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Research Article

Investigation of the Prevalence of Dermatophytes Causing Skin Lesions in Cats in Ankara Province by Different Diagnostic Methods

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Abstract

This study aimed to investigate the prevalence of dermatophytosis in domestic cats with skin lesions in Ankara province and to compare the effectiveness of different culture media for isolation. Skin and hair samples collected from a total of 195 cats were examined. For this purpose, a range of investigative techniques were employed, including direct microscopy, examination with Wood's lamp, cultivation, the slide culture method and the hair perforation technique. A comparative evaluation was conducted to ascertain the suitability of various media for the cultivation of dermatophytes. The prevalence of dermatophytosis was found to be 14.36% (28/195). Of the twenty-eight dermatophyte isolates, twenty-six were identified as *Microsporum canis* and two as *Nannizzia gypsea*. Moreover yeast and mycelial fungal agents such as *Aspergillus* spp., *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp., *Candida* spp., *Malassezia* spp., *Scopulariopsis* spp. were isolated and identified. The differential diagnosis of *Candida* species was conducted through the implementation of gram staining, germ tube tests and *Candida* Chromogenic Agar passage. The results of these tests enabled the identification of two different species: *Candida albicans* and *Candida krusei*. In addition to these results, it has been determined that Sabouraud Dextrose Agar and Potato Dextrose Agar are more suitable for use in the diagnosis of dermatophytes than other culture media. As a conclusion, this study suggests that further research should be conducted to report on the current epidemiology of dermatophytosis agents, which are important for public health, and to improve the media and techniques used in diagnosis.

Keywords: Cat, Dermatophytes, *Microsporum canis*, *Nannizzia gypsea*.

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Introduction

Dermatophytosis is a prevalent dermatological condition affecting both pets and farm animals. The public health significance of these zoophilic agents stems from their contagious nature within animal populations, the high cost and difficulty of treatment and control and their zoonotic potential (Chermette et al 2008, Akhoundi et al 2022). The fungal agents are a category of aerobic fungi that invade and infect keratinized skin, hair and nails. The classification of these organisms is determined by their host preference, which is categorised as follows: anthropophilic, zoophilic and geophilic (Moriello 2020). Also known as ringworm or tinea, dermatophytosis is a cosmopolitan infectious, cutaneous mycoses that affects humans, various mammals and to a lesser extent poultry. A wide variety of dermatophytes have the capacity to infect animals. The majority of these are zoophilic in nature although geophilic and exceptionally, anthropophilic dermatophytes are also present (Chermette et al 2008,Anikar et al 2022, Moskaluk and VandeWoude 2022). *Microsporum*, *Trichophyton* and *Epidermophyton* are keratinophilic fungi which the causative agents of cutaneous mycosis. Cats (*Felis catus*) are regarded as potential hosts and reservoirs for several dermatophyte species, most notably *Microsporum (M.) canis* (Fraga et al 2017). Transmission occurs predominantly through direct contact with an infected animal (Moriello 2020). Furthermore, indirect transmission via fomites such as contaminated collars, brushes, toys, storage boxes and transport cages represents a significant route of spread and is critical to understanding disease dynamics (Youssef et al 2023). In juvenile cats, the initial

lesions manifest on the nasal bridge, the auricular margin, and the auricular and caudal extremities (Chermette et al 2008, Moriello 2020). A wide variety of diagnostic methods have been developed for identifying dermatophytes. The methods above include the following: examination with Wood's lamp, direct examination of hair and skin, fungal culture in Sabouraud Dextore Agar (SDA), Potato Dextrose Agar (PDA), Dermasel Agar (DA) and Dermatophyte Test Medium (DTM), hair perforation test and evaluation of urease activity (Walsh et al 2018, Moriello KA 2021, Durdu and Ilkit 2021).

This study was designed to establish the prevalence and etiological profile of dermatophytosis in a feline population with skin lesions from Ankara, Türkiye, thereby generating critical contemporary epidemiological data for the region. In parallel, we conducted a methodological evaluation to assess the diagnostic performance and suitability of conventional culture media and phenotypic identification techniques in a clinical veterinary mycology context.

Material and Methods

This study was carried out after the animal experiment was approved by Kırıkkale University Local Ethics Committee with the decision numbered (2022/07 45, dated 28/12/2022). Samples were collected with the permission of the animal owners, obtained through an informed consent form, in accordance with the Local Ethics Committee decision. In this study, skin and hair samples were collected from a total of 195 cats of different ages and genders brought to veterinary clinics and hospitals with complaints of dermatological lesions.

Sampling

In this study, samples of skin and hair were collected from a total of 195 cats of varying ages and sexes with dermatological lesions. All samples were collected from veterinary clinics and animal hospitals over 16 months (from February 2023 to June 2024), following approval by the ethics committee. The cats were owned and maintained in domestic environments within the Ankara province. The skin lesion and its area were cleaned with 70% alcohol to collect samples of the skin and hair. The area was then dried and the hair, hair follicles were transferred into a sterile glass tube (Samanta 2015, Kidd et al 2016, Brun and Pihet 2021). In the context of this study, the animals from which the materials to be analyzed were collected were categorised according to six distinct life stages: less than 6 months old, 7 months to 2 years old, 3 to 6 years old, 7 to 10 years old, 11 to 14 years old, and 15 years old and above (Vogt et al 2010).

Inspection with Wood's lamp

Cats presenting at veterinary clinics and/or animal hospitals in Ankara province with a complaint of skin lesions were subjected to examination with a Wood's lamp (Archer, China) in darkened room (Samanta 2015, Kidd et al 2016, Brun and Pihet 2021).

Direct Microscopy

The materials collected from the animals were subjected to a 10% potassium hydroxide (KOH, Merck) treatment on a slide. Thereafter, the slides were covered with coverslips and subjected to slight heated. The fungal elements were examined under a microscope (Zeiss, Germany) at 10x and 40x magnification (Samanta 2015, Kidd et al 2016,Brun and Pihet 2021).

Fungal Culture, Isolation, Identification and Characterization

The collected specimens were initially inoculated onto SDA medium containing chloramphenicol (Condalab, Spain) and incubated at 25±2°C for 2 to 3 weeks (Samanta 2015, Kidd et al 2016; Brun and Pihet 2021). Following primary growth, isolates were subcultured onto PDA (Condalab, Spain) and DA (OXOID, UK) to enhance sporulation and incubated at 25°C for 4 to 7 days. Fungal isolates were identified based on macroscopic and microscopic colony characteristics. The conidia were identified after lactophenol cotton blue (LPCB, Merck) staining based on size, shape, septa, cell arrangement and thickness of the conidial wall. The positive samples were characterised through the implementation of slide culture method, in vitro hair perforation test, passage to DTM (Condalab, Spain) and urease test (Samanta 2015, Kidd et al 2016, Walsh et al 2018, Brun and Pihet 2021). After being passaged on DTM and Urease Agar (UA, Condalab, Spain) for evaluation based on colour changes, they were incubated at 25°C for 2 to 3 weeks. To assess the presence of growth in the culture media, colony formation was analyzed after subcultured from SDA to all other media.

Comparative Investigation of Dermatophytes on Culture Media

In this study, dermatophytes growing on SDA medium were subcultured on DA, DTM, and PDA media to evaluate their growth and colony formation after passage. The growth rate in the media was evaluated in detail in terms of false positives, false negatives, sensitivity and specificity. To evaluate these results, a cross-tabulation was created using various standards, as shown in Table 1. For this purpose, sensitivity (a/(a+b)) x 100; specificity = (d/(c+d)) x 100; false negativity = (b/(a+b)) x 100; false positivity = (c/(c+d)) x 100 were calculated using the formulas (TÜRKAK 2019; Magnusson and Tsimillis 2023).

Table 1. Cross-tabulation			
Medium	Negative culture tested	Positive culture tested	Total
Negative on SDA	a	b	a+b
Positive on SDA	c	d	c+d
Total	a+c	b+d	-

a; negative result for both culture media b; negative on SDA medium, positive on the tested medium c; positive on SDA medium, negative on the tested medium d; positive result for both culture media.

Comparative Evaluation of Yeasts

For the identification of *Candida* (*C.*) species, samples were inoculated onto *Candida* Chromogenic Agar (CKA, Condalab, Spain) and incubated at 25°C for 48 hours. Species were differentiated based on colony colour according to the manufacturer's instructions. Gram staining was performed to differentiate yeasts from bacteria. The germ tube test was performed as follows. For this purpose, a suspension of approximately 10^5–10^6 CFU/mL was prepared in sterile 0,9% saline from an 18–24-hour culture. Then, 0.5 mL of this was mixed with 0.5 mL of foetal bovine serum in a sterile tube. It was incubated at 37 °C for 2-3 hours. After incubation, the germ tubes were examined between a slide and cover slip and under a microscope for their presence (Kauffman et al 2011, Samanta 2015, Walsh et al 2018).

Statistical Analysis

To evaluate differences in age, sex, and lesion distribution among animal groups, chi-square and binary logistic regression statistical tests were performed using the SPSS 26 programme. Results with $p<0.05$ were considered statistically significant.

Results

Isolation and Identification Result

The obverse of colonies were yellowish-brown in the centre, white around the edges, with a puffy, hairy texture, and dark yellow reverse. Microscopic examination revealed numerous thick-walled, needle-shaped macroconidia containing an average of nine septa. The microconidia were stalked and occurred singly along the hyphae. These characteristics identified the isolate as *Microsporum (M.) canis* (Figure 1). The obverse of the colony presented a grey, powdery and the reverse is pigment, ranged from dark yellow to brown (Figure 2). Microscopy showed hyphae with rough-walled macroconidia that had thin walls, rounded tips, and an average of five septa (Figure 3). Based on these features, the isolate was presumptively identified as *Nannizzia (N.) gypsea*. A detailed analysis of the colony morphology of dermatophytes was conducted by means of the slide culture method (Figure 4).

Dermatophytes were isolated from 28 (14,36%) of the 195 samples examined. Following detailed analysis of the isolated agents, 26 of the 28 dermatophyte-positive animals were identified as *Microsporum canis* (92,86%) and two as *Nannizzia gypsea* (7,14%). The agents were isolated from 12 (14,12%) female cats and 16 (14,54%) male cats. Of the male cats from which dermatophytes were isolated, 3 (18,75%) were ≤ 6 months, 4 (25%) 7 months - 2 years, 7 (43,75%) 3-6 years, 2 (12,5%) 7-10 years old. The agent was not isolated in male cats aged 11-14 years and 15 years and older. The dermatophyte-positive female cats, 1 (8,33%) was ≤ 6 months old, 2 (16,66%) 7 months-2 years old, 6 (50%) 3-6 years old, 2 (16,66%) 7-10 years old, 1 (8,33%) 11-14 years old. In this study, it was determined that there was no statistically significant relationship between dermatophyte isolation and sex of the animals ($p>0.05$). When the distribution according to age was analyzed in detail, 20 (10,25%) were ≤ 6 months, 49 (25,13%) were 7 months-2 years, 105 (53,84%) were 3-6 years, 16 (8,20%) were 7-10 years, 4 (2,05%) were 11-14 years, and 1 (0,51%)

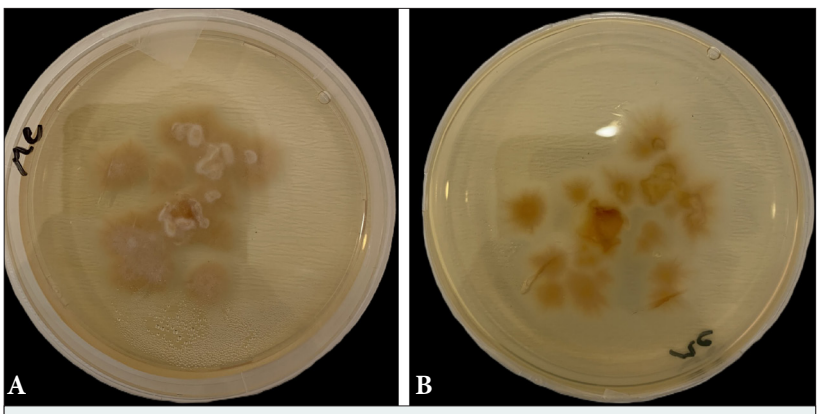

Figure 1. Macroscopic morphology of *M. canis*. A- Colony obverse on SDA, B- Colony reverse on SDA

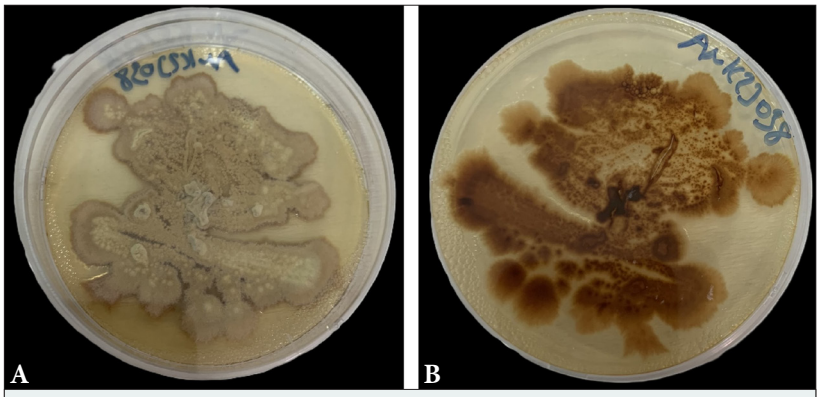

Figure 2. Macroscopic morphology of *N. gypsea*. A- Colony obverse on SDA, B- Colony reverse on SDA

was 15 years and older. Of the cats with dermatophyte-positive samples, 4 (14,28%) were ≤ 6 months old, 6 (21,43%) were 7 months to 2 years old, 13 (46,43%) were 3-6 years old, 4 (14,28%) were 7-10 years old, and 1 (3,57%) was 11-14 years old. A statistically significant relationship has been determined between dermatophyte isolation and the age group of animals ($p<0.05$). When the animals were analyzed according to the body region where the skin lesions were found, 70 (35,89%) of the cats had head and neck lesions, 8 (4,10%) of the cats had ear lesions, 62 (31,79%) of the cats had extremites lesions, 15 (7,69%) of the cats had intrascapular lesions, 17 (8,71%) thoracic, 25 (12,82%) abdominal, 5 (2,56%) perianal and 2 (1,02%) tail. A statistically significant relationship has been established between the distribution of lesions detected in animals and dermatophyte isolation ($p<0.05$). An evaluation of the clinical findings observed in animals testing positive for dermatophyte infection revealed that 21 out of 28 cats presented with alopecia, 18 exhibited erythema, 11 displayed crusting, 16 experienced itching and 9 exhibited scaling.

Other Fungal Agents Isolated and Identified

The isolation of non-dermatophyte yeast and fungal agents was conducted on 95 out of the total 195 samples, amounting to a percentage of 48,69%. A detailed analysis of the isolated agents revealed the following: *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Candida* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium* spp., *Malassezia* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., *Rhodotorula* spp. and *Scopulariopsis* spp (Table 2).

Comparative Evaluation of Yeasts

Yeast isolates forming green colonies on CKA were identified as *C. albicans*, while those exhibiting purple-pink colonies were identified as *C. krusei*. Gram-positive, oval-shaped microorganisms with germ tube formation were identified as *C. albicans*. In contrast, *C. krusei* presented as Gram-positive, oval to elliptical blastoconidia that did not produce germ tubes; instead, they formed pseudohyphae with distinctive arborescent, "tree-like" structures and long blastoconidia (Figure 5). The presence of agents that formed cream-coloured, smooth colonies on SDA, accompanied by the formation of a bud-like structure under microscopic examination, was indicative of suspected *Malassezia* spp. Conversely, yeast that formed orange-coloured, mucoid colonies on SDA, comprising Gram-positive bacteria, was identified as suspected *Rhodotorula* spp.

Diagnostic Methods Result

A detailed result of 28 cats that had tested positive for dermatophytes. The Wood's lamp examination of these cats yieled positive result in 18 cases and negative results in 10. Fungal elements were detected in 89 of the 195 cats examined by direct microscopy. A subset analysis of the 28 cats culture-positive cats revealed that 19 were also positive. Upon microscopic evaluation of the hair

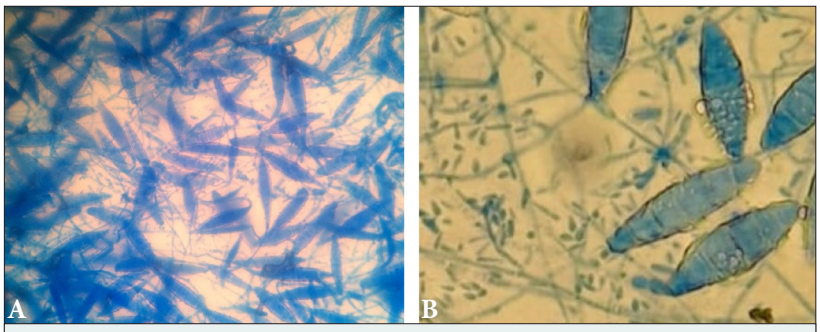

Figure 3. Microscopic examination A- Macroconidia and hyphae of *M. canis* at x400 magnification., B- Macroconidia, microconidia and hyphae of *N. gypsea* at x400

Table 2. Results of other fungal agents		
Isolates	Number	%
Alternaria spp.	17	8,72%
Aspergillus spp.	20	10,25%
Bipolaris spp.	2	1,02%
C. albicans	14	7,18%
C. krusei	2	1,02%
Cladosporium spp.	10	5,13%
Curvularia spp.	2	1,02%
Fusarium spp.	2	1,02%
Malassezia spp.	2	1,02%
Mucor spp.	2	1,02%
Penicillium spp.	15	7,70%
Rhizopus spp.	1	0,51%
Rhodotorula spp.	3	1,54%
Scopulariopsis spp.	3	1,54%
Total	97	48,69%

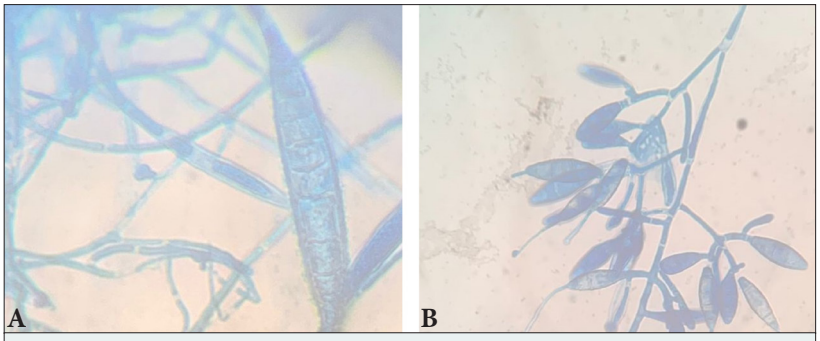

Figure 4. Slide culture method. A- Macroconidia of *M. canis* at x400 magnification., B- Macroconidia and hyphae of *N. gypsea* at x400 magnification

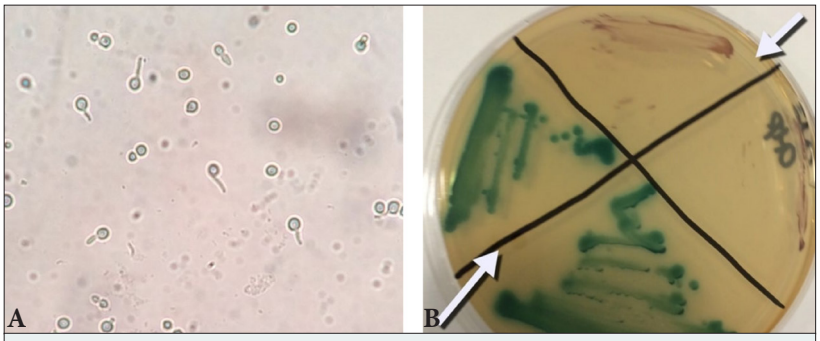

Figure 5. Differantial Diagnosis of yeasts. A- Germ tube (*C. albicans*) B- Colour detection on CKA (Lower left arrow: *C. albicans*; Upper right arrow: *C. krusei*)

perforation test findings, perforation was detected in all dermatophyte-positive isolates (Figure 6). Urease activity was detected in 21 out of the 28 isolates (Figure 7).

Comparison Results of Culture Media

A comparison of the results of the growth of 28 dermatophyte isolates identified as growing on SDA after being passaged to PDA, DTM, and DA media was undertaken. The results demonstrated that 28 (100%) of the 28 passaged isolates grew on PDA, 26 (92,86%) on DTM; and 22 (78,57%) on DA medium. When the comparison results of DTM and DA culture media are examined, the sensitivity rates are 71,43%, 21,43%, respectively; the specificity rates are 64,28%, 59,52%; false negative rates were 92,85% and 78,57%, respectively; false positive rates were 35,71% and 40,47%, respectively. The results of the studies conducted with SDA and PDA media and DA and DTM media were evaluated comparatively.

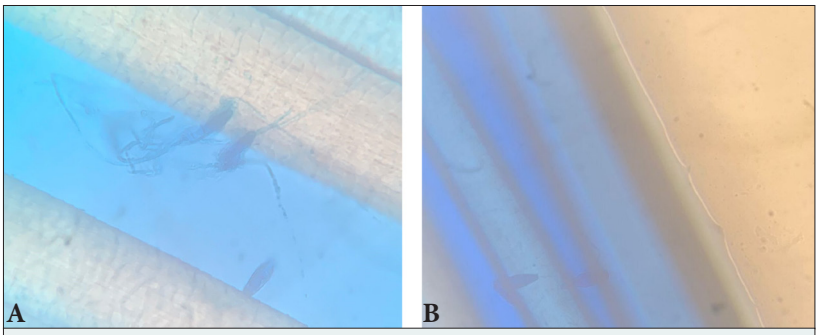

Figure 6. Hair perforation test result. A- Image of *N. gypsea* at x400 magnification., B- Image of *M. canis* at x400 magnification.

It was observed that DTM media provided more reliable results than DA media in terms of sensitivity, specificity, false positivity, and false negativity.

Discussion

Dermatophytosis is a significant feline health concern due to its pleomorphic clinical manifestation, infectious and contagious nature and potential zoonotic transmission (Lopes et al 2023, Mendes et al 2024). This study aimed to isolate dermatophytes from cats with skin lesions using various culture media, and to identify the isolates using different methods. Additionally, the suitability of the culture media for the obtained isolates was evaluated.

An examination of studies conducted in Türkiye reveals a wide range of dermatophytosis prevalence in cats from 17.44% to 47.1% (Seker and Dogan 2011, Ilhan 2015, Derincegöz and Parın 2016, Yapıcıer et al 2017, Diren Sığırcı et al 2019, Selvi and Yıldırım 2019, Sever et al 2021, Ince and Torun 2023). Consistent with regional findings, *M. canis* was the predominant etiological agent, corroborating its primary role in feline dermatophytosis (Yapıcıer et al 2017, Diren Sığırcı et al 2019). The prevalence of dermatophytes in cats exhibits considerable geographical variation. In the present study, 28 of 195 samples were positive. This result is consistent with the 16.2% prevalence reported in Thailand (Sanguansook et al 2024) and falls within the wide range (21.1%–70.27%) observed in India (Bagra et al 2024, Gautam et al 2024). Other studies have reported rates of between 30,2-39,1% in Azerbaijan, Iran and Switzerland (Bontems et al 2020, Katiraee et al 2021, Alasgarova and Omarov 2023). In Russia, higher prevalence rates ranging from 61,4% to 82% have been documented for *M. canis* (Ovchinnikov et al 2020, Manoyan et al 2024). In contrast, lower rates of 17,4% and 3,6% have been reported in Portugal (Lopes et al 2024, Afonso et al 2024), and 1,8% in the northwestern United States (DeTar et al 2019). In this study, it is hypothesised that the comparatively divergent prevalence observed in this study relative to other studies stems from geographical differences, environmental conditions, the collection of samples from stray animals, the diversity of animals in the sample, and the sample size.

The most frequent clinical manifestations observed were alopecia (75%), erythema (64,28%), and pruritus (57,14%), with itching (39,28%) and crusting (32,14%) also commonly noted. Our study examined in detail the body regions where lesions are most commonly seen, and determined that they are, in order, the head and neck, ears, extremities, scapular, thoracic, abdominal, perianal region, and tail. The statistically significant relationship between the location of the lesion and dermatophyte isolation may reflect factors such as the route of transmission, the density of hair follicles, and the animal's grooming habits. This clinical profile is consistent with

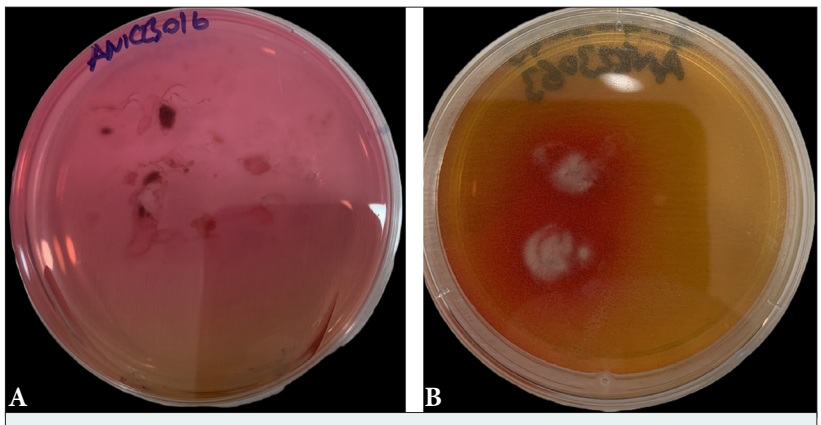

Figure 7. Positive biochemical test result A- *M. canis* on urea agar, B- *M. canis* on dermatophyte test medium.

the lesions and body regions where lesions were detected in other studies (Moriello 2020, Alasgarova and Omarov 2023). Consequently, the high prevalence of these lesions, and lesions region establishes them as key clinical findings for mycological examination in cats presenting with compatible dermatological signs.

The age distribution of the 28 positive cases was as follows: 4 cats (14,28%) were ≤6 months, 6 (21,43%) were 7 months–2 years, 13 (46,43%) were 3–6 years, 4 (14,28%) were 7–10 years, and 1 (3,57%) was 11–14 years. The highest prevalence was thus identified in the 3–6 year age group. This finding contrasts with several previous reports, which indicate a higher prevalence in younger cats. A statistically significant relationship was found between dermatophyte isolation and animal age ($p<0.05$). For instance, Diren Sığırcı et al (2019) reported a positivity rate of 41.6% in cats under two years of age, and other studies have documented prevalences ranging from 9.4% to 86.7% in cats under one year (Katiraee et al 2021, Bouza-Rapti et al 2023, Gautam et al 2024, Lopes et al 2024). Similarly, Saleem et al (2020) found the highest prevalence (42,22%) in cats under one year of age. Our results differ from those in the literature. The high rate of dermatophytosis diagnosis in adult cats may have affected the prevalence, as more samples were collected from cats in this age group in our study population. Regarding sex, dermatophytes were isolated from 12 of 85 females (14,12%) and 16 of 110 males (14,54%). No statistically significant association was found between sex and dermatophyte isolation ($p>0.05$). The literature on this association is conflicting. Some studies report a higher prevalence in females (Diren Sığırcı et al 2019, Selvi and Yıldırım 2019, Katiraee et al 2021, Gautam et al 2024), while others, including the present study, have observed a higher, though not statistically significant, prevalence in males (Saleem et al 2020, Lopes et al 2024). These comparisons indicate that the effects of age and gender on the prevalence of dermatophytosis are inconsistent. This discrepancy is hypothesised to originate from variations between the studies, including the type of sample and the age, sex, and sample size of the animals investigated in the studies.

Among 28 culture-positive cats, the diagnostic sensitivity of Wood's lamp examination was low, with a positive predictive value of 64,28%. Direct microscopic examination demonstrated a higher detection rate, identifying fungal elements in 19 of the 28 cases (67,86%). The results of the study demonstrated that 92,86% of the 28 dermatophyte-positive isolates exhibited growth on DTM, 78,57% on DA and 100% on PDA. In the urease test, 75% of the 28 dermatophyte-positive isolates yielded positive results. These findings present both alignments and discrepancies with the existing literature. The growth rate on DTM observed here (92,9%) is consistent with reports of 95.8% and 78,7% (Selvi and Yıldırım 2019, Mendes et al 2024). However, the growth rate on SDA in this study (78,6%) was higher than the 52% reported by Derincegöz and Parın (2016), though lower than the 89% reported by Tandon et al (2023). The superior performance of PDA observed in this study warrants further investigation, as it is not always the primary medium used in comparable studies. Similarly, the sensitivity of direct microscopic examination in this study falls within the broad range reported elsewhere, which varies from 24% to 84,6% (Derincegöz and Parın 2016, Katiraee et al 2021, Seker and Doğan 2011). This wide variation may have been influenced by factors such as sample quality, technical differences, and staff experience. Furthermore, it is thought that various factors such as the expiration date of the media, the method of preparation, water pH, and autoclaving may affect the results.

Ince and Torun (2023) isolated saprophytic fungal agents other than dermatophytes, including *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., and *Mucor* species. In a further study, *Alternaria* spp., *Cladosporium* spp., *Mucor* spp., *Aspergillus* spp., and *Fusarium* spp. were also isolated in addition to dermatophytes (Afonso et al 2024). The results obtained from isolated fungal species in this study are consistent with those obtained from mycelial fungal species isolated in other studies. This similarity is thought to stem from non-selective dermatophyte culture and sampling methods, as well as saprophytic agents in soil, air and organic debris.

Conclusion

Accurate and rapid diagnosis of dermatophytosis can facilitates the selection of the most effective treatment agent and successful treatment. It is further hypothesized that timely diagnosis may help prevent transmission to humans and the emergence of antifungal resistance. Direct diagnostic methods provide rapid results, however are insufficient for identifying species and genera. Consequently, molecular techniques are essential for precise species identification. The molecular studies are required to elucidate the full scope of regional diversity and genetic variation within dermatophyte species. In summary, this study has contributed to raising awareness regarding the epidemiology of dermatophytosis in Ankara and the suitability of culture media and methods used for diagnosis.

Declarations

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This study was derived from the PhD thesis of the first author.

Competing Interests
Authors declares that there are no conflicts of interest related to the publication of this article.

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Availability of Data and Materials
The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement
This study was carried out after the animal experiment was approved by Kirikkale University Local Ethics Committee with the decision numbered 2022/07 45, dated 28/12/2022. Samples were collected with the permission of the animal owners, obtained through an Informed Consent Form, in accordance with the Local Ethics Committee decision.

Author Contributions
Motivation / Concept: AUO, MY; Design: AUO; Control/ Supervision: AUO, MY; Data Collection and / or Processing: AUO; Analysis and / or Interpretation: AUO, MY; Literature Review: AUO, MY; Writing the Article: AUO, MY; Critical Review: AUO, MY

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