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Studying the Blood Virtues of Animals Spontaneously Infected with *Microsporum spp*. and the Scheme in the Treatment of the Fungal Disease *Microsporum spp*.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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 (*VetWELL Micoseb* Medicated Shampoo for Dogs & Cats - Medicated Dog Shampoo with *Miconazole*, Chlorhexidine & Aloe for Skin Infection Treatment of Skin Conditions 12oz). This study aimed to perform clinical characteristics, laboratory examination, isolation and identification of zoophilic dermatophytes due to *Microsporum spp*. In addition, to evaluate general and biochemical analyses of the blood of spontaneously infected pets and stray and shelter animals with *Microsporum spp*. in Baku and Ganja cities in Azerbaijan, and to focus the treatment includes systemic and topical therapy.

Keywords: Dermatophytes; miconazole, itraconazole; chlorhexidine.

ABBREVIATION

AST : Aspartate Aminotransferase Test
ITZ : Itraconazole
HGB : Hemoglobin Blood Test
HCT : Hematocrit Blood Test
MCH : Mean Corpuscular Hemoglobin level
MCHC :Mean Corpuscular Hemoglobin Concentration
RBCs : Red Blood Cell Count

1. 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[1-4]

The geographical location, exposure to stress factors, environmental conditions, and age play an essential 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 by its being one of the dermatologic problems in the veterinary field involving domestic and wild animals[5]. Dermatophytosis is a disease caused by dermatophytes, a group of fungi that can cause disease in humans and animals [6,7,4,8,9].

The clinical signs of ringworm appear 1-4 weeks after contact with fungal spores [5]. Infection with *Microsporum canis* 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 [10]. Fungal infections caused by *Microsporum canis*, followed by *Microsporum gypseum* and *Microsporum 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 [11-14]

Conventional methods, such as the direct microscopic examination of dermatophytes, are simple and can be rapidly carried on the collected skin-scrapping samples [15-17]. Mycological identification using specific culture media is one of the basic standard methods used to detect dermatophytes and identify the different species [18-22]. 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 [6].

The infected patients show hair loss with erythema. They are diagnosed as having dermatophytosis, but the transmission routes of M. canis from animals to others are sometimes unclear. However, they are critical to the treatment of patients and infection control [23-25]. The isolation rates of dermatophyte species from dogs and cats were 18.7% and 20.1%, respectively [26,27]. *Microsporum canis* (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 [26]. These pathogenic fungi flourish well at an estimated 25–28 °C temperature [28,29]. 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[30-33] This causes larger numbers of cases of eczema skin diseases in the tropics than in other regions[31,30] 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 [6,7,34]

For the treatment of dermatophytosis, griseofulvin, ketoconazole, itraconazole and terbinafine are the drugs most commonly used in veterinary medicine [35]. (Transmission of dermatophytosis occurs via direct contact with infective material originating from the skin and hair coat of infected animals [36-38]. 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 [18,19].

1.1 Objectives

The aims of this study were to perform clinical characteristics, laboratory examination, isolation and identification of zoophilic dermatophytes due to *Microsporum spp.* In addition, to evaluate general and biochemical analyses of the blood of spontaneously infected pets and stray and shelter animals with *Microsporum spp.* in Baku and Ganja, and to focus the treatment includes systemic and topical therapy.

2. MATERIALS AND METHODS

2.1 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) [39,40].

2.2 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, fifteen spontaneously infected animals were engaged for blood sampling. One peripheral blood sample and one venous blood were taken from each animal. Venous blood was taken from the tail vein, and peripheral blood was taken from the ear capillary. Peripheral blood was used to prepare a smear and stained with Romanowsky-Giemsa methods [41,42]and viewed under a binocular immersion microscope. Venous blood was used for the CANI V 4 test and 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. Faunae were grouped with clinical signs (Figs. 1, 2) according to age, breed, species, gender, and occupation of the owner. 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.

2.3 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 (Fig. 3). Positive hairs glow apple green. The glowing hairs were lifted and then collected for direct examination. Positive samples with a Wood's lamp were collected for further investigation. The mentioned samples were cleared with 10% Potassium hydroxide (KOH) before the examination, though a direct observation of a drop of mineral oil —infected animals considered to have positive glowing hairs. In our research, the Wood's lamp examination was a screening test, a direct observation under the microscope considered as a conformation test.

Second stage, 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.* [18,19,43], consequently mentioned agar was used to culture *Microsporum spp.* (HIMEDIA supplies Sabouraud Dextrose Agar, Granulated-GM063-500Gmedium/ Sabouraud Dextrose Agar,

Granulated) [15,28,44,45,46]. 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 (Fig. 4).

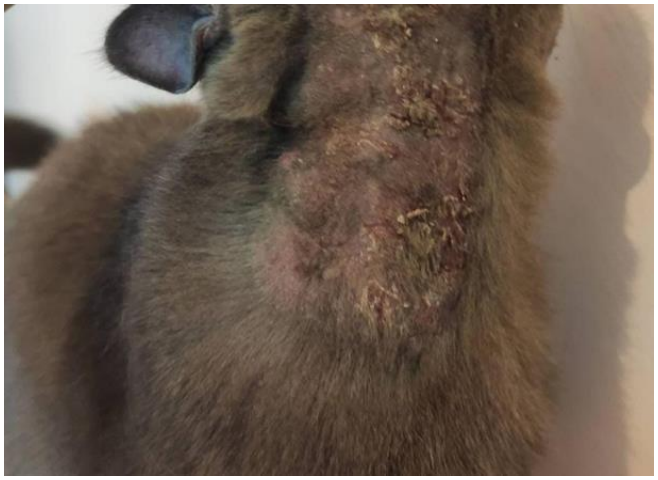

Fig. 1. Infected dog with Microsporum spp

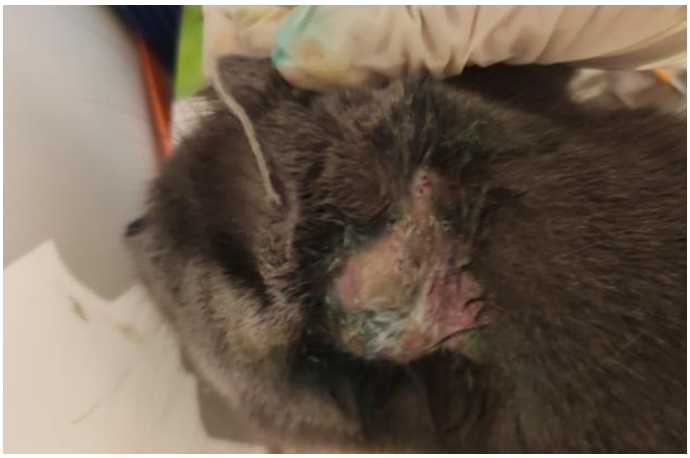

Fig. 2. Hair loss and skin damage in a sick animal

Fig. 3. Wood's lamps examination

Fig. 4. Direct examination of Microsporum canis-infected hairs
This is a 40X image of an infected hair (thick arrow)

Blood was taken from the 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.

2.4 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.

A total of 132 animals were examined; 85 (64%) faunae were cats, and 47 (36%) were dogs. 43 (32%) animals tested positive for *Microsporia spp.* diseases. The apparent prevalence from the population is 32.58% CI 95 [24.58; 40.57]. Considering the sensitivity and specificity of the diagnostic test, it corresponds to a true prevalence of 30.64%. The prevalence from population is between 24.58% and 40.57%. Because the confidence level is 95%, the proper population size is unidentified since the number of stray animals is unfamiliar.

Of 43 positive animals, 28 (65%) were cats, and 15 (35%) were dogs. All positive animals were infected spontaneously, and all were treated and recovered. Out of 43 positive animals, 38(88%) animals were stray (homeless), and 5(11%) animals were pet animals.

3. RESULTS AND DISCUSSION

The hair loss, scaling, crusting, erythema, blemishes, hyperpigmentation, and variable pruritus were manifested in the spontaneously infected dog (Fig. 1). Nodular lesions (kerion) reactions were developed in an examined dog.

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 localised mainly on the head (Fig. 2); some cats also observed the distal parts of the legs and the tail. Display lesions were localised to the muzzle. A papulo-crustous dermatitis was observed in a newborn mother cat.

3.1 General Analyses of the Blood of Spontaneously Infected Animals

Based on the results of RBC count, HGB, HCT, MCH blood tests and MCHC, low levels of healthy red blood cells, haemoglobin, and low results in MCHC blood tests were observed in the blood of all infected dogs and cats (Table 1).

3.2 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 1. Results of general blood analysis of spontaneously infected animals

Infected animals	Parameter	Results			Unit of measurement	Reference
		D01	D02	D03		
	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
1. Dog 01	*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
2. Dog 02	*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
3. Dog 03	*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

Table 2. Blood biochemical analyzes of spontaneously infected animals

Infected animals	Parameter	Results			Unit of measurement	Reference
		D01	D02	D03		
1. Dog 01	*GOT/AST*	24	61 H	24	U/l	17-44
2. Dog 02	*GPT/ALT*	27	21	36	U/l	17-78
3. Dog 03	*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

Table 3. Blood biochemical analyzes of spontaneously infected cats

Infected animals	Parameter	Results			Unit of measurement	Reference
		C01	C02	C03		
1. Cat 01	*GOT/AST*	33	50 H	22	U/l	17-44
2. Cat 02	*GPT/ALT*	25	24	28	U/l	17-78
3. Cat 03	*CRE*	1.28	1.60 H	0.75	mg/dl	0.40-1.40
	BUN	11.3	10.5	15	mg/dl	9.2-29.2

Cat 01 – Bumi, Cat 02 - Luna and Cat 03 - Oliver are sick cats mentioned in the Table 3
The GOT/AST index in the cat named Luna was higher than expected, and the CRE index was higher in the cat named Luna. Other blood indicators were within the norm

3.3 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 generalised dermatophytosis 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).

4. 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.

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"[47].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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