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The Prevalence of Animal Dermatophytosis in the Russian Federation and the Spectrum of Sensitivity to Antifungal Drugs

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Abstract

This article presents the results of studying the incidence of animal dermatophytosis in 8 regions of Russia, based on the study of etiological agents and the determination of sensitivity to antifungal drugs used, registered in the country. In the course of the research, 113 isolates of dermatomycetes were isolated and characterized by sensitivity to antifungal drugs, the species diversity was 4 species in 2 genera. Data on the sensitivity of isolates of M. canis, M. gypseum, T. verrucosum, mentagrophytes, T. equinum to the main antifungal drugs are presented. Separately, data on the number of isolates resistant to 2 or 3 drugs are presented.

Discipline: Veterinary epidemiology.

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1 Introduction

The causative agents of dermatophytosis in humans and animals are fungi of the genus Microsporum (microsporia) and the genus Trichophyton (trichophytosis). Until the middle of the 20th century, anthropophiles (Microsporum ferrugineum) were predominantly registered pathogens in Europe. Now Microsporum canis is the most frequently isolated fungus in microsporia of the scalp in children in Europe, the USA, South America, Japan, Israel, and a number of Arab countries [1, 2, 3].

At the same time, according to some sources, the dominant causative agent of microsporia in the United States and Western Europe is Microsporum audouinii.

The most common causative agent of microsporia in Russia is the zoophilic fungus Microsporum canis, and the second most common is the anthropophilic fungus Microsporum ferrugineum [4]. The incidence of microsporia in 2003 in the Russian Federation was 49 cases per 100,000 population.

Microsporum canis is a widespread zoophilic dermatophyte that causes disease in dogs, cats, monkeys, and, less commonly, other animals [5]. Microsporum canis causes dermatophytosis in animals more often than other species of the genus Microsporum: 61.1% of dermatophytosis in dogs, and 61.4% in cats. According to the same authors, the second most common causative agent of microsporia in dogs and cats is Microsporum gypseum (22.8% and 22.1%, respectively). Microsporum gypseum spreads mainly through the soil [6].

Trichophytosis in humans is currently inferior to microsporia in terms of frequency of occurrence in Russia. Thus, in 2006, 2.1 cases of trichophytosis per 100,000 population were registered on the territory of the Russian Federation. Trichophytosis occurs most frequently in the Southern Federal District (the incidence was 5.7 and 6.7 per 100,000 population in 2005 and 2006, respectively). Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton tonsurans, Trichophyton violaceum fungi are among the most common pathogens of trichophytosis [7].

Trichophyton verrucosum mainly infects cattle; diseases in other animals caused by this species are very rare. Accordingly, the main risk group for the development of trichophytosis caused by this species is employees of livestock farms or people who have contact with cattle. The main natural reservoir of Trichophyton mentagrophytes is mouse-like rodents (MD, Prof. VG Kornisheva).

In modern conditions, the animal organism is under constant pressure from a number of unfavorable external factors that cause a decrease in the body's natural resistance, and unsystematic, sometimes unjustified use of antimicrobial drugs. The number of breeds of animals with congenital dysfunction of the immune system has increased; exotic breeds and even animal species that are not typical for our climatic and geographical zone have become widespread. Due to the intensification of international relations, due to climate change, and socio-economic factors, the range of many pathogenic and potentially pathogenic fungi, including endemic species, has significantly expanded. These factors contribute to a change in the etiological structure of animal dermatomycosis.

A systematic study of the etiological structure of dermatomycoses in animals makes it possible to track the prevalence of fungal diseases, and changes in the species composition of pathogens, and to identify previously unseen etiological agents.

These studies are being conducted for the first time in the Russian Federation, the main purpose of which is to determine the incidence of animal dermatomycosis, to find its main etiological agents, and to determine sensitivity to commonly used antifungal substances (drugs).

Increasingly, there are reports of strains of pathogens of dermatophytosis resistant to antifungal drugs: strains of M. canis resistant to terbinafine [8] and azoles [9], strains of T. rubrum

resistant to terbinafine [10]. In addition, the possibility of the emergence of strains resistant to azole preparations in experiments in vitro has been experimentally shown. It should be noted that there are no similar data for the Russian Federation (or such data have not been published), so further work on the study of the resistance of dermatophyte fungi isolates is relevant.

2 Materials and Methods

The object of the study was samples of clinical material taken from domestic and farm animals (samples of wool, scabs, swabs from the ears and skin) with clinical signs of the disease or with suspicion of dermatophytosis.

Methods of microscopic examination.

To study the keratinized material, the generally accepted crushed drop method with preliminary clarification and softening of the material in a 10% KOH solution was used.

For the study of biopsy material separated from the ear canal and mucous membranes, fixed stained preparations are used. As dyes, lactophenol cotton blue (Lactophenol Cotton Blue), methylene blue, and neutral red are used, which stain the elements of mushrooms.

To identify fungal elements in any material, a luminescent dye, calcofluor white, was used.

Isolation and identification of isolates were carried out by seeding on solid nutrient media: Sabouraud (M063, Sabourand dextrose agar, HiMedia) with the addition of chloramphenicol (FD033, Chloramphenicol Selective Supplement, HiMedia) and Sabouraud with the addition of a selective additive containing cyclohexemide (Dermasel Selective Supplement, Oxoid), wort agar, Chapek-Doxa, potato-glucose agar.

To obtain isolated colonies on the above nutrient media, the culture under study was subcultured on each of them, inoculated by pricking into the center of each dish, and cultivated at different temperatures, the cultivation period was not specially limited.

In the process of identification, the following features of the colonies were studied:

- the color of the colony and the color of the reverse;
- the structure of the colony (folding, surface texture, structure);
- the growth rate (colony diameter) at a given temperature.

For dermatophytes, the need for some vitamins was additionally studied (for species identification of isolates of the genus Trichophyton). To do this, the growth rate of the culture was determined on a set of nutrient media, which made it possible to identify the need for vitamins of group B (Trichophyton media 1–7). HiMedia Laboratories Pvt. Limited" (India).

Additionally, the ratio of isolated cultures to temperature was studied; for this, all cultures were grown at temperatures of +26 °C and +37 °C.

To study the structural features of the vegetative mycelium and reproductive organs, a series of micropreparations were prepared using the tape-print method. The preparations were not stained; the study was carried out by bright field and phase contrast methods under a microscope.

At the final stage, the features of macro- and micromorphology were compared using the determinants of microscopic fungi [11].

The sensitivity to the three main antifungal substances (ketoconazole, terbinafie and enilconazole) was determined according to the standard, EUCAST e.def. 9.3.1.

3 Result and Discussion

A total of 202 samples were studied, and 113 dermatophyte isolates were isolated. Regions, the number of samples received and the number of isolated isolates is shown in Table 1.

Table 1: Study of clinical material

Region	Samples received	Isolates isolated	% allocation
Krasnodar region	23	11	47.83%
Rostov region	19	12	63.16%
Republic of Tatarstan	29	14	48.28%
Belgorod region	31	16	51.61%
Voronezh region	36	16	44.44%
Stavropol region	16	10	62.50%
Saratov region	12	9	75.00%
Kursk region	36	25	69.44%
TOTAL	202	113	55.94%

Microscopic fungi belonging to dermatophytes (genera Microsporum and Trichophyton) were isolated from 56% of the obtained samples and identified as Microsporum canis (M. c.), Microsporum gypseum (M. g), Trichophyton verrucosum (T. v.), Trichophyton mentagrophytes (T.m.). Identification was carried out by morphological and biochemical methods. Table 2 shows the number of different types of dermatophytes isolated from different regions.

Table 2: Isolated dermatophyte fungi

Region	M. canis	M. gypseum	T. verrucosum	T. mentagrophytes
Krasnodar region	6	4	0	1
Rostov region	5	5	1	1
Republic of Tatarstan	6	4	0	4
Belgorod region	4	6	4	2
Voronezh region	4	2	4	6
Stavropol region	3	3	1	3
Saratov region	1	1	4	3
Kursk region	5	5	7	8
TOTAL	34	30	21	28

A total of 34 M. canis isolates, 30 M. gypseum isolates, 21 T. verrucosum isolates, and 28 T. mentagrophytes isolates were isolated.

Sensitivity to antifungal drugs.

The determination of sensitivity was carried out by the method of serial dilutions to three antifungal drugs - ketoconazole, enilconazole and terbinafine, the method is described in the appropriate section. The choice of drugs was dictated by information about antifungal drugs registered in the Russian Federation for veterinary use.

Thiabendazole, enilconazole, miconazole and ketoconazole belong to the same group of drugs, and have a similar mechanism of action, which is to inactivate one of the enzymes of the late

stage of ergosterol synthesis (lanosterol-14α-demethylase). For this reason, only enilconazole and ketoconazole were used in the present study.

One group of drugs - allylamines - includes terbinafine and naftifine. They have a similar mechanism of action common to the drugs of this group, which is to inactivate squalene epoxidase, an enzyme in the early stage of ergosterol synthesis. For this reason, we limited ourselves to terbinafine in this study.

The number of isolates of M. canis, M. gypseum, T. verrucosum, T. mentagrophytes resistant to terbinafine, ketoconazole and enilconazole (imazilil) is shown in Figures 1-4.

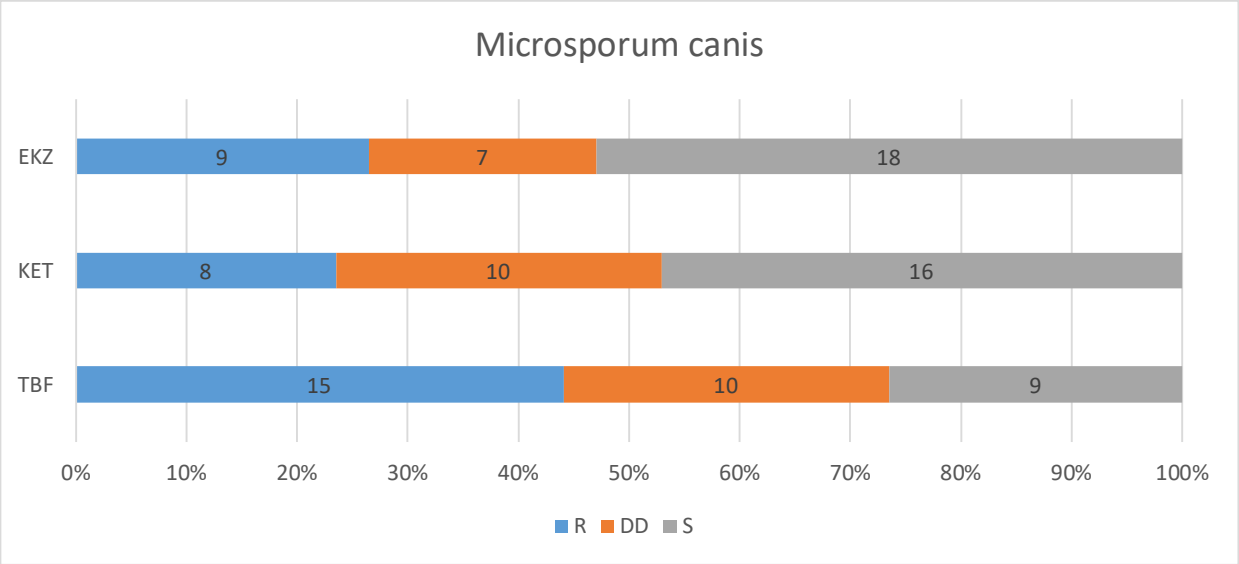

Figure 1: Number of M. canis isolates resistant to terbinafine, ketoconazole and enilconazole (R – resistance, DD – dose dependent, S – sensitive).

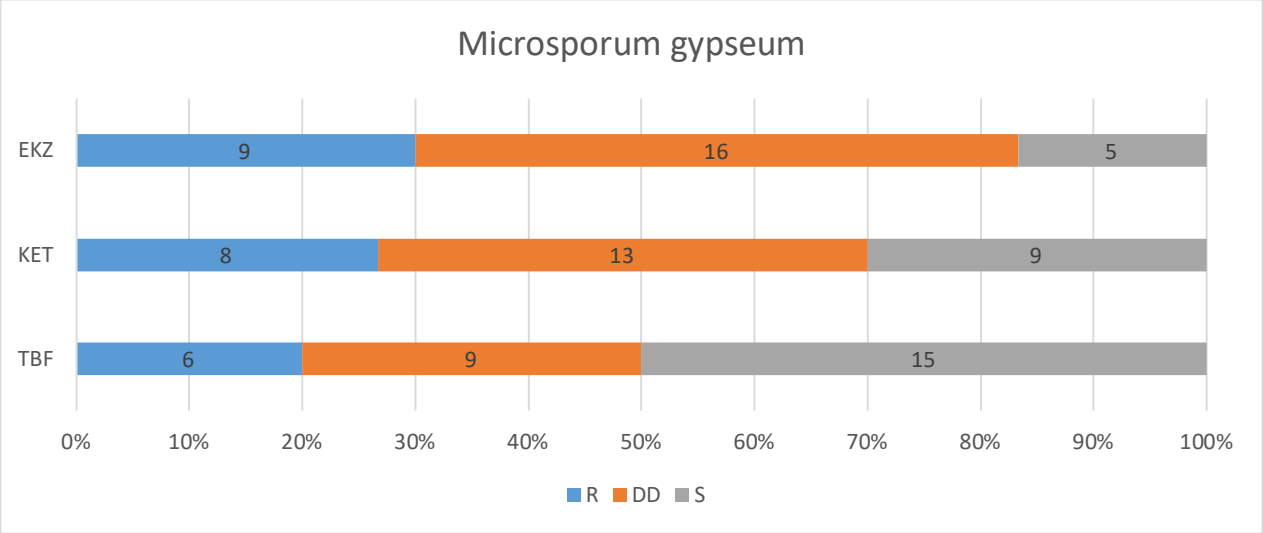

Figure 2: Number of M. gypseum isolates resistant to terbinafine, ketoconazole and enilconazole (R – resistance, DD – dose dependent, S – sensitive).

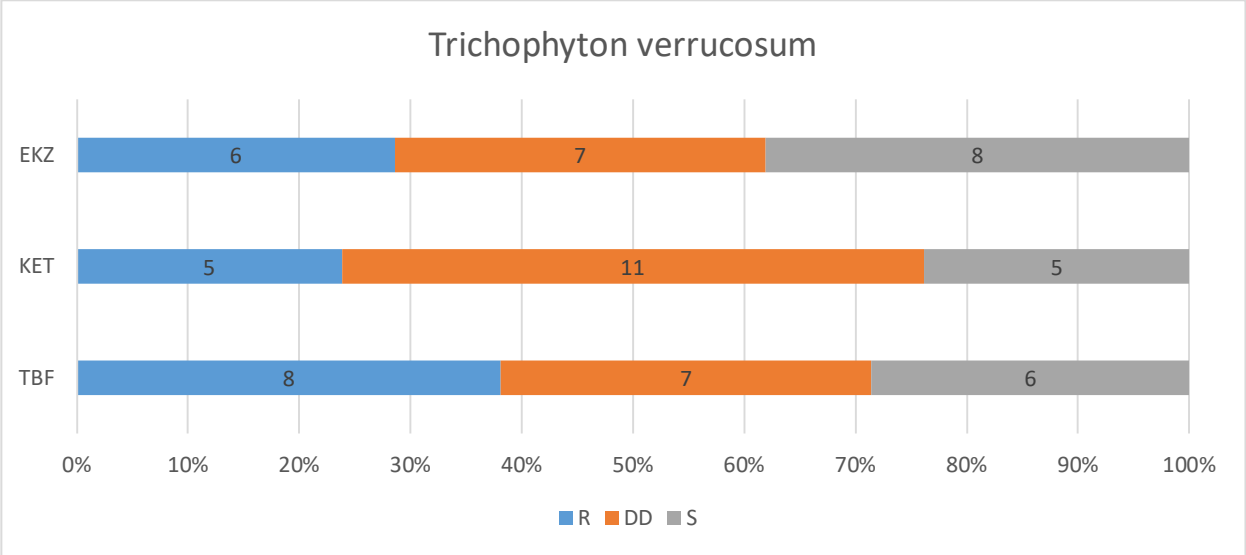

Figure 3: Number of T. verrucosum isolates resistant to terbinafine, ketoconazole and enilconazole (R – resistance, DD – dose dependent, S – sensitive).

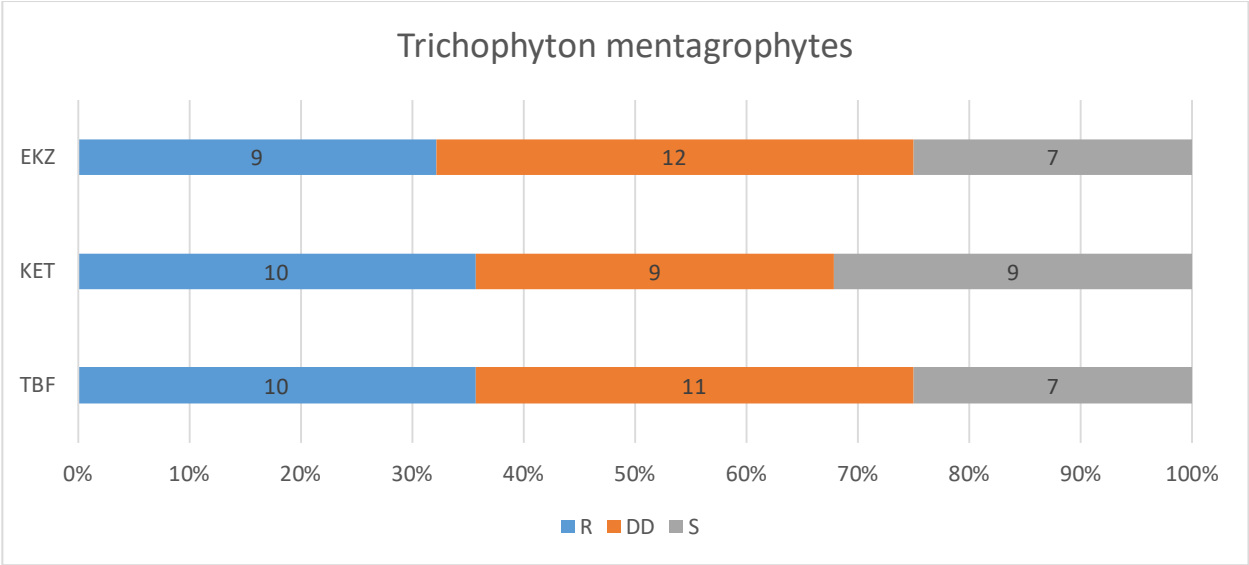

Figure 4: Number of T. mentagrophytes isolates resistant to terbinafine, ketoconazole and enilconazole (R - resistance, DD - dose dependent, S - sensitive).

The distribution of resistant, dose-dependent and susceptible isolates by regions where sampling was carried out is presented in Figures 5-8. Separately, data are presented on the number of isolates resistant to 2 or 3 drugs by region (Figure 9). The numbers indicate the regions: 1 - Krasnodar Territory, 2 - Rostov Region, 3 - Republic of Tatarstan, 4 - Belgorod Region, 5 - Voronezh Region, 6 - Stavropol Territory, 7 - Saratov Region, 8 - Kursk Region.

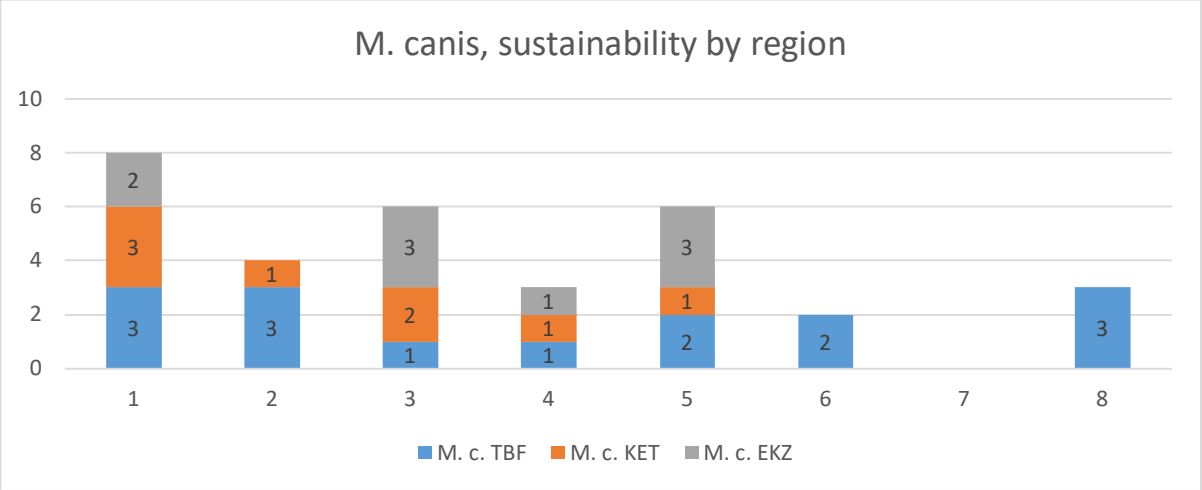

Figure 5: Distribution of antifungal resistant M. canis isolates by region. M. c. TBF, M. canis isolates resistant to terbinafine; M. c. KET, M. canis isolates resistant to ketoconazole; M. c. EKZ - M. canis isolates resistant to enilconazole.

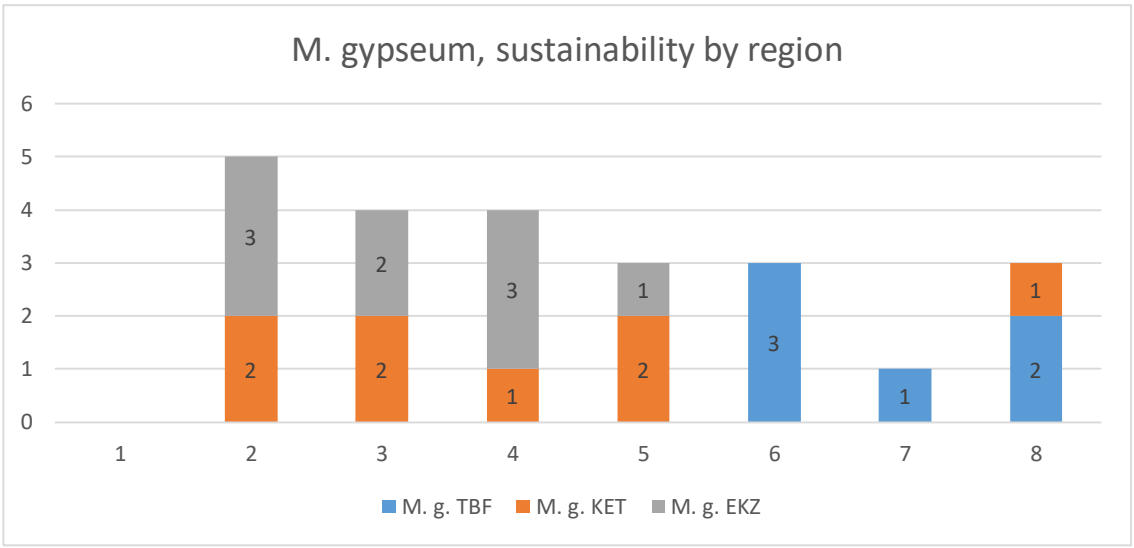

Figure 6: Distribution of antifungal resistant M. gypseum isolates by region. M.g. TBF, M. gypseum isolates resistant to terbinafine; M.g. KET, M. gypseum isolates resistant to ketoconazole; M.g. EKZ, M. gypseum isolates resistant to enilconazole.

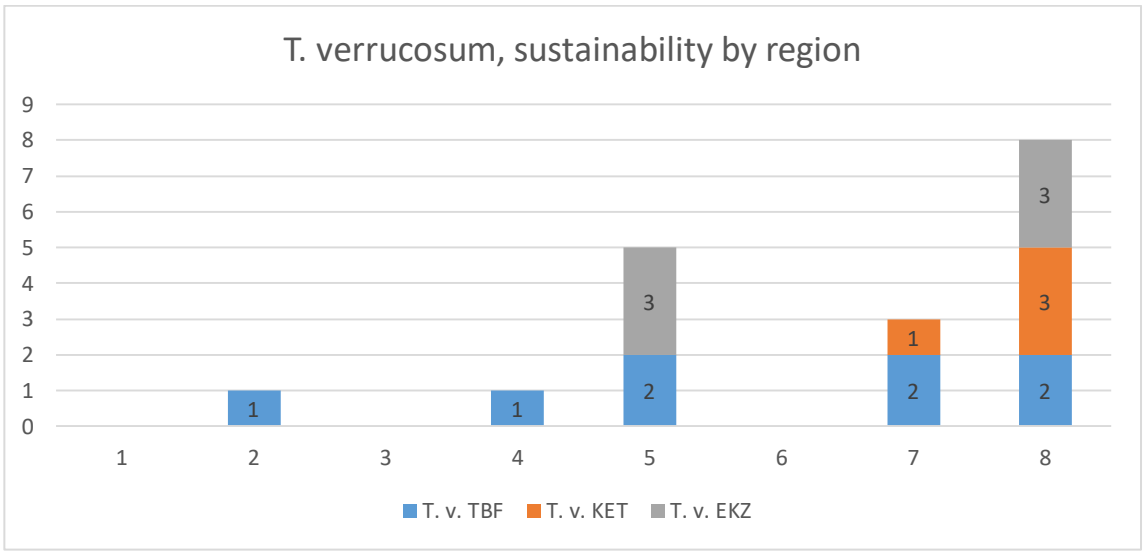

Figure 7: Distribution of antifungal resistant T. verrucosum isolates by region. T.v. TBF, T. verrucosum isolates resistant to terbinafine; T.v. KET, T. verrucosum isolates resistant to ketoconazole; T.v. EKZ, isolates of T. verrucosum resistant to enilconazole.

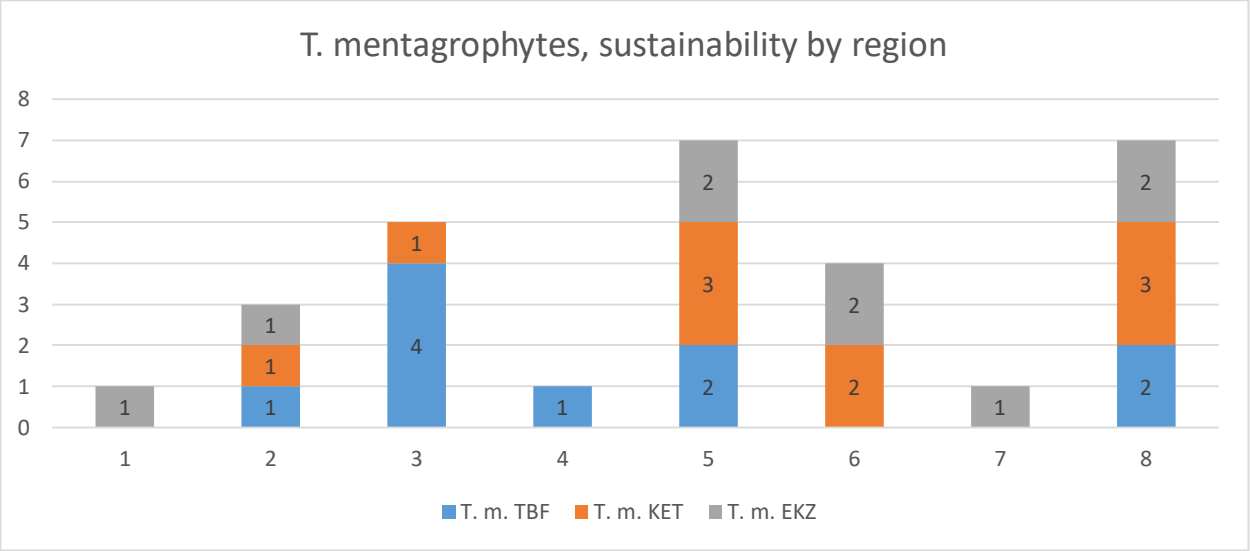

Figure 8: Distribution of antifungal resistant T. mentagrophytes isolates by region. T.m. TBF, isolates of T. mentagrophytes resistant to terbinafine; T.m. KET, isolates of T. mentagrophytes resistant to ketoconazole; T.m. EKZ, isolates of T. mentagrophytes resistant to enilconazole.

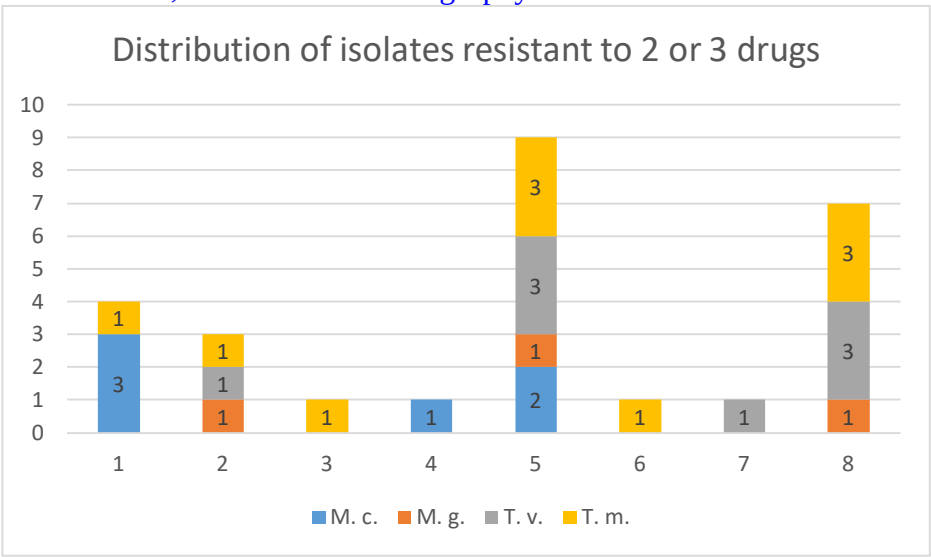

Figure 9: Distribution of isolates resistant to 2 or 3 antifungals by region. M. c. – Microsporum canis, M. g. – Microsporum gypseum, T.v. – Trichophyton verrucosum, T. m. – Trichophyton mentagrophytes.

4 Conclusion

The proportion of Microsporum canis isolates resistant to terbinafine reached 44%, to ketoconazole - 24%, to enilconazole - 26%. The proportion of Microsporum gypseum isolates resistant to terbinafine reached 20%, to ketoconazole - 30%, to enilconazole - 27%. The proportion of Trichophyton verrucosum isolates resistant to terbinafine reached 38%, to ketoconazole - 19%, to enilconazole - 29%. The proportion of Trichophyton mentagrophytes isolates resistant to terbinafine reached 26%, to ketoconazole - 36%, to enilconazole - 32%. The proportion of Trichophyton verrucosum and T. mentagrophytes isolates resistant to 2 or 3 drugs reached 38% and 36%, respectively. The number of clinically significant microscopic fungi resistant to antifungal drugs was observed in large cities. To conduct effective, adequate therapy, it is necessary to introduce methods for determining the sensitivity of pathogens to antifungal drugs into the

practice of veterinarians, which will exclude the possibility of the emergence of resistant strains of dermatophytes.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

6 Acknowledgement

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