Identification of Dermatophyte and Nondermatophyte Molds Isolated from Animal Lesions Suspected to Dermatomycoses

Abstract

Background: Dermatomycoses contain superficial fungal infections of keratinized layers of the body such as skin, hair, and nail that affect more than 20%-25% of people and animals worldwide. Some fungi can cause superficial infections in animals after accidental penetration and colonization on injured skin and can be transmitted to humans by exposure. The infection caused mainly by dermatophyte species and may also be caused rarely by yeasts and nondermatophytic molds. Materials and Methods: Eighty-two skin scrapings and hair samples were collected from animals (sheep, cow, cat, camel, calf, goat, horse, and dog) in three specialized pet clinics and three livestock and slaughterhouses. The isolates were identified using direct microscopy, culture, and polymerase chain reaction-sequencing of ITS1-5.8SrDNA-ITS2 region. Results: Thirteen mold strains out of 82 clinical samples (15.8%) were isolated from animal lesions. Acremonium exuvarum (n = 4; 30.7%), Sarocladium implicatum (n = 2; 15.4%), Arthroderma otae (n = 2; 15.4%), Chaetomium iranianum (n = 1; 7.7%), Trichothecium roseum (n = 1; 7.7%), Lichtheimia ramosa (n = 1; 7.7%), Penicillium chrysogenum (n = 1; 7.7%), and Microsporum equinum (n = 1; 7.7%) were isolated from clinical specimens. Conclusion: Since opportunistic fungi are increasing as etiological agents of dermatomycoses, isolation of these molds from wounds can be a warning to veterinarians, and daily cleaning of wounds with a proper disinfectant is recommended for the prevention of fungal colonization.

Keywords: Animal lesions, dermatomycoses, dermatophytes, opportunistic molds, zoonosis

Introduction

Dermatomycoses contain superficial fungal infections of the skin, hair, and nail that affect more than 20%-25% of the people and animals worldwide, particularly in tropical and subtropical regions. These infections caused by yeasts, dermatophyte species, and hyaline or dematiaceous molds. The frequency of infection and the distribution of causative agents can alter substantially according to geographical region, population migration profiles, climate, socioeconomic status, condition of animal husbandry, and cultural factors. Opportunistic mycoses are uncommon, most regularly revealed as cutaneous infections in cats or as systemic hyalohyphomycosis in dogs. The prevalence of dermatomycoses has increased considerably in animals over the past 20 years. Some fungi can cause superficial infections in animals after accidental penetration and colonization on injured skin, particularly when immunologic defects exist in the host. The aim of the present study was to identify the molds isolated from animal lesions suspected to dermatomycoses.

Materials and Methods

In this cross-sectional study, 82 skin scrapings and hair samples were collected from animals in three specialized pet clinics located in Mardavij street, Northern Sheikh Sadough street, and Second Apadana street, Isfahan, and three livestock and slaughterhouses in Borkhar County, Fasaran, a village in Baraan-e Shomali Rural District, in the Central District of Isfahan County, and Najafabad County, Isfahan Province, Iran, from August 2018 to April 2019. All specimens were divided into two parts: one portion used for direct microscopic examination with potassium hydroxide 20%, and another part subcultured on sabouraud glucose agar (Difco, Detroit, MI, USA) with

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Access this article online
Website: www.advbiores.net
DOI: 10.4103/abr.abr_230_19

How to cite this article: Rahimi T, Mohammadi R. Identification of dermatophyte and nondermatophyte molds isolated from animal lesions suspected to dermatomycoses. Adv Biomed Res 2020;9:2.

Received: 20 October 2019; Revised: 30 October 2019; Accepted: 12 November 2019; Published: 21 January 2020

Address for correspondence:
Dr. Rasoul Mohammadi, Ph.D., Associate Professor of Medical Mycology, Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: drrasoul_mohammadi@yahoo.com

Tahereh Rahimi¹, Rasoul Mohammadi¹,²
From the ¹Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, ²Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

© 2020 Advanced Biomedical Research | Published by Wolters Kluwer - Medknow
chlamphenicol (0.04 g/L) and cycloheximide (0.5 g/L) for dermatophytes and sabouraud glucose agar (Difco, Detroit, MI, USA) with chlamphenicol (0.04 g/L) and without cycloheximide for nondermatophyte molds and incubated at 35°C for 3 weeks.

- The inclusion criteria: resistant lesions to antibacterial agents
- The exclusion criteria: antifungal consumption and bacterial growth on culture media.

### Molecular identification

DNA was extracted using phenol/chloroform method.[7] ITS1-5.8SrDNA-ITS2 region was amplified for sequence analysis.[8] Briefly, polymerase chain reaction (PCR) mixture including 2.5 μL of 10 × reaction buffer, 0.4 mM dNTPs, 1.5 mM MgCl₂, 1.25 U of Taq polymerase, 30 pmol of both ITS1 (5’-TCC GTA GGT GAA CCT GCG G-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’) primers, and 2 μL of extracted DNA were applied in a final volume of 25 μL. The PCR cycling conditions were an initial denaturation phase at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension phase at 72°C for 7 min. Seven microliters of PCR products was loaded on 1.5% agarose gel, and stained with 0.5 μg/mL ethidium bromide, then visualized by gel documentation system (UVITEC, UK) and photographed. PCR products were purified, and cycle sequencing reactions in forward direction were performed (Bioneer, South Korea). The sequencing products were analyzed with Chromas 2.6.6 (https://technelysium.com.au/wp/chromas/) and were evaluated using of NCBI BLAST searches against fungal sequences existing in DNA databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

### Results

Clinical specimens were obtained from sheep (29.3%), cow (25.6%), cat (12.2%), camel (9.7%) (from Borkhar), calf (7.3%), goat (6.1%), horse (4.9%) (from Najafabad), and dog (4.9%). Thirteen mold strains out of 82 clinical samples (15.8%) were isolated and identified. Male-to-female sex ratio was 2/22, 7/14, 6/4, 1/7, 3/3, 4/1, 1/3, and 1/3, for sheep, cow, cat, camel, goat, horse, and dog, respectively. Age range was 1–3 years, 2–4 years, 6 month–3 years, 2–5 years, 5–8 months, 2–3 years, 3–6 years, and 4 months–4 years for sheep, cow, cat, camel, calf, goat, horse, and dog, respectively. Lesions were located on the ear (35.4%), abdomen (21.9%), neck (20.7%), trunk (10.9%), tail (4.9%), foot (2.4%), eyelid (2.4%), and muzzle (1.2%). The ITS1-5.8SrDNA-ITS2 region was amplified, and PCR products were sent for sequence reaction in forward direction. Acremonium exuviam [Figure 1], Sarocladium implicatum [n = 2; 15.4%], Arthroderma otae [n = 2; 15.4%] [Figure 3], Chaetomium iranianum [n = 1; 7.7%], Trichothecium roseum [n = 1; 7.7%], Lichtheimia ramosa [n = 1; 7.7%] [Figure 2], Penicillium chrysogenum [n = 1; 7.7%], and Microsporum equinum [n = 1; 7.7%] [Figure 3] were isolated and identified from clinical specimens. Table 1 shows the characteristics of animals with lesions suspected to dermatomycosis in the present study.

### Discussion

Dermatomycoses have a worldwide distribution, with high frequency in the industrialized countries. Etiologic agents contain opportunistic fungi (Aspergillus, Trichosporon, Rhodotorula, Acremonium, Scopulariopsis, Rhizopus, Candida, Cryptococcus) and dermatophyte species.[9][10] In the present study, we isolated rare molds from various lesions in animals mimicking dermatophytosis. Three isolates belonged to dermatophyte genus including A. otae (2 isolates; obtained from cats), and M. equinum (1 isolate; obtained from a horse). Interestingly, all A. exuviam strains were isolated from sheep. This uncommon mold isolated by Sigler et al.[12] from shed reptile skins for the first time and identified based on β-tubulin and ribosomal internal transcribed spacer sequences. Acremonium is a large fungal genus that contains almost 160 species, most of them are in soil and phytopathogens and others are considered as humans and animals opportunistic pathogens.[13] Infections in mammals generally caused by traumatic implantation of the mold into the eye and skin; however, the role of Acremonium genus as a causative agent of onychomycosis has also been reported.[14] Sarocladium genus was previously classified in the Acremonium complex, however, regarding recent molecular investigation, the taxonomy of Acremonium was altered and some important animal and phytopathogenic species transferred to Sarocladium as a separate genus.[15] S. implicatum (A. implicatum) has been isolated.
from different clinical specimens such as sputum, bronch wash, sinus, bone, and bronchoalveolar lavage, but we isolated this fungi from ear lesions in sheep and cow. *A. otae* complex comprises three species of *Microsporum*, including *M. canis* as a zoophilic species, the anthropophilic *M. ferrugineum*, and *M. audouinii* species. *Microsporum canis* is a zoophilic species with worldwide distribution. Dogs, cats, and horses are natural reservoirs, and humans can be infected after contact with infected animal or human. We isolated two *M. canis* strains from muzzle and trunk of two cats referred to a specialized pet clinic. The genus *Chaetomium* is an olivaceous nondermatophytic mold found in plant debris, soil, and environment as opportunistic fungus. *Chaetomium* species are scarcely involved in human and animal infections, however, it can cause superficial (onychomycosis), subcutaneous, and disseminated infections in immunosuppressed patients. *C. iranianum* is a member of the *C. carinthiacum* species group, characterized by hairs and fusiform ascospores and spirally coiled ascomatal hairs. We obtained one isolate *C. iranianum* from ear lesion of a sheep in the present investigation. *T. roseum* is an ascomycetous fungus first reported in 1809, which produces different kinds of mycotoxins, such as trichothecenes and roseotoxins, which
The characteristics of animals with lesions suspected to dermatomycosis in the present study

| Number | Kind of animal | Sex  | Age (year) | Location of lesion | Etiologic agent       |
|--------|----------------|------|------------|--------------------|-----------------------|
| 1      | Cat            | Female | 1          | Muzzle             | A. otae               |
| 2      | Cat            | Female | 2          | Trunk              | A. otae               |
| 3      | Sheep          | Female | 2          | Neck               | A. exuviarum          |
| 4      | Sheep          | Female | 3          | Ear                | A. exuviarum          |
| 5      | Sheep          | Female | 2          | Ear                | A. exuviarum          |
| 6      | Sheep          | Female | 1          | Ear                | S. implicatum         |
| 7      | Sheep          | Female | 2          | Ear                | A. exuviarum          |
| 8      | Sheep          | Female | 3          | Ear                | C. iranianum          |
| 9      | Cow            | Female | 2          | Ear                | S. implicatum         |
| 10     | Cow            | Female | 2          | Ear                | T. roseum             |
| 11     | Calf           | Female | 5 months   | Neck               | L. ramosa             |
| 12     | Calf           | Female | 6 months   | Ear                | P. chrysogenum        |
| 13     | Horse          | Male   | 6          | Neck               | M. equinum            |

*A. otae: Arthroderma otae, A. exuviarum: Acremonium exuviarum, S. implicatum: Sarocladium implicatum, C. iranianum: Chaetomium iranianum, T. roseum: Trichothecium roseum, L. ramose: Lichtheimia ramose, P. chrysogenum: Penicillium chrysogenum, M. equinum: Microsporum equinum*

can spoil fruit crops. So far, this mold has not been isolated from clinical samples of human or animals, thus isolation of this phytopathogen mold from ear lesion in the present study is questionable. Another rare isolated mold was *P. chrysogenum* obtained from a 6-month-old calf’s ear lesion. Infection due to *Penicillium* species is rare; however, a number of superficial or systemic infections have been reported in human, such as otomycosis, onychomycosis, keratitis, alveolitis, esophagitis, endocarditis, and peritonitis. Wigney *et al.* reported an osteomyelitis associated with *P. verruculosum* in a German shepherd dog. *Lichtheimia (Absidia)* belongs to the order Mucorales containing six species, namely *L. corymbifera, L. ramosa, L. hyalospora, L. brasiliensis, L. sphaerocystis, and L. ornata*. Roden *et al.* showed that *Lichtheimia* spp. accounted for nearly 5% of all mucormycosis in the USA, but it was identified as the second most prevalent causative agent of mucormycosis in Europe (19%–29%). We isolated this fungus from neck lesion of a calf. The animal’s wound had become chronic and was resistant to topical antifungal agents. *M. equinum* was another dermatophyte species caused a single lesion in the neck of a 6-year-old horse. The infection was treated using chlorhexidine as a common disinfectant agent and daily washing by ketoconazole shampoos after 8 weeks. Shokri and Khsoravi isolated 255 fungal cases from 1011 suspected animals to dermatomycoses. The most prevalent fungal infections were dermatophytosis (49.7%), Malassezia dermatitis (45.4%), candidiasis (2.5%), aspergillosis (2.2%), and zygomycosis (0.2%). In the present study, we isolated 3 out of 13 dermatophyte spp. (23%) from infected animals. Khsoravi and Mahmoudi reported *Microsporum canis* as the most frequent dermatophyte isolate from domestic animals in Iran between 1994 and 1998. We also isolated *A. otae (M. canis)* as the most common dermatophyte from two cats. Aghamirian and Ghiasian identified *Trichophyton verrucosum* as the causative agent of dermatophytoses among infected cows, however, none of the cows in our study had dermatophytosis.

**Conclusion**

Zoophilic dermatophytes have public health implications and can transmit to humans by frequent contacts, so complete care must be considered when dealing with the infected animals, especially for immunosuppressed patients. Since opportunistic fungi are increasing as etiological agents of dermatomycoses, isolation of these molds from wounds can be a warning to veterinarians, and daily cleaning of wounds with a proper disinfectant is recommended for prevention of fungal colonization.

**Acknowledgments**

The authors thank Dr. Moradi for his cooperation for collection of clinical specimens.

**Financial support and sponsorship**

This investigation was financially supported by Isfahan University of Medical Sciences (No. 397178).

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Silva L, De Oliveira D, Da Silva B, De Souza R, da Silva P. Ferreira-Paim K, *et al*. Identification and antifungal susceptibility of fungi isolated from dermatomycoses. J Eur Acad Dermatol Venereol 2014;28:633–40.
2. Simonnet C, Berger F, Gantier JC. Epidemiology of superficial fungal diseases in French Guiana: A three-year retrospective analysis. Med Mycol 2011;49:608–11.
3. Abamni A, Bakheshwain S, El Khizzi N, Zouman AR, Hantrah S, Al Harthi F, *et al*. Characteristics of superficial fungal infections in the Riyadh region of Saudi Arabia. Int J Dermatol 2008;47:229–35.
4. Archer TM, Boothe DM, Langston VC, Fellman CL,

---

**Table 1: The characteristics of animals with lesions suspected to dermatomycosis in the present study**
Lunsford KV, Mackin AJ. Oral cyclosporine treatment in dogs: A review of the literature. J Vet Intern Med 2014;28:1-20.

5. Dedeaux A, Grooters A, Wakamatsu-Utsuki N, Taboada J. Opportunistic fungal infections in small animals. J Am Anim Hosp Assoc 2018;54:327-37.

6. Casadevall A, Pirofski LA. Host-pathogen interactions: Basic concepts of microbial commensalism, colonization, infection, and disease. Infect Immun 2000;68:6511-8.

7. Gnat S, Nowakiewicz A, Ziolkowska G, Troscianczyk A, Major-Dziedzic B, Zięba P. Evaluation of growth conditions and DNA extraction techniques used in the molecular analysis of dermatophytes. J Appl Microbiol 2017;122:1368-79.

8. Makimura K, Tamura Y, Mochizuki T, Hasegawa A, Tajiri Y, Hanazawa R, et al. Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Clin Microbiol 1999;37:920-4.

9. Dias MF, Quaresma-Santos MV, Bernardes-Filho F, Amorim AG, Schechtman RC, Azulay DR. Update on therapy for superficial mycoses: Review article part I. An Bras Dermatol 2013;88:764-74.

10. Neoff P, Krüger C, Schaller J, Ginter-Hanselmayer G, Schulte-Beerbühl R, Tietz HJ. Mycology – An update part 2: Dermatomycoses: Clinical picture and diagnostics. Dtsch Dermatol Ges 2014;12:749-77.

11. Malik NA, Raza N. Non-dermatophyte moulds and yeasts as causative agents in onychomycosis. J Pak Assoc Dermatol 2016;19:74-8.

12. Sigler L, Zuccaro A, Summerbell RC, Mitchell J, Paré JA. Acremonium exuviarum sp. nov., a lizard-associated fungus with affinity to Emericellopsis. Stud Mycol 2004;6:409-13.

13. Guarro J, Gams W, Pujol I, Gené J. Acremonium species: New emerging fungal opportunists-in vitro antifungal susceptibilities and review. Clin Infect Dis 1997;25:1222-9.

14. Gupta AK, Jain HC, Lynde CW, MacDonald P, Cooper EA, Summerbell RC. Prevalence and epidemiology of onychomycosis in patients visiting physicians’ offices: A multicenter Canadian survey of 15,000 patients. J Am Acad Dermatol 2000;43:244-8.

15. Summerbell RC, Gueidan C, Schroers HI, de Hoog GS, Starink M, Rosete YA, et al. Phylogenetic overview and revision of Gliomastix, Sarocladium, and Trichothecium. Stud Mycol 2011;68:139-62.

16. Perdomo H, Sutton DA, Garcia D, Fothergill AW, Cano J, Gené J, et al. Spectrum of clinically relevant Acremonium species in the United States. J Clin Microbiol 2011;49:243-56.

17. Kobyjak N, Bykowski B, Kurzyk E, Nowicki R, Brillowska-Dabrowska A. PCR and real-time PCR approaches to the identification of Arthroderma otae species Microsporum canis and Microsporum audouinii/Microsporum ferrugineum. J Eur Acad Dermatol Venereol 2016;30:1819-22.

18. Serrano Falcón C, Serrano Falcón MD, Delgado Ceballos J, Delgado Florencio V, Crespo Erchiga V, Serrano Ortega S. Onychomycosis by Chaetomium sp. Mycoses 2009;52:77-9.

19. Kim DM, Lee MH, Suh MK, Ha GY, Kim H, Choi JS. Onychomycosis Caused by Chaetomium globosum. Ann Dermatol 2013;25:232-6.

20. Anandi V, John TJ, Walter A, Shastry J, Lalitha M, Pathy A, et al. Cerebral phaeohyphomycosis caused by Chaetomium globosum in a renal transplant recipient. J Clin Microbiol 1989;27:2226-9.

21. Asgari B, Zare R. The genus Chaetomium in Iran, a phylogenetic study including six new species. Mycologia 2011;103:863-82.

22. Gong D, Bi Y, Li Y, Zong Y, Han Y, Prusky D. Both Penicillium expansum and Trichothecium roseum infections promote the ripening of apples and release specific volatile compounds. Front Plant Sci 2019;10:338.

23. Lyratopoulos G, Ellis M, Nerringer R, Denning DW. Invasive infection due to Penicillium species other than P. marneffei. J Infect 2002;45:184-95.

24. Park HS, Jung KS, Kim SO, Kim SJ. Hypersensitivity pneumonitis induced by Penicillium expansum in a home environment. Clin Exp Allergy 1994;24:383-5.

25. López-Martínez R, Neumann L, González-Mendoza A. Case report: Cutaneous penicilliosis due to Penicillium chrysogenum. Mycoses 1999;42:347-9.

26. Chander J, Sharma A. Prevalence of fungal corneal ulcers in Northern India. Infection 1994;22:207-9.

27. Hoffman M, Bash E, Berger SA, Burke M, Yust I. Fatal necrotizing esophagitis due to Penicillium chrysogenum in a patient with acquired immunodeficiency syndrome. Eur J Clin Microbiol Infect Dis 1992;11:1158-60.

28. Huang SN, Harris LS. Acute disseminated penicilliosis: Report of a case and review of pertinent literature. Am J Clin Pathol 1963;39:167-74.

29. Wigney D, Allan G, Hay L, Hobbing A. Osteomyelitis associated with Penicillium verruculosum in a German shepherd dog. J Small Anim Pract 1990;31:449-52.

30. Alastruey-Izquierdo A, Hoffmann K, de Hoog GS, Rodriguez-Tudela JL, Voigt K, Bibashi E, et al. Species recognition and clinical relevance of the zygomycetous genus Lichtheimia (syn. Absidia pro parte, Mycocladus). J Clin Microbiol 2010;48:2154-70.

31. Schwartzke VU, Jacobsen ID. Mucormycoses caused by Lichtheimia species. Mycoses 2014;57 Suppl 3:7-38.

32. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: A review of 929 reported cases. Clin Infect Dis 2005;41:634-53.

33. Shokri H, Khorasani AR. An epidemiological study of animals dermatothecomycoses in Iran. J Mycol Med 2016;26:170-7.

34. Aghamirian MR, Ghiasian SA. Dermatophytes as a cause of epizoonoses in dairy cattle and humans in Iran: Epidemiological and clinical aspects. Mycoses 2011;54:e52-6.