Studying the blood virtues of animals spontaneously infected with *Microsporum spp.* and the scheme in the treatment of the fungal disease *Microsporum spp.*

Abstract :

This article focuses on performing clinical, laboratory examination, isolation, and identification of Microsporum spp., general and biochemical analyzes of the blood of spontaneously infected animals and the successful treatment of Microsporum spp. infection in cats and dogs using miconazole/chlorhexidine combination and itraconazole. Skin scraping, hair samples and blood samples were collected from a total of 132 animals.Out of 132 examined dogs and cats, 43 animals were diagnosed with the disease, 28 of the sick animals were cats and 15 were dogs. Based on the results of RBCs (Red Blood Cell) Count, HGB (Hemoglobin), HCT (Hematocrit), MCH (Mean Corpuscular Hemoglobin) blood tests and MCHC (Mean Corpuscular Hemoglobin Concentration), low levels of healthy red blood cells, hemoglobin, and low results in MCHC blood tests were observed in the blood of all of infected dogs and cats, a high level of AST (Aspartate Aminotransferase) is also observed in some infected animals. Among the sick animals, there were also animals with normal AST (Aspartate Aminotransferase) levels.In our study, 29 infected cats and dogs were treated with itraconazole and one of two topical therapies including 2% chlorhexidine and 2% miconazole shampoo (VetWELLMicoseb Medicated Shampoo for Dogs & Cats - Medicated Dog Shampoo with Miconazole, Chlorhexidine & Aloe for Skin Infection Treatment of Skin Conditions 12oz).

PLEASE INCLUDE THE OBJECTIVE IN THE ABSTRACT

Keywords: dermatophytes; miconazole, itraconazole;chlorhexidine

Abbreviation

ITZ- Itraconazole

RBCs (Red Blood Cell) Count

HGB (Hemoglobin) Blood Test

HCT (Hematocrit) Blood Test

MCH (Mean Corpuscular Hemoglobin) level

MCHC (Mean Corpuscular Hemoglobin Concentration)

AST (Aspartate Aminotransferase Test)

Introduction

Dermatophytes, referred to as the ringworm fungi, are traditionally divided into three closely related genera, including *Epidermophyton, Trichophyton,* and *Microsporum spp.Trichophyton spp.* and *Microsporum spp.* cause skin diseases in animals, such as *T. mentagrophytes, T. verrucosum,* and *M. canis,* which are known as zoophilic dermatophytes (Che-Cheng Chang, WittawatWechtaisong, et al., 2022). Please review journal citation guidelines for references*.

The geographical location, exposure to stress factors, environmental conditions, and age play an important role in the spread of dermatophytes. Economically, the increasing concern of dermatophytosis is not triggered by its worldwide public health problems in terms of affecting millions of individuals annually, but also being one of the dermatologic problems in the veterinary field affecting domestic and wild animals (*E.M. Fawzi, M.M. Abd-Elmegeed, 2022). Dermatophytosis is a disease caused by dermatophytes, a group of fungi that can cause disease both in humans and animals (Vena Chupia, JirapatNinsuwon et al., 2022*).

The clinical signs of ringworm appear 1-4 weeks after the contact with fungal spores (E.M. Fawzi, M.M. Abd-Elmegeed, 2022). Infection with *M.canis please review writing of scientific names* is usually associated with alopecia, and infection has been diagnosed by isolation of fungus, which has characteristic hyphae or arthroconidia, from the patients' hair lesions (Hsiao YH, Chen C, Han HS, et al., 2018). Fungal infections caused by *M.canis*, followed by *M.gypseum* and *M.hominis*, involving skin and its appendages, represent one of the most common diseases worldwide and a recalcitrant problem in dermatology that demands appropriate diagnostic and treatment strategies (MihaelSkerlev MD, PhD, Paola Miklić MD, 2009*).

Conventional methods such as the direct microscopic examination of dermatophytes are simple and can be rapidly carried on the collected skin scrapping samples. Mycological identification using specific culture media is one of the basic standard methods used to detect dermatophytes and identify the different species (Moriello KA, DeBoer DJ, 2012*). The pathogen can be found in the hair of cats with and without skin lesions, owners, keepers, veterinarians, and others who come into contact with these animals are at risk of infection if they are not aware or do not take precautions after contact with them (Vena Chupia, JirapatNinsuwon et al., 2022*).

The infected patients show hair loss with erythema and are diagnosed as having dermatophytosis, but the transmission routes of *M. canis* from animals to others are sometimes unclear, although they are critical to the treatment of patients and infection control (Maya Hariu, Yuji Watanabe et al., 2021). The isolation rates of dermatophyte species from dogs and cats were 18.7% and 20.1%, respectively (Esra Seker, Nurhan Dogan, 2010). *Microsporumcanis* (57.1%) was the most common species isolated from dogs and cats. The isolation rate of dermatophytes was relatively high in the spring and winter for dogs, and in the spring, summer and autumn for cats in western Turkey (Esra Seker, Nurhan Dogan, 2010). These pathogenic fungi flourish well at an estimated temperature of 25–28 °C. Large and crowded populations cause easy exposure to the infection. Pet animals, which can easily cause fungal infections to be passed from animals to people. This causes larger numbers of cases of eczema skin diseases in the tropics than in other regions. Pathogen transmission depends on many factors, especially spore contraction through direct contact with a carrier cat, which is the main factor that causes the spread of the disease (Vena Chupia, JirapatNinsuwon, et al., 2022).

For the treatment of dermatophytosis, griseofulvin, ketoconazole, itraconazole and terbinafine are the drugs most commonly used in veterinary medicine (Boothe, 2012).Transmission of dermatophytosis occurs via direct contact with infective material originating from the skin and hair coat of infected animals. Thus, the purpose of topical therapy is to decrease the infectious, contagious and zoonotic risks associated with this disease by disinfecting the hair coat and minimizing contamination of the environment (Karen A. Moriello, Kimberly Coyner, Susan Paterson, Bernard Mignon, 2017*).Please include the objective of the study here.

Materials and methods

Ethical approve

Samples from animals were collected in accordance with the bioethical and standard procedures of the "Bioethics Committee of the Azerbaijan National Academy of Sciences".

Biosecurity and biosafety regulations

Collection, packaging, and transportation of samples were carried out in accordance with biosafety rules (Chapter 1.1.4 Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities).

Animals and clinical samples

From June 2021 to September 2023, a total of 132 dogs and cats were admitted to a veterinary clinic operating under the Veterinary Scientific Research Institute, Baku, Azerbaijan and a veterinary clinic of Azerbaijan State Agrarian University, Ganja, Azerbaijan. For the study, two **fifteen** blood samples were taken from the dogs - from the ear and venous. Peripheral blood was used to prepare a smear, stained with Romanowsky-Giemsa stain and viewed under a binocular immersion microscope THIS PARAGRAFH IS NOT CLEAR, PLESE RE-STATE THE SENTENCES. Venous blood was used for the CANI V 4 test, as well as for general and biochemical blood tests, determining the level of AST, ALT, creatinine and urea. The CANI V 4 test and VH3VET-07249 and DRI CHEM NX 600 analysers from Hasvet were used. We grouped the animals with clinical signs (Figure 1, 2) according to age, breed, species and other important dataPLEASE BE MORE SPECIFIC. Skin scraping, hair samples and blood samples were collected from 132 animals. Skin lesions were collected from all animals suspected to be infected with *Microsporum spp.* Skin lesions of sick dogs and cats were cleaned with a cotton swab soaked in 70% alcohol. Skin scrapings from infected animals were collected using a sterile scalpel, and a new, unopened toothbrush.

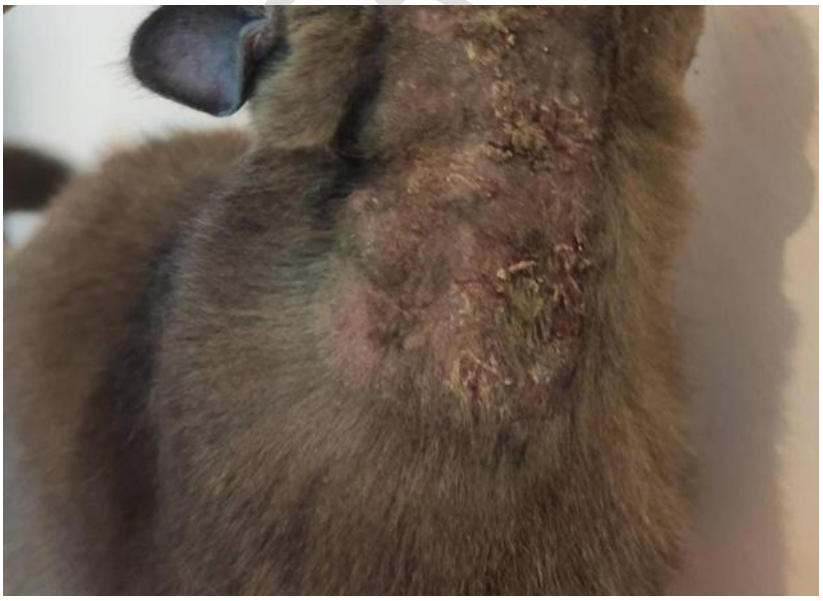

Figure 1: Infected dog with Microsporumspp

The hair loss, scaling, crusting, erythema, blemishes, hyperpigmentation, and variable pruritus were manifested in the spontaneously infected dog (Figure 1). Nodular lesions (kerion) reactions were developed in an examined dog. Move this last paragraph to results??

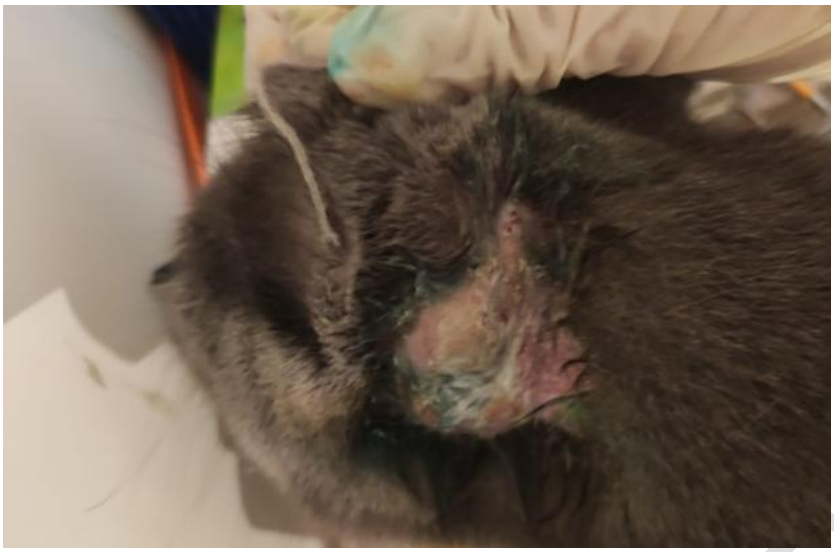

Figure 2: Hair loss and skin damage in a sick animal

The circular alopecia was observed with hair breakage, desquamation, and sometimes an erythematous margin and central healing. A small lesion was detected around the ears. Multiple exaggerated parts are localized mainly on the head (Figure. 2); some cats also observed the distal parts of the legs and the tail. Display lesions were localized to the muzzle. A papulo-crustous dermatitis was observed in a newborn mother cat. Results??

Examination of the collected samples

Our study used a Wood's lamp (320 to 400 nm) examination to detect *Microsporum* infections. The affected area of infected animal skin changed colour under ultraviolet light (Figure 3).Positive hairs glow apple green.The glowing hairs were lifted and then collected for direct examination. Samples cleared 10% KOH??before the examination, though a direct observation of a drop of mineral oil —infected animals considered to have positive glowing hairs PARAGRAPH DIFFICULT TO UNDERSTAND, PLEASE RE-STATE.

Figure 3: Wood's lamps examination

A new, unopened toothbrush is scrubbed over the lesions and then inoculated onto a fungal culture medium. Culture on Sabouraud dextrose agar is generally supposed to be the gold standard for detecting *Microsporum spp.* (Moriello et al., 2017), consequently mentioned agar was used to culture *Microsporum spp.* (HIMEDIA supplies Sabouraud Dextrose Agar, Granulated-GM063-500Gmedium/ Sabouraud Dextrose Agar, Granulated). Inoculated Perti dishes *(*Sabouraud's dextrose agar) were incubated at 30^0C for four weeks. The colonies formed on the surface of Sabouraud's dextrose agar were observed, and firstly, it was determined that the territories belong to *Microsporum spp*. according to their colour and structure. Later, the smears prepared from those colonies were subjected to microscopy, and the result was confirmed. The microscopic identification was done by examining *M. canis*-infected hairs (Figure 4).

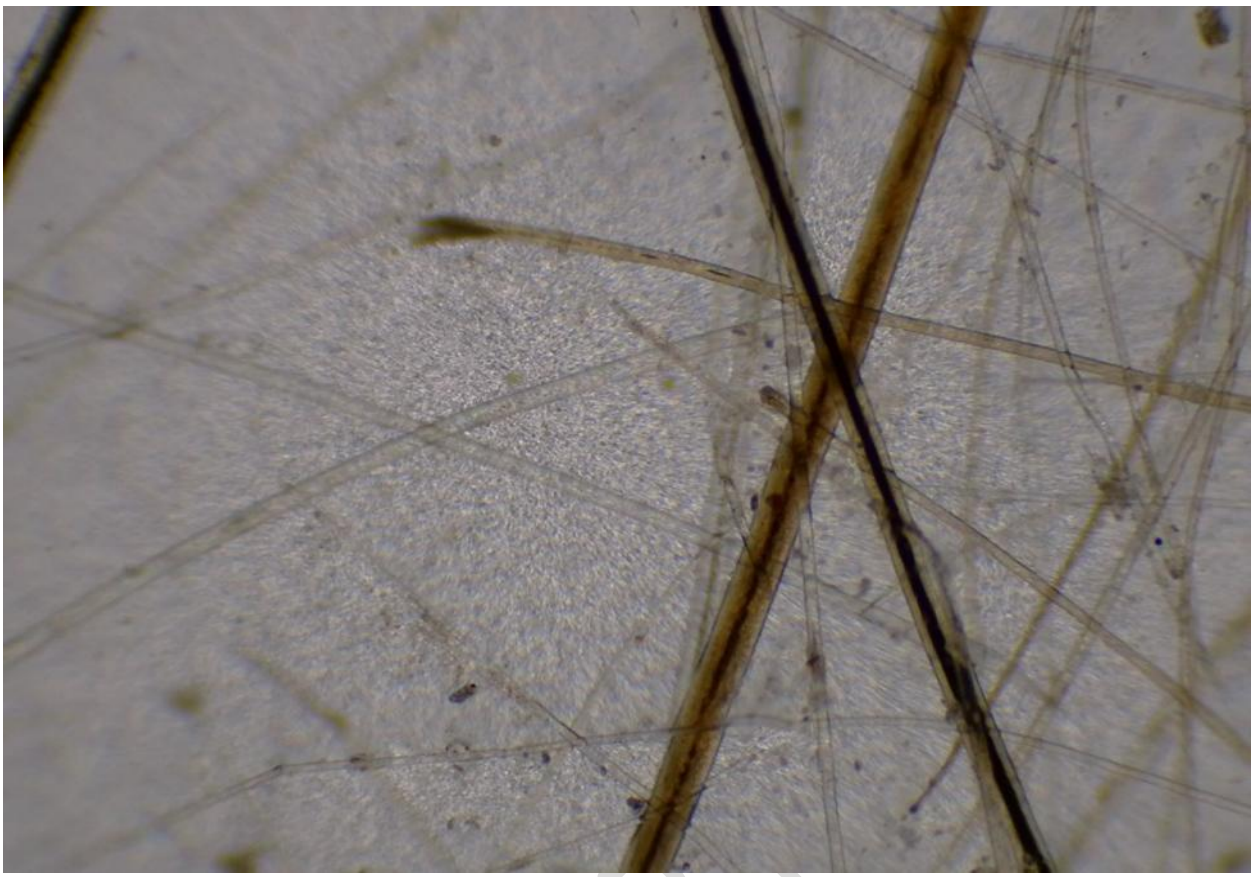

Figure 4: Direct examination of Microsporumcanis-infected hairs

This is a 40X image of an infected hair (thick arrowWHERE IS THE ARROW??).

Blood was taken from the slanderously **spontaneously** infected animals and typical and biochemical analyses were carried out in the laboratory of the Veterinary clinic operating under the Azerbaijan Veterinary Scientific Research Institute in 2023.

RBCs (Red Blood Cell) Count, HGB (Hemoglobin) Blood Test, HCT (Hematocrit) Blood Test and MCH (Mean Corpuscular Hemoglobin) level, MCHC (Mean Corpuscular Hemoglobin Concentration) and Aspartate Aminotransferase Test (AST) are used.The CANI V 4 test, VH3VET-07249, and DRI CHEM NX 600 analysers from Hasvet were used to check the above-mentioned indicators.

Treatment of infected dogs and cats

Itraconazole (ITZ) with twice-weekly and chlorhexidine/miconazole shampoo treats spontaneously infected dogs and cats.Shampoos contain 2% chlorhexidine and 2% miconazole. Itraconazole in dogs and cats: 5 mg/kg to two times per day orally was implemented. 5 Days were considered as one cycle, and 3…5 cycles were applied and infections were resolved after 10…21 days. Itraconazole is given with feed. The absence of clinical symptoms initially assessed the effectiveness of the treatment. The clinical cure is the resolution of all lesions and the lack of new lesions. A Wood's lamp examination was used to look for areas of residually infected hairs in animals with the infections. Organic material and hair were cleaned via a vacuum cleaner. After cleaning, carpet disinfectants were finished for disinfection. Bathroom

disinfectant was implemented for conservational cleaning of infective material from the environment.

PLEASE INCLUDE THE STATISTICAL ANALYSIS OF THE DATA.

Results&discussion :

Out of 132 examined dogs and cats, 43 animals were diagnosed with the disease.The apparent Prevalence from the Population ARE CAPITAL LETTERS NEEDED HERE? is 32.58%, and considering the sensitivity and specificity of the used diagnostic test, it corresponds to a True Prevalence equal to 30.64%. 28 of the sick animals were cats, and 15 were dogs.

General analyses of the blood of spontaneously infected animals

Based on the results of RBCs (Red Blood Cell) Count, HGB (Hemoglobin), HCT (Hematocrit), MCH (Mean Corpuscular Hemoglobin) blood tests and MCHC (Mean Corpuscular Hemoglobin Concentration)WHY INCLUDING DEFINITION OF ABREVIATIONS AGAIN HERE??, low levels of healthy red blood cells, hemoglobin, and low results in MCHC blood tests were observed in the blood of all of infected dogs and cats (Table 1).

Table 1: Results of general blood analysis of spontaneously infected animals

Infected animals	Parameter	Results			Unit of measurement	Reference
		D01	D02	D03		
1. Dog 01 2. Dog 02 3. Dog 03	***WBC***	9.3	16.4	10.3	10^9/L	6.0-17.0
	LYM%	23.6	21.2	11.8 L	%	12.0-30.0
	MID%	8.8	9.0	5.0	%	2.0-9.0
	GRAN%	67.6	69.8	83.2 H	%	60.0-83.0
	LYM#	2.1	3.4	1.2	10^9/L	0.8-5.1
	MID#	0.8	1.4	0.5	10^9/L	0.0-1.8
	GRAN#	6.4	11.6	8.6	10^9/L	4.0-12.6
	RBC	7.53	5.34 L	2.69 L	10^9/L	5.50-8.50
	HGB	15.1	9.5 L	5.5 L	g/dl	11.0-19.0
	HCT	54.5	36.2 L	24.0 L	%	39.0-56.0
	MCV	72.4 H	67.9	89.4 H	fl	62.0-72.0
	MCH	20.0	17.7 L	20.4	Pg	20.0-25.0
	MCHC	27.7 L	26.2 L	22.9 L	g/dl	30.0-38.0
	RDW-CV	11.4	11.6	14.8	%	11.0-15.5
	RDW-SD	40.1	37.3	54.6	fl	
	PLT	362	159	13 L	10^9/L	117-460
	MPV	7.0	7.5	8.6	fl	7.0-12.0
	PDW	9.3	10.8	9.9	fl	
	PCT	0.25	0.11	0.01	%	
	P-LCR	12.0	14.8	20.8	%	

	P-LCC	43	23	2	10^9/L	

Dog 01 - Samuray (Samurai), Dog 02 - Cora and Dog 03 - Sherlock are sick animals mentioned in Table 1.

MCV indicator of dog#03 was an overestimated amount; however, the MCHC pointer was underestimated nevertheless, other vital indicators were within normal limits.

Biochemical analyses of the blood of spontaneously infected animals

A high level of AST (Aspartate Aminotransferase) is also observed in some infected animals, among the sick animals, there were also animals with normal AST (Aspartate Aminotransferase) levels (Table 2).

Table 2: Blood biochemical analyzes of spontaneously infected animals

Infected animals	Parameter	Results			Unit of measurement	Reference
		D01	D02	D03		
1. Dog 01 2. Dog 02 3. Dog 03	*GOT/AST*	24	61 H	24	U/l	17-44
	GPT/ALT	27	21	36	U/l	17-78
	CRE	1.36	0.75	1.27	mg/dl	0.40-1.40
	BUN	13.1	13.5	45.7 H	mg/dl	9.2-29.2

Dog 01 – Samuray (Samurai), Dog 02 - Cora and Dog 03 - Sherlock are sick animals mentioned in the Table 2.

The GOT/AST index in the dog named Samurai was 1.3 times higher than normal, and the BUN index was 1.6 times higher in the dog named Sherlock. Other blood indicators were within the norm.

WHY THE CAT DATA IS NOT SHOWN IN THE RESULTS, INCLUDE THE JUSTIFICATION FOR SUCH OMISSION, IF IT IS THE CASE, OTHERWISE, STATE THE REASONS FOR NOT INCLUDING THEM.

Treatment efficacy

Itraconazole (ITZ) with twice-weekly chlorhexidine/miconazole shampoo was more effective than ITZ alone to treat *Microsporum*infections in cats and dogs.Twice weekly application of miconazole/chlorhexidine shampoos recommended effective topical therapies in treating generalisedermatophytosis in cats and dogs.As a result of our study, 29 infected cats and dogs were treated with itraconazole and one of two topical therapies, including 2% chlorhexidine and 2% miconazole shampoo. The median time to clinical cure was under 5 cycles and the median time to mycological cure was 4 cycles (range 7–21 weeks).

Conclusion

Our research determined that sick pets, stray animals and shelter animals have an exceptional role in the spread of *Microsporum* infections. We can assume that the second main factor in the space of the disease is the objects with which sick animals come into contact.

To diagnose the disease, in addition to skin scraping and hair samples, it is essential to take blood samples from infected dogs and cats, as well as general examination and biochemical analyses of blood along with mycological examination methods. It gives us a clue for future investigations.

It was found that the disease is spread in Baku and Ganja cities of our republic in all seasons of the year, but the condition is observed more often in the spring, autumn and winter seasons of the year.

According to the information provided by dermatologists (face-to-face interviews) in Baku and Ganja, *Microsporum spp.* infection in people is mainly spread among children and adolescents aged 0-14 years. Corresponding to face-to-face interviews, it was determined that the vast majority of children and adolescents infected with the disease were in close contact with sick and carrier pets and street animals. This study clearly shows that *Microsporum spp*. infections among dogs and cats are a public health concern in Baku, the capital city of our republic, and Ganja, one of our 3 largest industrial cities. Considering these, the treatment of the disease is a vital issue.

Our research shows that the end point of treatment includes systemic treatment and topical therapy and is required to clean the hair coat and disinfect the environment. Regular use of common bath detergents is effective and environmentally friendly, rather than using toxic chemicals for conservational disinfection.

References:PLEASE USE THE JOURNAL CITATION GUIDELINES FOR ALL REFERENCES CITED HERE AND IN THE TEXT.

1. Yamada S, Anzawa K, Mochizuki T. An epidemiological study of feline and canine dermatophytoses in Japan. *Med Mycol J.* 2019;60:39–44. [PubMed] [Google Scholar]

2. Thakur R, Kalsi AS. Outbreaks and epidemics of superficial dermatophytosis due to trichophyton mentagrophytes complex and microsporumcanis: Global and Indian scenario. *ClinCosmetInvestigDermatol.* 2019;12:887–93. [PMC free article] [PubMed] [Google Scholar]

3. Hubka V, Dobiašova, Dobiaš R, et al.Microsporumaenigmaticum sp. nov. from *M. gypseum* complex, isolated as a cause of tinea corporis. *Med Mycol.* 2014;52:387–96. [PubMed] [Google Scholar]

4. Gräser Y, Scott J, Summerbell R. The new species concept in dermatophytes – a polyphasic approach. *Mycopathologia.* 2008;166:239–56. [PubMed] [Google Scholar]

5. Mock M, Monod M, Baudraz-Rosselet F, et al. Tinea capitis dermatophytes: Susceptibility to antifungal drugs tested in vitro and in vivo. *Dermatology.* 1998;197:361–67. [PubMed] [Google Scholar]

6. Aste N, Pau M. *Tinea capitis caused by microsporumcanis treated with terbinafine mycoses.* 2004;47:428–30. [PubMed] [Google Scholar]

7. Hsiao YH, Chen C, Han HS, et al. The first report of terbinafine resistance *Microsporumcanis* from a cat. *J Vet Med Sci.* 2018;80:898–900. [PMC free article] [PubMed] [Google Scholar]

8. Faggi E, Pint G, Campisi E, et al. Application of PCR to distinguish common species of dermatophytes. *J ClinMicrobiol.* 2001;39:3382–85. [PMC free article] [PubMed] [Google Scholar]

9. Dobrowolska A, Debska J, Kozłowska M, et al. Strains differentiation of *Microsporumcanis* by RAPD analysis using (GACA)4 and (ACA)5 primers. *Pol J Microbiol.* 2011;60:145–48. [PubMed] [Google Scholar]

10. Brosh-Nissimov T, Ben-Ami R, Astman N, et al. An outbreak of *Microsporumcanis* infection at a military base associated with stray cat exposure and person-to-person transmission. *Mycoses.* 2018;61:472–76. [PubMed] [Google Scholar]

11. Havlickova B, Czaika VA, et al. Epidemiological trends in skin mycoses worldwide. *Mycoses.* 2008;51:2–15. [PubMed] [Google Scholar]

12. Subelj M, SvetičičMarinko J, Učakar V. An outbreak of *Microsporumcanis* in two elementary schools in a rural area around the capital city of Slovenia, 2012. *Epidemiol Infect.* 2014;142:2662–66. [PMC free article] [PubMed] [Google Scholar]

13. Cornut J, De Respinis S, Tonolla M, et al. Rapid characterization of aquatic hyphomycetes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Mycologia.* 2019;111:177–89. [PubMed] [Google Scholar]

14. de Respinis S, Tonollia M, Pranghofer S, et al. Identification of dermatophytes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Med Mycol.* 2013;51:514–21. [PubMed] [Google Scholar]

15. Lau AF, Walchak RC, Miller HB, et al. Multicenter study demonstrates standardization requirements for mold identification by MALDI-TOF MS. *Front Microbiol.* 2019;10:2098. [PMC free article] [PubMed] [Google Scholar]

16. Hariu M, Watanabe Y, Oikawa N, et al. Usefulness of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify pathogens, including polymicrobial samples, directly from blood culture broths. *Infect Drug Resist.* 2017;10:115–20. [PMC free article] [PubMed] [Google Scholar]

17. Hariu M, Watanabe Y, Oikawa N, et al. Evaluation of blood culture broths with lysis buffer to directly identify specific pathogens by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry methods. *Infect Drug Resist.* 2018;11:1573–79. [PMC free article] [PubMed] [Google Scholar]

18. Seki M, Hariu M, Watanabe Y. Critical points of direct pathogens identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry methods. *J Infect Dis Ther.* 2020;8:1–4. [Google Scholar]

19. Moriello K.A. Diagnostic techniques for dermatophytosis. *Clin. Tech. Small Anim. Pract.* 2001;16:219–224. doi: 10.1053/svms.2001.27597. [PubMed] [CrossRef] [Google Scholar]

20. Moriello KA, DeBoer DJ. Dermatophytosis: Infectious diseases of the dog and cat. 4th ed. St. Louis: Elsevier; 2012. 588-602 p.

21. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Description of medical fungi. 2nd Ed. Adelaide: University of Adelaide; 2007. 61-167 p. DOI: 10.1.1.694.2167&rep=rep1&type=pdf

22. https://www.petplace.com/article/drug-library/drug-library/library/itraconazole-sporanox-intrafungol-for-dogs-and-cats#:~:text=Dosing%20Information%20of%20Itraconazole%20for%20Dogs%20and%20Cats&text=The%20dose%20for%20cats%20is,should%20be%20given%20with%20food)