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Research article

Types of dermatophyte on rabbit skin in rabbit cafés in Chiang Mai province

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Abstract

Dermatophytosis or ringworm is a superficial cutaneous infection caused by fungi of the genera *Microsporum*, *Trichophyton and*, *Epidermophyton*. It also has a zoonotic characteristic which can transmit skin diseases between animals and human. While dermatophytes have been investigated extensively in several animal species, the information specifically on rabbits is limited. The aim of this study was to identify species of dermatophytes in rabbit cafés in Chiang Mai. A total of 66 hair samples was collected from rabbits in 3 cafés using hairbrush diagnosis technique. Sabouraud's Dextrose agar plates containing cycloheximide and chloramphenicol were used as a fungal culture. Types of fungi were identified based on colony characteristics and fungal morphology observed under light microscope. Dermatophytes were detected in 8 from 66 hair samples (12.11%) composed of *Microsporum gypseum* (7.58%), *Trichophyton mentagrophytes* (3.03%), and *Trichophyton verrucosum* (1.51%) which were isolated from both rabbits with healthy skin and those with dermatological problems. To prevent transmission to human, good hygiene management in cafés are suggested.

Keywords: : Dermatophyte, *Microsporum gypseum*, Rabbit, *Trichophyton mentagrophytes*, *Trichophyton verrucosum*

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INTRODUCTION

Dermatophytes are fungi belong to Ascomycota division which commonly cause skin disease in both animals and human. These anamorphic mold composed of 3 important genera: *Microsporum*, *Trichophyton* and *Epidermophyton*, which are distributed worldwide (Rippon, 1988). These fungi obtain nutrients mainly from keratin (Powers, 2000) to grow and multiply themselves in the stratum corneum of skin, hair, and nails (Midgley, Moore Mk Fau - Cook, Cook Jc Fau - Phan, & Phan, 1994). The severity of symptoms of an infected animal depends on the fungus species and its strain (Powers, 2000). Skin diseases caused by dermatophytes such as *M. canis*, *M. gypseum*, and *T. mentagrophytes* are common in many companion animals such as dogs, cats (Chupia, 2010), especially in dogs with the most common species being *M. canis* (Dai-Jun et al., 2012). Beside those animals mentioned before, other mammals such as ruminants and horses have also been reportedly infected by dermatophytes (Dey et al., 2016; Maurice et al., 2016).

As pet rabbits became popular, business involving rabbits such as rabbit farms and rabbit cafés have been growing rapidly. Forming a large population in short period without knowledge on the species and proper environment, they can easily catch diseases. Skin disease, which are frequently found in domestic rabbits, can be caused by parasites, fungi, bacteria or viruses (White, Bourdeau, & Meredith, 2002). Although skin disease is commonly caused by fungi, it is not as much as occurred in healthy rabbits. Dermatophytes can be found in juvenile or immunocompromised ones, and they can be transmissible to human.

Trichophyton spp. and *Microsporum* spp. are the main cause of fungal skin diseases in rabbit, with *T. mentagrophytes* being the most common. *M. canis*, *M. gypseum*, *M. audouinii*, *T. verrucosum*, and *T. schoenleinii* can cause infection, but young rabbits are at less risk. Animals infected by these fungi usually show lesions of alopecia, red skin, and yellowish crusting, which are often seen on the head area. Rabbits infected with *T. mentagrophytes* may show no symptoms at all (Powers, 2000), but they can still be important sources of transmission to other animals and human. The transmission from rabbits to human may be from whether direct or indirect contact via infected rabbit's hair or scaly skin.

Fungal skin disease can be generally found worldwide, with outbreaks in developing countries that have begun to face health management issues. Therefore, understanding the epidemiology of fungal skin disease in rabbits will play an important part on reducing its incidence in both human and rabbits (Dai-Jun et al., 2012). There have been many studies on fungal skin disease in various species, nevertheless epidemiological studies about the disease formation in rabbits are limited (Cafarchia et al., 2010). Of all the epidemiological studies reporting fungal skin disease in rabbits, none of them concerned Thailand. Other studies reported fungal skin disease in a cat farm and animals from animal hospital in Bangkok (Niyomtham et al., 2011). Three coffee shops that have been feeding rabbits outdoor (rabbit café) might be the sources of fungal skin disease transmission between rabbits and human. The aim of this study is to investigate types of fungus causing skin disease in rabbits that live in rabbit cafés in Chiang Mai, Thailand.

METERIALS and METHODS

Animals

Hair of 66 mixed breed rabbits (31 males and 35 females, all were older than 2 months) were collected from 3 rabbit cafés (RC) in Chiang Mai, Thailand. All procedures related with animals in this study were approved by Animal Ethic Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (S15/2560) (Permission number U1-00630-2558).

Sample selection

Convenience sampling was applied to select 40 to 60 percent of rabbit population from each rabbit café. Rabbit hair samples were collected using Mackenzie brush technique. Twenty five samples were collected from the first café (RC1) while 19 and 22 samples were collected from second café (RC2) and third café (RC3) respectively.

Data and sample collection

We gathered each rabbit's individual data such as age, sex, weight, lesion characteristics, preliminary health checks, vaccination history, etc. As animal rearing and management may be predisposing factors for fungal skin disease, general information about rabbit rearing and management were also inquired (e.g. frequency of soil contact, rabbit cleaning, and contact with the customers).

Hair-brushing was carried out more than 1/3 of the body, covering the head, ears, body and feet using hairbrush diagnosis technique (Mackenzie, 1963) by a 1x2 centimeters sterile toothbrush with 1 centimeter of bristle. The collection was performed in 2 areas; where there were skin lesions and where there were none. Skin with lesions was cleaned with 70% alcohol before collecting a sample to prevent bacterial decontamination (Loewenthal, 1961). Labeled samples and brushes were then kept in a sterile plastic bag at room temperature. Gloves worn by the collector were changed with each individual collection. The samples were cultured within 24 hours after collection (Moriello, 2001).

Fungal identification

The samples were cultured on Sabouraud's Dextrose agar (SDA) supplemented with cycloheximide 0.05 g/l and chloramphenicol 0.5 g/l and incubated at 25°C for 7 to 10 days before performing the culture slide (Vanittanakom, 1999; Sutapaha, 2008).

Types of fungi were identified by macroscopic examination using criteria such as colony morphology, color, colony diameter, and color changing of the agars. Any plate that showed more than 1 type of fungi; the colonies will be collected, subcultured, and incubated for 7 to 10 days.

Microscopic examination was performed by using lactophenol cotton blue. We examined fungal morphology under light microscope at 100X and 400X magnification. Size and shape of fungal hyphae, microconidia, and macroconidia were noted and identified by using laboratory identification of pathogenic fungi as a reference (Sutapaha, 2008).

Statistical analysis

Descriptive statistics analysis was applied in the study to estimate the percentage of fungi detected from the hair. Then, classified them by fungi strain, source, and other factors such as age, gender of rabbit, etc.

Chi-square statistics, with significance at $P \leq 0.05$, was used to analyze the relationship between fungus and predisposing factors (age, sex, location, frequency of contact with soil, rabbit cleaning, and rabbits being touched by customers).

RESULTS

Data of 66 rabbits collected from 3 rabbit cafés was divided into 3 categories based on location (Table 1). To classify a juvenile and adult rabbit depends on rabbit's breed; generally rabbit is fully grown at the age of 5 to 8 months. Since café's owner could not provide an accurate information, data collection concerning rabbit's age was difficult. Thus we roughly divided rabbit into two group with one year old as a benchmark. We divided sample groups into two according to an appearance of skin lesions. Any rabbit with hyperemia and/or alopecia was categorized into a group with skin lesion (Figure 1) as well as rabbit without any skin lesion into another group. The body cleaning of rabbit in each café was related to any hygienic procedure on the animals such as hair brushing or bathing. Environmental disinfection is the application of any disinfectant on surface where rabbits were raised or kept.

Fungal identification

Dermatophytes were found in 8 out of 66 samples (12.12%) from 3 rabbit cafés, comprising 5 out of 25 samples (20.0%) in RC1, 1 out of 19 samples (5.26%) in RC2, and 2 out of 22 samples (9.09%) in RC3 (Table2).

From macroscopic and microscopic examination: *M. gypseum* was found in 3 samples collected from RC1 and 2 samples from RC3. *T. mentagrophyte* was found in each sample from RC1 and RC2. While *T. verrucosum* was only identified in 1 sample from RC1 (Table 3, Figure 2).

Using Chi-square statistic analysis to interpret the relationship between factors inducing fungal skin disease, 5.3% of rabbits under one year old and 14.9% of rabbits over one year old were infected with dermatophytes. The infection in male and female rabbits was 16.1% and 8.6%, respectively. Fifty percent of rabbits with dermatophyte infection displayed skin lesions, whereas 8.3% of such rabbits showed none ($P \leq 0.05$) (Table 4).

Table 1 Data of rabbit hair samples distribution.

Data		Location		
		RC1 (n=25)	RC2 (n=19)	RC3 (n=22)
Age				
	<1 year	12	6	1
	>1 year	13	13	21
Sex				
	Male	15	11	5
	Female	10	8	17
Lesion				
	Yes	1	3	2
	No	24	16	20
Body cleaning				
	Yes	25	0	0
	No	0	19	22
Environmental disinfection				
	Yes	25	0	15
	No	0	19	7
Ground contact				
	Yes	25	19	7
	No	0	0	15
Human contacted				
	Yes	25	0	7
	No	0	19	15

RC = rabbit café, n = number

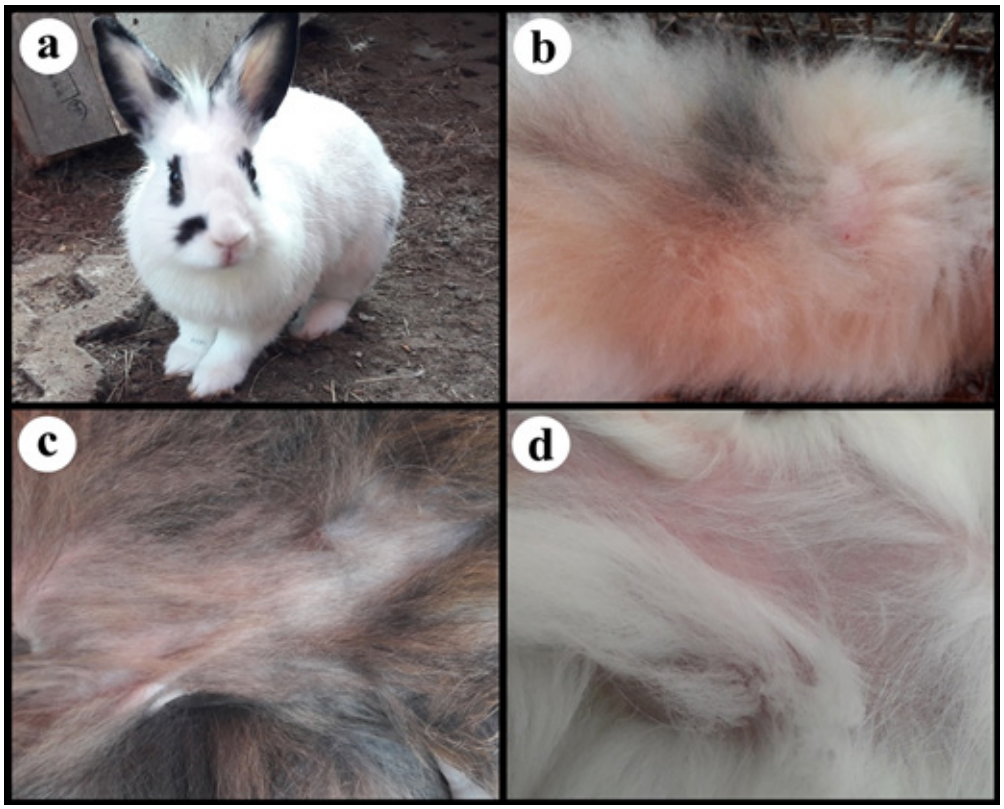

Figure 1 Rabbit without skin lesion (a) and a skin lesion of rabbit (b, c, d). Hyperemia skin (b), alopecia (c) and alopecia with mild hyperemia skin (d).

Table 2 Number and percentage of rabbit hair samples positive for dermatophytes.

Rabbit café	Number of samples	Number of positive samples	Percentage of positive samples
RC1	25	5	20.00
RC2	19	1	5.26
RC3	22	2	9.09
Total	66	8	12.12

Table 3 Distribution of dermatophytes isolated from rabbit hair samples.

Dermatophytes	Number of positive samples			Number of samples	Percentage
	RC1	RC2	RC3		
M. gypseum	3	0	2	5	62.5
T. mentagrophytes	1	1	0	2	25.0
T. verrucosum	1	0	0	1	12.5
Total	5	1	2	8	100.0

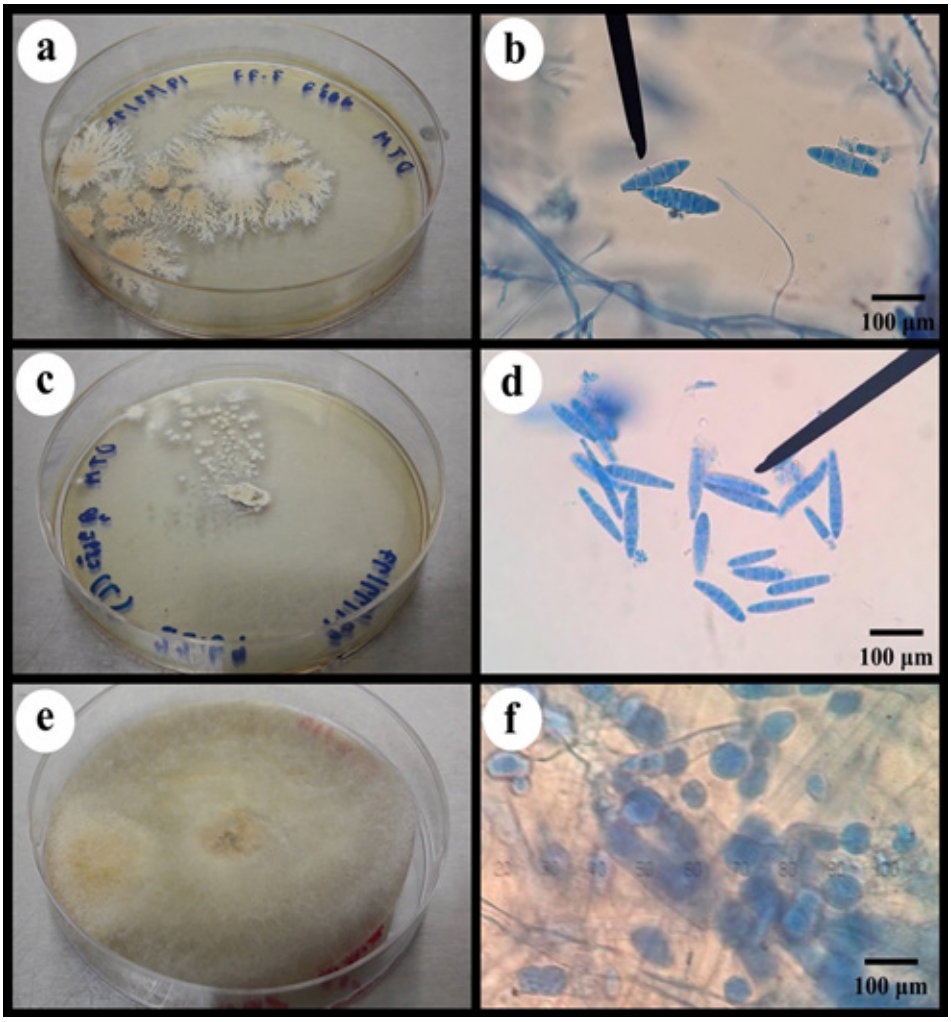

Figure 2 Fungal macroscopic (a,c,e) and microscopic (b,d,f) findings: a-b = *Microsporum gypseum*, c-d = *Trichophyton mentagrophyte*, and e-f = *Trichophyton verrucosum*.

Table 4 Numbers and percentages of rabbits that were positive for dermatophytes between different variables.

Variables		Numbers of positive samples (%)	P-value
Age			0.28
	<1 year (n=19)	1 (5.3%)	
	>1 year (n=47)	7 (14.9%)	
Sex			0.35
	Male (n=31)	5 (16.1%)	
	Female (n=35)	3 (8.6%)	
Lesion			0.01
	Yes (n=6)	3 (50.0%)	
	No (n=60)	5 (8.3%)	
Place			0.29
	RC1 (n=25)	5 (20.0%)	
	RC2 (n=19)	1 (5.3%)	
	RC3 (n=22)	2 (9.1%)	
Body cleaning			0.12
	Yes (n=25)	5 (20.0%)	
	No (n=41)	3 (7.3%)	
Environmental cleaning			0.37
	Yes (n=40)	6 (15.0%)	
	No (n=26)	2 (7.7%)	
Ground contact			0.46
	Yes (n=51)	7(13.7%)	
	No (n=15)	1 (6.7%)	
Human contacted			0.11
	Yes (n=32)	6 (18.8%)	
	No (n=34)	2 (5.9%)	

From data regarding location factor, the maximum percentage of rabbits with dermatophyte infection was 20% in RC1, following by 9.1% in RC3 and 5.3% in RC2. When the factors of management were compared such as animal cleaning systems, environment management, and frequency of soil and customer exposure; rabbits in RC1 were exposed to soil and customers yet were often brushed clean and the environment was hygienic. The rabbits in RC2 exposed to soil, though did not contact with customers, their hair and environment were not properly cleaned. Fifteen rabbits in RC3 stayed in clean cages and did not expose to soil, whereas 7 rabbits were exposed to soil. Their hair and environment were not cleaned accordingly.

These results showed that 20.0% of the rabbits resided in rabbit café were infected with dermatophyte despite being cleaned, whereas 7.3% of the rabbits were infected, despite not being cleaned. Rabbits with ground contact (13.7%) were infected with dermatophyte, while 6.7% of those not exposed to soil were infected. In cafés where customers were allowed to touch the rabbits, 18.8% of the rabbits suffered from dermatophyte infection whereas cafés that did not allow human contact, only 5.9% were infected (Table 4).

DISCUSSION

All cafés investigated in this study possessed rabbits infected with dermatophyte. *M. gypseum* was the most common infection in these rabbits, following by *T. mentagrophytes* and *T. verrucosum.*

In accordance with previous studies (Khosravi & Mahmoudi, 2003), *M. gypseum* was the most typical infection found in the rabbits. This type of fungus can commonly grow in a geophilic environment, where the soil is its primary source (Chermette, Ferreiro L Fau - Guillot, & Guillot, 2008). Therefore, rabbits contact or raised on ground can be infected with this organism. This study also discovered that *T. mentagrophytes* was the second most common infection in rabbits, unlike previous studies, as the most common strain (Cabañes, Abarca, & Bragulat, 1997). It was also the main source of infection in zoophilic species detected in animals (Chermette et al., 2008). Prevalence of fungal skin disease varies by geographical location (Cabanes et al., 1997; Cafarchia et al., 2010; Dai-Jun et al., 2010; Khosravi and Mahmoudi, 2003), and previous studies usually found this type of fungus in experimental rabbits and rabbit cafés (White et al., 2002). The least common fungus identified in this study was *T. verrucosum*, which is accordant to Donnelly et al. (2000), indicating its low occurrence in rabbits.

Using statistical analysis to identified factor correlated with skin fungal disease prevalence in rabbits, skin lesion was the statistically significant factor associating with fungal skin disease in rabbits. This is consistent with previous studies that 71.7% of rabbits with skin lesions were infected with fungus (Cafarchia et al., 2010).

In addition, factors related to management could be associated with fungus detection. The majority of rabbits in RC1 was infected with pathogenic fungi, which may be the result from different management; for example, contact with soil, hair brushing, and exposure to customers. Using the same brush on different rabbits or handling from customers could spread fungal skin diseases. Therefore, clean brushes for use on individual rabbits are on the list of options together with clean cages and rearing premises that may help to reduce fungal skin disease. In a previous study, applying vaporization procedures (enilconazole or sulfur and/or iodine compounds and/or potassium peroxymonosulfate) more than once per week, can reduce fungal skin disease (White et al., 2002). Thus, rabbit cafés should provide hand washing areas for customers before and after touching rabbits.

Pathogenic fungi were detected in rabbit cafés that allowed their customers to touch the rabbits. These fungi may cause zoonosis in human via direct contact with infected rabbit's skin, scales or hair. Rabbits that recovered from fungal skin disease and those infected without skin lesions, may be important pathogen carriers to other animals and human.

However, this trial was not designed to seek factors associated with fungal skin disease directly; there were limitations in sample size and timing to perform the research. Further studies should be designed with more samples and other relevant factors such as temperature, humidity, other pets cohabited in the cafés, samples from customers, and from the environment where rabbits live. This would enable to analyze risk factors correlated with fungal skin disease and create suggestions on how to reduce infection risk.

CONCLUSION

Fungal skin disease in rabbits can be transmitted to humans by both direct and indirect contact. Hence, people who touch or get close to rabbits may be vulnerable to infection. This study is only a preliminary information that can be utilized for study of skin fungal types, factors associated with fungal skin disease in rabbits, as well as a contribution to suggest protection practice and management against transmission of the disease. The advantage of this study was to obtain data that will be profitable for controlling and preventing the disease dispersion in both human and rabbits.

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