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Research Article

Epidemiological studies and antifungal sensitivity pattern of clinical isolates of dermatophytes from localized cases of canine dermatophytosis

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Abstract

Dermatophytosis is one of the most common skin diseases in dogs caused by keratinophilic fungi of the family Arthrodermataceae. Dermatophytes of veterinary importance belong to genus *Microsporum*, *Trichophyton* and *Epidermophyton*. Lesser treatment options and frequent use of non-prescription antifungal drugs resulted in development of resistance to the commonly used drugs. The research was conducted to study the epidemiology of cases of localized canine dermatophytosis and to assess the antifungal sensitivity pattern of the isolated organisms. Animals tentatively diagnosed with dermatophytosis based on the presence of endothrix or ectothrix on trichogram were selected for the study. Occurrence rate of dermatophytosis was 17.66% during the study period. The dermatophytes isolated in the current study were *Microsporum canis*, *Trichophyton mentagrophytes* and *Microsporum gypseum*. The sensitivity pattern of the dermatophytes varied depending on the species isolated. Majority of the *M. canis* isolates were mainly sensitive to nystatin, *M. gypseum* to amphotericin B and *T. mentagrophytes* to itraconazole, whereas resistance was shown by all three isolates against fluconazole. Statistically significant difference was noticed in the culture results obtained on two different fungal specific medias *viz*, dermatophyte test medium and sabouraud dextrose agar.

Keywords: Canine, Dermatophytosis, *Microsporum*, *Trichophyton*

Highlights

- The incidence rate of canine dermatophytosis was higher during winter, followed by other seasons.
- Dogs in the age group of 1 to 3 years were mainly affected, and the incidence was greater in non-descript breed of dogs.
- The dermatophyte species isolated were *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes*.
- Dermatophyte test medium was found to be better than sabouraud dextrose agar for isolation of the dermatophytes.

INTRODUCTION

Canine dermatophytosis is a superficial fungal disease with variable clinical presentation and affecting almost all parts of the body. The disease is more prevalent in warm climatic zones, and development of the disease requires a favourable condition in exposed animals. The disease as such is self-limiting, but weakened immune status of the animals and a conducive microenvironment result in wide spread of the disease (Moriello and Newbury, 2006). Untreated animals act as the source of infection to other animals as well as in contact humans. Manifestation of the disease includes primary or secondary lesions comprising of crusts, scales, erythema, pruritus, kerions, alopecia, papules, hyperpigmentation or nodules (Silver, 2011). Limited treatment options and increasing incidence of drug resistance demand the wise use of available treatment choices. Selection of the drug of choice should better depend on sensitivity results for an improved response, reduction in treatment duration and prevention of development of drug resistance. The media selection

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for culture and sensitivity also affects the genuineness of the results obtained. The present study envisages the epidemiological pattern and better diagnostic options for canine dermatophytosis.

MATERIALS AND METHODS

Epidemiological study

Epidemiological study of cases of canine dermatophytosis was conducted in dogs presented for dermatological consultation at the dermatology outpatient units of Veterinary Hospitals under Kerala Veterinary and Animal Sciences University, Kerala from June 2022 to January 2024. Dogs presented with scales, crusts, erythema, hyperpigmentation, alopecia, pruritus or nodules at a maximum of two locations on the body were included in the study population. Detailed signalment and anamnesis which included age, sex, breed of the affected animals, seasonal distribution of the cases, age of onset, previous medications if any, concurrent infections, management aspects including diet, nutrient supplementation, grooming and bathing practice, housing and contact with other animals was studied in animals which produced a positive dermatophyte growth on either of the fungal culture medias, sabouraud dextrose agar (SDA) or dermatophyte test medium (DTM).

Trichogram

Hair pluck was collected using artery forceps, and skin scrapping was collected using a sterile scalpel from the periphery of the lesions into separate sterile containers. Samples collected were subjected to trichogram. The samples were placed on microscopic slide, and liquid paraffin was added as mounting medium. The mounted slides were observed under 10X, followed by 40X of light microscope for the presence of either ectothrix or endothrix infection.

Wood's lamp examination

Wood's lamp examination of the presented cases was performed in a dark room. After adaption to the dark, the lesions were observed with a Wood's lamp with ultra violet light in the wavelength range of 320 to 400 nm with 5X magnification to detect the presence of apple green fluorescence (Klatte *et al*., 2015).

Microbiological characterization

Sample collection and isolation: Samples were collected from the suspected lesions for culture and sensitivity using Mackenzie brush technique (Colombo *et al*., 2010). Mycologically sterile brushes were swabbed on the periphery of the lesions for a minimum of one minute and were stored in individual sterile containers until inoculation, which was performed on the same day. The samples were inoculated on ready-to-use sterile Sabouraud dextrose agar plates with chloramphenicol and gentamicin (90 mm; Himedia, MP 5332) and Dermatophyte test medium (DTM) plates (90 mm; Himedia, MP 188), the temperature of which was brought to room temperature from refrigerated conditions. The bristles of the brushes were gently pressed on the surface of the selected mediums. The plates were incubated at room temperature (27-30°C) for a minimum period of seven days and observed regularly for the presence of growth or colour change of the media in case of DTM plates. Plates with no growth were discarded after a period of three weeks (Kaufmann *et al*., 2016).

Macroscopical and microscopical identification: The identification of the dermatophyte growth on the inoculated plates was done by examining both macroscopic and microscopic characteristics. Colony morphology of the dermatophytes on the media which included the surface texture and pigmentation was observed on both obverse and reverse side of the culture plates.

The dermatophytes were identified microscopically after lactophenol cotton blue staining (Himedia S016-100 ML). A drop of the stain was placed on a microscopic slide. A clear cellophane tape strip was gently pressed on the colony growth and placed on the slide with stain and kept for two minutes. The slides were then observed under 10X followed by 100X of the light microscope. Presence of hyphae, microconidia and macroconidia was observed (Moskaluk and VandeWoude, 2022).

Antifungal sensitivity profile

A pure colony was selected from the culture plate. Using a sterile swab, the colony was transferred to normal saline in a centrifuge tube and vortexed. It was then centrifuged at 5000 rpm for 5 min to remove the sediments. The supernatant obtained was adjusted to 0.5 McFarland standard (HiMedia R092), which contains approximately $0.4–5\times10^6$ conidia or sporangiospores/mL. A sterile swab was dipped into the well-mixed saline supernatant containing sporangiospores and swabbed on SDA plates (Dogra *et al*., 2019). An antifungal sensitivity disc of six antifungal agents (Himedia- hexaantimyco - 01 disc; HX 104) *viz* itraconazole, fluconazole, amphotericin B, ketoconazole, clotrimazole and nystatin was placed on the swabbed plates and incubated at room

temperature. The plates were observed daily, and the zone of inhibition around each disc was measured.

RESULTS

A total of 3799 dogs were presented for dermatological consultation during the study period of which 671 animals were diagnosed with fungal dermatitis. Generalized infection was noticed in 491 animals, and 180 animals had localized lesions in a maximum of two locations.

Seasonal distribution of the cases was done into four major official seasons classified by the Indian Meteorological Department (IMD) as post-monsoon (October to December), winter (December to early April), summer or pre-monsoon (April to June) and rainy or monsoon (June to September). Maximum number of cases were reported in the winter season (35/108), followed by monsoon (33/108), post-monsoon (23/108) and the least reports in summer/ pre-monsoon season (17/108).

Localized cases of dermatophytosis were reported in animals from 3 weeks of age to 12 years of age. Highest incidence was seen in animals in the age group of 1 to 3 years (28.70%), followed by animals up to one year of age (27.78%), 6 to 9 years of age (22.22%) and above 9 years of age (12.04%). Least incidence was noticed in animals in the age group of 3-6 years of age (9.26%).

Males were found to have a higher incidence rate of 61 per cent, followed by females (47%). The breeds which were presented with localized dermatophytosis in the present study were Rottweiler, Labrador Retriever, non-descript dog, Dachshund, Pug, Belgian Malinois, Pomeranian, German Shepherd, Doberman, Shih Tzu and Beagle with the highest occurrence in non-descript dogs (27.78%) and least incidence in Belgian Malinois (1.85%) (Table 1). The dogs affected in various groups according to American Kennel Club classification were, sporting group (27 animals), working group (14), toy group (14), hound group (13) and herding group (10). Thirty dogs were non-descript; those were not included in any of the groups.

Details about the management and environmental aspects collected included data about diet of the pet, skin supplements given, grooming frequency, housing of pet (indoor/outdoor), cleaning of the kennels, presence of ectoparasites and endoparasites and contact with other animals. The majority of the animals were fed with a combination of regular commercial food and a homemade diet (59.26%). Other diet types practised included commercial diet alone (19.44%) and homemade diet alone (21.30%). The common inclusions in the homemade diet were rice, chapati, fish, egg, chicken, red meat, vegetables, especially carrots, pumpkin, potatoes and fruits. Fifty-four per cent of the animals were fed with multi vitamin mineral tonics on a regular basis. Seven dogs (6.48%) were administered topical coat conditioners on a monthly basis, which was mainly moisturizer spot-on. Eighty-two per cent (88 no.) of the animals were groomed regularly, of which 62 animals were groomed at home daily, and 26 animals were taken to grooming centres. No proper grooming was practised in 18 per cent of the animals.

Table 1. Breed wise distribution of the cases

Sl. No.	Breed	No. of cases	Per cent
1	Non-descript	30	27.78
2	Labrador Retriever	27	25
3	Rottweiler	9	8.33
4	Beagle	9	8.33
5	German Shepherd	8	7.42
6	Pug	5	4.63
7	Doberman	5	4.63
8	Pomeranian	5	4.63
9	Shih Tzu	4	3.70
10	Dachshund	4	3.70
11	Belgian Malinois	2	1.85
	Total	108	100

Only 29 per cent of the animals were maintained strictly indoors. Forty-four per cent of the animals were kept in kennel outside, and 27 per cent of the animals were permitted both indoor and outdoor. The animals kept in kennel were given access to outside environment, which included sit out of the houses, car parking areas, lawn and surroundings of the houses. Ninety-three per cent of affected dogs were taken out regularly for walking. Commonly used substances to clean the kennels as well as the inside of the houses included kennel wash (52%), lemongrass oil (29%), diluted dog shampoo (7%), plain water (11%) and agents including Dettol and Lizol. Thirty-three per cent of cases were maintained indoor were given separate blankets which were washed with regular detergents. Twelve per cent (13 no.) of the animals had concurrent presence of ectoparasites, especially fleas (4 animals) and ticks (9 animals). Three animals had ancylostomiasis which was confirmed by the presence of ova on direct microscopical examination of the faecal sample. A greater percentage of the animals were maintained alone (74%). Nine per cent of the dogs were maintained with cats, and 15 per cent of the animals had dogs as their kennel mates.

Wood's lamp examination findings: Examination of the lesions under ultraviolet light in the range of 320 to 400 nm wavelength emitted apple green fluorescence in 21 cases (19.44%), and no fluorescence was noticed in 87 cases. All the cases positive for fluorescence in Wood's lamp examination had *M. canis* dermatophyte growth on sabouraud dextrose agar (Fig. 1).

Isolation of the dermatophytes: The dermatophyte species grown either on SDA with chloramphenicol and gentamicin or DTM from hair samples were *M. canis* (71.29%), *M. gypseum* (16.67%) and *T. mentagrophytes* (12.04%). Other fungal organisms identified were *Aspergillus* spp. (12 of 180 samples), *Malassezia* spp. (8 of 180 samples) and *Fusarium* spp. (4 of 180 samples). Out of the 180 samples positive for fungal spores on trichogram, only 108 samples yielded growth of the dermatophytes.

Growth of dermatophytes: Of the hair samples collected from 180 selected cases, 108 samples produced dermatophyte growth on DTM, whereas only 96 samples were positive on SDA with chloramphenicol and gentamicin. Significant difference was noticed in the growth pattern of the isolated dermatophytes in DTM and SDA when analyzed by Wilcoxon paired rank test (Table 2). The average days taken for the growth is depicted in Table 3. A significant difference was also noticed in the days taken for growth in SDA and DTM by *M. canis*, *M. gypseum* and *T. Mentagrophytes* on analysis using paired 't' test.

Macroscopic and microscopic characteristics: Flat yellowish buff powdery colonies with a feathered white hyphal border were noticed for *M. gypseum* growth on the obverse side and light yellow shaded on the reverse side. A white fluffy and silky colony on the obverse side was noticed in the growth of *M. canis*, and a yellowish to light tan pigmentation was observed on the reverse side. *Trichophyton mentagrophytes* had a white to yellowish granular colony appearance on the obverse side and yellowish on the reverse side (Fig. 2, 3). *Microsporum canis* produced thick-walled, spindle-shaped, large macroconidia with a knob-like end and septate hyphae. *Microsporum gypseum* produced thin-walled, large and spindle-shaped macroconidia. Apical ends were rounded, and the base was truncated. Macroconidia has a rough verrucose surface. Microscopic characteristics of *T. mentagrophytes* observed were cigar-shaped, thin-walled and long macroconidia with about seven cells. Microconidia were spherical in shape and sessile and were seen as grape-like clusters or dense along the septated hyphae (Fig. 4).

Antifungal sensitivity pattern: Antifungal sensitivity pattern of the dermatophytes against amphotericin B, clotrimazole, fluconazole, itraconazole, ketoconazole and nystatinis given in Table 4 and Fig. 5. Out of the 77 *M. canis* isolates, 67.53% were sensitive to nystatin followed by ketoconazole (61.04%), clotrimazole (55.84%), itraconazole (53.25%), amphotericin B (36.36%) and fluconazole (20.78%). The antifungal drug against which the *M. canis* isolates showed more resistance was fluconazole (79.22%). Similarly, the sensitivity pattern of *M. gypseum* isolates against dermatophytes was amphotericin B (66.67%), ketoconazole and itraconazole (61.11%), clotrimazole (55.56%), nystatin (50%) and fluconazole (44.44%). *Trichophyton mentagrophytes* isolates were sensitive to itraconazole (76.92%) followed by clotrimazole (69.23%), nystatin (61.54%), amphotericin B (53.85%), ketoconazole (38.46%) and fluconazole (15.38%).

Table 2. Growth pattern of dermatophytes in culture media

Sl. No	Dermatophyte species	No. of cases in which growth observed			Z value	Sig. (2 tailed)
		Total	SDA (%)	DTM (%)		
1	*M. canis*	77	90.90	100	2.646	0.008**
2	*M. gypseum*	18	72.22	100	2.236	0.025*
3	*T. mentagrophytes*	13	100	100	0.000	1.000

** Significant at 0.01 level; * Significant at 0.05 level

Table 3. Average days taken for growth

Sl. No.	Dermatophyte species	Average days taken for growth		t- value	p-value
		SDA	DTM		
1	*M. canis*	7.27±0.011	5.94±0.09	9.763**	0.001
2	*M. gypseum*	7.62±0.24	6.23±0.23	4.185**	0.001
3	*T. mentagrophytes*	7.31±0.21	6.31±0.24	3.950**	0.002

** Significant at 0.01 level ($p<0.01$)

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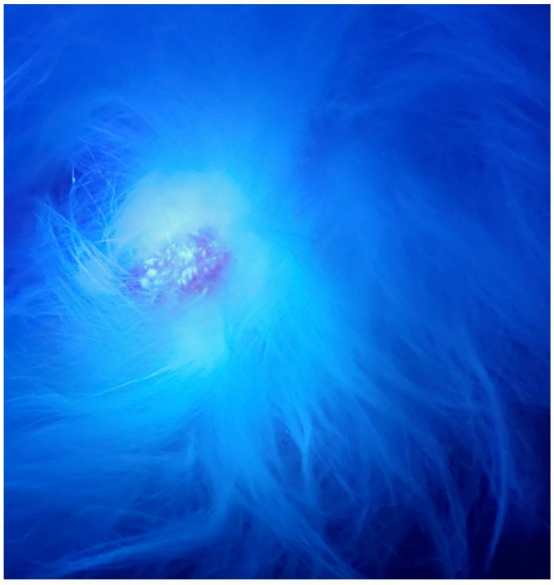

Fig. 1. Apple green fluorescence on Wood's lamp examination

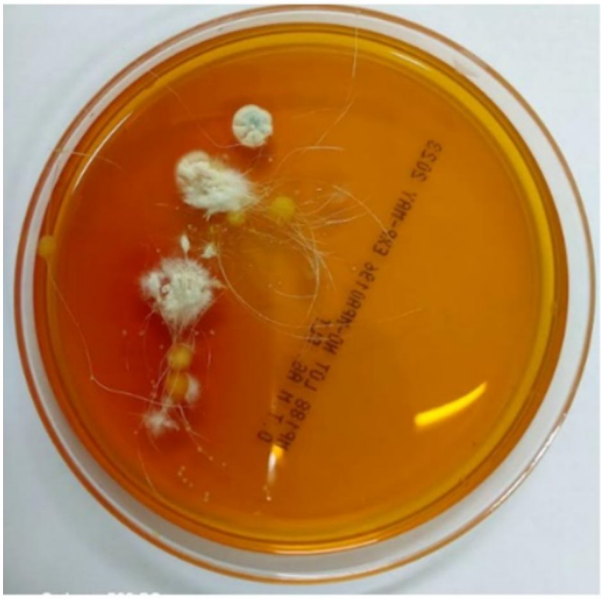

Figure 2 (a)

Figure 2 (b)

Figure 2 (c)

[Fig. 2(a). *M. canis* growth on DTM obverse side; Fig. 2(b). M. canis growth on SDA obverse side; Fig. 2(c). *T. mentagrophytes* on SDA obverse side]

Fig. 2. *M. canis* growth on DTM and SDA obverse side

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Figure 3 (a)

Figure 3 (b)

Figure 3 (c)

[Fig. 3(a). *M. canis* growth on DTM reverse side; Fig. 3(b). *M. canis* growth on SDA reverse side; Fig. 3(c). *T. mentagrophytes* on SDA reverse side]

Fig. 3. *M. canis* growth on DTM and SDA reverse side

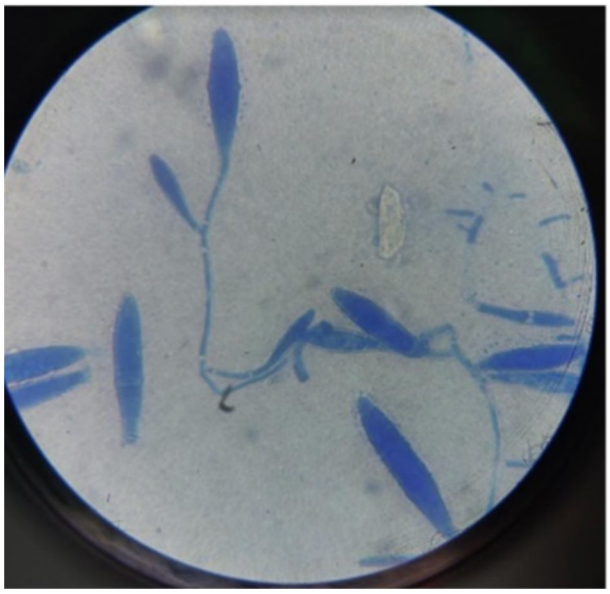

Figure 4 (a)

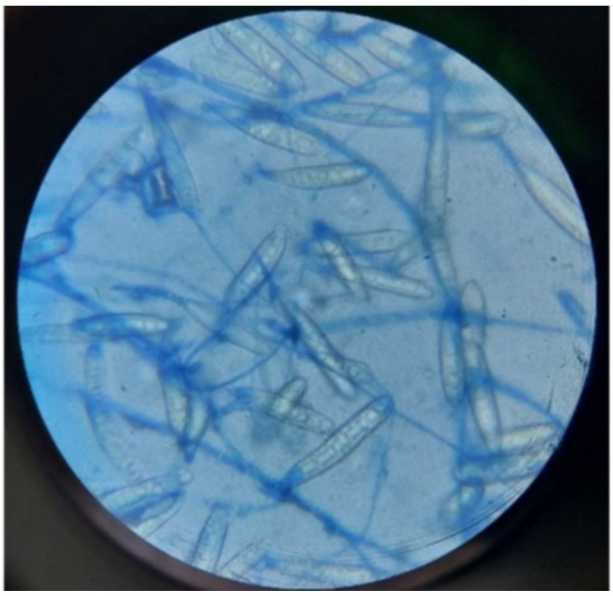

Figure 4 (b)

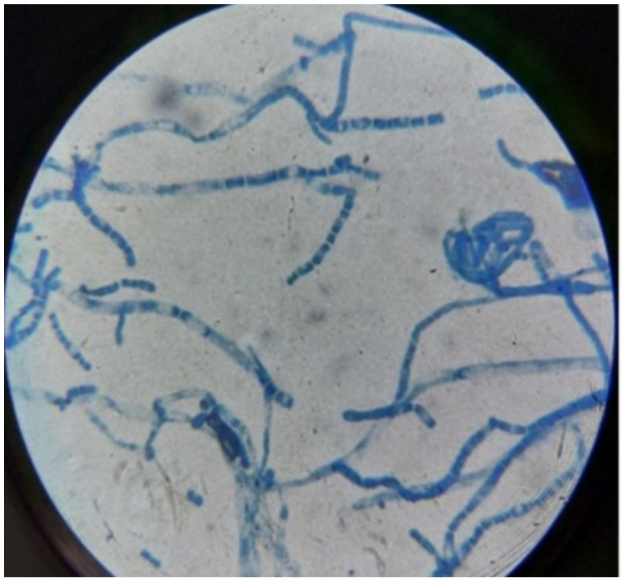

Figure 4 (c)

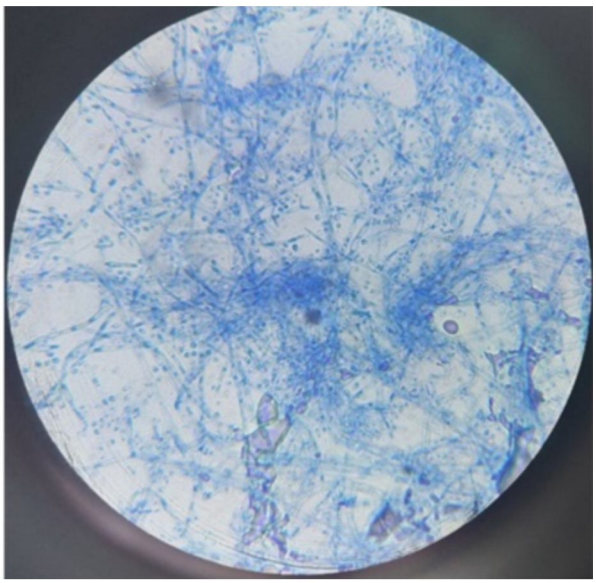

Figure 4 (d)

[Fig. 4(a). Macroconidia of *M. canis*; Fig. 4(b). Macroconidia of *M. gypseum*; Fig. 4(c). Macroconidia of *T. Mentagrophytes*; Fig. 4(d). Microconidia of *T. Mentagrophytes*]

Fig. 4. Macroconidia of *M. canis, M. gypseum* and *T. mentagrophytes*

Table 4. Antifungal sensitivity pattern of the isolated dermatophytes

Sl. No.	Antifungal drug	*M. canis* (n=77)		*M. gypseum* (n=18)		*T. mentagrophytes* (n=13)	
		S	R	S	R	S	R
1	Amphotericin B	28	49	12	6	7	6
2	Clotrimazole	43	34	10	8	9	4
3	Ketoconazole	47	30	11	7	5	8
4	Fluconazole	16	61	8	10	2	11
5	Itraconazole	41	36	11	7	10	3
6	Nystatin	52	25	9	9	8	5

DISCUSSION

Fungal dermatitis reported in veterinary patients could be either due to yeast or dermatophytes. Dermatophytes invade the keratinized tissues and degrade them. Incidence of dermatophytosis depends mainly on species of animal affected, virulence of dermatophyte species, contact with contaminated surfaces, immune status and age of the host (Moriello *et al*., 2017). Increase in the level of pro-inflammatory cytokines and cortisol also increases the development of infection in individual animals. Dermatophytosis is commonly reported in warm and humid climates since moisture and warmth are important in the pathogenesis of fungal organisms. The increased incidence reported in winter season followed by monsoon season in the present study might be due to the high environmental humidity during the period in Kerala. Increased presence of ectoparasites during this season can cause microtrauma, which could favour penetration and germination of spores (Debnath *et al*., 2016).

Animals of all age groups could be affected by this disease, and the higher incidence in young animals in the current study might be due to the deficiency of the fungistatic linoleic acid in young animals or due to a poorly developed immune system (Debnath *et al*., 2016). The reason for the difference in incidence in both sexes might be due to the variation in the composition of sebum in both sexes. Aggressive behaviour of male dogs may result in microtrauma on the skin, which might also lead to disease development (Malleswari *et al*., 2022). The difference in the incidence rate reported in various breeds worldwide may be due to the difference in the population presented for consultation and region-wise distribution of the dogs. In the present study, it was noticed that animals fed a commercial diet showed the lowest incidence. Marchegiani *et al*. (2020) suggested that a deficiency of vitamins, minerals, fatty acids and essential amino acids could alter skin structure and function, thus predisposing to various diseases. This deficiency is rare with standard commercial pet foods. Since most of our dogs are fed with an imbalanced homemade diet, disease development might be due to

Fig. 5. Antifungal sensitivity pattern

the deficiencies aroused. The presence of ectoparasites, especially ticks, was noticed in 12% of the animals. Ectoparasites can act as a carrier of spores from infected dogs. The optimal condition for dermatophyte infection can also be produced by the presence of microtrauma resulting from pruritus due to ectoparasites or any other causes. Moriello and Newbury (2006) and Zineldar *et al*. (2023) reported the presence of ectoparasites as a predisposing factor for the development of dermatophytosis.

Even though fungal culture is considered as the gold standard test for the confirmatory diagnosis of dermatophytosis in dogs, only 60% of the samples positive for fungal spores on trichogram yielded positive culture results in the present study. False positives and false negatives are common in fungal cultures. Scherer and Kinmon (2000) also reported a low correlation between the DTM culture results and positive microscopic results, which was attributed to the absence of a proper incubation environment. This disparity in results could also be due to the difference in the sampling site of the affected animal and may also be due to sampling from an animal undergoing antifungal therapy where there is a possibility for the

presence of dead spores (Moriello *et al*., 2017).

In the present study, there was a significant difference in the days taken for growth of the dermatophytes in both SDA and DTM, and also, there was a difference in the percentage of samples which had growth in DTM and not in SDA. Ninety-seven per cent agreement in the results obtained in DTM and SDA was reported when the incubation and storage instructions were strictly followed, and macroscopic morphology and microscopic characteristics were taken into account (Kaufmann *et al*., 2016). The absence of growth of the suspected samples in the media could be attributed to sampling differences or due to the sample obtained from an animal undergoing antifungal therapy. Statistically significant difference was also noticed in the growth of *M. canis* and *M. gypseum* in SDA and DTM, suggesting DTM as a better media for the culture of the dermatophytes. Dermatophyte test medium contains phenol red indicator. Dermatophytes which metabolize the proteins preferably produce an alkaline environment which changes the media colour to red from yellow before the saprophytes, and this provides a more reliable result than growth in SDA (Salkin *et al*., 1997; Kaufmann *et al*., 2016). Ease of interpretation and a faster turnaround time in comparison with other mycotic medias make DTM a better choice. Among the isolated dermatophytes, a greater percentage of *M. canis* were sensitive to nystatin (67.53%), *T. mentagrophytes* to itraconazole (76.92%) and *M. gypseum* to amphotericin B (66.67%). The difference in sensitivity pattern might differ depending on the difference in the commonly used topical preparations worldwide and development of resistance.

Conflict of interest: Authors have no conflict of interest in the present study.

Author's contribution: MKM, NMU, SA, AG, VR, SRS: Design, supervision and compilation; MKM: Performed the study and collected the data.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

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