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Abstract. Dermatophytosis (ringworm) is the most important infectious and contagious skin disease of cats in shelters. Wherein, the diagnosis, identification and treatment of dermatophytosis is not always effective. Here we report an epidemiological study of the outbreak of feline dermatophytosis in animal shelter in Moscow region. At least 11% of cats kept in the shelter were affected. Seventeen cats suspected dermatophytosis were isolated for mycological examination and treatment, 82% of them were culture-positive. These cats received a course of oral terbinafine, with no topical treatment. Of the dermatophyte-positive cats, 36% demonstrated skin lesions, while 64% were asymptomatic. No mycological control of recovery was carried out after treatment. It was found that 44% of clinically recovered cats turned to be asymptomatic carriers. When examining environmental objects (bedding, cat houses, floors, etc.), dermatophytes were isolated from 80% of the samples. The only causative agent of dermatophytosis was Microsporum canis. The MIC (minimal inhibitory concentration) of terbinafine for clinical strains of M. canis was determined to be 0.001-0.002 μg/ml. The study confirms the need for large-scale mycological screening of cats in shelters using DTM-type diagnostic media. Oral therapy alone is not sufficient for effective treatment; it should include the whole-body antifungal treatment. Mycological control of cure is also mandatory.

1. Introduction

Dermatophytosis (ringworm) is the most important infectious and contagious skin disease of cats in shelters. Furthermore, feline dermatophytosis is of particular significance because of zoonotic risk factors associated public health concerns [1].

The main causative agent of feline dermatophytosis is a zoophilic dermatophyte Microsporum canis, which can also affect a variety of mammalians including humans. M. canis transmission occurs through direct contact with sick or subclinically infected asymptomatic animals, or with arthrospores, that remain viable in the environment for up to 18 months. Asymptomatic animals are considered to be spreaders of the disease in about 50% of the infected humans [2].

Due to the highly contagious nature of M. canis infections, treatment protocols are mandatory to prevent potential transmission to other receptive hosts. A variety of oral and topical antifungal agents is available and drugs such as griseofulvin, terbinafine, itraconazole, and fluconazole are used to cure severe infections in humans and animals. However, M. canis infections characterized by recurrence and treatment failure has been recorded in 25–40% of treated patients, potentially due to lack of patient compliance, lack of drug penetration into tissue, variable medication bioavailability, and resistance phenomena [3]. In vitro analysis of the antifungal agents allows comparing different antimycotics, which in turn may assist clinicians in exploring resistance phenomena and choosing an effective therapy for their patients [4].

Here we present the results of epidemiological study of the outbreak of feline dermatophytosis among cats in an animal shelter. The study included mycological examination of affected animals, identification of the pathogen, environmental detection of fungi, determination of terbinafine MIC for clinical strains of dermatophytes.

2. Materials and methods

Animals: the study was conducted in winter 2019 in big animal shelter in Moscow region. The clinical signs of ringworm were detected in cats by shelter personnel, and suspected animals were isolated in separated ward (isolator) for antifungal treatment. Cats were kept in separate cages. This group of cats was included in the study. No specific diagnostic tests were performed in shelter before the study.

Sample collection: all cats (affected and asymptomatic) were brushed for 60 seconds with a plastic toothbrush (Makenzie method) over the whole body, with the main focus on the head and fore limbs. Brushing was alternately performed by two researchers, which were wearing disposable overalls, overshoes and hairnets. After brushing the animal, each brush was kept in a separate envelope and transported to the laboratory at the same day. Environmental samples were taken by the same method.

Data collection: a general examination of the cats was performed after the brush sample was taken. The following data was recorded: estimated age, estimated breed, hair length, skin lesions and other abnormalities in the health check. Furthermore, their story of antifungal treatment was recorded.

Laboratory methods: the samples were taken for mycological culture to FSC VIEV (Moscow), Laboratory of mycology and mycotoxicology. All toothbrushes were inoculated on diagnostic media DTM-Expert (VIEV, Russia) and Dermatophyte test agar (Himedia, India) by pressing the bristles into the medium. Hairs were picked off the brush with a sterile forceps and gently pressed into the medium. The plates were incubated at 28^0C in the dark up to 21 days and examined every day. When suspect colonies were found, microscopic examination was performed to identify the colonies basing on mycological atlas of clinical fungi [5].

Antifungal susceptibility testing: MIC (minimum inhibitory concentration) of terbinafine for clinical strains of dermatophytes was determined by broth dilution method on the basis of NCCLS document M38-A [6].

3. Results and discussion

At the period of the study, a total of about 150 cats were housed in the shelter. Seventeen of them (11%) were placed in the isolation ward as suspected for dermatophytosis and was included in the study. The status and conditions of admitted cats are presented in table 1.

Table 1. Clinical status and conditions of cats included in the study.

No	Clinically affected / asymptomatic	State of therapy	Shorthair / longhair	Age
1	Clinically affected	Terbinafine therapy in progress	Shorthair	11 mo
2	Clinically affected	Terbinafine therapy in progress	Shorthair	6 mo
3	Asympthomatic	Terbinafine therapy in progress	Shorthair	7 mo
4	Asymptomatic	Terbinafine therapy in progress	Shorthair	1,3 y
5	Asymptomatic	Terbinafine therapy complete	Shorthair	2 y
6	Asymptomatic	Fluconazole therapy in progress	Shorthair	3 y
7	Asymptomatic	Terbinafine therapy in progress	Shorthair	5 mo
8	Asymptomatic	Terbinafine therapy complete	Shorthair	9 mo
9	Asymptomatic	Terbinafine therapy in progress	Shorthair	3 mo
10	Asymptomatic	Terbinafine therapy in progress	Shorthair	4 mo
11	Clinically affected	Terbinafine therapy in progress	Shorthair	2 mo
12	Clinically affected	Terbinafine therapy in progress	Shorthair	2 mo
13	Asymptomatic	Terbinafine therapy complete	Longhair	1,2 y
14	Asymptomatic	Terbinafine therapy complete	Shorthair	3 y
15	Clinically affected	Terbinafine therapy in progress	Shorthair	2,7 y
16	Asymptomatic	Terbinafine therapy in progress	Shorthair	6 mo

17	Clinically affected	Terbinafine therapy in progress	Shorthair	6 mo

Out of 17 cats admitted to the study, 13 received antifungal therapy. Animals received terbinafine orally by pulses of 7 days (with the exception of cat No. 6, which received fluconazole). In 4 remaining cats (No 5, 8, 13, 14) the course was completed, they were kept in the isolation ward under clinical observation. Topical treatment of the coat and culture control of recovery was not carried out.

At the mycological examination, dermatophytes were isolated in 14 of 17 cats (82%) (see figure 1). Of these, 5 (36%) manifested clinical signs of dermatophytosis, and 9 (64%) had no skin lesions. At the same time, 4 out of 9 dermatophyte-positive asymptomatic cats (44%) had already completed the course of therapy (figure 2). Thereby, these cats can be judged as asymptomatic carriers.

All but one surveyed cats were shorthair. Eighty-five percent of dermatophyte-positive cats were younger than 1 year. Negative results of mycological assay were obtained in 2 cats with no clinical signs (No 6, 16) and one cat with mild skin lesions (No 2).

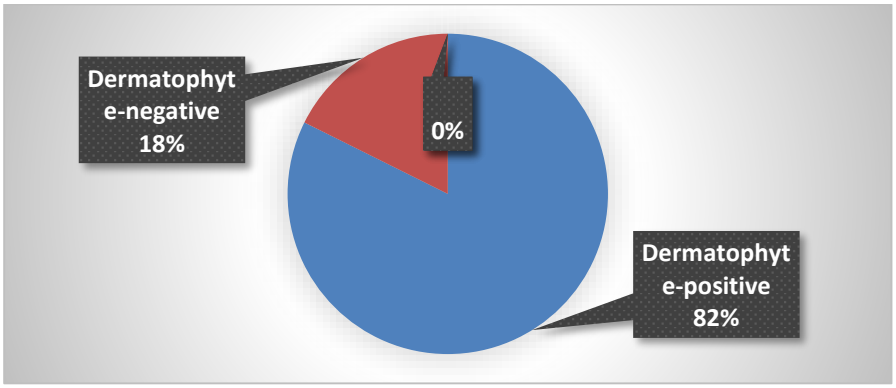

Figure 1. Prevalence of dermatophytosis in clinically suspected cats.

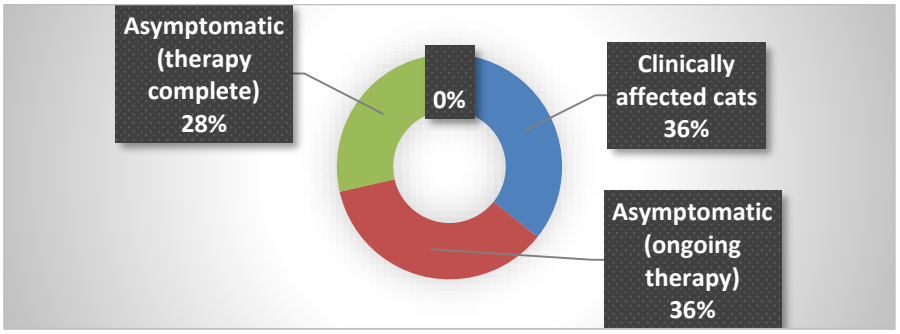

Figure 2. Clinical status of dermatophyte-positive cats.

Environmental samples taken from cat houses, pads, hammocks, mats etc picked at the shelter facilities were subjected for mycological examination. Results are presented in table 2.

Table 2. Detection of dermatophytes in environmental samples.

No	Sample	Detected dermatophytes
1	Hammock in a cage	M. canis
2	Cat house	M. canis
3	Cage pad	M. canis
4	Claw-point	Not detected
5	Mat	M. canis

Dermatophyte M. canis was detected in 4 of 5 examined environmental samples (80%). Moreover, during the course of this study, skin lesions resembling dermatophytosis were noticed in one of the shelter employees. With the consent of the employee, sample was taken from the affected areas for mycological analysis. Isolated fungal culture was also identified as Microsporum canis.

Thus, the only dermatophyte species found in the study was M. canis. During cultivation on diagnostic media visible growth of dermatophyte colonies was usually observed on days 3–5. Reddening of the medium was observed simultaneously with the colony growth or 1-2 days later. The fungus showed characteristic light colonies (white, beige, yellowish, woolly or velvety) (figure 3). These characteristic features allowed to diagnose dermatophytosis easily in short terms. Mold non-dermatophytic colonies appeared sporadically and did not interfere with the growth of dermatophytes.

When subculturing on Saubouraud media, M. canis produced a number of multicelled spindle-like spores (macroconidia), which are the key features of species affiliation (figure 4).

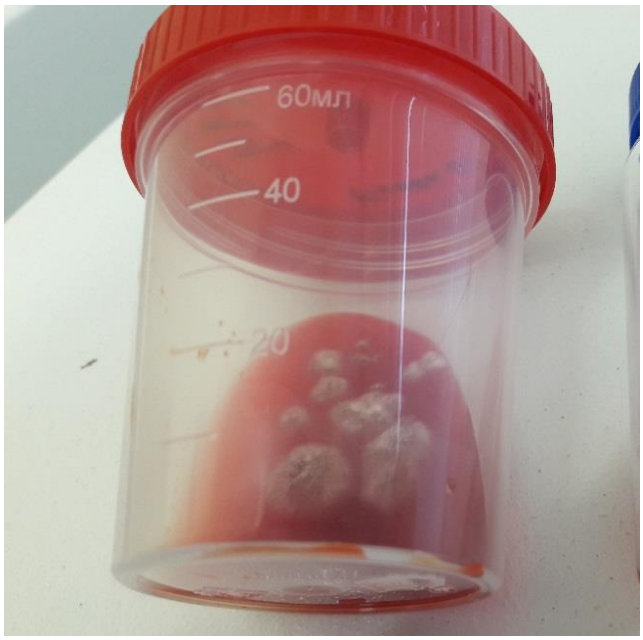

Figure 3. Characteristic colonies of Microsporum canis causing redness of diagnostic media “DTM-Expert”.

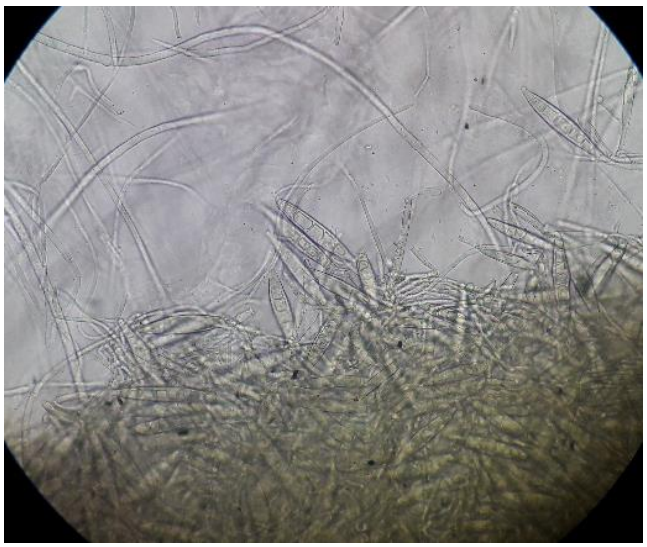

Figure 4. Numerous multicelled spindle-like spores (macroconidia) of M. canis.

Antifungal susceptibility testing of fungal pathogens is of great practical importance. In this study, 3 strains of M. canis isolated from cats were randomly selected, in which MICs were determined for terbinafine, the most commonly used antimycotic in veterinary practice. The results are presented in table 3.

Table 3. MIC of terbinafine for clinical strains of M. canis isolated from cats.

No	Strain	MIC, µg/ml
1	M. canis VC02-19	0,002
2	M. canis VC05-19	0,001
3	M. canis VC11-19	0,002

MIC of terbinafine was found to be 0,002 µg/ml in 2 strains and 0,002 µg/ml in one clinical strain of M. canis.

4. Discussion

In this study, dermatophytosis was mycologically confirmed in 11% of cats kept at the shelter at the time of the survey. It can be assumed that the true number of affected and asymptomatic animals is much higher. However, to identify them, a wide screening examination of the whole shelter is needed. For comparison, in one USA shelter the overall occurrence of dermatophytosis in cats was 1,8%. In affected cats demonstrating skin lesions dermatophytes were diagnosed in 69% [7], what is comparable with current results (82%).

Diagnostic media used in this study (DTM-Expert and Dermatophyte test agar) showed themselves as practical and effective tool for point-of-care diagnosis of dermatophytosis. Visible growth on DTM-Expert was observed at 3-5 days of the incubation. In similar study, infected cat cultures showed colony growth consistent with M. canis by day 7 of inoculation, with a median of 4 days [7]. Kaufmann et al. compared the results of point-ofcare dermatophyte cultures with those from a diagnostic laboratory. When fungal culture storage and incubation instructions were followed along with use of macro- and microscopic identification characteristics, there was 97% agreement between the two [8]. Fungal culture on diagnostic dermatophyte media has been considered the gold standard for dermatophytosis detection, has been used by shelter clinicians to help distinguish infected from fomite carrier cats and remains the best method for determining mycological cure [7].

Our study confirms that Microsporum canis is the most commonly isolated fungal pathogen from cats in shelters. It is likely that many cats present to shelters are already infected with dermatophytosis. Effective screening is a strong preventive measure since cases identified at intake or before other critical movement. Ideally, fungal cultures should be processed in-house [1]. To prevent and contain spread, shelters strive to recognize infected cats at intake, by using inexpensive point-of-care screening tools [7]. We believe that efficient and cost-effective diagnostic media (such as DTM-Expert) is highly appropriate for shelters for purposes of incoming screening, confirming diagnostics, and post-therapeutic mycological control. Eighty-five percent of dermatophyte-positive cats in our study were younger than 1 year. As reported by [7], kittens were 8.0 times more likely to be diagnosed with dermatophytosis than cats over 6 months of age. Previous studies found that cats under 1 year of age had an increased risk. Increased susceptibility to dermatophytosis in young animals can be explained by a number of factors, including immune system immaturity, lack of previous immunity, skin microtrauma from siblings or ectoparasites, and frequent close contact with other cats during socialization periods. Terbinafine is a fungicidal allylamine with documented in vitro and in vivo efficacy against Microsporum canis in pet cats and in cats with experimental infections [9]. Oral itraconazole and terbinafine are judged to be the most effective and safe treatments for dermatophytosis. However, for the most effective treatment of dermatophytosis, a combination of systemic and topical therapy is necessary. The purpose of topical therapy is to decrease the infectious, contagious and zoonotic risks associated with dermatophytosis by disinfecting the hair coat and minimizing contamination of the environment [10]. If no such measures are taken, the spores of the fungi may remain on the haircoat, and the animal will remain mycocarrier.

The most significant result of current study is the finding that 44% of clinically recovered cats turned to be asymptomatic carriers. Obviously, this is a consequence of the fact that topical therapy of animals was not performed, as well as mycological control of recovery at the end of therapy. The use

of two negative consecutive cultures as mycological cure and cfu/plate for screening and monitoring of infections is widely used in shelters abroad [10], but in Russia such practice should be beneficially introduced in the nearest future. Compared to itraconazole, fluconazole, ketoconazole and griseofulvin, terbinafine has the lowest MIC for Microsporum sp. and Trichophyton spp. In the study with 300 veterinary isolates, terbinafine MICs ranged from 0.002 to 0.25 μg/mL, but MIC values were within a range of 0.008–0.03 μg/mL in over 90% of fungal isolates [10]. Our MIC values (0.001-0.002 μg/ml) are comparable with the data of published studies. The indigenous M. canis strains we studied are relatively sensitive to terbinafine. The decontamination of the exposed environments is highly required for controlling the dermatophytosis in animals and preventing zoonotic human infection [4]. Eighty percent of environmental samples examined in the current study were contaminated with M. canis. Similarly, environmental contamination was detected in all households (100%) with M. canis infected cats [2]. It's not surprising that a typical case of zoonotic M. canis infection was diagnosed in one shelter employee during our survey.

5. Conclusion

In conclusion, the study confirms necessity for large-scale mycological screening of cats in shelters using DTM-type diagnostic media. Oral terbinafine therapy alone is not sufficient for effective treatment of dermatophytosis; it should include also the whole-body antifungal treatment. Cultural control of cure is also mandatory.

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