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Original Article
Özgün Araştırma

Isolation and identification of dermatophytes in cats in Balıkesir province

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Abstract: Dermatophytosis can be observed in cats of all ages. However, it is more common in young, sick, elderly, and immunocompromised animals, and the recovery time may be prolonged in these cases. Humid environments and group housing are significant factors contributing to the onset of the disease. The diagnosis of dermatophytosis is primarily made through direct microscopy and culture. For this purpose, hair and skin scrapings taken from the lesion sites are used as materials. A total of 56 samples (hair, skin scrapings, etc.) collected from suspected of dermatophytosis cats by clinical veterinarians and sent to the laboratory for mycological diagnosis were examined. Out of these, 44 samples showed growth, while 12 did not. Based on the isolation and identification results, the most commonly isolated pathogens were *Penicillium* spp. (%23,21) and total *Aspergillus* spp. (25%), followed by *Candida* spp. (8,9%), *Microsporum* spp. (7,14%), *Trichophyton* spp. (7,14%), and *Alternaria* spp (5,35%). These fungal pathogens were isolated and identified from suspected dermatophyte samples in cats in Balıkesir province. One reason for dermatophyte's lower prevalence in this study could be that the samples were taken from pet cats under the care of clinical veterinarians. This study provides the first report on the prevalence of fungal pathogens observed in cats in Balıkesir province. Future studies should focus on monitoring and tracking fungal pathogens in cats, especially considering the One Health principle. Therefore, it was considered beneficial to conduct further research to determine the species-level agents responsible for dermatophytosis.

Keywords: cat, dermatophyte, identification, isolation

Balıkesir ilinde kedilerde görülen dermatofitlerin izolasyonu ve identifikasyonu

Özet: Dermatofitozis her yaştaki kedilerde görülebilmektedir. Ancak gençlerde, hasta, yaşlı hayvanlarda ve immun sistemi baskılanmış hayvanlarda daha sık görülür ve bu hayvanlarda iyileşme süresi de uzayabilmektedir. Nemli ortamlar ve grup halinde barındırma da hastalığın çıkışında önemli faktörlerdir. Dermatofitozisin tanısı direk mikroskobik ve kültür olmak üzere iki temel şekilde yapılır. Bu amaçla lezyonlu bölgelerden alınan kıl ve deri kazıntısı materyal olarak kullanılır. Dermatofitozis şüpheli kedilerden klinik veteriner hekimleri tarafından alınan ve mikolojik teşhis amacıyla laboratuvara gönderilen 56 materyalin (kıl, deri döküntüsü vb.) izolasyon ve identifikasyon bulgularına göre 44 materyalde üreme görüldü. 12 materyalde üreme görülmedi. İzolasyon ve identifikasyon sonucunda en çok üreyen etkenler toplam *Aspregillus* spp. (25%) ve *Penicillium* spp. (%23,21) olarak bulundu. *Candida* spp. (%8,9), *Microsporum* spp.(%7,14), *Trichophyton* spp. (%7,14) ve *Alternaria* spp. (%5,35) Balıkesir ilinde kedilerin dermatofit şüpheli materyallerinden izole ve identifiye edildi. Bu çalışmada dermatofitlerin daha düşük oranda görülmesinin bir sebebi olarak çalışma materyallerinin klinik veteriner hekimleri tarafından sahipli ev kedilerinden alınmış olması düşünüldü. Bu çalışma ile Balıkesir ilinde ilk defa kedilerde görülen mantar etkenlerinin prevalansı ortaya koyuldu. İleriki çalışmalarda özellikle tek sağlık prensibi düşüncesiyle mantar etkenlerinin kedilerde monitorize ve takip edilmesi gerektiği düşünüldü. Bu nedenle, dermatofitoza neden olan etkenlerin tür düzeyinde belirlenmesi için daha fazla araştırma yapılmasının faydalı olacağı düşünüldü.

Anahtar kelimeler: dermatofit, identifikasyon, izolasyon, kedi

Introduction

Dermatophytes are common pathogenic agents that cause dermatophytosis, an infection where zoophilic, geophilic, and anthropophilic fungal agents infect the skin (stratum corneum), hair, and nails of cats. Dermatophytosis is one of the most prevalent skin diseases worldwide. Due to the circular nature of the lesions they cause, these infections are also referred to as "ringworm" in both humans and animals (Babacan et al., 2011; Fratti et al., 2023; Frymus et al., 2013; Zorab et al., 2023). Dermatophtes also have zoonotic potential (Boehm and Mueller, 2019).

The most important causative agents of dermatophytosis in cats are *Microsporum* spp., *Epidermophyton* spp., and *Trichophyton* spp. (Mattei et al., 2014; Tel and Akan, 2008; Torti and Pinter,

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2009). *Epidermophyton* spp. are less commonly encountered (Spazamberg et al., 2023). Cats acquire these pathogens through direct contact with infected animals or by coming into contact with arthrospores present in the soil. Minor skin lesions play a crucial role in the entry of these pathogens into the body (Bilgili and Hanedan, 2022; Şahan Yapıcıer et al., 2017).

In pets, 95% of dermatophytosis infections are caused by *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton mentagrophytes* (Spazamberg, 2023). Dermatophytosis is also a zoonotic disease, making it a significant concern for human health. Particularly, *Microsporum canis* is responsible for infections in humans and is one of the main causative agents of human dermatophytosis (Bilgili and Hanedan, 2022; Kano et al., 2023; Şahan Yapıcıer et al., 2017).

Dermatophytosis can occur in cats of all ages. However, it is more frequently observed in young, sick, elderly, and immunocompromised animals, and the recovery period may be prolonged in these animals. Humid environments and group housing are also important factors in the development and transmission of the disease (Bilgili and Hanedan, 2022).

In cats, the lesions observed in dermatophytosis include crusting, focal, multifocal, or generalized alopecia, erythema, and miliary dermatitis (Şahan Yapıcıer et al., 2017). Lesions typically begin on the face, ears, and nose, and as the infection progresses, they can also appear on the paws and tail. Hair loss, papules, crusting, scaling, erythema, follicular obstruction, and increased pigmentation of the skin may be seen. Pruritus in affected animals is variable (Bilgili and Hanedan, 2022). These symptoms usually appear within 2-4 weeks, but they can be detected within 7 days post-exposure using a Wood's lamp (Babacan et al., 2011).

The diagnosis of dermatophytosis is primarily made through direct microscopy and culture. For this purpose, hair and skin scrapings collected from lesion sites are used as materials. Direct microscopy is performed using potassium hydroxide, and structures such as hyphae and spores of dermatophyte pathogens are examined under the microscope. Culture is conducted for pathogen isolation by inoculating Sabouraud's Dextrose Agar. After inoculation, the samples are incubated at 25°C for 5-7 days. Following incubation, the colonies are evaluated morphologically and microscopically for identification. Lactophenol cotton blue is used to stain fungal structures for microscopic identification. (Khosravi and Mahmoudi, 2003; Moriella, 2001; Proper Gary et al., 2020).

The aim of this study is the isolation and identification of dermatophytes in cats in Balıkesir province. The zoonotic nature of dermatophytosis, the fact that this study is the first of its kind in Balıkesir Province, the widespread practice of adopting and keeping cats, and the novelty of investigating the presence of dermatophyte pathogens and infections in Balıkesir make this research of unique value.

Materials and Methods

In this study, between October 2023 and August 2024, 56 samples (hair, skin scrapings, etc.) collected from cats suspected of dermatophyte infections by clinical veterinarians for mycological diagnosis at pet animal veterinary clinics were investigated. These samples were sent to the laboratory under cold chain conditions for mycological diagnosis by veterinarians, with the aim of isolating and identifying dermatophytes in cats.

The materials sent to the laboratory by clinical veterinarians for mycological diagnosis were subjected to microscopic examination, isolation, and identification.

This study is not subject to ethics committee approval according to Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik 8-k, ethics committee permission is not required for this study.

Microscopic examination

The microscopic examination was performed using a 10% KOH solution. A drop of 10% KOH was placed on a microscope slide, and the samples were carefully placed on top using sterile forceps. A cover slip was applied, and the slide was gently heated from below. The preparation was then left to stand at room temperature for 30-60 minutes. Afterward, the sample was examined under a microscope using a 40x objective lens, where spores and hyphae of the causative agents were searched for (Babacan et al., 2011; Bilgehan, 2004; Quinn et al., 2002).

Isolation and identification of dermatophytes

For the isolation of dermatophytes from all materials, Sabouraud Dextrose Agar (SDA) containing chloramphenicol (50mg/l) (Merck, Germany) was used. The samples were taken using sterile forceps or a scalpel and placed on the surface of the SDA

agar, embedding them to facilitate inoculation. The inoculated agars were incubated in an aerobic environment at 25°C for 5-7 days. The growth was monitored, and the results were recorded (Babacan et al., 2011; Bilgehan, 2004; Quinn et al., 2002).

During the incubation period, colonies of the growing pathogens were identified based on their macroscopic and microscopic characteristics. Macroscopically, the growth status and duration, structure, and pigmentation characteristics on both the front and back surfaces of the colonies growing on SDA agar were evaluated. For microscopic examination, a preparation was made from the colonies growing on SDA using Lactophenol Cotton Blue solution. A drop of Lactophenol Cotton Blue solution was placed on a slide, and a small piece from the outer edge of the growing colony was carefully collected using a sterile scalpel and placed on the solution. The preparation was covered with a cover slip, and under the microscope, the hyphae, macroconidia, microconidia, and spore structures of the fungal colonies were examined. Dermatophytes were evaluated at the genus level (Babacan et al., 2011; Bilgehan, 2004; Quinn et al., 2002; Moriello, 2001; Şeker and Doğan, 2011).

Results

According to the isolation and identification findings of 56 samples (hair, skin scrapings, etc.) collected from cats suspected of dermatophytosis by clinical veterinarians and sent to the laboratory for mycological diagnosis, fungal growth was observed in 44 samples, while no growth was detected in 12 samples. The findings of the isolated and identified dermatophytes and other fungal pathogens from the samples are presented in Table 1.

Table 1. Isolation and identification of dermatophytes and other fungal agents

Materials (n)	*Penicillium spp.*	*Other Aspergillus spp.*	*Aspergillus niger*	*Trichophyton spp.*	*Microsporum spp.*	*Alternaria spp.*	*Mucor spp.*	*Candida spp.*	**No growth**
56 (%100)	13 (%23,21)	4 (%7,14)	10 (%17,85)	4 (%7,14)	4 (%7,14)	3 (%5,35)	1(%1,78)	5(%8,9)	12(%21,42)

As a result of the isolation and identification, the most frequently growing pathogens were found to be *Penicillium* spp. and *Aspergillus* spp.. *Candida* spp., *Microsporum* spp., *Trichophyton* spp., and *Alternaria* spp. were also isolated and identified from dermatophyte-suspected samples collected from cats by clinic veterinarians in Balıkesir Province (Figure 1,2,3,4,5,6)

Figure 1. The macroscopic appearance of fungal colonies (*Aspergillus niger*, *Penicillium* spp. etc.) growing on SDA

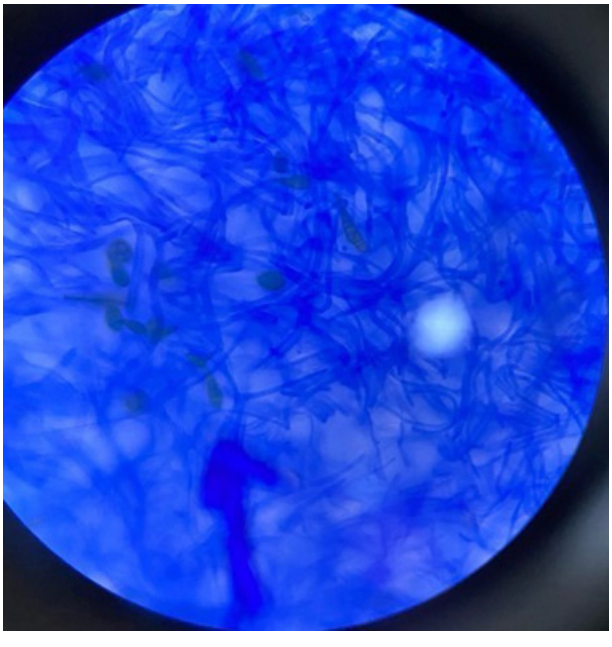

Figure 2. The microscopic appearance of *Trichophyton* spp. colonies growing on SDA

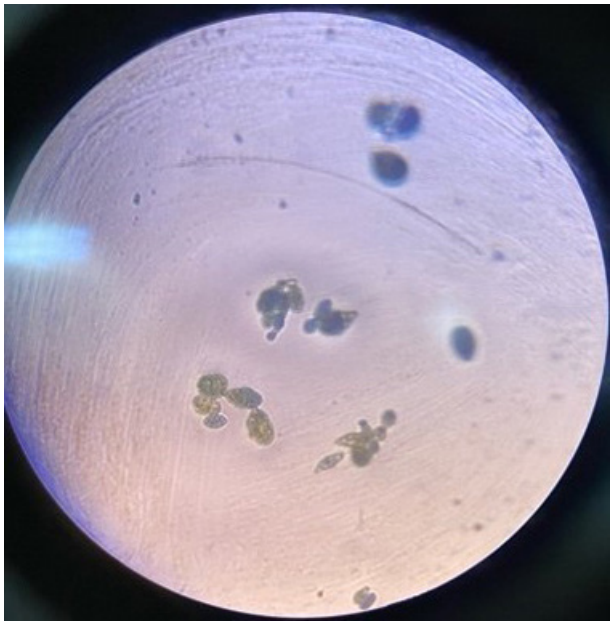

Figure 3. The microscopic appearance of *Alternaria* spp. colonies growing on SDA

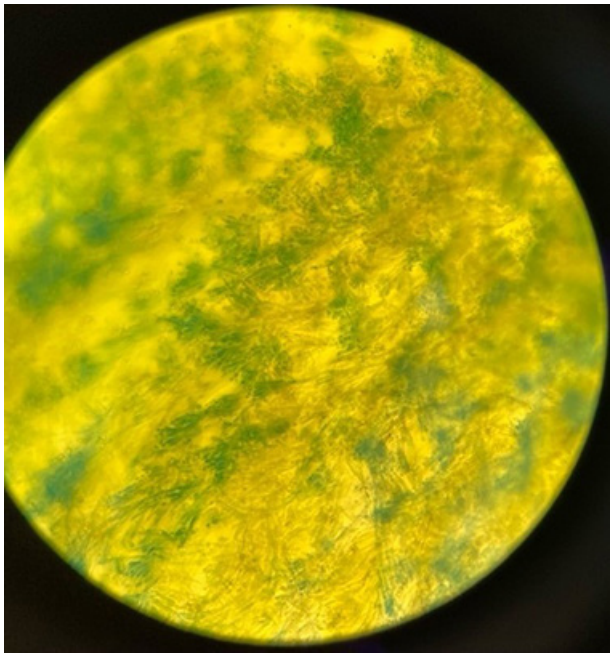

Figure 4. The microscopic appearance of *Penicillium* spp colonies growing on SDA.

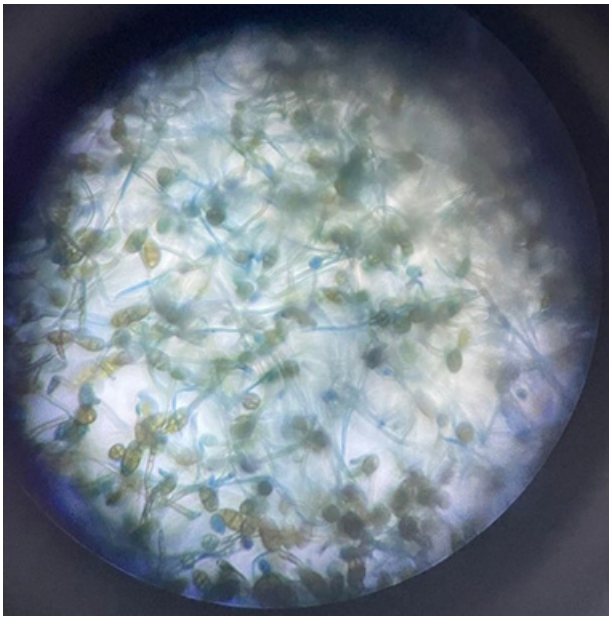

Figure 5. The microscopic appearance of *Microsporum* spp. colonies growing on SDA (Sabouraud Dextrose Agar).

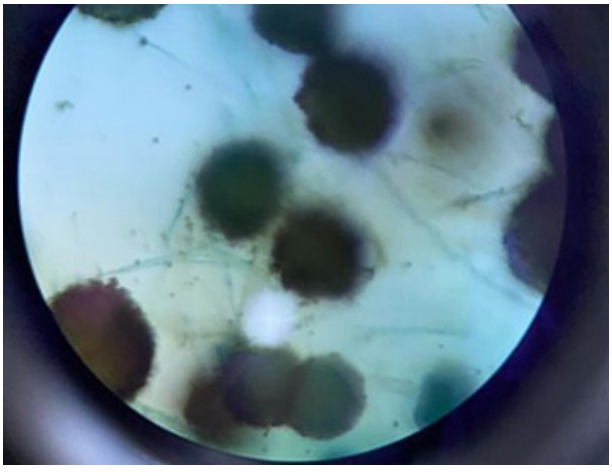

Figure 6. The microscopic appearance of *Aspergillus* spp. colonies growing on SDA (Sabouraud Dextrose Agar).

Discussion and Conclusion

The aim of this study was to determine the isolation, identification, and prevalence rates of dermatophytes and other fungal pathogens in cats in Balıkesir Province.

Studies conducted in our country have reported that the most commonly isolated dermatophyte pathogens in cats and dogs are *Microsporum* spp., *Trichophyton* spp., and *Candida* spp. in various studies (Hanedan et al., 2021).

When reviewing studies conducted worldwide, it has been reported that the most commonly isolated dermatophyte pathogens in cats and dogs are *Microsporum* spp. and *Trichophyton* spp. (Hanedan et al., 2021).

In Italy, it has been reported that the prevalence of dermatophytes in cats is 13% in owned cats, while it reaches 100% in stray cats (Iorio et al., 2007; Hanedan et al., 2021). In Brazil, dermatophytes were detected in 37.1% of the samples taken from 70 pet animals with dermatophytosis lesions (Neves et al., 2018; Hanedan et al., 2021). In East India, 37.33% of cats were found to be positive for dermatophyte spores (Debnath et al., 2016; Hanedan et al., 2021).

Debnath et al. (2016) reported that they were identified *M. canis* (55.34%), *M. gypseum* (31.07%) and *T. mentagrophytes* (13.59%) of 292 samples from cats.

Nichita and Marcu (2010) reported that *Microsporum* spp. was isolated in 11.8% of cats, while *Trichophyton* spp. was isolated in 13.8% of cats.

Şeker and Doğan (2011) in their study reported that the dermatophyte prevalence in dogs (198) and cats (164) in Ankara and Izmir provinces was 18.7% and 20.1%, respectively. They also identified the isolated dermatophyte species as *M. canis*, *M. gypseum*, *M. nanum*, *T. mentagrophytes*, and *T. terrestre*.

Şahan Yapıcıer et al. (2017) reported that in Burdur Province, the dermatophyte prevalence was 20.83% (5/24) in cats and 60.25% (47/78) in dogs, with *Microsporum* spp. and *Trichophyton* spp. being isolated.

In contrast to other studies, the most frequently isolated pathogens in this study were *Aspergillus* spp. and *Penicillium* spp.. The dermatophytosis pathogens isolated from cats were *Trichophyton* spp. (4 samples, 7.14%), *Microsporum* spp. (4 samples, 7.14%), *Alternaria* spp. (3 samples, 5.35%), and *Candida* spp. (5 samples, 8.9%) in this study.

When compared with the results of the studies conducted by Şeker and Doğan (2021) and Şahan Yapıcıer et al. (2017), it was observed that the isolation rates of *Microsporum* spp. and *Trichophyton* spp. were lower in this study.

Dermatophytes can spread to other susceptible animals and humans through direct contact with the fur of infected animals, contaminated tools, equipment, and objects (fomites), or environmental factors such as soil (Babacan et al., 2011).

When compared to the prevalence rates of dermatophyte pathogens reported in studies conducted in our country, the results of this study indicate that the isolation rates of Microsporum and Trichophyton species were lower.

Studies have indicated that fungal pathogens are isolated at much higher rates in stray cats (Hanedan et al., 2021). The lower prevalence of fungal pathogens observed in this study may be attributed to the fact that the samples were collected from owned domestic cats by clinical veterinarians.

The results indicate that dermatophyte infections, particularly *Microsporum spp. and Trichophyton spp.*, were seen in cats in Balıkesir province. According to the findings of this study, it was thought that important to monitor dermatophyte infections in both stray and owned cat populations, particularly in line with the One Health approach, in order to prevent transmission and zoonotic risks.

This study reveals, for the first time, the presence of fungal pathogens in cats in Balıkesir Province. Because of this, it was thought that this study provides valuable insights into the distribution of dermatophytes among the feline population in this region. It was thought that to minimize the spread of dermatophyte infections among cats and to protect public health, regular veterinary examinations, prompt laboratory diagnosis and treatment of infected animals, and the implementation of control and preventive measures targeting environmental factors are essential. It is thought that further research is needed to explore the species diversity and genetic variability of dermatophytes in this area.

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Author contribution: O.B: Laboratory analyses and controls, article writing, G.D.C: Laboratory analyses, G.İ: Laboratory analyses, N.G: Laboratory analyses

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