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Prevalence of Non-dermatophytic Fungi in affected Skin of Dogs and Cats

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Abstract

THE global trend of keeping pets in households is on the rise, especially in Egypt. This study aimed to ascertain the incidence of saprophytic molds and yeasts in dogs and cats in Egypt. Hence, a grand total of 100 samples, comprising 50 from dogs and 50 from cats, exhibiting skin abnormalities such as alopecia and desquamation, were thoroughly studied from March 2021 to February 2023. Through direct microscopic examination, it was found that 35 out of the 100 samples analyzed showed evidence of fungal infection. The prevalence rate of fungal infection in cats was 20 out of 50 cases examined at 40%. While the prevalence rate in dogs was 27 out of 50 cases examined at 54%. While using PCR analysis revealed prevalence rates of 16%, and 14% from cats and dogs, respectively. The direct microscopic examination revealed detection fungal elements of dermatophytes at 16.66% and 28.57% from hair and skin samples in cats. The rates were 20% from both hair and skin samples in dogs. However, the isolation rates of non-dermatophytes were 33.33% and 71.42% from hair and skin samples in cats. Such rates were 40% from both hair and skin samples in dogs. The non-dermatophytes identified in the present study were Aspergillus flavus, Aspergillus fumigates, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Cladosporium liminforme., Penicillium chrysogenum, Penicillium rubens and Saccharomyces cerevisiae. In conclusion, combination of direct microscopic, culture and PCR analysis is highly recommended for accurate diagnosis of fungal infections in cats and dogs especially of non-dermatophyte fungi.

Keywords: Dermatophytes, non-dermatophytes; Dog; cats.

Introduction

Fungal diseases pose a significant risk to dogs and cats. It leads to a skin infection called superficial cutaneous mycosis; there are over 50,000 different species of fungi. However, in nature, the majority of fungi do not pose a threat to cats and dogs. Only a small number of fungi, numbering in the hundreds, have the potential to cause diseases in these animals. The world is ill-prepared to confront these serious challenges because of a scarcity of laboratories, low awareness, and the complexities associated with handling mycoses, particularly in developing nations [1]. Certain cats with a strong immune system may not show any symptoms of the disease, but they can still transmit it to other healthy cats, which are known as asymptomatic carriers. The International Society for Human and Animal Mycology (ISHAM) has established a worldwide outreach initiative in collaboration with national affiliated mycology societies and other regional groups [2].

Skin infections called dermatophytosis are common and caused by dermatophytes, which are certain types of fungi from the genera Microsporum, Trichophyton, and Epidermophyton. Dermatophytosis affect over 20–25% of the global pet population, and the number of cases has increased in both humans and animals [1].

Dermatophytes are a collection of filamentous fungi that have proteolytic enzymes capable of degrading keratin in many tissues, including the epidermis, hair, nails, feathers, horns, and hooves. This enzymatic activity leads to superficial mycoses in both people (known as tinea) and animals (referred to as ringworm) [3]. These fungal infections' primary clinical characteristics include consistent hair loss accompanied by redness, which typically does not

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cause itching. However, varying levels of inflammation can alter the appearance.

Dermatophytoses are a prevalent worldwide issue and pose a significant public health concern [4]. The incidence of infection exhibits variability based on factors such as temperature, humidity, season, and geographical location [5]. In several countries, the epidemiology of dermatophytosis in dogs and cats is well understood. M. canis is the predominant dermatophyte responsible for tinea capitis and corporis in humans [6]. Nevertheless, there has been a lack of focus on the epidemiological situation of canine and feline dermatophytosis in Egypt.

Thus, this study attempted to study the prevalence of saprophytic mould and yeast infecting cats and dogs in Zagazig city, Egypt. In addition, identification of the species of non-dermatophyte moulds that impact domestic and stray dogs and cats.

Material and Methods

Sample collection

This investigation was carried out in Zagazig city, Egypt during March 2021 to February 2023. Samples were collected from various sites in Zagazig city, including private clinics, shelters, and stray cats and dogs that were treated with care.

A total of 66 (36 cats and 30 dogs) that had skin lesions classified as being caused by a fungal infection were included. One hundred samples were collected including 50 samples from 36 cats (36 hair, 72 %, and 14 skin scrapes, 28 %) and 50 samples were collected from 30 dogs (30 hair sample, 60 %, and 20 skin scrapes, 40 %).

Sampling procedures

Skin Samples were collected using a sterile blunt scalpel or blade then the crust placed in sterile petri dish, hair samples were collected by using clean scissors or forceps, following strict aseptic procedures. In order to mitigate the risk of sample contamination or the transmission of infectious pathogens, these samples were treated with caution and promptly sent to the mycological laboratory at Faculty of Veterinary Medicine, Zagazig University in a sterile manner within a 24-hour timeframe [7].

Direct microscopic examination:

The samples were analyzed for fungi using a light microscope at a magnification of 40X, with the addition of a single drop of 20% KOH [7].

Cultural identification

The hair and skin scraping samples were cultured on Sabouraud's dextrose agar (SDA) medium, which included 0.5 mg/ml chloramphenicol and 1 mg/ml cyclohexamide. The identical samples were introduced to Dermatophytes test media (DTM) supplemented with FD176, which contains 5 mg of Amphotericin B, chlortetracycline at a dosage of 50 mg and gentamicin at a dosage of 50 mg. The plates and tubes were placed in an incubator at a temperature of 25 °C and observed on a daily basis for a period of three weeks. Each of the isolates was identified using lactophenol cotton blue, both macroscopically and microscopically by examination of hyphae, macroconidia, and microconidia [7].

Molecular identification

DNA extraction of the non-dermatophytes isolates

DNA extraction from culture isolates

The DNA was extracted from cultures of non-dermatophyte isolates using the QIAamp DNeasy Plant Mini kit (Catalog no.69104), crushed 100 mg of mycelia in liquid nitrogen, and then processed according to the manufacturer's instructions [8]. A NanoDrop 1,000 spectrophotometer from Thermo Fisher Scientific was used to ascertain the DNA content and purity. The DNA concentration was about 50 ng and the purity ranged between 1.8 to 2.0 spectral quality at 260/280 wave length. As a positive control, DNA isolated from fungal cultures was subjected to PCR amplification of the internal transcribed spacer (ITS) region.

Methods for polymerase chain reaction (PCR) and DNA sequencing

The ITS1 and ITS4 primer pairs were used to amplify the ribosomal region that includes 28S ribosomal DNA (rDNA) and the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCC TCC GCT TAT TGA TAT GC) regions, respectively. The Emerald Amp GT PCR mastermix (×2), 12.5 µL of template DNA, 1 µL of each primer (20 pmol), and 4.5 µL of PCR-grade water were all components of the 25 µL mixture that was used to conduct the reaction. Denaturation at 94°C for 5 minutes, followed by 35 cycles of annealing at 56°C for 40 seconds, extension at 72°C for 45 seconds, and finally a final extension at 72°C for 10 minutes, were the conditions used in the amplification in a thermal cycler (Biometra T3, Göttingen, Germany) [8].

Each 10 µL aliquot was electrophoresed on a 1.5% agarose gel (Applichem GmbH; Darmstadt, Germany) in ×1 Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer at room temperature for about 30 min in order to detect PCR-amplified products. The gel was then stained with 0.5 µg/mL ethidium bromide. For this purpose, a 100 bp gene ruler DNA ladder (cat. no. SM0243) was used to measure the individual fragment sizes. Alpha Innotech's GelDoc UV gel documentation equipment (San Leandro, CA, USA) was used to photograph the bands and establish their sizes [9].

Using the QIAquick PCR Product extraction kit (Qiagen Inc. Valencia CA), fourteen isolates' PCR products were purified. Then, the ITS1 and ITS4 primers were used for forward and reverse sequencing, respectively. An automated sequencer model 3130 (ABI, 3130, DNA USA) was used for the sequencing process. With the use of a Bigdye Terminator V3.1 cycle sequencing kit (Cat. No. 4336817) manufactured by Perkin-Elmer/Applied Biosystems in Foster City, CA. The BLAST tool, which is accessible at http://www.ncbi.nlm.nih.gov/BLAST/, was used to compare nucleic acid sequence data with previously published data in GenBank.

Statistical analysis

The collected data were encoded, input, organized, and examined utilizing SPSS (Statistical Package for Social Science) version 25 (IBM Corporation, Armonk, NY, USA). The qualitative data was presented as a categorized set, with the frequency, percentage, or proportion of each category. A proportion test (Z test) was conducted to compare the percentages of two groups. A significance level of $p<0.05$ was used [10].

Results and Discussion

The frequency of several saprophytic fungi affecting cats and dogs was examined in this research. The current research made advantage of the convenience sampling strategy. Some independent factors were included in the research model, such as the use of additive in DTM media and chloramphenicol in SDA media. This is in line with what reported before that dermatophytoses can be more accurately diagnosed with the use of direct microscopic examination, which in turn helps veterinary practitioners start treatment sooner [11, 12]. In the present study, mycological examination was performed for 100 skin scraping and hair samples from cats and dogs. Animal samples collected from the affected dogs and cats showed scaly skin with or without a red outline and broken hairs (Fig. 1a).

The overall prevalence rates of fungal infection in cats and dogs using the traditional culture and microscopic examination were 40% (20 out of 50 cats), and 54% (27 out of 50 dogs), respectively (Fig. 2). Further examination for the prevalence of non-dermatophytes in the tested positive samples was done. The obtained results revealed isolation of dermatophytes at 16.66% and 28.57% from hair and skin samples in cats. Such rates were 20% from both hair and skin samples in dogs. However, the isolation rates of non-dermatophytes were 33.33% and 71.42% from hair and skin samples in cats. Such rates were 40% from both hair and skin samples in dogs (Table 1).

Further identification of the recovered fungal species revealed that *Aspergillus flavus* was the most frequently isolated species, followed by *Fusarium oxysporum, Cladosporium limoniform*, *Saccharomyces cervisea*, *Penicillium chrysogienum*, *Penicillium rubens, Fusarium solani and Fusarium proliferatum* those were the most recovered fungal spp. at variable rates as shown in Table 2. The detailed prevalence of the recovered fungal species from either hair or skin of the examined cats and dogs was described in Tables 3 and 4.

These isolates were identified on the basis of phenotypic and molecular characteristics. *Aspergillus flavus* growing on SDA was characterized by greenish yellow colony (Fig. 1b, c), whereas *Fusarium oxysporum* characterized by whitish to yellow, red shades (Fig. 1d-E).

However, PCR was used for confirmation; PCR amplifications of the 28S rDNA fragment of dermatophyte species gave a single product of 480 to 780 bp (Fig. 2). The selected sequence was unique and species specific for all isolates of *Aspergillus flavus, Fusarium oxysporum, Cladosporium limoniform*, *Saccharomyces cervisea*, *Penicillium chrysogienum*, *Penicillium rubens, Fusarium solani and Fusarium proliferatum* .

Nucleotide sequence accession numbers.

The dermatophyte 28S rDNA sequences described in this study have been deposited in the GenBank database and assigned accession no. PQ394668 (*P. chrysogenium*), PQ394671 (*A.flavus*), PQ400038 (*A.flavus*), PQ394714 (*F.oxysporum)*, PQ419152(*A.f umigatus*), PQ394717 (*P.chrysogenium*), PQ403592 (*Saccharomyces cerevisiae*), PQ394723(*Saccharomyces cerevisiae*)), PQ409448 (*P, rubens*), PQ409454 (Cl. Liminiforme), PQ409505 (*P. chrysogenium*), PQ409510 (*F. solani*), PQ409525 (*F.proliferatum*) and PQ409568 (*F. oxysporum*).

Consistent with the current study's results, Visitsunthorn et al. [13] identified *Cladosporium spp* in 29% of cat samples and 28% of dog samples. Furthermore, Velázquez-Jiménez et al. [14] validated the isolation of *Cladosporium spp* from a cat by PCR. Moosavi et al. [15] documented the frequency of dermatophytosis and other keratinophilic fungus on the skin and fur of stray cats in rural Meshkin-shahr, Iran. Their results showed that *Aspergillus spp, Alternaria spp, Rhizopus spp, Penicillium spp* and *paecilomyces spp* in descending frequency were the most predominantly identified saprophytic fungi. In numerous nations, dermatophytosis is recognized

as a significant public health issue, with intimate animal contact, poor hygiene, and climatic conditions being the predominant contributors in its transmission. They observed that asymptomatic cats significantly contribute to the potential transmission of dermatophytosis to people, with a prevalence of 14.5% affecting the skin and fur of cats in the examined region. The variability in the occurrence of dermatophytosis in cats is attributed to various factors, including climate, humidity, season, and geographical region. Kilic et al. [16] reported in their study identified a total of 145,099 spores/m^3 from 20 fungal taxa in the atmosphere surrounding Elazig city, Turkey in 2018. The predominant fungal spores belonged to the following genera: *Cladosporium spp* (75.5%), *Alternaria spp* (6.1%), *and Aspergillus spp, Penicillium spp, Fusarium spp,* and *Drechslera spp* (1.3%). Mavrommatis et al. [17] indicated that *Aspergillus spp, Penicillium spp,* and *Fusarium spp* are isolated from felines and canines, with mycotoxins generated by these three fungal taxa. Tatfeng et al. [18] discovered that DNA sequencing revealed 16 non-dermatophytic mold species across 8 genera: *Aspergillus spp, Talaromyces spp, Curvularia spp, Cladosporium spp, Pestalotiopsis spp, Fusarium spp, Penicillium spp,* and *Absidia spp*. Following PCR and PCR sequencing, the identified fungi were as follows: *Penicillium citrinum* (4%), *Aspergillus fumigatus* (4.7%), *Aspergillus terreus* (2%), *Aspergillus welwitschiae* (15%), *Aspergillus flavus* (11.1%), *Aspergillus aculeatus* (7.3%), *Aspergillus sydowii* (3.6%), *Fusarium solani* (2.2%), *Cladosporium tenuissimum* (4.5%), *Fusarium linchenicola* (19.4%), *Fusarium succisae* (12.5%), and *Absidia species* (14%). *Fusarium linchenicola* was the most often isolated species, followed by *Aspergillus welwitchiae* and *Absidia spp* species, while *Aspergillus terreus* and *Fusarium solani* were the least isolated. Of the 24 samples exhibiting lesions within the State, ten isolates were identified, comprising *Penicillium citrinum* (5%), *Aspergillus fumigatus* (2%), *Aspergillus welwitschiae* (21.3%), *Aspergillus aculeatus* (5%), *Aspergillus flavus* (5%), *Aspergillus sydowii* (3.3%), *Cladosporium tenuissimum* (7%), *Fusarium lichenicola* (13.1%), *Fusarium succisae* (20%), and *Absidia species* (20%). Sudipa et al. [19] collected samples from 15 free-roaming Bali dogs suspected of fungal infection. Identification was conducted through microscopic and macroscopic observation methods which revealed the presence of *Aspergillus spp* in Bali dog skin, which is in coincidence with our results.

Conclusion

The present study indicated that cats and dogs can be affected by non dermatophyte fungi rather than dermatophyte. Dogs and cats, in close proximity to people, may pose a possible health risk due to the zoonotic significance of species derived from them. Understanding the epidemiology of dermatomycosis in pets is crucial for mitigating the transmission of zoophilic fungal infections to humans. Consequently, subsequent research may concentrate on the isolation of dermatophytes as well as non-dermatophyte from both asymptomatic and sick animals.

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Funding statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study was done according to the ethical guidelines of Zagazig University, Egypt, and received the ethical approval number of ZU-IACUC/2/F/253/2024.

Authors' contributions

All authors contributed equally to this study.

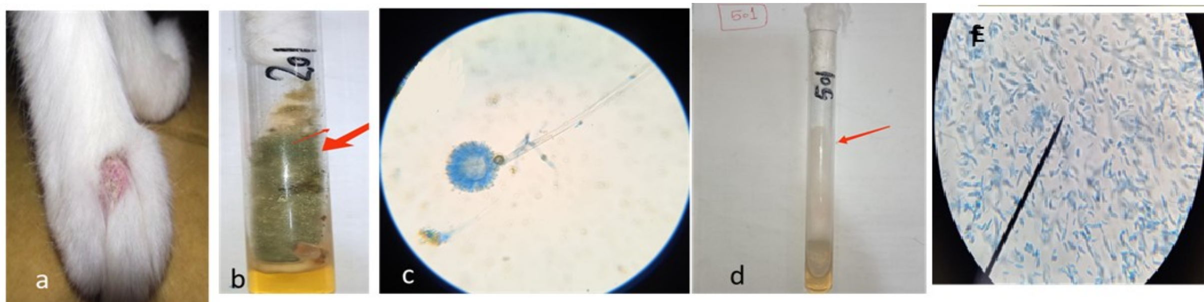

Fig. 1. Traditional (Culture, macroscopic and microscopic) diagnosis of fungal infection in cats and dogs.

TABLE 1. Frequency of positive KOH for dermatophytosis and non-dermatophyte from the studied samples obtained from cats and dogs

Type of positive KOH isolates	Source of the studied samples (n=100)							
	Cat samples (n=50)				Dog samples (n=50)			
	Hair sample (n=36)		Skin scrap (n=14)		Hair sample (n=30)		Skin scrap (n=20)	
	N	%	n	%	n	%	N	%
Dermatophytes	6	16.66	4	28.57	6	20	4	20
Non-dermatophytes	12	33.33	10	71.42	12	40	8	40
Z test	9.773				0.000			
P value	0.002*				1.000			

*Statistically significant (P<0.05)

TABLE 2. Identification of non-dermatophyte fungi in the present study:

Type of fungi isolated from samples	Source of the studied samples (n=62)				Z test	P value
	Dog samples (n=30)		Cat samples (n=32)			
	n	%	N	%		
Aspergillus flavus	4	13.3	5	15.6	8.837	0.003*
Aspergillus fumigatus	4	13.3	0	0		
Fusarium oxysporum	5	16.6	2	6.25		
Fusarium proliferatum	0	0	2	6.25	8.837	0.003*
Fusarium solani	0	0	1	3.1		
Cladosporium liminiforme	0	0	3	9.3	0.165	0.684
Saccharomyces cervisea	4	13.3	2	6.2	0.220	0.639
Penicillium rubens	0	0	3	9.3	1.274	0.259
Penicillium chrysogenum	3	10	4	12.5		

*Statistically significant (P<0.05)

TABLE 3. Identification of non-dermatophytes fungi isolated from skin scrapings and hair samples of the studied cats

Type of positive KOH isolates	Type of sample from the studied cats (n=32)				Z test	P value
	Hair samples (n=18)		Skin scrap samples (n=14)			
	n	%	n	%		
Aspergillus flavus	5	27.7	4	28.5	8.275	0.004*
Fusarium oxysporum	2	11.1	0	0	1.966	0.161
Fusarium solani	0	0	2	14.2		
Fusarium proliferatum	0	0	1	7.1		
Cladosporium limoniforme	1	6.25	2	14.2	6.419	0.011*
Saccharomyces cervisea	4	25	2	14.2	4.736	0.029*
Penicillium rubens	6	33.3	1	7.1	8.927	0.003*
Penicillium chrysogenum	0	0	2	14.2		

*Statistically significant (P<0.05)

TABLE 4. Identification of non-dermatophytes fungi isolated from skin scrapings and hair samples of the studied dogs

Type of positive KOH isolates	Type of sample from the studied dogs (n=30)				Z test	P value
	Hair samples (n=18)		Skin scrap samples (n=12)			
	n	%	n	%		
Aspergillus flavus	6	33.3	5	41.6	8.275	0.004*
Aspergillus fumigatus	1	5.5	1	8.3	6.419	0.011*
Fusarium oxyspprum.	3	16.6	1	8.3	1.966	0.161
Saccharomyces cervisea	4	22.2	2	16.6	4.736	0.029*
Penicillium chrysogenium	4	22.2	3	25	8.927	0.003*

**Statistically significant (P<0.05)*

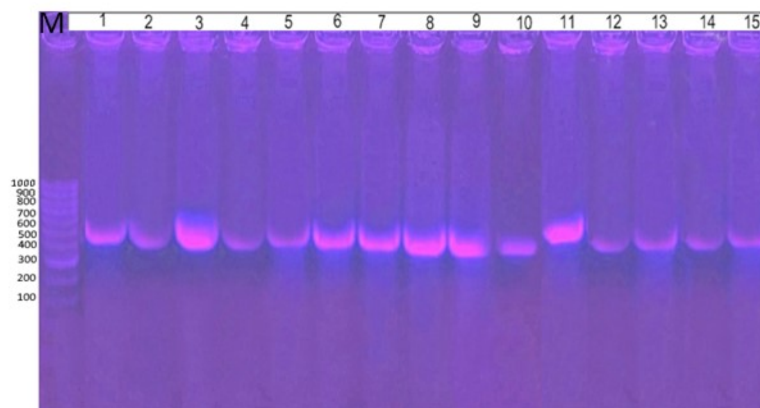

Fig. 2. Analysis of DNA from non-dermatophyte isolates using intergenic spacer (ITS)-based polymerase chain reaction (PCR) products. Lane M refers to one molecular size marker per ladder 100 bp); lanes: 5,6,7: *Penicillium chrysogenium* at 558 bp; 1,2: *Aspergillus flavus* at 572 bp; 3,15: *Fusarium oxysporum* at 688 bp; and lane 4,14*: Saccharomyces cerevisiae* at 546 bp., lane 11, *Penicillium rubens* at 789, lane 12,*Cladosporum liminiforme* at 509, lane 13, 8, *Fusarium solani* at 544,lane 10:*Fusarium proliferatum* at 490 and lane 9, *Aspergillus fumigatus* at 400.

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مدي انتشار الفطريات غير الجلدية في اصابات الجلد في الكلاب والقطط

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قسم الفطريات، كلية الطب البيطري، جامعة الزقازيق، جمهورية مصر العربية.

الملخص

الاتجاه العالمي للحفاظ على الحيوانات الأليفة في المنازل في تزايد، خاصة في مصر. هدفت هذه الدراسة إلى تحديد مدى انتشار الفطريات السابروفية والخمائر في الكلاب والقطط في مصر. لذا، تم دراسة ما مجموعه 100 عينة، تتكون من 50 عينة من الكلاب و50 عينة من القطط، التي تظهر تشوهات جلدية مثل تساقط الشعر وتقشر الجلد، بشكل شامل من مارس 2021 إلى فبراير 2023. من خلال الفحص المجهري المباشر، وُجد أن 35 من أصل 100 عينة تم تحليلها أظهرت دليلًا على الإصابة الفطرية. معدل انتشار العدوى الفطرية في القطط كان 20 من أصل 50 حالة تم فحصها بنسبة 40%. بينما كانت نسبة الانتشار في الكلاب 27 من أصل 50 حالة تم فحصها بنسبة 54%. بينما أظهرت تحليل تفاعل البلمرة المتسلسل معدلات انتشار بنسبة 16% و14% من القطط والكلاب، على التوالي. أظهر الفحص المجهري المباشر الكشف عن العناصر الفطرية للفطريات الجلدية بنسبة 16.66% و28.57% من عينات الشعر والجلد في القطط. كانت النسب 20% من كل من عينات الشعر والجلد في الكلاب. ومع ذلك، كانت معدلات عزل الفطريات غير الجلدية 33.33% و71.42% من عينات الشعر والجلد في القطط. كانت هذه النسب 40% من عينات الشعر والجلد في الكلاب. الفطريات غير الجلدية التي تم تحديدها في الدراسة الحالية كانت الاسبيرجلس فلافس ، الاسبيرجلس فيمجيتس ، الفيوزاريوم سولاني ، كلادوسبوريوم ليمينيفورم ، بينسيليوم روبينز ، بينسيليوم كريزوجينم ، وساكاروميسيز سيرفيسس لذا يُوصى بشدة باستخدام كل من الفحص المجهري المباشر، وطرق العزل التقليدي للفطريات، وتحليل تفاعل البلمرة المتسلسل للتشخيص الدقيق للعدوى الفطرية في القطط والكلاب، خاصةً الفطريات غير الجلدية.

الكلمات الدالة: الفطريات غيرالجلدية، العدوي الجلدية، الكلب، القطط.