



Detection of bacterial and fungal agents in the skin of various domestic animals

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Abstract: It is important for public health to monitor infectious agents in domestic animals. This study aimed to detect of bacterial and fungal agents in the skin of various domestic animals. A total of 263 skin samples were collected during 2021-2023 from 147 cats, 102 dogs, 10 cows, 3 rabbits, and 1 iguana. Bacteriological examination indicated that 37.6% of the samples were positive. *S. pseudintermedius* (35.4%) was the dominant bacteria in all isolates, followed by *P. aeruginosa* (23.2%). *S. aureus* was isolated from one rabbit sample and *P. aeruginosa* was isolated from one iguana sample. These findings add to the limited number of studies on these species. As a result of mycological examinations, 61.6% of all samples were identified as positive by cultural examination. Saprophytes were found in 41.1% of all samples, specifically *A. niger* (30.6%), *Penicillium* spp. (28.7%), *A. fumigatus* (16.7%), *Alternaria* spp. (15.7%), *Mucor* spp. (4.6%), and *A. flavus* (3.7%). The high prevalence of saprophytes was correlated with environmental contamination. Dermatophytes were isolated in 20.5% of all samples. *M. canis* was the dominant dermatophyte (64.8%), followed by *T. mentagrophytes* (31.5%) and *M. ferrugineum* (3.7%). The higher prevalence of dermatophytosis in spring (51.9%), was associated with rainy seasonal conditions in Turkey.

Keywords: dermatophytosis, *M. ferrugineum*, pyoderma, saprophyte, *S. pseudintermedius*.

Farklı evcil hayvanların derisindeki bakteri ve mantar etkenlerinin tespiti

Özet: Evcil hayvanlarda enfeksiyöz etkenlerin izlenmesi halk sağlığı açısından önemlidir. Bu çalışma, farklı evcil hayvanların derisindeki bakteri ve mantar etkenlerini saptamayı amaçlamıştır. Bu amaç doğrultusunda, 2021-2023 döneminde 147 kedi, 102 köpek, 10 inek, 3 tavşan ve 1 iguanadan olmak üzere toplam 263 adet deri numunesi toplandı. Bakteriyolojik inceleme, numunelerin %37,6'sının pozitif olduğunu gösterdi. Tüm izolatlar arasında *S. pseudintermedius* (%35,4) baskın bakteri iken, takiben *P. aeruginosa* (%23,2) izole edildi. Bir adet tavşan numunesinden *S. aureus* ve bir adet iguana numunesinden *P. aeruginosa* izole edildi. Bu bulgular, bu türleri ele almış sınırlı sayıda literatüre katkıda bulunmaktadır. Yapılan mikolojik incelemeler sonucunda, tüm numunelerin %61,6'sı kültürel inceleme ile pozitif tespit edildi. Tüm numunelerin %41,1'inde saprofit etken, *A. niger* (%30,6), *Penicillium* spp. (%28,7), *A. fumigatus* (%16,7), *Alternaria* spp. (%15,7), *Mucor* spp. (%4,6) ve *A. flavus* (%3,7), saptandı. Saprofitlerin yüksek prevalansı, çevresel kontaminasyon ile ilişkilendirildi. Tüm numunelerin %20,5'inde dermatofit izole edildi. Baskın dermatofit *M. canis* (%64,8) iken, takiben *T. mentagrophytes* (%31,5) ve *M. ferrugineum* (%3,7) izole edildi. İlkbaharda dermatofitoz prevalansının daha yüksek olması (%51,9), Türkiye'deki yağışlı mevsim koşulları ile ilişkilendirildi.

Anahtar kelimeler: dermatofitozis, *M. ferrugineum*, piyoderma, saprofit, *S. pseudintermedius*.

Introduction

Dermatophytosis is the most frequent dermatological infection of keratinized tissues in animals worldwide (Nweze 2011). This infection is caused by *Microsporum*, *Trichophyton*, and *Epidermophyton* genera, which can use host keratin as a nutritional substrate. The most common observed dermatophytes are *Microsporum canis* (*M. canis*), *Microsporum gypseum* (*M. gypseum*) and *Trichophyton mentagrophytes* (*T. mentagrophytes*) responsible for more than 95% of all dermatophytosis in domestic animals (Roshanzamir et al. 2016).

Humans can be directly infected with dermatophytes through close contact with infected domestic animals (Nweze 2011), which are considered the main reservoir for dermatophytes (Seker and Dogan 2011). Many studies have demonstrated that dermatophytes are transmitted from domestic animals to humans and dermatophytosis is common among patients in contact with domestic animals (Ben-Ziony and Arzi 2000; Murmu et al. 2015; Maraki and Mavromanolaki 2016). Therefore, monitoring of dermatophytosis in domestic animals is critical to control infections and reduce transmission to hu-

mans (Roshanzamir et al. 2016). In addition, dermatophytes accompany various saprophytic fungi, such as *Aspergillus* spp., *Penicillium* spp., and *Alternaria* spp., while saprophytes mainly cause infections in immunosuppressive hosts (Ben-Ziony and Arzi 2000; Moosavi et al. 2019). Due to their infectious potential, it is important for public health to monitor of saprophytes like dermatophytes in domestic animals.

In addition to fungal agents, bacterial agents are frequently isolated from skin infections in domestic animals. *Staphylococcus pseudintermedius* (*S. pseudintermedius*) is the bacterium most frequently isolated from pyoderma in cats, dogs, and humans and is even considered the primary cause of pyoderma in companion animals (Somayaji et al. 2016; Müştak et al. 2019). *Pseudomonas aeruginosa* (*P. aeruginosa*) is another common opportunistic pathogen in animal and human skin (Hillier et al. 2006; Petersen et al. 2002). Following these bacteria, *Staphylococcus aureus* (*S. aureus*), *Staphylococcus intermedius* (*S. intermedius*), *Staphylococcus simulans* (*S. simulans*), *Streptococcus* spp., and *Escherichia coli* (*E. coli*), etc. are rarely isolated from skin infections (Hanselman et al. 2009; Moon et al. 2022). As with dermatophyte and saprophyte epidemiology, pathogenic bacteria may be transmitted to humans through direct contact with infected domestic animals (Moon et al. 2022).

Accordingly, the present study aimed to determine the factors that may pose risks of transmission to humans by detecting bacterial and fungal agents in the skin of various domestic animals, during 2021-2023.

Materials and Methods

Sample collection

The samples were analyzed at Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, in 2021-2023. The sampling period was divided into 4 seasons: autumn (September-November), winter (December-February), spring (March-May) and summer (June-August). A total of 263 swap samples were obtained from the skin of cats (n=147), dogs (n=102), cows (n=10), rabbits (n=3), and iguana (n=1) that had not been treated with any antibacterial or antifungal drugs. Prior to sampling, the infection site was sterilized with 70% alcohol. The samples were collected according to the Kirk and Bister scraping procedure using sterile scalpels, petri dishes, and tweezers (Bister and Ford

1995) before being aseptically stored at +4°C until the analyses.

Bacteriological examination

Each sample was inoculated on 5% sheep blood agar plate. The plates were incubated at 37°C for 24-48 h under aerobic conditions. All colonies were identified using standard bacteriological methods (Markey et al. 2013). Following Gram staining, the isolates were identified using catalase, oxidase, oxidation-fermentation, coagulase, citrate, DNase, indole, hemolysis, motility, nitrate, novobiocin (5 µg/disc), polymixin-B (300 U/disc), Voges-Proskauer, and urease tests. The isolates were cultured on *Eosin Methylene Blue* (EMB) Agar, MacConkey Agar (MCA), Mannitol Salt Agar (MSA), and Triple Sugar Iron (TSI) Agar (Oxoid, UK). Discrimination of *S. pseudintermedius* and *S. intermedius* species was performed by sequencing of the RNA polymerase B (*rpoB*) gene, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2008). Polymerase chain reaction (PCR) and sequencing were performed using 31F and 830R primers (Drancourt et al. 2004). PCR mix consisted of 2.5 µL of 10xbuffer, 0.5 µL 10 mM dNTPs (Thermo Fisher Scientific, USA), 3 µL MgCl₂ (Thermo Fisher Scientific, USA), 1 µL of each 10 mM primer, 0.2 µL Taq polymerase (2U/µL; Thermo Fisher Scientific, USA), and 2 µL template DNA. The mix increased to total 25 µL volume with PCR-grade water. PCR was run under the following conditions: pre-denaturation at 94°C for 3 min, 30 cycles of 94°C for 1 min, 58°C for 30 s, 72°C for 1 min, and final extension at 72°C for 7 min. Amplicons were purified with Exosap-IT (Thermo Fisher Scientific, USA). BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used for sequencing, while Sephadex (Oxoid, UK) gel filtration was used for purification. Sequencing was performed on an ABI 3500 genetic analyzer system (Applied Biosystems, USA). The obtained sequences were analyzed by CLC Main Workbench version 8.1.0 software (Qiagen, USA).

Mycological examination

The samples were investigated by direct microscopy and cultural examination. Direct microscopy was performed using a sterile lancet, lam, and 10% solution of potassium hydroxide (KOH). All preparations were examined under a light microscope at 40x magnification to detect fungal components, such as hyphae, spore, and conidia. Cultural examination was conducted using Sabouraud Dextrose Agar (SDA) supplemented with 0.05 mg/ml chloramphen-

icol (Oxoid, UK). The plates were incubated at 25°C for 15 days under aerobic conditions. The fungal colonies were examined with both macroscopically and microscopically. Macroscopic examination was conducted to detect cultural features, while microscopic examination was performed by lactophenol cotton blue staining (Merck, Germany) under a light microscope at 40x magnification to identify fungal components. The fungal isolates were identified according to the guide of Larone (Walsh et al. 2006). The urease test was also used to identify of *T. mentagrophytes*.

Results

Bacteriological examination

Overall, 37.6% (99/263) of the samples were positive for bacterial agents. The highest prevalences were in the cat and dog: 36.1% (53/147) and 43.1% (44/102), respectively. No bacteria were isolated from the cow samples (Table 1).

Table 1. Bacterial isolation prevalence by host

Isolation	No. of isolates (%)					
	Cat	Dog	Cow	Rabbit	Iguana	Total
Negative samples	94 (63.9)	58 (56.9)	10 (100.0)	2 (66.7)	0	164 (62.4)
Positive samples	53 (36.1)	44 (43.1)	0	1 (33.3)	1 (100.0)	99 (37.6)
Total	147 (100.0)	102 (100.0)	10 (100.0)	3 (100.0)	1 (100.0)	263 (100.0)

S. pseudintermedius (35.4%, 35/99) was the dominant bacteria in all isolates, followed by *P. aeruginosa* (23.2%, 23/99). The dominant Gram-positive

and Gram-negative bacteria were the same in cats and dogs (Table 2).

Table 2. Distribution of bacterial agents by host

Bacteria	No. of isolates (%)					
	Cat	Dog	Cow	Rabbit	Iguana	Total
<i>S. pseudintermedius</i>	19 (35.8)	16 (36.4)	0	0	0	35 (35.4)
<i>S. aureus</i>	11 (20.8)	3 (6.8)	0	1 (100.0)	0	15 (15.2)
<i>S. intermedius</i>	4 (7.5)	7 (15.9)	0	0	0	11 (11.1)
<i>S. epidermidis</i>	3 (5.7)	1 (2.3)	0	0	0	4 (4.0)
<i>Streptococcus</i> spp.	0	2 (4.5)	0	0	0	2 (2.0)
<i>E. coli</i>	3 (5.7)	4 (9.1)	0	0	0	7 (7.1)
<i>P. aeruginosa</i>	12 (22.6)	10 (22.7)	0	0	1 (100.0)	23 (23.2)
<i>P. mirabilis</i>	1 (1.9)	1 (2.3)	0	0	0	2 (2.0)
Total	53 (100.0)	44 (100.0)	0	1 (100.0)	1 (100.0)	99 (100.0)

Mycological examination

None of the samples tested by direct microscopy were positive for fungal agents. In contrast, 61.6% (162/263) of all samples tested by cultural examination were positive for fungal agents, while 38.4% (101/263) were negative. Dermatophytes (*M. canis*,

M. ferrugineum, and *T. mentagrophytes*) and saprophytes (*A. flavus*, *A. fumigatus*, *A. niger*, *Alternaria* spp., *Mucor* spp., and *Penicillium* spp.) were detected in 20.5% (54/263) and 41.1% (108/263) of all samples, respectively (Table 3).

Table 3. Fungal isolation prevalence by host

Isolation	No. of isolates (%)					Total
	Cat	Dog	Cow	Rabbit	Iguana	
Dermatophytes	33 (22.4)	19 (18.6)	2 (20.0)	0	0	54 (20.5)
Saprophytes	64 (43.5)	42 (41.2)	2 (20.0)	0	0	108 (41.1)
Negative	50 (34.0)	41 (40.2)	6 (60.0)	3 (100.0)	1 (100.0)	101 (38.4)
Total	147 (100.0)	102 (100.0)	10 (100.0)	3 (100.0)	1 (100.0)	263 (100.0)

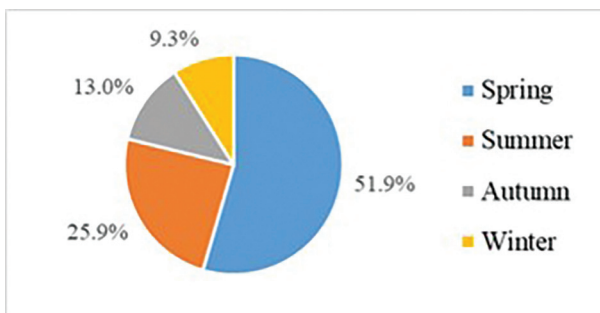
A wide spectrum of nine different fungal agents were detected. Regarding the dominant agents, 64.8% (35/54) of the dermatophytes were *M. canis*,

while 30.6% (33/108) of the saprophytes were *A. niger* (Table 4).

Table 4. Distribution of dermatophytes and saprophytes by host

Fungal agents	No. of isolates (%)				Total
	Cat	Dog	Cow	Total	
<i>M. canis</i>	21 (63.6)	14 (73.7)	0	35 (64.8)	
<i>T. mentagrophytes</i>	10 (30.3)	5 (26.3)	2 (100.0)	17 (31.5)	
<i>M. ferrugineum</i>	2 (6.1)	0	0	2 (3.7)	
Total Dermatophytes	33 (100.0)	19 (100.0)	2 (100.0)	54 (100.0)	
<i>A. niger</i>	15 (23.4)	18 (42.9)	0	33 (30.6)	
<i>A. fumigatus</i>	7 (10.9)	11 (26.2)	0	18 (16.7)	
<i>A. flavus</i>	4 (6.3)	0	0	4 (3.7)	
<i>Penicillium</i> spp.	21 (32.8)	8 (19.0)	2 (100.0)	31 (28.7)	
<i>Alternaria</i> spp.	12 (18.8)	5 (11.9)	0	17 (15.7)	
<i>Mucor</i> spp.	5 (7.8)	0	0	5 (4.6)	
Total Saprophytes	64 (100.0)	42 (100.0)	2 (100.0)	108 (100.0)	

Dermatophytes were most frequently isolated (51.9%) during spring (Figure 1), specifically 54.5% in cats, 42.1% in dogs, and 100.0% in cows.

**Figure 1.** Distribution of dermatophytes by season

Discussion and Conclusion

According to the 'One Health' concept, animal health, public health, and the environment should be evaluated together to obtain optimal results (Atusing-wize et al. 2020). Domestic animals are important sources in the transmission of zoonoses to humans. Therefore, it is important for public health to monitor infectious agents in domestic animals (Moon et al. 2022). In this study, we detected the bacterial and fungal agents in skin of various domestic animals to evaluate the risk of transmission to humans.

Regarding bacteria, *S. pseudintermedius* was 35.4% of all bacterial isolates, and was the dominant agent in cats and dogs. *S. pseudintermedius* is defined as an opportunistic pathogen that is the primary cause of pyoderma in dogs (Markey et al. 2013; Nomoto et al. 2020). Moreover, it is common-

ly associated with pyoderma in humans (Somayaji et al. 2016). The transmission of *S. pseudintermedius* from dog to human was reported by Somayaji et al. (2016) and Nomoto et al. (2020). Somayaji et al. (2016) reported that the majority of human *S. pseudintermedius* clinical cases (91.7%) were caused by contact with dogs, and concluded that dogs are an important reservoir for transmission of *S. pseudintermedius*. Similar to our study, Ma et al. (2020) reported that *S. pseudintermedius* was the dominant agent (46.2%) in dogs, and second-most dominant agent (8.8%) in cats. Moon et al. (2022) also reported that *S. pseudintermedius* (12.5%) was one of the dominant agents in cats.

In our study, *P. aeruginosa* (23.2%) was the dominant Gram-negative bacteria in all isolates, which confirms previous findings regarding the high prevalence of this agent. For example, Petersen et al. (2002) reported that *P. aeruginosa* was the only agent isolated in 33.1% of dog skin samples while Hillier et al. (2006) found that 30% sampled of dogs had pseudomonal pyoderma. Nocera et al. (2021) reported that *P. aeruginosa* accounted for 36% of the Gram-negative bacteria isolated from the skin of cats and dogs.

We also isolated *S. aureus* from one rabbit sample and *P. aeruginosa* from one iguana sample. White et al. (2002) also reported that *S. aureus* was the most common secondary infectious agent isolated from rabbits, as well as *T. mentagrophytes*. They concluded that asymptomatic rabbits infected with dermatophytes, especially from pet stores, may pose risks to the health of other animals and humans. In contrast, we did not detect any dermatophytes in the rabbits in the present study. On the other hand, our isolation of *P. aeruginosa* from the iguana sample, is important because it is a dominant bacterial agent with zoonotic potential in cold-blooded animals. There have been few reports of bacteriological analysis of skin in iguanas. In a case report, Supic et al. (2021) described a fatal *P. aeruginosa* infection in a case of extensive dermatitis in an iguana. Hence, our finding adds to the limited number of studies on this species. Apart from *S. pseudintermedius* and *P. aeruginosa*, we isolated *S. aureus* (15.2%), *S. intermedius* (11.1%), *S. epidermidis* (4.0%), *Streptococcus* spp. (2.0%), *E. coli* (7.1%) and *Proteus mirabilis* (2.0%) from all samples. This finding is not unexpected given that previous studies also isolated these agents from skin infections of domestic animals (Chaudhary et al. 2019; Li et al. 2021; Nocera et al. 2021).

Our mycological analyses indicated that 20.5% of all samples were positive for dermatophytes. Previous studies have shown that dermatophyte prevalence varies widely (Maraki and Mavromanolaki 2016). For example, Moosavi et al. (2019) reported 14.5% prevalence in cats; Dworecka-Kaszak et al. (2020) reported 23.5% prevalence in various domestic animals; and Roshanzamir et al. (2016) reported 56% prevalence in cats and dogs. In our study, dermatophytes were more prevalent in cats (22.4%) than in dogs (18.6%), which is similar to previous studies. For example, Cafarchia et al. (2004) reported 28.2% and 20.5% positivity in cats and dogs, respectively; Seker and Dogan (2011) reported 20.1% and 18.7% positivity in cats and dogs, respectively; and Murmu et al. (2015) reported 55.5% and 37.8% positivity in cats and dogs, respectively.

At 64.8%, *M. canis* was the most prevalent dermatophyte. This finding is important, given that *M. canis* is also the dominant dermatophyte in humans (Aneke et al. 2022). Similarly, Cafarchia et al. (2004) reported that *M. canis* (77.7%) was the dominant dermatophyte in domestic animals. The second-most dominant dermatophyte isolated in our study was *T. mentagrophytes* (31.5%). Similarly, Nweze (2011) found that *M. canis* (37.4%) was the dominant dermatophyte in domestic animals, followed by *T. mentagrophytes* (22.9%). Seker and Dogan (2011) found that *M. canis* (57.1%) was the dominant dermatophyte in domestic animals, followed by *T. mentagrophytes* (20.0%). Murmu et al. (2015) reported that *M. canis* (60.0%) and *T. mentagrophytes* (15.8%) were the dominant dermatophytes in cats, dogs, and humans, while Dworecka-Kaszak et al. (2020) reported that they were also dominant (59.25% and 40.7%, respectively) in domestic animals. Consistent with these studies, our findings are important because *M. canis* and *T. mentagrophytes* are also responsible for dermatophytosis in humans.

Regarding other species, Moosavi et al. (2019) reported that *T. verrucosum* (86.66%) was the dominant dermatophyte, while Roshanzamir et al. (2016) reported that *M. gypseum* (27.5%) was dominant in cats and dogs. However, we did not isolate either species. On the other hand, we detected *M. ferrugineum* in 6.1% of cat samples. This finding is not surprising as the species is phylogenetically related to *M. canis*. Rezaei-Matehkolaei et al. (2012) noted that *M. ferrugineum* is rarely isolated in routine analysis due to its limited endemicity.

While we isolated *T. mentagrophytes* from cow samples, we did not isolate any bacteria. Dermatophytosis is an important risk for animal health due

to its high morbidity. In addition, infected cows can be reservoirs for humans and healthy animals (Papini et al. 2009). Various reports indicate that cows can spread infection and human dermatophytosis among workers on infected farms (Ming et al. 2006; Papini et al. 2009). Our finding is in line with those of Ranganathan et al. (1998), Yildirim et al. (2010), and Nweze (2011) in terms of isolating of *T. mentagrophytes* from cows. Dalis et al. (2018) and Mohamadifard et al. (2022) also found that *T. mentagrophytes* is one of the most common isolated dermatophytes from cows.

Considering saprophyte and dermatophyte isolation together, we detected saprophytes (41.1%) more frequently than dermatophytes (20.5%). High saprophytes prevalence is correlated with soil contamination. Such environmental contamination is considered an effective factor in isolating saprophytes (Moosavi et al. 2019; Dworecka-Kaszak et al. 2020). In our study, the detected saprophytes included *Aspergillus* spp. (50.9%), *Penicillium* spp (28.7%), *Alternaria* spp. (15.7%), and *Mucor* spp. (7.8%). Moosavi et al. (2019) reported that all cat samples were positive (100.0%) for saprophytes. Consistent with our findings, the dominant agents in their study were *Aspergillus* spp. (18.4%) followed by *Alternaria* spp. (17.4%), and *Penicillium* spp. (12.0%).

Research has produced different findings regarding the correlation between season and dermatophytosis prevalence in domestic animals. We correlated the high prevalence in samples collected during spring (51.9%) with rainy seasonal conditions in Turkey. Similarly, Murmu et al. (2015) found that the highest prevalence of dermatophytosis (74.5%) in cats and dogs was correlated with the rainy season, while Şahan Yapıcıer et al. (2017) also reported that dermatophytosis in both cats (57.1%) and dogs (86.9%) was more common in the spring.

Ethical statement: Ethical approval was not required for the study.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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