

## Research Article

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# Prevalence of dermatophytosis and efficacy of antifungal agents against *Microsporum canis* in cats

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### Abstract

Dermatophytosis or ringworm is an infectious fungal disease that can be caused by three species of dermatophytes. *Microsporum Canis* (*M. canis*) appears to be the most common cause of ringworm. Dermatophytes invade and live in the keratinized layer of the dermis and can be transmitted to humans and animals through direct contact with infected animals or through the ground. The dermatophyte *M. canis* is the most common fungal agent of dogs and cats. The cat is commonly kept as pet all over the world and in Pakistan. The present study noted the prevalence of *M. canis* in pet cats in the area of district Lahore and the chemotherapeutic effect of antifungal agents against the *M. canis*. The animals found positive with *M. canis* were divided into three groups named, group A (griseofulvin), group B (ketoconazole) and group C (terbinafine). All the drugs were given orally, and the efficacy of each drug was observed by collecting the samples on 15 days interval after the commencement of treatment until the mycological cure was achieved up till day 90<sup>th</sup>. The prevalence of *M. canis* infestation was 31%. The breed wise prevalence was highest in Persian cats as compared to Siamese, and local breed and the prevalence were also higher in young ones as compared to old age cats. Moreover, the calculation of efficacy revealed that griseofulvin was the best drug among the three drugs used.

**Keywords:** Dermatophytosis; Griseofulvin; Ketoconazole; Ringworm; Terbinafine

### Introduction

Dermatophytosis is the most common contagious and fungal infection in cats, and

there are almost 50 species of fungi which are pathogenic for animals throughout the world. Dermatophytosis is a superficial skin

infection of the keratinized tissues including nails, claws, hairs and stratum corneum of skin. The fungi that cause these infections are known as dermatophytes. Three genera of fungi cause dermatophytosis which are *Epidermophyton*, *Microsporum* and *Tricophyton* [1]. They can be divided into three groups on the basis of natural habitat which are Geophilic dermatophytes which inhabit the soil and serve as a source of infection for both animals and humans [2], Anthropophilic dermatophytes found mainly in humans and usually infect the humans but less commonly animals [3, 4], and Zoophilic dermatophytes found mainly in animals but are capable of infecting other animals and human beings [5, 6]. These pathogenic fungi are found worldwide, and all domestic animals are susceptible to this fungus. But the most important is *Microsporum* with species like *M. canis*, *Microsporum gypseum*, *Microsporum nanum* and in cats 98% infection is caused by *M. canis* [7, 8]. Dermatophytosis (ringworm) also carries zoonotic importance that infects the humans and infected 9% of USA's human population at the end of the 1980s [9]. Cats represent the primary source of infection to people, and 20% of human diseases can be result of close contact with cats and dogs [10, 11]. Dermatophytosis has a broad spectrum of hosts, but the cat represents the most effective carriers due to high frequency of asymptomatic infections [12]. As the cat is an asymptomatic carrier of dermatophytes and its habit to sleep on beds and divans that prone the human to get this infection [13]. In these places' spores shed by infected animals are difficult to be removed and can remain viable in the environment for up to 18 months. The cats affected by *M. canis* may undergo spontaneous remission, but the infection can be persistent for 18 months to 4 years [13]. Lesions are most commonly on the head, ears, nails, and tail. The classic symptom is small, round lesion

that is devoid of hair and will often have scaly skin in the center. Cats of any age can suffer from dermatophyte infection, but ringworm infection is more frequent in young because older animals are less susceptible and if previously exposed to dermatophytosis, have developed immunity [7].

Many antifungal drugs like griseofulvin, ketoconazole, enilconazole, itraconazole, terbinafine, lufenuron, etc. are commonly used worldwide [14, 15]. Griseofulvin is a fungistatic antifungal agent that inhibits the nucleic acid synthesis and cell mitosis metaphases by interfering with the functions of spindle microtubules [16]. It is used as oral administration in tablet formulation which is reportedly safe for use in puppies and kittens [17].

Oral treatment with Terbinafine is reported to be effective. It is fungicidal that suppresses the biosynthesis of ergosterol through inhibition of the fungal enzyme squaline epoxidase [18]. Ketoconazole is also fungicidal and also effective for the treatment of dermatophytosis in cats [18]. It is available in market in tablets, ointments, creams and shampoos formulations [19]. Itraconazole is triazole derivative that works by altering fungal cell membrane permeability through inhibition of ergosterol synthesis. At low doses, itraconazole is fungistatic and at higher dose it is fungicidal [20].

Keeping in view the importance of dermatophytosis in cats and degree of damage they cause, the present study was designed to evaluate the comparative efficacy of three most common medicines like griseofulvin, terbinafine, and ketoconazole to determine their effectiveness and to check the prevalence of dermatophytosis in cats.

## Materials and methods

### Study area

In the present study, the presence of *M. canis* was studied in pet cats in the area of district Lahore. For the collection of samples three most busy clinics of Lahore district were

selected randomly (Pets care and cure clinic Model town, Pet center University of Veterinary and Animal Sciences Lahore, and Pet care clinic Akbar chowk). A trial of three most commonly available medicines, i.e. griseofulvin, ketoconazole and terbinafine in Pakistan was conducted.

### **Experimental design**

A total number of 200 animals were examined during a period of 3 months from the clinics in district Lahore. The samples were collected from all the cats brought to the clinics. The animals positive for the dermatophytosis were categorized into three groups named, group A, B and C which were comprised of five animals each. Group A animals were treated with Griseofulvin, group B with Ketoconazole, group C with Terbinafine.

### **Clinical signs**

The cats were examined for clinical signs and symptoms depicted with an infestation. The signs included, round lesions devoid of hairs small pustules, erythema, crusts, scales, itching and patches of alopecia on head, ears, tail, and feet and sometimes the whole body involved. All the positive cats showed above mentioned signs. The most prominent signs recorded were, round lesions devoid of hairs and alopecia on head, ears, tail, and feet (Fig. 1).

### **Sample collection and preparation**

The animal and equipment were prepared before sample collection. Area of the head, ears, and tail which are the most common sites of infection in dermatophytosis was disinfected with 70% ethyl alcohol. Sample collection was performed by using sterilized tweezers and scalpel. Hairs (broken, frayed, twisted or hair with healthy appearance) were wrested with tweezers from the edges and center of the lesions. The scales were collected with a scalpel after the lesion scratching [21, 22]. The samples were labeled for their identification. Then hair and scale samples were cleared by clearing agent,

i.e., chloral lactophenol which can break the tissue and released the spores from the hairs and scale sample for identification and a drop of methylene blue was added on slide to get a clear view. A coverslip was placed on the hair and scale sample and the slide was allowed to stand for 24 hours at room temperature which was stabilized the spores on the slide. Preparation was observed under microscope for identification of spores.

### **Fungal culture**

For fungal culture, standardized [23] was utilized. The area of head, ears and tail was disinfected with 70% ethyl alcohol as in microscopic technique, to remove surface adhering organisms. Samples were collected with the help of sterilized tooth brushing technique [23, 24]. With this technique, each cat was brushed vigorously to ensure hairs trapped in the bristles of the toothbrush and the areas mentioned above were brushed twice. Fungal culture was performed by pressing the bristles of the brush on to the plates of Mycobiotic agar containing Sabouraud's dextrose agar, chloramphenicol (0.05 g) and cycloheximide (0.5 g) and incubated at 28 °C for 2-5 weeks [25]. *M. canis* was identified by its macro and microscopic features (Fig. 2), following the criteria published by [26].

### **Identification and characterization of *Microsporum canis***

For identification of *M. canis*, the slide culture method described by Harris [27] was followed. Petri dishes, microscopic slides, and coverslips were sterilized before preparation of slide culture. A small square block 1 cm square from a plate of mycobiotic agar was taken with a sterile spatula and then placed it in the center of slide. Then a small amount of suspected dermatophyte was taken and inoculated on the solid medium. After that put a coverslip on it and left a piece of cotton soaked with water in the bottom of petri dish to provide the humidity which prevented the media from dehydration. Then

incubated the petri dish at 25-28 °C. When growth took place, another sterilized microscopic slide was taken and put a drop of lactophenol blue dye in the center of the slide and took the coverslip from the grown slide culture and mounted it on the drop of dye. After fixation of the coverslip, the slide culture was seen under low and medium magnification for identification of the reproductive structures, i.e., microconidia, macroconidia, and spiral hyphae of the required dermatophyte (Fig. 3). *M. canis* was identified by its macro and microscopic features, following the criteria published by [26].

#### Medication protocol

The animals positive for the dermatophytosis were treated according to their groups as already mentioned. In group A, griseofulvin (Fungivin, Ethical Laboratories Pvt. Ltd) was given orally at dose rate of 50 mg/kg body weight (250 mg) for 4-6 weeks in the form of tablet [28, 29]. Group B animals were treated with ketoconazole (Conaz, Atco Laboratories Ltd) at a dose rate of 10 mg/kg body weight (50 mg) orally for 4-6 weeks in the form of tablet [18, 30]. Group C animals were treated with terbinafine (Terbin, Martin Dow Pharmaceuticals, Pakistan, Ltd) at dose rate of 30 mg/kg body weight (125 mg) orally for 4-6 weeks [31–33]. Samples of all above groups were collected at 0, 15, 30, 45, 60, 75 and 90 days. Mycological cure was obtained on the basis of scoring system for assessment of lesion severity and with two consecutive negative cultures [20, 28, 34].

#### Statistical analysis

The prevalence of *M. canis* infestation was calculated by using the formula as described by [35]. The data obtained as a result of different medications of various groups were analyzed according to the completely randomized design through analysis of variance technique and means were compared for significance according to the least significant difference [36].

## Results

### Prevalence *Microsporum canis* in different sex, breed, and age

Prevalence of *M. canis* infestation was recorded in pet cats of different sex, breed and age. It was noted that out of the total 200 animals, 62 were found positive whereas, 138 were negative for *Microsporum canis* infestation the prevalence was 31% (Table 1). Moreover, 62 positive cases 40.32% (n=25) were recorded in male cats and 59.67 % (n=37) in female cats.

The prevalence of dermatophytosis was also calculated in individual breeds and gender-wise and it was found that 38 were positive with *M. canis* out of 96 Persian cats. However, in Siamese 18 out of total 66 cats tested were recorded positive, whereas, in the local breeds 6 were positive out of 38 (Table 2). Therefore, prevalence was 39.58% in Persian, 27.27% in Siamese and 15.78% in local breeds. Moreover, the data of gender revealed 25 positive cases from 80 male cats and 37 from 120 female cats presented in the clinics irrespective of their breeds. The prevalence in male and female cats was found to be almost equal that was 31.25 % and 30.83% respectively (Table 2).

The prevalence of *M. canis* calculated in cats in relation to their age revealed that 90 cats were tested at 12 months of age, it was noted 38 cats positive with *M. canis*. Whereas 1-5 years, 50 cats were tested and 14 found positive (Table 3). Moreover, in 5-8 years 10 were positive out of 60 presented in the clinics (Table 3). Therefore, the prevalence was recorded 42.22% in 12 months, 28% in 1-5-year, and 16.66% in 5-8 years of age in cats (Table 3).

### Therapy and antifungal susceptibility profile of *Microsporum canis*

The cats found positive for the *M. canis* infestation were categorized into three groups named, treatments A, B, and C. In treatment A the animals were treated with griseofulvin, B with ketoconazole and C with terbinafine.

The efficacy of the above-mentioned drugs was determined on day 15, 30, 45, 60, 75 and 90 during the study trial. In all the three treatments the medicines were administered orally to the cats. For this all the drugs were given to the animals of three mentioned groups until achieved the mycological cure. The efficacy of the three-drug trials was calculated numerically. In treatment A, the efficacy of griseofulvin was found to be 25% at day 15, 80% at day 30 and 100% at day 45 and medication were stopped because mycological cure was achieved at day 45. In treatment B ketoconazole was used, the recorded efficacy was 10% on day 15, 20% on day 30, 40% on day 45, 60% on day 60, 70% on day 75 whereas it was 85% on day 90 when the drug trials were completed. However, in treatment C, the efficacy of terbinafine was recorded 20%, 45%, 75% and 100% on day 15, 30, 45 and 60 respectively. Statistically griseofulvin has no significant difference with terbinafine while significant difference ( $P < 0.05$ ) was observed among griseofulvin, ketoconazole, and terbinafine.

### Discussion

The cat is commonly kept as a pet for its fine nature, nice learning and a good sense of essence and worldwide population of cats exceeds 500 million [37]. Cats serve as a reservoir of *M. canis* and since no work has been done to determine the prevalence of dermatophytosis and chemotherapy against *M. canis* infestation in pet cats in Pakistan [22, 38]. Keeping in view the importance of *M. canis* infestation in cats and degree of damage they cause, the present study was designed to determine the prevalence of *M. canis* infestation in pet cats (*Felis catus*) in the area of district Lahore to establish their efficacy [2, 11].

During the present study a total of 200 cases were recorded, among them, 62 (31%) were found positive whereas, 138 were negative for *Microsporium canis* infestation (Table 1). The finding of present investigation was

lower as compared to the former reports that the prevalence of *Microsporium canis* infestation in cats was 53.5% and 55.9% respectively because of environmental and managerial conditions [25, 39]. The higher percentage reported by these workers may be due fact that they worked on both pet and stray cats and our study was conducted only on pet cats because pet animal is in good condition as compared to stray one. Out of the total 62 positive cases in cats, 31.25% and 30.83% prevalence was recorded in male and female cats, and the similar findings were recorded by [25, 40].

In the present study, the prevalence of *M. canis* infestation was recorded in three different breeds of cats which were kept as pets and brought to the clinics. Breed wise the highest prevalence was recorded in Persian cats (39.58%), and the second-highest was recorded in Siamese cats (27.27%), whereas the lowest prevalence of *M. canis* was recorded in local breed (21.05%) (Table 2). These findings are different as compared with the results reported by [41], according to report the prevalence of *M. canis* infestation in Siamese cat was bit higher than the Persian cats (14.3%, and 12.5% respectively), because Persian has long hair coat and long hairs trap more spores and has fungal friendly environment as compared to short hair cat and local breed showed lowest result of prevalence percentage because this breed is immunologically more competent as compared to exotic breeds. While [34, 42] had similar findings to our study that the prevalence of *M. canis* infestation in long hair cats was more as compared to short hair cats.

In this study cats received in the clinics were divided into three age groups (1 month to 12 months, 1-5 years, and above 5 years), and the total number of cats which were studied ranged from 1 month to 8 years of age. It was observed that a maximum prevalence of *Microsporium canis* infestation

was recorded in young cats ranging from 1 to 12 months of age (42.22%) and the second-highest prevalence was in age group from 1-5 year (28%), whereas the lowest prevalence was recorded in 5-8 years of cats (Table 3). The same findings were recorded by [25, 41] that prevalence of *M. canis* infestation mostly occurs in cats less than 1 year. The highest prevalence of *M. canis* in the age group up to 12 months of age indicated that such cats were not immunologically competent to protect themselves against the disease due to their young age and it also depended upon the degree of interaction in the fellow cats present in the same home. The second highest prevalence in 1-5 years of age could be due to the fact that such cats had contact with stray cats as they were let outdoors by the clients. The lowest prevalence was recorded in maximum age group of cats above 5 years indicating development of resistance and competent immunological response against the disease and this is similar to the findings of [41] that the prevalence of *M. canis* in cats with age range from 1 month to more than 5 years.

Cats positive to *M. canis* were also observed for a depiction of clinical signs. The signs recorded were round lesions devoid of hair, small pustules were often found in lesion and patches of alopecia with erythema and greyscales (Fig. 1). Lesions were most common on the head, ears, and tail, it often spread across the face, lips, chin or nose and the same findings were observed by [43]

In treatment A, griseofulvin was given orally at the dose rate of 50 mg/kg body weight once in a day up to a 4-6 week in the form of tablets. The efficacy of griseofulvin was observed by collecting the samples on day 15, 30, 45, 60, 75 and 90 after the commencement of treatment, which was 25%, 80%, and 100% respectively on day 15, 30 and 45 and the further treatment was stopped when 100% results and mycological cure were obtained (Table 4), which is in

agreement with the findings of [44], it showed that efficacy of griseofulvin was 100% on day 41 and obtained mycological cure. Similarly, [28] used griseofulvin along with 2% miconazole shampoo twice weekly and obtained mycological cure 100% at day 42.

Whereas treatment B, ketoconazole was given orally at a dose rate of 10 mg/kg bodyweight for 4-6 weeks in the form of tablet. The efficacy of ketoconazole was observed by collecting the samples on day 15, 30, 45, 60, 75 and 90 after the commencement of treatment, which was 10%, 20%, 40%, 60%, 70% and 85% respectively (Table 4), which is in agreement with the findings of [45], he obtained successful result only in 8 cats out of 12 which was successfully treated with resolution of lesions and mycological cure after 2 to 10 weeks of drug trial. According to [18] also found the same result in his study but he used ketoconazole along with food to obtain an optimal absorption.

However, treatment C, terbinafine was given orally at the dose rate of 30 mg/kg body weight once in a day up to a 4-6 week in the form of tablets. The efficacy of terbinafine was observed by collecting the samples on day 15, 30, 45, 60, 75 and 90 after the commencement of treatment, which was 20%, 45%, 75% and 100% respectively on day 15, 30, 45 and 60 because we obtained mycological cure at day 60 (Table 4) which is in agreement with the findings of [32] the investigation finds that terbinafine in cats and showed that efficacy of terbinafine was 100% on day 60. Similarly, [46] studied that terbinafine showed mycological cure on day 63. Moreover, the present study indicated that the efficacy of ketoconazole was not more than 85% while 100% was recorded in griseofulvin and terbinafine.

Griseofulvin was found to be a most effective medicine to treat the *M. canis* infestation as maximum efficacy of 100% was recorded on

day 45, which was significantly higher than terbinafine which showed 100% efficacy on day 60 and much higher than ketoconazole which showed 85% efficacy after the completion of whole drug trial. In this study, we found that there was no significant difference in the efficacy between the groups treated with griseofulvin and terbinafine but

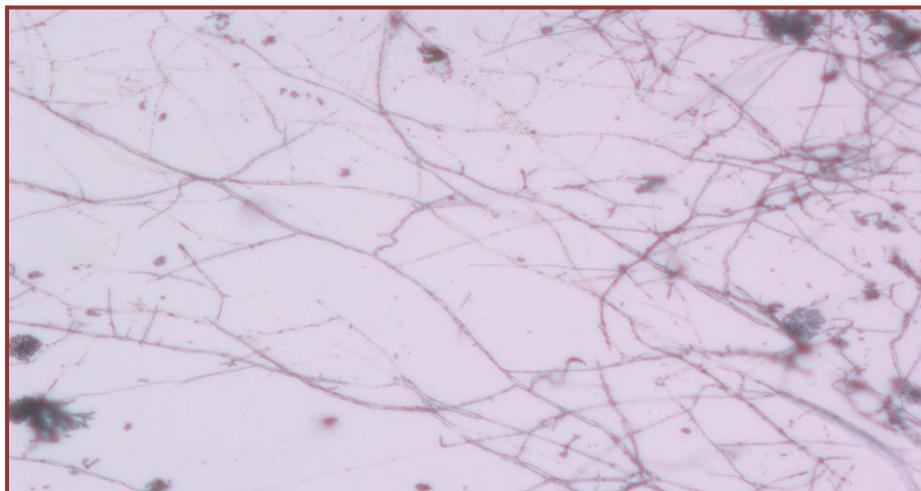
found significant difference ( $P < 0.05$ ) between griseofulvin and ketoconazole and was a significant difference ( $P < 0.05$ ) between terbinafine and ketoconazole at the end of trial statistically but overall all the drugs has a significant effect against *M. canis* infestation in cats.



**Figure 1.** The cat's tail having complete alopecia



**Figure 2.** Fungal colony of *Microsporium canis*



**Figure 3.** Microscopic structure of *Microsporium canis*

**Table 1.** Prevalence of *Microsporium canis* infestation in Pet Cats

No. of cats (n)	Positive with <i>Microsporium canis</i> (n)	Negative with <i>Microsporium canis</i> (n)	Prevalence of <i>Microsporium canis</i> (%)
200	62	138	31

**Table 2.** Prevalence of *Microsporium canis* infestation in different breeds and sex

Particulars	Breeds			Sex	
	Persian	Siamese	Local	Male	Female
Dermatophytosis positive (n)	38	18	6	25	37
Dermatophytosis negative (n)	58	48	32	55	83
Total (n)	96	66	38	80	120
Prevalence (%)	39.58	27.27	15.78	31.25	30.83

**Table 3.** Prevalence of *Microsporium canis* infestation in different age groups

Particulars	Upto12 months	1 year - 5 year	5 year – 8 year
<i>Microsporium canis</i> Positive (n)	38	14	10
<i>Microsporium canis</i> negative (n)	52	36	50
Total (n)	90	50	60
Prevalence (%)	42.22	28	16.66

**Table 4. Post oral treatment efficacy of Griseofulvin, Ketoconazole and Terbinafine against *Microsporum canis* infection in cats. Mean values followed by the same letter in the same column do not vary significantly (P < 0.05)**

Treat ments	Drugs	Efficacy at day 15 (%)	Efficacy at day 30 (%)	Efficacy at day 45 (%)	Efficacy at day 60 (%)	Efficacy at day 75 (%)	Efficacy at day 90 (%)
A	Griseofulvin	25 <sup>a</sup>	80 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup> *	100 <sup>a</sup> *	100 <sup>a</sup> *
B	Ketoconazole	10 <sup>c</sup>	20 <sup>c</sup>	40 <sup>c</sup>	60 <sup>c</sup>	70 <sup>c</sup>	85 <sup>c</sup>
C	Terbinafine	20 <sup>ab</sup>	45 <sup>b</sup>	75 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup> *	100 <sup>a</sup> *

\* = NO further treatment when achieved 100% efficacy

### Conclusion

The cat is commonly kept as a pet all over the world. The dermatophyte *M. canis* is the most common fungal agent of dogs and cats. In the present study, the prevalence of *M. canis* was recorded in pet cats in the area of district Lahore. It was found that *M. canis* infestation having moderate prevalence in domestic cats and Griseofulvin is the most effective and readily available product in Pakistan. Moreover, there is no predilection of sex, whereas, the high prevalence was found in young and long coat cats.

### Authors' contributions

Conceived and designed the experiments: KU Rehman & AK Mahmood, Performed the experiments: MI Saleem & A Qudus, Analyzed the data: M Akbar & MT Naveed, Contributed materials/ analysis/ tools: A Nadeem. M Tanveer, A Noor & M Attique, Wrote the paper: KU Rehman.

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