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Use of Miconazole/Chlorhexidine Shampoo and Itraconazole to Treat Dogs and Cats Naturally Infected with *Microsporum Spp.* in Ganja, Shamkir and Goygol, Azerbaijan, in 2021-2023

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Abstract

This article focuses on performing clinical, laboratory examination, isolation, and identification of M. canis and the successful treatment of M. canis infection in cats and dogs in Ganja, Shamkir and Goygol, Azerbaijan, during 2021-2023. The source of infection (SOI) was demonstrated to be cats and dogs in small animal hospitals, Ganja, Azerbaijan. Samples were collected from a total of 37 animals. 15 of the sampled animals are dogs and 22 are cats. 14 of the 37 suspected animals we examined had the disease. The skin lesions observed in the M. canis infected cats and dogs were erythema, alopecia, scaly, and crusty distributed to the ear, body, neck, back and tail of cats, respectively. As a result of our study, 6 infected cats and 3 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 six weeks and the median time to mycological cure was six weeks (range 7–21 weeks).

Keywords: dermatophytes; miconazole, itraconazole; zoonosis;

Abbreviation

FLZ – fluconazole, IT- Itraconazole, GRI- griseofulvin, TER - terbinafine

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 (Chang, 2022; Moriello, 2019).

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 (Fawzi et al., 2022; Subel et al., 2012; Havlickova et al., 2008). Dermatophytosis is a disease caused by dermatophytes, a group of fungi that can cause disease both in humans and animals (Vena et al., 2012; Hsiao et al., 2018).

The clinical signs of ringworm appear 1-4 weeks after the contact with fungal spores (Fawzi et al., 2022; Mock et al., 1998). Infection with *M.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 (Chang., 2022; Hsiao et al., 2018; Hao., 2014). 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 (Skerlev & Miklić, 2010; Yamada et al., 2019).

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., 2019; Ellis, 2007). 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 (Hsiao et al., 2018; Seki et al., 2020).

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 (Hariu et al., 2017). The isolation rates of dermatophyte species from dogs and cats were 18.7% and 20.1%, respectively (Seker & Dogan, 2011). *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 (Seker & Dogan, 2011). These pathogenic fungi flourish well at an estimated temperature of 25–28 °C. Large and crowded populations cause easy exposure to the infection.

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 et al., 2012; Seki et al., 2020).

For the treatment of dermatophytosis, griseofulvin, ketoconazole, itraconazole and terbinafine are the drugs most commonly used in veterinary medicine (Boothe, 2006; Murray, 2022). 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 (Moriello, 2019).

1. 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" (https://science.gov.az/).

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) (https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.04_BIOSAFETY_BIOSECURITY.pdf)

Mendeley reference manager software was used for the bibliography, and the IEEE citation style was implemented.

Animals and clinical samples

Samples were collected from spontaneously infected stray dogs, cats, and domestic pets (dogs and cats) in small animal hospitals Ganja, Azerbaijan. Totally 15 dogs, 22 cats were caught, 37 samples were collected during our research. For each animal, a complete clinical examination was performed. The animals were sampled using the toothbrush technique (Moriello, 2001). We observed patchy hair loss and the infected areas were round, oval, or irregular and 1 to 4 cm in diameter, and multiple patches are common in sick animals. Skin scrapped from cats and dogs that clinically showed lesions of dermatitis i.e. combination of alopecia, erythema,

papules, pustules, scaly, and crusty were used in this study (Table 1). Hair samples are handled in a manner to preserve any epithelial cells adhering to the hair shaft. Samples were collected into glassine evidence bags, dry-mount on microscope slides. Hair samples lifted with a conventional fingerprint tape was placed sticky side down on paper for shipping (https://vgl.ucdavis.edu/forensics/evidence-collection-sample-type). Infected animals were selected based on the clinical signs we observed. 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 1).

Figure 1. 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, 2019; Moriello, 2007), 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. Out of 14 samples of 37 patients were with culturally proven *Microsporum canis* infections.

Later, the smears prepared from those colonies were subjected to microscopy, and the result was confirmed. The microscopic identification was done by examining *Microsporum*-infected hairs (Figure 2).

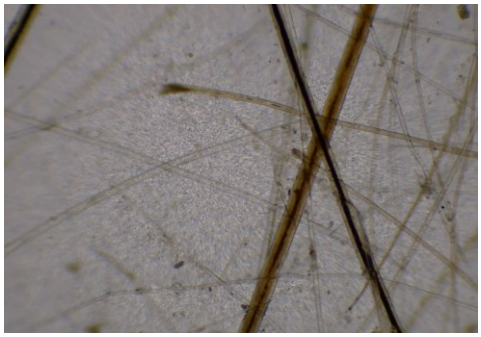

Figure 2. Direct examination of Microsporum canis-infected hairs

This is a 40X image of an infected hair (thick arrow).

2. Conventional Therapy for Animals and Humans

The choice of proper treatment is determined by the site and extent of the infection, as well as by the efficacy, safety profile, and pharmacokinetics of the available drugs. A vast range of antifungal classes, such as the first oral imidazole (e.g., ketoconazole-KTZ) and GRI have been used in human and veterinary medicine to treat dematophytoses. Later on, other azoles (i.e., FLZ, ITZ, efinaconazole, and luliconazole), allylamines (i.e., TER, butanafine, and naftifine) and amorolfine, and ciclopiroxolamine were employed. In animals, topical therapies (i.e., weekly application of lime sulphur, enilconazole, or a miconazole/chlorhexidine shampoo) are currently recommended..

3. Topical antifungal treatments

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 (Moriello 2019; Moriello, 2001).

4.1 Miconazole/chlorhexidine formulations

After a clinical report showed efficacy of a combination miconazole/ chlorhexidine shampoo in the treatment of dermatophytosis, two in vitro studies investigated the antifungal efficacy of stock solutions of miconazole, chlorhexidine, or a 1:1 combination of both against Microsporum spp. and Trichophyton spp. The

minimum inhibitory concentrations (MIC) of chlorhexidine, miconazole and chlorhexidine/miconazole ranged from 12.5 to 25 μL/mL, 0.29 to 1.17 μL/mL, and 0.14 to 0.39 μL/mL, respectively. For nine of 10 of the isolates, the miconazole/chlorhexidine combination was more effective than either agent alone; there was either a synergistic (n = 5 isolates) or additive (n = 4 isolates) effect. This study protocol was repeated but this time evaluated these agents against T. mentagrophytes (n = 9), T. erinacei (n = 9) and M. persicolor (n = 5). The MIC of chlorhexidine, miconazole and miconazole/chlorhexidine ranged from 12.5 to 50 μL/mL, 0.24 to 1.56 μL/mL, and 0.11 to 1.66 μL/mL, respectively. The mean MICs did not vary significantly between the three dermatophyte species tested, but the MICs of miconazole alone and in combination with chlorhexidine for T. erinacei were significantly greater than for T. mentagrophytes and M. persicolor. A synergistic or additive effect was seen in 15 of 23 isolates tested.

4.2 Vaccine Polivac-TM (Вакцина ПОЛИВАК-TM)

We contacted the veterinarians at the small animal clinics in Baku and Ganja and found out that the Polivac-TM vaccine is used for the treatment and prevention of infection with *M. canis* in our country. Veterinary doctors were also informed that the mentioned vaccine is used in the treatment of all dermatophytosis. Polivac-TM prevents the infection of trichophytosis and microsporia during the year, therefore, annual double revaccination of animals with an interval of 10-14 days at the indicated doses is indicated. For therapeutic purposes, cats are vaccinated two or three times: at 1-5 months at a dose of 1.5 ml from 6 months at a dose of 2 ml If the animal is in the incubation period of fungal diseases, then immunization with Polivac can provoke the appearance of single or multiple lesion of disease. In this case, 10-14 days after the main course, the vaccine at a therapeutic dose is repeated. The main drawback of this method is that same vaccine is used in the treatment of Dermatophyte infections (*Trichophyton, Microsporum, and Epidermophyton*) and no means are used to eliminate the clinical signs directly on the skin during this treatment method.

5. Results

Microsporum canis is a fungal species that causes numerous forms of disease. It is part of a group of fungi known as Dermatophytes. Though mostly well known for ringworm in pets and other animals, it is also known to infect humans. This fact makes this pathogen both anthrophilic and zoophilic in nature. *Microsporum canis* is a contagious pathogen (https://www.viroxanimalhealth.com).

Table 1. Clinical manifestation of the diseases

Animals	erythema	alopecia	scaly	crusty
Cat GA 01	-	+	+	+
Cat GA 02	-	+	+	+
Cat GA 03	-	+	+	+
Cat GO 04	-	+	-	-
Cat SH 05	+	+	-	-
Cat SH 06	+	+	-	-
Dog BA 07	-	-	+	+
Dog BA 08	-	-	+	+
Dog BA 09	+	+	-	-
Dog BA 10	+	+	-	-
Dog GA 11	-	-	+	+
Dog GA 12	-	-	-	-
Dog GO 13	+	+	-	+
Dog SH 14	-	-	+	+

The animals shown in the table are conventionally numbered. The mentioned first letters indicate the city, district and residential areas where the samples were taken (GA- Ganja, GO- Goygol, SH- Shamkir. The sign **"+"** indicates the presence of a clinical sign, and **"-"** indicates its absence. As can be seen from the table, samples were collected from a total of 14 animals. 8 of the sampled animals are dogs and 6 are cats. Erythema was observed in 5 animals, and crusty was observed in 8 animals, alopecia was observed in 9 animals, scaly was observed in 7 animals.

Table 2. Clinical dissemination of the pathogen in the body areas of the spontaneously infected animals

Host animal	head	neck	nose	patchy hair loss
Cat GA 01	+	+	-	+
Cat GA 02	+	+	-	+
Cat GA 03	+	+	-	+
Cat GO 04	+	+	-	+
Cat SH 05	+	+	-	+
Cat SH 06	+	+	-	+
Dog BA 07	-	-	-	-
Dog BA 08	-	-	-	-
Dog BA 09	+	+	+	+
Dog BA 10	+	+	+	+
Dog GA 11	-	-	-	-

Dog GA 12	-	-	-	-
Dog GO 13	+	+	-	+
Dog SH 14	-	+	-	-

The animals shown in the table are conventionally numbered. The mentioned first letters indicate the city, district and residential areas where the samples were taken (GA- Ganja, GO- Goygol, SH- Shamkir. The sign "+" indicates the presence of a clinical sign, and "-" indicates its absence in the mentioned parts of the body (head, nose). The clinical signs of the disease were observed on the head of 9 animals (6 cats and 8 dogs), on the neck of 10 animals (6 cats and 4 dogs), and on the nose of 2 animals (2 dogs) and patchy hair loss was observed in 9 animals (6 cats and 3 dogs).

The skin lesions observed in the *M. canis* infected cats and dogs were erythema, alopecia, scaly, and crusty distributed to the ear, body, neck, back and tail of cats and dogs, respectively (Table 2). 14 of the 37 samples (38%) were identified as *M. canis* clinically (Figure 3) and microscopically. Results were approved by laboratory findings.

The cats and dogs were successfully treated using itraconazole (IT) with oral therapy and miconazole with topical therapy and disinfecting of washable textiles via mechanical washing. Contamination was monitored over time, and cleaning procedures were stopped when fungal culture results were negative. As a result of our study, twice weekly application miconazole/ chlorhexidine shampoo are currently recommended effective topical therapies in the treatment of generalized dermatophytosis in cats and dogs.

Miconazole shampoos are effective *in vitro* but *in vivo* are most effective when combined with chlorexidine.

In our study, 9 infected animals were treated with itraconazole and one of two topical therapies including 2% chlorhexidine and 2% miconazole shampoo (Figure 4). The median time to clinical cure was six weeks and the median time to mycological cure was six weeks (range 7–21 weeks).

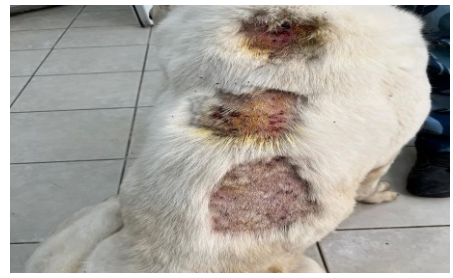

Figure 3. A dog infected by M.canis (before the treatment)

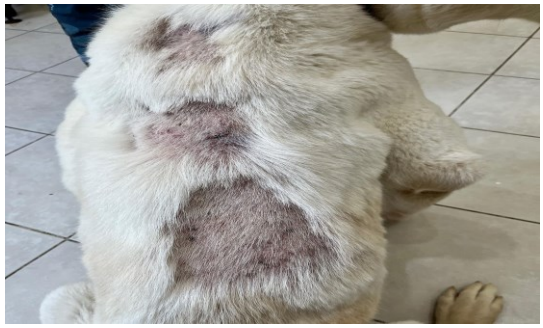

Figure 4. A dog after the treatment

6. Conclusion

Our research determined that sick pets, stray animals have an exceptional role in the spread of *Microsporum spp.* 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.

It was found that the disease is spread in Ganja, Shamkir and Goygol 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 Ganja, one of our 3 largest industrial cities, Shamkir and Goygol regions. 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.

7. Discussion

The natural reservoir of *M. canis* is dogs and cats, and secondary infection in humans causes tinea capitis and tinea corporis. These hair and skin lesions have been observed in severe cases when *M. canis* infects immunocompromised hosts

(Mock et al, 1998). In this present case, the origin was directly identified, so the source of infection (SOI) was demonstrated to be cats and dogs in Ganja, Shamkir and Goygol, Azerbaijan. These cats were very likely the reservoir of the *M. canis* and the origin of transmission to the family. *M. canis* has a characteristic morphology and produces septate hyphae and macro-conidia that are spindle-shaped and have asymmetrical, apical knobs. According to our research, this disease is also observed in neighboring countries in certain seasons of the year and infected cats play an important role in the spread of the disease in other countries, as well as in Azerbaijan. The disease is especially common in autumn, winter and spring for dogs and cats in Azerbaijan but 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 (Seker & Dogan, 2011).

The main cons of this method are that, Vaccine Polivac-TM is used in the treatment of Dermatophytes (Trichophyton, Microsporum, and Epidermophyton) and no means are used to eliminate the clinical signs directly on the skin during this treatment method. In contrast, the method of treatment (using itraconazole (IT) with oral therapy and miconazole with topical therapy) that we apply is designed only for the treatment of infection with *Microsporum spp.* In addition, the application of a miconazole/chlorhexidine shampoo has an important role in faster elimination of problems caused by *Microsporum spp.* on the skin.

Outcomes

1. Fungal infections were spread among stray cats and dogs and stray animals are main reservoirs of infection in Ganja, Shamkir and Goygol.
2. 2. M.canis was one of the main infections among dermatophytes in Baku, Ganja, Shamkir and Goygol.
3. 3. Antifungal drugs were demonstrated differently in “in vitro” performance.
4. 4. “in vitro” concerts were excused on the spontaneously infected animals.
5. 5. Combination of antifungal prescriptions demonstrated more effective treatment of mycosis.

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