. 2007). These insects have been shown to feed on secretions and other human and animal wastes, thus becoming ideal carriers for transmitting various pathogenic microorganisms. Not only has the association of insects and bacterial diseases been documented but also transmission of fungi has been confirmed by several reports (Zarrin *et al.* 2007, Gilliam *et al.* 1974, da Costa *et al.* 1998). Da Costa and Oliveira (da Costa *et al.* 1998) isolated various species of *Penicillium* from mosquito vectors of tropical diseases. Zarrin and colleagues (Zarrin *et al.* 2007) verified the predominance of fungi from the genera *Aspergillus* and *Penicillium* in adult insects from the family *Muscidae* captured in an abattoir. Interestingly, two species of dermatophytes, i.e. *Trichophyton mentagrophytes* and *Microsporum gypseum*, were identified among filamentous fungi in their study. Our study has demonstrated that the housefly can be a carrier of *Trichophyton benhamiae* fungal spores. The epidemiological danger of this situation is associated with the fact that these flies were caught on the alpaca farm, where there was an outbreak of dermatophytosis caused by this etiological factor. These results are in contradiction to the findings reported by Ysquierdo and colleagues (Ysquierdo *et al.* 2017) suggesting that dermatophyte arthroconidia are not acquired and disseminated by houseflies. While adherence of arthroconidia to human skin is strongly time dependent, it is unclear whether this holds true for cuticular surfaces (Ysquierdo *et al.* 2017). It is worth noting that the host is most contagious prior to hair loss, while denuded lesions are definitely less contagious. The housefly collection in the study conducted by Ysquierdo and colleagues (Ysquierdo *et al.* 2017) was sampled from cattle with hair loss, which was less than optimal in terms of contagion. Given these circumstances, the formulated conclusions do not have to be definitive.

Analyses of various superficial mycosis cases based on the severity of infection have shown that dermatophyte strains may vary in their ability to cause infection in animals and humans (Sharma *et al.* 2007, Gnat *et al.* 2019b). This phenomenon can be associated with the synthesis of enzymes that enhance dermatophyte survival in tissues of different hosts (Gnat *et al.* 2018c, Figueredo *et al.* 2011). Therefore, the pathogenic potential of a dermatophyte depends on its ability to produce various enzymes (Sharma *et al.* 2007). In turn, variations in the enzymatic profile and its potential might be responsible for the differences in the pathogenic effects in different hosts (Dalis *et al.* 2014). In this context, interesting data are provided by studies of the enzymatic activity of strains isolated from another source. Some investigators suggest that the diversified enzymatic activity of dermatophytes is an adaptive trait and can be partially explained by the effect of substrate availability (Figueredo *et al.* 2011). However, in our study, statistically significantly higher phospholipase and protease activity was obtained in the case of the alpaca infection in comparison to strains isolated from the cowshed and insects. In turn, the keratinase activity was much lower in the dermatophyte from the insects. Probably, there is a relationship between the enzymatic activity and the affinity of dermatophytes for species-specific keratin, and every change in the life niche weakens the fungal virulence. In this case, there is a time to adapt between the transfer of infectious dermatophyte structures from the vector to the sensitive individual and the occurrence of the infection symptoms (Gnat *et al.* 2019a). Appropriate hygienic activities provided to the animal at this time can prevent the development of an infection. This issue requires more extensive research and deeper discussion.

Zoophilic dermatophytes tend to be contagious; therefore, collaboration between a dermatologist and a veterinary is advisable as soon as the source of infection has been identified. This report implies that rodents and insects can be a reservoir or vector for zoophilic dermatophyte infection, also in the case of breeding animals. The treatment of the patients alone is insufficient, and cleaning of contaminated items and areas needs to be performed simultaneously in every case of dermatophytosis. Therefore, one should be aware that only elimination of all fungal sources ensures a long-term success and is the key point of therapy.

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