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Descriptive epidemiology of dermatophytosis in rodents

Donya Nikaein[1,2] | **Pegah Yaghuti**[3] | **Aghil Sharifzadeh**[1,2] | **Alireza Khosravi**[1,2] | **Asad Balal**[1,2]

[1]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[2]Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[3]Doctor of Veterinary Medicine graduate, Faculty of Veterinary Medicine, Razi University of Kermanshah, Kermanshah, Iran

Correspondence
Donya Nikaein, Azadi St. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: dnikaein@ut.ac.ir

Abstract

Introduction: Dermatophytosis is a zoonotic disease caused by a group of keratinophilic fungi called dermatophytes.

Objectives: Since the epidemiology of diseases revolves over time, this research studies the incidence of dermatophytosis among rodents referred to mycology laboratory during 2019–2021.

Methods: A total of 163 rodents including rabbits, guinea pigs, and hamsters suspecting having dermatophytosis were sampled by scraping lesions. Direct microscopic examination, culture, and polymerase chain reaction were done for diagnosis of dermatophytosis and identification of the etiologic agent.

Results: The results of this study showed that 37.4% of rodents were involved with dermatophytosis, among which 41.13% of rabbits, 25% of guinea pigs, and 26.3% of hamsters were included. *Microsporum canis* (52.7%) was the most isolated agent. Incidence of dermatophytosis was higher in female in rabbits while in hamsters and guinea pigs male were mostly infected. Rodents less than 6 months were more susceptible for dermatophytosis except for hamsters in which 6–12 months animals had a higher prevalence.

Conclusion: In conclusion, it is significant to update our knowledge about the epidemiology of dermatophytosis in rodents and other animals every few years to define valid preventive strategies. Moreover, since dermatophytes are contagious and zoonotic, it is also a priority to apply preventing methods for dermatophytosis and treat infected rodents with appropriate antifungal agents to decrease the risk of infection.

KEYWORDS
dermatophytosis, rodents, *Trichophyton mentagrophytes*, *Microsporum canis*, *M. gypseum*

Clinical significance

Dermatophytosis is zoonotic fungal infection considered as a public health hazard in many countries. Rodents are among hosts of dermatophyte that can transfer the disease to human and other animals. It is important to update our knowledge about the epidemiology of diseases every few years, since there has not been a recent research on dermatophytosis of rodents and its changing epidemiology and the interest to keep small rodents as house pets has increased in late years, this study could highlight the importance and etiologic agents of

dermatophytosis in rodents and might assist in prevention and treatment strategies.

1 | INTRODUCTION

Dermatophytes are a group of fungi that are capable to digest keratin as food source. They attack keratinized tissues of skin including hair, nails, claws, and feathers, and cause dermatophytosis (commonly known as ringworm). Dermatophytosis is the most prevalent superficial fungal infection, which occurrence is increasing worldwide along with difficult to treat cases. This zoonotic disease has been identified as a public health hazard in recent years (Gnat et al., 2021). Among animals that can be infected with different types of these fungi are rodents, they transmit the infection to humans through hair, body shells, and fomites. The most common isolated pathogens in animals with dermatophytosis are *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Microsporum canis* (Cafarchia et al., 2010).

Trichophyton mentagrophytes and recently molecularly recognized *T. benhamiae* are the most common fungal species isolated from rabbits, and some researchers consider rabbits to be asymptomatic carriers of the organism (Donnelly et al., 2000; Fehr, 2015; Tekin et al., 2019). Most rodents involving with dermatophytes have no clinical symptoms. According to published data, infection is common in rabbits and guinea pigs and is uncommon in chinchillas, mice, and rats (Donnelly et al., 2000).

Arthroconidia of dermatophytes will remain on hair and skin scrapings of infected animals or in the environment (Torres-Rodriguez et al., 1992; Van Rooij et al., 2006). Infected rodents especially rabbits might often act as asymptomatic carriers of zoophilic dermatophytes and spread arthroconidia, which are able to remain viable in the environment for a 2–3 years period. As a result, these exotic pets could be reservoirs of infection for humans and animals (Debnath et al., 2016; Kazemi-Moghaddam et al., 2019; Papini et al., 2008). While dermatophytosis has been extensively studied in many animal species, including dogs and cats (Hernandez-Bures et al., 2021; Ibrahim et al., 2021), information on their occurrence and epidemiology in rodents is limited. However, the industrialization of rabbit breeding in some countries has led to an increase in the incidence of dermatophytosis in these animals (Mishra et al., 2022). Age of rabbits and habitat management (temperature, humidity, and method and rate of disinfection) are known as the most important risk factors for dermatophytes (Cafarchia et al., 2010; Mishra et al., 2022). Young rabbits are most sensitive to dermatophytes due to the immaturity of the immune system as well as immune-compromised rabbits. The presence of external parasites, especially fleas and mites, can also be important in the development and spread of dermatophytosis (Mishra et al., 2022). Dermatophytosis can also lead to malnutrition, stunted growth, and reduced feed conversion rates in rabbits or even death (Kushwaha & Guarrro, 2000). This research studies the incidence of dermatophytosis among rodents referred to mycology laboratory during 2019–2021.

2 | MATERIALS AND METHODS

2.1 | Place and time of study

This study was performed on 163 cases of rodents suspecting of involving with dermatophytosis referred to Mycology laboratory, Faculty of veterinary Medicine, University of Tehran between the years 2019 and 2021. Rodents' species included guinea pig, hamster, and rabbit.

2.2 | Sampling procedure

Animals with dermatological lesions including alopecia, erythema, and scaling were sampled. Samples were collected by skin scraping of the lesions after disinfecting the lesions with 70% ethanol or scrubbing the skin using toothbrush technique (Gnat et al., 2021). Rodents were distributed to three age groups, <6 months, 6–12 months, and >6 months.

2.3 | Direct microscopic examination (DME)

Direct light microscopy was performed using potassium hydroxide (KOH) 10% mount. In brief, skin scrapings and hairs were mounted on a drop of 10% KOH and examined under 100 and 400X magnification for dermatophyte hyphae/arthroconidia.

2.4 | Culture

Sabouraud's dextrose agar (SDA) (Merck Co., Germany) plates containing chloramphenicol (Sigma-Aldrich Co., USA) 0.05 mg/l, cycloheximide (Sigma-Aldrich Co., USA) 0.5 mg/l (SC & SCC) and Dermatophyte test medium (DTM) (Merck Co., Germany) were used. Samples (hair, scales) were inoculated on SC (SDA plus chloramphenicol), SCC (SDA plus chloramphenicol and cycloheximide), and DTM media and incubated at 28 and 37°C (SC & SCC media) for at least 21 days. Cultures were evaluated for dermatophyte growth every day. Each fungal isolate was sub-cultured for further experiments.

2.5 | Identification of dermatophyte isolates

Dermatophyte species were identified using macroscopic and microscopic characteristics of the colonies. In microscopic examination, the colonies were stained with Lactophenol cotton blue and then examined (Debnath et al., 2016). Morphological identification was mainly based on colony macroscopic characteristics (color, texture, etc.) as well as macro and micro conidia presence, shape, size, and hyphae properties under light microscope. Molecular identification was done

using pan-dermatophyte primers based on Internal Transcribed Spacer region (ITS) (Gnat et al., 2021). In brief, total DNA was isolated from fresh cultures of dermatophyte colonies using trizol reagent (Ambion Life Technologies, USA) according to manufacturer's instructions. The quantity and purity of extracted DNA were assessed using nanodrop 2000 (Thermo Fisher Scientific, USA). A previously confirmed *M. canis* isolate (PTCC5069) was also used as the positive control (data not shown).

Primers used were ITS1 (5′TCCGTAGGTGAACCTGCGG3′) and ITS4 (5′TCCTCCGCTTATTGATATGC3′). Polymerase chain reaction (PCR) was done at 95°C 3 min, followed by 30 cycles of 95°C 1 min, 50°C 1 min, 72°C 1 min, and then 72°C 8 min for final extension. Amplicons were electrophorized on 2% agarose gels. PCR products were further sequenced using automated Sanger method with ABI 3730XL DNA Analyzer (Thermo Fisher Scientific, USA). The identified isolates were registered on NCBI GenBank (accession numbers: OP614984-5, OP615073, OP615076, OP615083, and OP615094).

2.6 | Statistical analysis

Differences in occurrence of dermatophytosis relating to age, sex, and species were analyzed using SPSS version 25. Chi-squared test was used to evaluate significant differences between occurrence of disease and different variables. A *p*-value of less than 0.05 was considered significant.

3 | RESULTS

A total of 163 samples were taken from three species of rabbits, guinea pigs, and hamsters, of which 74 (51%) were male and 71 (49%) were female. Number of culture positive and DME positive cases are shown in Table 1.

Out of 124 suspected rabbits, dermatophyte hyphae/arthroconidia was observed in DME of 51 cases (41.13%), while dermatophytes were only isolated in culture of 46 individuals (37.1%) and 6 rabbits were found infected with *Malassezia* spp on DME examination (4.8%). Prevalence of dermatophytosis was significantly higher in female rabbits than males (58.8% vs. 41.2%) ($p<0.05$). Incidence of dermatophytosis was significantly higher (78.5%) in rabbits between 0 and 6 months of age ($p<0.05$) (Table 2). In association with site of infection, disseminated dermatophytosis (involving of more than one area) was most common (21.57%) and neck was the less common site of infection (9.8%) and this difference was significant ($p<0.05$); however, there were no significant differences between other sites of infections ($p>0.05$). Figure 1 summarizes the frequency of infections sites in different rodents in this study.

TABLE 1 Number of microscopically and culture positive cases of dermatophytosis among rodents referred to mycology laboratory

Rodent	Number	Positive cases		
		DME*	Culture	DME and culture
Rabbit	124	51 (41.13%)	46 (37.1%)	46 (37.1%)
Guinea pig	20	5 (25%)	4 (20%)	4 (20%)
Hamster	19	5 (26.3%)	5 (26.3%)	5 (26.3%)
Total	163	61 (37.4%)	55 (33.7%)	55 (33.7%)

*DME: Direct microscopic examination.

Total number of Guinea pigs in this study was 20 from which 5 (25%) found positive on DME and 4 (20%) on culture. Interestingly, 15% of guinea pigs were positive for *Malassezia* spp. and 10% (Cafarchia et al., 2010) possibly had dermatitis due to *Candida spp* according to observing yeast and budding cells and true hyphae on DME. *Scopulariopsis brevicaulis* was also isolated from the ear skin of one guinea pig (5%). Among positive cases, 80% were male and 20% were female. Male had significantly higher prevalence of dermatophytosis ($p<0.05$). In age groups, dermatophytosis was significantly more present in <6 months of age guinea pigs ($p<0.05$). None of the cases had disseminated dermatophytosis and occurrence of the disease was mostly seen in ear and body (40% each) and infection of these sites was significantly higher in comparison with limbs (20%) ($p<0.05$). No significant differences was observed between breed of guinea pigs ($p>0.05$).

In hamsters, 26.3% were both DME and culture positive from which 60% were male and 40% were female ($p<0.05$). *Malassezia* spp was observed in 10.5% of cases and 5.27% had *Candida* dermatitis on their ears. Hamsters had significantly higher prevalence of dermatophytosis in age groups 6–12 months (60%) ($p<0.05$) and occurrence of dermatophytosis in <6 months was 40%. In hamsters like guinea pigs, the incidence of dermatophytosis was significantly higher in males ($p<0.05$) and body was the most prevalent site of infection ($p<0.05$).

Confirmation of phenotypic identification of dermatophyte species was performed based on ITS-based PCR and DNA sequence analysis. Amplicons size of the identified species ranged between 650 and 740 bp as presented in Figure 2. *Microsporum canis* was the most isolated species (52.7%) after molecular identification of culture positive dermatophytes followed by *M. gypseum* (25.4%) and *Trichophyton mentagrophytes* (18.3%) and *T. benhamiae* (3.6%). *M. gypseum* was the most common isolate in guinea pigs (75%) while it was not isolated in hamsters; and *Trichophyton* spp. was not seen in culture of guinea pigs. *T. benhamiae* was only observed in culture of rabbits' specimens (4.35%).

4 | DISCUSSION

The affection to keep small rodents as pets has increased in recent years, this has led to an increase in occurrence of infectious diseases and growing risk of human infection (Tekin et al., 2019). Dermatophytosis is the most important fungal infection in small rodents (Pollock, 2003). Despite its low incidence, the zoonotic nature of the disease has made it a public health hazard, and it is among notifiable infections in some countries including Norway (Lund et al., 2014). Moreover, rodents can be asymptomatic carriers of the infection and transfer the disease to human and other animals (Pollock, 2003). Kazemi-Moghaddam et al. (2019) examined rodent related diseases in Iran,

TABLE 2 Frequency and relative frequency of dermatophyte species isolated from rodents in this study and confirmed by ITS-PCR and DNA sequencing according to age groups along with frequency and relative frequency of dermatophyte infected rodents in relation with sex

Rodent	Age (month)	Males	Females	*M. canis*	*M. gypseum*	*T. mentagrophytes*	*T. benhamiae*
Rabbit	<6	16 (57.2%)	24 (72.8%)	17 (58.6%)	11 (78.6%)	6 (60%)	2 (100%)
	6-12	3 (10.8%)	5 (15.2%)	7 (24.1%)	0	1(10%)	0
	>12	2 (7.1%)	1 (3%)	1(3.4%)	0	1 (10%)	0
Guinea pig	<6	2 (7.1%)	1 (3%)	1(3.4%)	1 (7.1%)	0	0
	6-12	2 (7.1%)	0	0	2 (14.3%)	0	0
	>12	0	0	0	0	0	0
Hamster	<6	1 (3.6%)	1 (3%)	2 (7.1%)	0	0	0
	6-12	2 (7.1%)	1 (3%)	1 (3.4%)	0	2 (20%)	0
	>12	0	0	0	0	0	0
Total		28 (100%)	33 (100%)	29 (100%)	14 (100%)	10 (100%)	2 (100%)

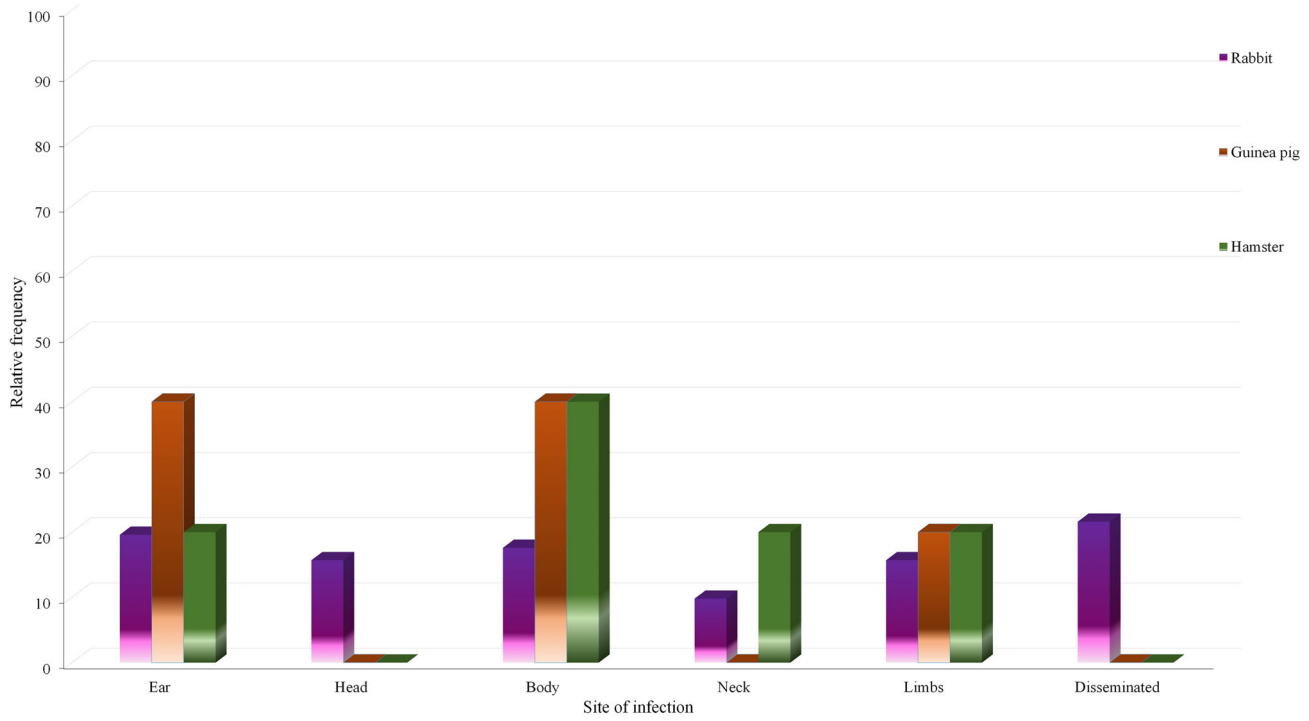

FIGURE 1 Relative frequency of different areas of body involved with dermatophytosis in rodents

they reported rodents as reservoirs and carriers of dermatophytosis and other infectious diseases. They described that dermatophytosis in rodents is almost spread in all parts of Iran and they named Golestan, Guilan, and Lorestan as provinces documented to be involved with dermatophytosis (Kazemi-Moghaddam et al., 2019). Shokri and Khosravi (2016) studied epidemiology of dermatophytosis in animals in Iran, along with of 1011 cases in their study 5 were rabbits and prevalent of dermatophytosis was 0.4% (Shokri & Khosravi, 2016). In their study similar to ours occurrence of dermatophytosis in female rabbits was more than males (60% vs. 40%). They declared *T. mentagrophytes* as the etiologic agent of dermatophytosis in rabbits, while in our study *M. canis* was the most prevalent species in rabbits. The difference in our results shows that epidemiology of diseases change by year. The more isolation of *M. canis* in our study might be as the result of increasing exposure to cats and dogs in today's life; and this also indicates the ability of *M. canis* to survive in rodent's skin and disease induction while *T. mentagrophytes* might have become less infective in rodents skin due to host adoptation however this opinion must be proven by further experiments on infectivity and virulence of these isolates.

In a recent study in Switzerland, *T. mentagrophytes*, *T. benhamiae*, and *M. canis* were the most prevalent zoophilic species in children and young adults, according to their investigation they considered cats and

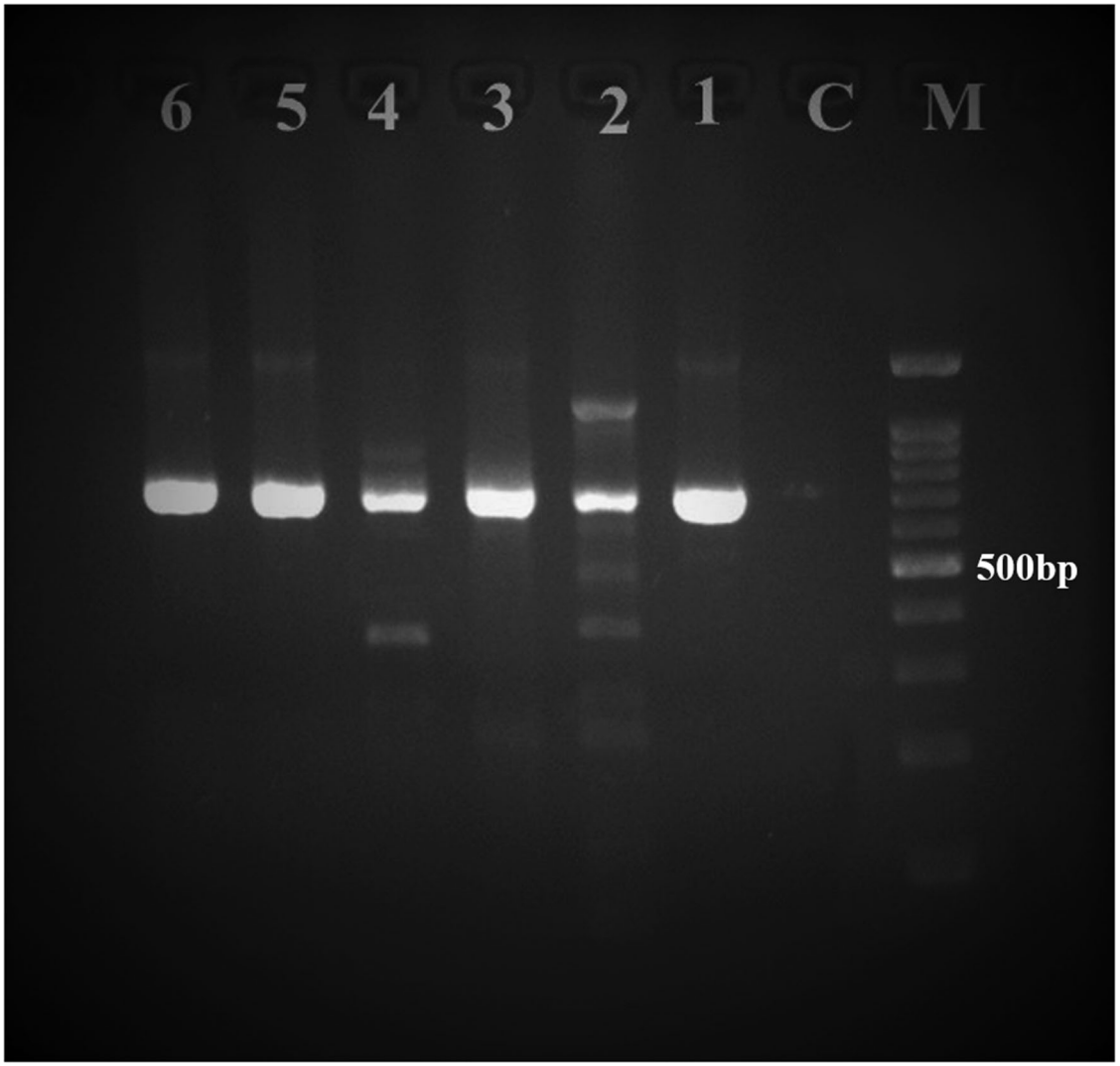

FIGURE 2 PCR products on 2% agarose gel using pan-dermatophyte primers based on Internal Transcribed Spacer region (ITS); Host: Rabbit, Lane M: Molecular Marker, Lane C: negative control, Lanes 2&4: *Trichophyton benhamiae* (657 bp), Lanes 1,3,5: *Microsporum gypseum* (686 bp), Lane 6: *Microsporum canis* (718 bp)

dogs as reservoirs of *T. mentagrophytes* and *M. canis* and guinea pigs as reservoirs of *T. benhamiae* (Bontems et al., 2020). They reported *T. mentagrophytes* and *T. benhamiae* in two and one cases of rabbits, respectively. In our study, however, *M. gypseum* was the most prevalent dermatophyte in guinea pigs and we could not isolate *T. benhamiae* from these animals. *M. gypseum* is a geophilic dermatophyte found in soil (Taha et al., 2018). Isolation of this microorganism could be a sign of bed contamination with soil or as a result of keeping of guinea pigs in soil environment. By the way in our study rabbits were the most common rodent infected with dermatophytosis and this might be due to more acceptance of rabbits in Iranian households. In this study, *M. canis* was mostly isolated from rabbits which was not observed in previous studies in Iran (Kazemi-Moghaddam et al., 2019; Khosravi et al., 1994), this might be the results of increasing contact with other pets, especially cats.

T. benhamiae has also reported in humans in Iran which had low but increasing prevalence (Abastabar et al., 2013; Ansari et al., 2016; Ebrahimi et al., 2019; Farokhipor et al., 2018; Rezaei-Matehkolaei et al., 2016; Zareshahrabadi et al., 2021). In another study in 2019, *T. benhamiae* has been reported in 38% of guinea pigs and 6% of hamsters and 0% of rabbits and they recommended guinea pigs as the main reservoir of zoophilic *T. benhamiae* (Tekin et al., 2019). Ansari et al. (2021) reported two cases of dermatophytosis in a father and daughter who had been infected by *T. benhamiae* transmitted from a guinea pig in Iran (Ansari et al., 2021). Nevertheless, in our study *T. benhamiae* was only isolated in rabbits; however, it must be considered that there were only 20 and 19 cases of guinea pigs and hamsters, respectively, in our study, while there were 124 cases of rabbits and this might be the cause of no isolation of *T. benhamiae* in these two in species. Another reason could be that in our study all cases had symptoms of dermatophytosis but in the described study samples were collected from apparently healthy rodents.

A study in 2014 in Iran, investigated the fungal flora of golden hamster, one of their objectives was to determine the presence of

dermatophyte species, but on the contrary they could not isolate any dermatophyte species (Shariatzadeh et al., 2014), in our study all hamsters had clinical signs of dermatophytosis. Their results along with ours, reinforces this issue that hamsters do not act as reservoirs of dermatophyte agents.

In an old study in Korea, dermatophytes and keratinophilic fungi in wild rodents were studied (Hong et al., 1998). They reported a prevalence of 16.3%. *T. mentagrophytes* was the most prevalent species (12.2%) in *Apodemus agrarius* and ventral hairs and feet were the most prevalent site of infection. They reported *Scopulariopsis* spp in 10.2% of their cases as well. Also, this fungi was the main keratinophilic species isolated from wild rodents in Kimpo-gun. In our study, *M. canis* was mainly isolated from rodents (52.7%), this finding suggests that geographical region, climate, and housing conditions could affect the dominant strain of dermatophytes in rodents. Interestingly, *Scopulariopsis brevicaulis* was isolated from ear skin of one of the guinea pigs (5%) in this study too.

Trichophyton quinckeanum is another zoophilic dermatophyte naturally found in rodents and camels and is rather isolated from mice (Lysková et al., 2021). Since mice are not remarked as pets in Iran, we did not observe this species in our cultures.

In a review article in 2000, ringworm has been described in rodents (Donnelly et al., 2000). *T. mentagrophytes* has been described as the main cause of dermatophytosis in hamsters, guinea pigs, and rabbits. It has been mentioned that *M. canis* and *M. gypseum* occasionally infect rabbits and guinea pigs but infection with *Microsporum* spp is common in hamsters. Yet, the results of our study showed that causative agents of diseases could change over time, as in our study *M. canis* was the main etiologic agent isolated while *T. mentagrophytes* was only seen in 18.3% cases. There must be an adaptation to *T. mentagrophytes* and this agent might not have the same virulence as before in rodents. Rodents might have become silent carriers of *T. mentagrophytes* and play role in infecting human with this dermatophyte as studies have previously shown the role of rodents in farms in infection of animals to *T. mentagrophytes* (Tartor et al., 2020; 2016).

It should also be noted that we observed cases of dermatitis related to *Malassezia* spp and *Candida* spp on DME. Both *Malassezia* and *Candida* genus are yeasts that inhabit the skin of humans and animals (microbiota) and are potential pathogens in convenient conditions. since our study was focused in dermatophytosis we just to confined to reporting them, but our data shows that yeasts could also been important agents of dermatitis in rodents and should be considered in future studies.

In conclusion, the results of this study suggest that the occurrence of dermatophytes in rodents has changed in recent years, and we should be aware of the new emerging species in rodents in the future. It is significant to update our knowledge about the epidemiology of dermatophytosis every few years. Since dermatophytes are contagious and zoonotic, it is also a priority to apply preventing methods for dermatophytosis and treat infected rodents with appropriate antifungal agents to decrease the risk of infection.

AUTHOR CONTRIBUTIONS

Donya Nikaein: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Writing – original draft; Writing – review and editing. Pegah Yaghuti: Methodology; Writing – original draft. Aghil Sharifzadeh: Resources; Supervision; Validation. Alireza Khosravi: Project administration; Supervision; Visualization. Asad Balal: Investigation; Methodology.

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All authors contributed to this study conception and design. Material preparation, data collection and analysis were performed by Donya Nikaein, Aghil Sharifzadeh, Alireza Khosravi, and Asad Balal. The first draft of the manuscript was written by Donya Nikaein and Pegah Yaghuti and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL

This is an observational study. The Faculty of Veterinary Medicine Research Ethics Committee has confirmed that no ethical approval is required.

ORCID

Donya Nikaein https://orcid.org/0000-0003-2333-3361

PEER REVIEW

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