Prevalence of dermatophytosis and *Malassezia* infection in dogs and cats in Thonglor Bangkok Pet Hospital in Ho Chi Minh City, Vietnam

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ARTICLE INFO

Research Paper

Received: September 04, 2023

Revised: September 25, 2023

Accepted: October 03, 2023

Keywords

ATI methods

Dermatitis

Dermatophytosis

Malassezia

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ABSTRACT

Malassezia yeasts are occasional human and animal skin organisms that commensally act as pathogens, while dermatophytes are common fungi in many clinics and hospitals. The aim of this study was to evaluate the prevalence of dermatophytosis and *Malassezia* infections in dogs and cats, and the effect of treatments. The study was carried out from December 2022 to May 2023 in Thonglor Bangkok Pet Hospital, Ho Chi Minh City, Vietnam. Total 208 cases visited the hospital, in which 32 cases of otitis and 53 dermatitis were recorded. Sterile cotton swabs were used to collect the aural samples from 32 cases, then observed under the microscope to detect the presence and population of *Malassezia* yeasts. In 53 dermatitis cases, adhesive tape impressions (ATI) methods and fungal culture were used to identify the species of dermatophytes and *Malassezia* dermatitis. The results showed that *Malassezia* was isolated from 24 cases, including 23 *Malassezia otitis* and 1 *Malassezia* dermatitis. However, there was only one positive case with dermatophyte by two methods. Therefore, dermatophytosis could not be concluded due to the small number of positive cases. Meanwhile, *Malassezia* infection occurred mainly in dogs rather than cats at every age, and the infected rates were found more in exotic animals than in domestic animals and usually in males than females. The effect of treatments illustrated the significant improvement in *Malassezia* otitis by using Epiotic solution combined with Oridemyl ear drop. Moreover, great improvement was evident in dermatophytosis therapy with Itraconazole.

Cited as: Nguyen, T. T., Dinh, K. N., & Nguyen, T. T. (2023). Prevalence of dermatophytosis and *Malassezia* infection in dogs and cats in Thonglor Bangkok Pet Hospital in Ho Chi Minh City, Vietnam. *The Journal of Agriculture and Development* 22(6), 42-54.

I. Introduction

Malassezia yeasts had been known common commensal organisms on the skin of humans and animals that could be secondary infections under various influences of predisposing factors (Cafarchia et al., 2005). In comparison, dermatophyte was a pathogenic keratinolytic fungus in animals and humans. The common *Malassezia* in companion animals was *Malassezia pachydermatitis*, and the common dermatophyte species were *Microsporum* and *Trichophyton* spp. (Begum & Kumar, 2021). Both diseases were recorded as highly risks of skin diseases in dogs and cats, which were commonly found in veterinary clinic. The diseases gradually became a special attention in public health, particularly in dogs and cats. Various diagnostic methods could be used in *Malassezia* infection and dermatophytosis, including cytological examination and fungal culture, such as cotton swab methods, adhesive tape impressions (ATI), and dermatophyte test medium (DTM) culture (Bouza-Rapti et al., 2023). *Malassezia* otitis could be detected through cotton swab methods and microscopic examination. While dermatophytosis could be suspected through different methods, such as Wood's lamp, ATI, and DTM culture (Moriello, 2019). Topical treatment, including Epiotic cleanser and Oridermyl ear drop, could be applied for *Malassezia* otitis, while systemic therapy with Itraconazole was used for *Malassezia* dermatitis and dermatophytosis. Therefore, the objective of this study was to determine the prevalence of dermatophytosis and *Malassezia* infections in dogs and cats, and evaluate the effect of treatments.

2. Materials and Methods

2.1. Determination of *Malassezia* infection and dermatophytosis cases in Thonglor Bangkok Pet Hospital

2.1.1. Clinical examination

In total of 208 cases visited the Thonglor Bangkok Pet Hospital from December 2022 to May 2023, 85 cases including dermatitis and otitis externa were collected through clinical examination. In which, 32 cases were detected with otitis externa, and 53 cases were detected with different kinds of dermatitis through clinical examination. Otitis external cases were collected based on the changes in ear pinna, including alopecia, excoriation, crusting, erythema, and hyperpigmentation. Other signs can be observed in the external ear canal, consisting of hyperemia, ulceration, discharge, masses, and stenosis (Bajwa, 2019). Otherwise, dermatitis cases were collected according to general clinical signs, such as pruritis, alopecia, erythema, crust, hyperpigmentation, and many other dermatological lesions in various areas, including lip margins, axilla, groin, ventral neck, interdigital skin, facial or tail folds, and other signs.

2.1.2. Cytological examination

Malassezia dermatitis and *Malassezia* otitis could be detected by using skin cytological examinations, including sterile cotton swabs and adhesive tape impression (ATI). With *Malassezia* otitis, a sterile cotton swab was rolled on a clean slide and stained with Diff-Quik solution for microscopic examination. While ATI was applied for *Malassezia* dermatitis by using transparent tapes to collect samples at the skin lesions suspected of fungi infection (Sudipa et al., 2021). Afterward, the samples were stuck on the slides, stained with a few drops of Giemsa or Diff-Quik solution, and observed under the microscope. *Malassezia* spp. could be identified through microscopic examination with the characteristic of a round to oval or classical peanut form with monopolar budding (Sudipa et al., 2021). The

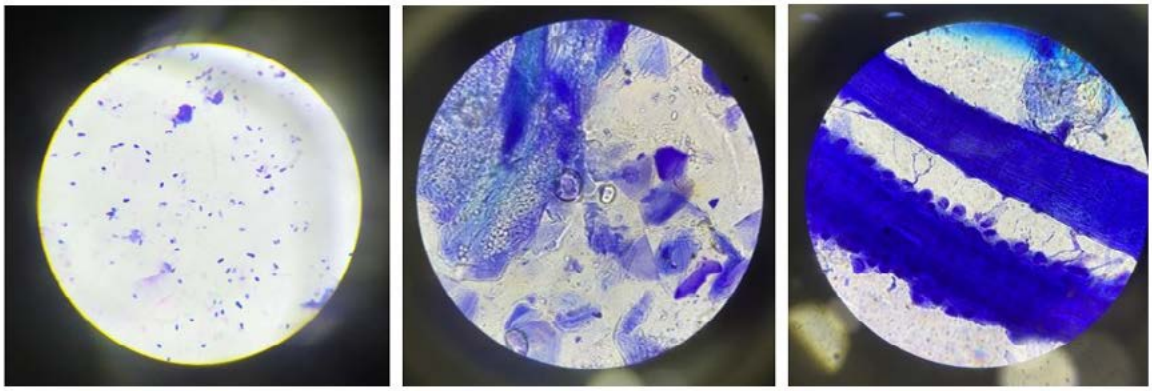


Figure 1. *Malassezia* and *Arthroconidia* were observed under the microscope.

result was identified to be positive if more than 5 *Malassezia* cells in one random field at 100x magnification; and the number of less than 2 *Malassezia* cells in one random field was detected in the negative result (Sudipa et al., 2021).

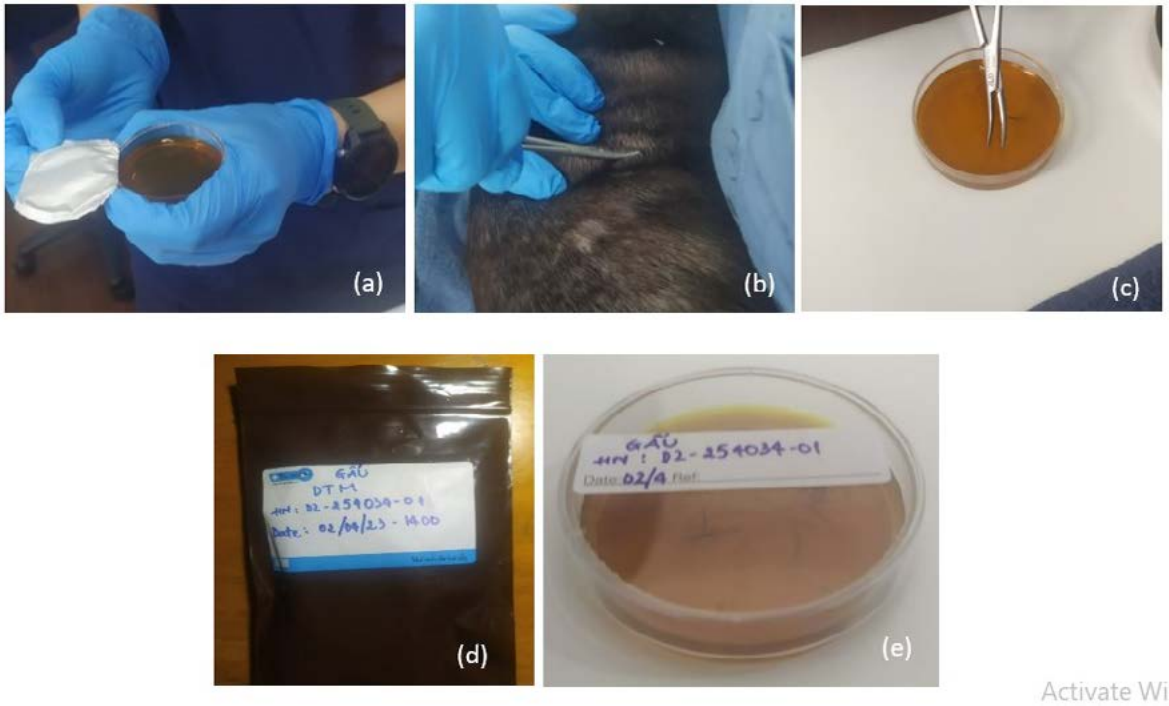
Dermatophytosis should be identified by rapid diagnostic tests for early treatment and prevention. At least two diagnostic tests were essential for dermatophyte detection, as no single test could be considered the "gold standard" (Bouza-Rapti et al., 2023). So the most common techniques used for dermatophyte identification were the hair plucking and fungal culture. And another method was ATI that was the valuable and sensitive diagnosis to detect dermatophytes in dogs and in cats with kerion for quick test (Bouza-Rapti et al., 2023). After collecting samples by impressing three times on the suspected lesions, then the slide was stained with a few drops of Giemsa or Blue Diff-Quik and observed under the microscope with 40x or 100x magnification to identify the presence of fungal hyphae, macroconidia, or arthrospores (Figure 1) (Begum & Kumar, 2021). Therefore, in Thonglor Bangkok Pet Hospital, both ATI technique and fungal culture have been applied to detect dermatophytosis in dogs and cats.

2.1.3. Fungal culture

The indirect examination for identification of dermatophytosis was fungal culture, which commonly used Sabouraud dextrose agar (SDA) or Dermatophyte test media (DTM) (Bouza-Rapti et al., 2023). The DTM was an innovative SDA adding cycloheximide, gentamycin, and chlortetracycline to prevent fungal and bacterial contamination (Kaufmann et al., 2016). At Thonglor Bangkok Pet Hospital, DTM was used to identify two species of dermatophytes *Microsporum* spp. and *Trichophyton* spp. The exact species needed to be re-check with microscopes for the positive or negative results. The medium should be kept at room temperature for 21 days, and the colony appearred within 7 - 10 days (Diren et al., 2019) within a 15-year period, in the city of Istanbul, Turkey. Dermatological specimens were collected from 1504 dogs and 846 cats, which were presented clinical signs of ringworm. Direct microscopy and mycological cultures were performed. The fungal growth rate was detected at 8.2% and 22.8% from dogs and cats, respectively. Microsporum canis was the most frequently isolated species followed by *Trichophyton* spp., *M. gypseum*, *T. mentagrophytes*, *M. nanum*, other *Microsporum*

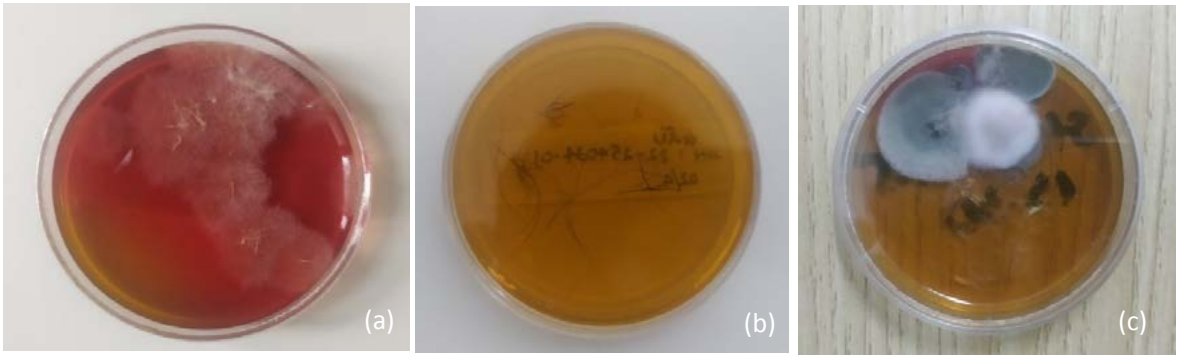
spp. moreover *T. tonsurans*. The cats less than two-year age and more than ten-year age showed a statistically significant higher isolation rate of infection ($P < 0.05$). There were four main steps in DTM culture diagnosis: (1) inoculate plates by plucking infected hair onto the surface in four or five areas, the infected hair can be collected through the assessment of Wood's lamp; (2) incubate the plates at 25°C to 30°C and store them in plastic bags to avoid dehydration; (3) monitor daily and assess the growth once weekly for contamination, Microsporum, or Trichophyton development; and (4) count the colonies on the plate when the pathogen was identified (Moriello, 2019) (Figure 2). The color of the DTM agar was changed from yellow to red, and the white or buff color colony grew on the agar when the result was positive (Jarjees & Issa, 2022) (Figure 3). However, the green or grey colony developing on the surface of the agar and changing the color of DTM to red would be due to contamination from other factors.

After observing the DTM agar daily, the colony growed on the agar after 7 to 10 days. Specific species of dermatophytes were identified by re-checking under the microscope through ATI method to collect the samples (Figure 4).

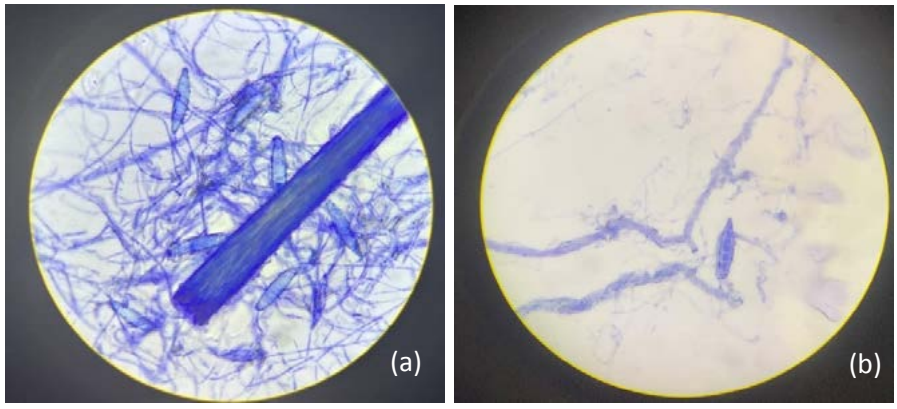


(a) DTM agar preparation, (b) Hair plucking, (c) Culture,
(d) Storage in dark plastic bag, (e) Observation from 7 to 10 days.

Figure 2. Procedure of Dermatophyte test media (DTM) culture.



(a) Positive result: the agar will change from yellow to red color,
(b), (c) Negative result: no change or contaminated colony on the agar.
Figure 3. Results of Dermatophyte test media (DTM) agar after 7 - 10 days.



(a) *Microsporum Canis* in day 6th, (b) *Microsporum Canis* in day 10th
Figure 4. The morphology of dermatophytes identified under the microscope.

2.2. Predisposing factors of *Malassezia* infection and dermatophytosis between dogs and cats in groups of ages, breeds, and gender

After the evaluation of identification of *Malassezia* infection and dermatophytosis through clinical and subclinical examination, the data were analyzed the risk factors associated with the diseases, including types of animals, groups of ages, breeds, and gender. Dogs and cats were the primary animals that collected the information in the research. The ages of animals were divided into three groups, including groups of age under one year, from one to three years, and over three years. The age groups were divided due to the susceptibility of the immunity at different ages, with various predisposing factors easily affecting the hosts. The breeds of animals were analyzed based on two groups, including domestic and exotic groups, because some exotic breeds were considered to be more common with *Malassezia* infections (Sudipa et al., 2021) and dermatophytosis (Moriello et al., 2017). The differences between males and females in dogs and cats contribute to the influence of *Malassezia*

infection and dermatophytosis (Negre et al., 2009; Paryuni et al., 2020).

2.3. Effectiveness of treatment

All cases with *Malassezia* otitis, *Malassezia* dermatitis, and dermatophytosis were treated with some specific medicines combined with others for clinical treatment. With *Malassezia* otitis, Epiotic cleanser and Orydemyl ear drops were applied to control and treat *Malassezia* infection. Dermatological problems with *Malassezia* and dermatophytes were controlled by cleaning with chlorhexidine and treated with oral Itraconazole. For *Malassezia* otitis, the frequency of Epiotic and Orydemyl was different depending on the severity of the otitis. The low pH of the acid components in most ear cleansers helped inhibit bacterial growth (Swinney et al., 2008). The high population of *Malassezia* in the ears was controlled and treated with Epiotic and Orydemyl twice daily. Mild cases were applied once a day. With *Malassezia* dermatitis, topical and systemic therapy could improve the clinical signs. Therapy should be continued for 7 - 10 days. Follow-up examinations were necessary after 3 - 4 weeks for evaluation of clinical responses (Bajwa, 2019) and cytological examinations again. The treatment for dermatophytosis included topical antifungal shampoos or cleansers combined with systemic antifungal treatments (Moriello, 2019). Topical treatment could be used for the whole body or local lesions. In this research, systemic antifungals applied in dogs and cats were Itraconazole (5 mg/kg) orally once daily for one week.

3. Results and discussion

3.1. Determination of *Malassezia* infection and dermatophytosis cases in Thonglor Bangkok Pet Hospital

Total 208 cases were examined for treatment in Thonglor Bangkok Pet Hospital from December 2022 to May 2023. In which, fifty-three cases showed skin disease clinical signs, making up 25.48% (Table 1). Compared with the survey of the prevalence, diagnosis, and treatment of dermatological conditions in small animals in general practice in the United Kingdom, the percentage of dermatological problems was 21.4% (Hill et al., 2006), which was nearly the same as our rate in this study. Otherwise, 32 cases were identified with otitis externa, comprising 15.38% of the total cases in Thonglor Bangkok Pet Hospital (Table 1). In comparison with the study of the frequency and predisposing factors for canine otitis externa was conducted on 905,554 dogs in the UK during 2016, our rate was higher than 7.3% cases in this research (O'Neill et al., 2021). The difference in the rate depended on the predisposing factors affecting the statistical proportion. Our investigation was conducted in District 2, where the life conditions were better for pets and animals; however, the rate of otitis externa and dermatological problems was still higher than it in other surveys in foreign countries. This result showed the risk of otitis externa and dermatological problems in our local area.

According to Table 2, 24 cases (11.53%) were positive with *Malassezia* infection, while 61 cases were negative with *Malassezia* spp., and 123 cases were diagnosed with other diseases. Our *Malassezia* infected rate was 11.53% which was much lower than 48.26% in the research of *Malassezia* spp. in the external ear canal of the healthy and otitis dogs and cats performed by Carfachia et al. (2005). Tyler et al. (2020) evaluated 129 *Malassezia* cases in 341 total cats in the study of otoscopy and aural cytological findings in a population of rescue cats and cases

Table 1. The rate of dermatitis and otitis external cases in Thonglor Bangkok Pet Hospital (n = 208)

	Numbers of cases	Percentage (%)
Otitis externa	32	15.38
Dermatitis	53	25.48
Others	123	59.13
Total cases	208	100

Table 2. The rate of *Malassezia* infection in Thonglor Bangkok Pet Hospital (n_1 = 32, n_2 = 53, n_3 = 123, n = 208)

	Results	Otitis externa (%) n_1 = 32	Dermatitis (%) n_2 = 53	Total cases (%) n = 208
Malassezia infection	Positive	23 (71.8)	1 (1.9)	24 (11.53)
	Negative	9 (28.2)	52 (98.1)	
Other diseases (n_3 = 123)		-	-	184 (88.47)

n_1: total of otitis externa cases, n_2: total of dermatitis cases, n_3: total of other diseases, n: total cases came to the hospital.

Table 3. The rate of dermatophytosis in Thonglor Bangkok Pet Hospital (n_1 = n_2 = 53, n_3 = 155, n = 208)

	Results	ATI n_1 = 53 (%)	DTM culture n_2 = 53 (%)	Total cases n = 208 (%)
Dermatophytosis	Positive	3 (5.7)	1 (1.9)	1 (0.48)
	Negative	50 (94.3)	52 (98.1)	
Other diseases (n_3 = 155)		-	-	207 (99.52)

n_1, n_2: total of dermatitis cases, n_3: total of other diseases, n: total of cases came to the hospital; ATI: adhesive tape impression; DTM: dermatophyte test media.

in a referral small animal hospital in England and Wales, made up 37.8% which is higher than our ratie in Thonglor Bangkok Pet Hospital. Our results illustrated the low prevalence of *Malassezia* infections at Thonglor Bangkok Pet Hospital. This difference depended on the number of cases, the research methods, environmental conditions, and various predisposing factors at different places.

The results of the diagnostic tests of 53 animals with dermatological problems were presented in Table 3. In 53 animals, 3/53 animals were positive with the ATI method. All 53 dermatological cases were collected the sample and performed DTM culture. After 10 days, the colony in DTM agar grew, then it was collected and observed under the microscope to evaluate the species of dermatophyte. The results showed that 1/53 cases was positive with both ATI method and DTM culture, which detected species was *Microsporum canis*. Thus, in a total of 208 cases in Thonglor Bangkok Pet Hospital, dermatophytosis constituted 1/208 cases (0.48%) resulting in a low rate of dermatophytosis in our hospital.

3.2. Predisposing factors of *Malasssezia* infection and dermatophytosis at Thonglor Bangkok Pet Hospital

The study showed that dermatophytosis rate in dogs was 100% and in cats was 0% (Table 4). According to the observed data, dermatphytosis was detected in dogs more than in cats. This could be due to the small quantity of dermatophytosis in dogs and cats identified in hospital. The rate of *Malassezia* infection recorded in dogs is 91.7%, higher than 8.3% in cats. Some research papers studied that *Malassezia* mainly occurred in dogs more than cats, while others had higher results in cats than in dogs (Cafarchia et al., 2005). Our data were suitable with much research and dependent on two factors as (1) good hygiene and the life-being of dogs and cats in the local area, and (2) frequent ear cleaning for dogs and cats for *Malassezia* prevention.

Dermatophytosis occurred in 1 dog over three years old, while the high percentage of *Malassezia* infection rounded up the cases from 1 to 3 years, constituting 45.8% of *Malassezia* cases (Table 4). Based on the observed data, dermatophytosis occurred in old age, and *Malassezia* infection occurred primarily from 1 to 3 years old. Dhoot et al. (2021) carried out the study of prevalence of *Malassezia* infection in dogs of Nagpur City recorded that the percentage of Malassezia infection under one year was 0.39%, from 1 to 3 years was 3.92%, and gradually increased infection to 49.8% from 3 to 9 years old, then decreased to 2.7% at the age above 12 years old. Our data from 1 to 3 years old were 45.8%, higher than 3.92% compared with the study above. Otherwise, our data of cases over three years old is 33.3%, relatively analogous with the data from the study above (12.94 - 49.8%) and other study performed by Sudipa in Badung, Balo with 40% of *Malassezia* infection in dogs above three years (Sudipa et al., 2021).

The dermatophytic infection occurring in exotic animals was one case and there were no cases in domestic animals. The percentage of Malassezia infection in domestic breeds was 25.0%, lower than 75.0% in exotic animals (Table 4). Based on the observed data, dermatophytosis and Malassezia infection occurred mainly in exotic animals. However, in survey duration, we recorded two factors affecting the data results as (1) the number of exotic dogs and cats that came to Thonglor Bangkok Pet Hospital was much higher than domestic dogs and cats, and (2) genetic factors related to immunity and sensitivity were lower in exotic dogs and cats. Compared with other research about breed

Table 4. Dermatophytosis and *Malassezia* infection in Thonglor Bangkok Pet Hospital

Factors n		Dermatophytes (n_1 = 1)		*Malassezia* infection (n_2 = 24)	
		%	n	%	
Animal	Dogs	1	100	22	91.7
	Cats	0	0	2	8.3
Age	< 1 year	0	0	5	20.8
	1 - 3 years	0	0	11	45.8
	> 3 years	1	100	8	33.3
Breed	Domestic	0	0	6	25.0
	Exotic	1	100	18	75.0
Gender	Male	1	100	13	54.2
	Female	0	0	11	45.8

factors in dermatophytosis and *Malassezia*, Moriello et al. (2017) noted that 75.0% of Persian cats had just four cases diagnosed with dermatophytosis, and 23.6% of Yorkshire Terrier dogs were identified as being predisposing commonly to dermatophytosis. *Malassezia* infection could occur in dogs and cats of any age, breed, and sex. However, some dog breeds were identified to be predisposed, including American cocker spaniels, West Highland white terriers, basset hounds, poodles, and Australian silky terriers, while commonly in Sphynx and Devon Rex cats (Sykes et al., 2014). So our data were suitable with some studies that dermatophytosis and *Malassezia* could occur mainly in exotic breeds.

Both dermatophytosis and *Malassezia* infection occurred in males more than in females, which were 100% and 54.2% compared with 0% and 45.8%, respectively. Jarjees & Issa (2022) investigated the first study on molecular epidemiology of dermatophytosis in cats, dogs in the Kurdistan region of Iraq, the prevalence of dermatophytosis in male cats was higher than in female cats, 47.6% compared with 41.4% with no statistical significance, while this percentage was the same in male and female dogs. Dhoot et al. (2021) showed that *Malassezia* infection in males was 60% against 40% in females. Our data was relatively like the data collected in the study. However, Nardoni et al. (2004) in the study of occurrence of *Malassezia* species in healthy and dermatologically diseased dogs said that the prevalence of *Malassezia* infection between male and female were nearly the same. However, our data showed that dermatophytosis was only one case in males and zero in females, with a percentage of 100% compared with 0%. At the same time, the rate of *Malassezia* infection in males was also higher than in females.

3.3. Result of effectiveness in treatments

The effectiveness of Epitotic and Oridermyl ranged from good (1 - 2 weeks) to intermediate (2 - 4 weeks) response (Table 5). The efficacy of the treatment was recorded based on the recovery of clinical signs after 7 - 10 days and re-examination under the microscope. In 23 cases of *Malassezia* otitis, Epiotic played a significant role in improving all otitis cases. Compared to Swinney et al. (2008) in the study of the comparative in vitro antimicrobial efficiency of commercial ear cleaners, their results were recorded with the improvement of otitis in 16/31 dogs used Epiotic, which was effective in more than 50% of *Malassezia* otitis cases. Another study from Roy et al. (2012), they carried out with comparative short-term efficacy of Oridermyl auricular ointment and Revolution selamectin spot-on against feline otodectes cynotis and its associated secondary otitis externa, the results identified that 18/24 cases evaluated the number of *Malassezia* from day 0 to day 10 using Oridermyl. Our results were much better than these research.

Table 5. The effectiveness of treatments

Medication	Infection	The effectiveness of treatments	
		Time of treatment	n
Epiotic cleanser + Oridermyl	*Malassezia* otitis (n=23)	1 - 2 weeks	19
		2 - 4 weeks	4
		> 4 weeks	0
Itraconazole	*Malassezia* dermatitis	7 - 8 weeks	1
	Dermatophytosis	7 - 8 weeks	1

With *Malassezia* dermatitis and dermatophytosis, Itraconazole was used to treat fungal infections. The results illustrated the cases detected with *Malassezia* dermatitis and dermatophytosis. The effectiveness of these therapy was evaluated due to the improvement of the clinical signs after four weeks and recovered until 7 - 8 weeks (Table 5). According to Moriello (2019), the study of dermatophytosis in cats and dogs, and the practical diagnosis and treatment, the systemic therapy should follow during 8 weeks until clinical resolution of lesions.

4. Conclusions

During this study, the *Malassezia* infection occurred mainly in dogs rather than cats at every age. It was also found more in exotic animals than in domestic animals and usually in males than in females. The effectiveness of treatment illustrated the significant improvement in *Malassezia* otitis when using Epiotic solution combined with Oridemyl ear drop. Moreover, great improvement was evident in dermatophytosis therapy with Itraconazole. Due to low number cases of dermatophytosis, the predisposing factors of this fungus could not be evaluated.

Conflict of interest

The authors have no conflicts of interest to declare.

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