



ORIGINAL RESEARCH

ISOLATION OF DERMATOPHYTES FROM INFECTED STRAY DOGS IN SELANGOR, MALAYSIA

Isolasi Dermatofita dari Anjing Liar yang Terinfeksi di Selangor, Malaysia

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ABSTRACT

Background: Dermatophytosis is one of the most common skin diseases that affects cats and dogs. Geographic factors play an important role in determining prevalence, showing high rates of prevalence in warm and humid environments. **Purpose:** The aim of this study was to determine the macroscopic and microscopic identification of different types of dermatophytes present on stray dogs. **Methods:** The design of this study was laboratory-based research. Each sample was collected from an infection site (nail, hair, or skin) that was identified by conducting a physical examination of a stray dog that was infected with fungi. The skin scraping, nail clipping, and fur cutting samples were collected from infected dogs and then cultured on Sabouraud Dextrose Agar (SDA). The cultures were incubated at 26°C for five weeks. The isolates of fungi were then examined macroscopically and microscopically. The Lactophenol Cotton Blue (LPCB) staining technique was used for fungi morphology identification. **Results:** Overall, the most common type of dermatophytes that affected the dogs were *Trichophyton spp.* (64.70%), *Aspergillus spp.* (10.10%), *Microsporum spp.* (7.20%), and *Curvularia spp.* (5.60%). **Conclusion:** This study revealed the most common dermatophyte infections found on stray dogs in Selangor, Malaysia. This study can assist investigators in understanding the prevalence of the dermatophyte burden in stray dogs and help prevent further complication, such as the spread of illness, especially zoonotic infection.

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ABSTRAK

Pendahuluan: Dermatofitosis adalah salah satu penyakit kulit yang sering menyerang kucing dan anjing. Faktor geografi juga berperan dalam menentukan prevalensi yaitu prevalensi lebih tinggi pada daerah yang hangat dan lembap. **Tujuan:** Penelitian ini bertujuan untuk mengidentifikasi perbedaan tipe dermatofita pada anjing liar baik secara makroskopis maupun mikroskopis. **Metode:** Desain dari studi ini adalah penelitian berbasis laboratorium. Sampel ditentukan dengan mengevaluasi hasil pemeriksaan fisik dari anjing liar yang terinfeksi jamur dan dipilih berdasarkan situs infeksi (kuku, bulu, dan kulit) anjing terinfeksi. Sampel potongan kulit, potongan kuku, dan potongan bulu dikumpulkan dari anjing terinfeksi dan dibiakkan pada Sabouraud Dextrose Agar (SDA). Biakan diinkubasi pada suhu 26°C selama 5 minggu. Isolasi memeriksa fungi secara makroskopis maupun mikroskopis. Fungi diwarnai menggunakan teknik Lactophenol Cotton Blue (LPCB) untuk mengidentifikasi struktur morfologi. **Hasil:** Secara keseluruhan tipe dermatofita yang sering menyerang anjing adalah *Trichophyton ssp.* (64.70%), *Aspergillus spp.* (10.10%), *Microsporum spp.* (7.20%), diikuti oleh *Curvularia spp.* (5.60%). **Kesimpulan:** Penelitian ini mengungkap infeksi dermatofita pada anjing liar di Selangor Malaysia. Penelitian ini dapat membantu peneliti untuk memahami prevalensi dermatofita pada anjing liar dan mencegah komplikasi lebih lanjut seperti penyebaran penyakit terutama pada infeksi zoonosis.

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INTRODUCTION

Dermatophytes are one of the most common skin diseases of pets and livestock. Transmissibility among animal communities, public health consequences, high cost of treatment, and difficulty of control measures of animal ringworm explain the importance of these diseases. Ringworm is an important skin disease because it is contagious, infectious, and can be transmitted to people. Dermatophytes in living hosts usually remain in superficial tissues such as the epidermis, hair, and nails. Serious consequences are uncommon, and infections can be self-limiting; however, the illness may be disfiguring and uncomfortable, especially when the lesions are widespread. Infrequently, dermatophytes may invade subcutaneous tissues and very rarely invade other sites, especially in immuno-compromised hosts (K. A. Moriello, Coyner, Paterson, & Mignon, 2017).

The dermatophytes will be transmitted by close direct contact with infected animals or contaminated fomites including arthropods, such as houseflies, and the hair coats of animals. Dermatophytes are subdivided into three main groups: *geophilic*, *zoophilic*, and *anthropophilic*.

Geophilic is a type of dermatophyte or microorganism from soil. *Microsporum gypseum* is a type of geophilic dermatophyte that most commonly infects dogs or cats. *Microsporum gypseum* is widespread in warm, humid, tropical and subtropical environments. *Zoophilic* dermatophytes are found in animal hosts and are rarely found in soil. This type of dermatophyte affects almost 60% dogs, compared to cats with almost 90% (Abdalla, Wisal, 2018). *Anthropophilic* dermatophytes are adapted to human hosts and cannot survive in soil. Species included in this type are *Microsporum audouinii*, *Trichophyton tonsurans*, *Trichophyton rubrum*, and *Epidermophyton floccosum*. This species rarely infects dogs or cats that have a history of close contact with infected humans (K. A. Moriello et al., 2017).

The disease burden of chronic-relapsing and therapy-refractory superficial dermatophytosis has dramatically increased in India within the past 5–6 years. A study by Gupta et al (2016) showed that 149 out of the 201 samples (74.13%) showed a dermatophyte-positive culture result. Out of this, 138 (92.62%) samples were identified as

Trichophyton (T.) mentagrophytes and 11 (7.38%) as *Trichophyton rubrum*.

Tinea capitis is a lesion on the scalp that is divided into two parts, which are ectothrix, with follicular destruction, and arthroconidia formation on the outside of hair shafts. This form is often caused by zoophilic species such as *M. canis*, *M. gypseum*, *T. equinum*, and *T. vericosum*. Endothrix arthroconidia occurs within the hair shaft. This form is caused by anthropophilic species and favus a crusting form of scalp dermatophytosis caused by anthropophilic strains (Hay, 2017).

Dermatophytic infections are very common worldwide with incidence rates increasing every day because of the increasing coexistence of humans and pets. *Microsporum canis* is the most frequent cause of infection in domestic animals with a high risk of transmission to humans. Dermatophytosis is one of the most frequent skin diseases in dermatologic observance in México. It can affect any sex, age, and any part of the body (Rodríguez, Guzmán, García, Asz-Sigall, & Arenasa, 2018). Dermatophytosis is also the most common pet associated and occupational zoonotic disease, where in the United States almost 2 million people are infected each year due to contact with animals. Dermatophytic infections are common in pediatric and geriatric patients due to decreased host immunity. Diagnosis is easy if performing a good clinic history; a mycological examination is the gold standard because it is quick and cheap (Sahni, Singh, & Dogra, 2018).

Dermatophytosis is a contagious zoonotic disease, the most common mycosis in dogs and cats in the western hemisphere, and can be transmitted to humans (Minarikova, Hauptman, Jeklova, Knotek, & Jekl, 2015; Pasquetti, Min, Scacchetti, Dogliero, & Peano, 2017). Dermatophyte species are classified into three genera: *Epidermophyton*, *Microsporum* and *Trichophyton* (Hayette & Sacheli, 2015). It has been reported that the prevalence of infection varies depending on temperature, humidity, season, and geographical area (Magdum et al., 2016). Although in many countries the epidemiology of dermatophytosis from dogs is well understood, studies of the dermatophytes on stray dogs are limited, especially in Malaysia. Therefore, the aim of this study was to determine the macroscopic and microscopic identification of different types of dermatophytes present on stray dogs.

METHODS

This research was conducted from January to May 2019 in Selangor, Malaysia. Designed as a descriptive study based on laboratory research, samples were collected from an infection site (nail, hair, or skin) as determined by conducting physical examinations of stray dogs which were infected with fungi. Direct physical examination was conducted using a random sampling method.

Sabouraud Dextrose Agar (SDA) was used and prepared using 300 petri dish plates to culture 100 samples. The SDA was prepared by mixing 65 g of SDA powder into 1,000 ml of distilled water. The mixture was autoclaved and poured onto the petri dish under the flame to avoid contamination. The SDA mixtures were then kept in the chiller below 4°C for 24 hours for future use. The samples were cultured on SDA and were incubated at 26°C for two weeks to allow for sample growth. After the incubation period, the samples were stained using the Lactophenol Cotton Blue wet mount technique. Dermatophyte species are known to support gross and microscopic morphology. The macroscopic fungi identification was done by identifying the fungi colony, color, and texture. Microscopic identification was done using a light microscope to identify the fungi morphology.

The collected data were grouped and analyzed univariably. The univariable analysis was displayed in descriptive form with frequency tables. The data were then interpreted and compared with the existing theories, standards, guidelines, and provisions.

RESULTS

A total of 306 samples, which consisted of 102 samples each of nail, skin, and fur were obtained. The direct microscopy showed 63 positive sample results for *Trichophyton spp.*, 11 positive sample results for *Aspergillus spp.*, and 10 positive sample results for *Microsporum spp.* on a stray dog's hair. Almost similar prevalence was demonstrated on a stray dog's nails and skin.

Table 1 shows the type of dermatophytes that are present on the dog's fur. The most common type of dermatophyte was *Trichophyton spp.* which appeared in 63 out of 102 samples (62%). *Trichophyton spp.* was the highest type of dermatophyte isolated from dog fur. The lowest type of dermatophyte present on dog fur was *Bipolaris spp.* which only appeared in 1 out of 102 samples (1%). The dermatophytes that were

isolated on the dog's nail also showed *Trichophyton spp.* as the most common dermatophyte isolated with 70 out of 102 samples (68.60%). Similar statistics were demonstrated on dog skin with *Trichophyton spp.* being the dominant dermatophyte isolated from infected skin with 65 out of 102 samples (63.70%), followed by *Aspergillus spp.* (10%), *Curvularia spp.*, and *Microsporum spp.* (6%).

Table 1

Type of Dermatophytes Isolated from Stray Dog's Hair, Nail, and Skin in Selangor, Malaysia

| Type of Dermatophytes | % |
|--------------------------|-------|
| Stray Dog's Hair | |
| Bipolaris | 1.00 |
| Trichoderma | 3.00 |
| Absidia | 3.00 |
| Fusarium | 3.00 |
| Alternaria | 3.00 |
| Curvularia | 5.00 |
| Microsporum | 10.00 |
| Aspergillus | 11.00 |
| Trichophyton | 63.00 |
| Stray Dogs's Nail | |
| Bipolaris | 1.00 |
| Trichoderma | 1.00 |
| Absidia | 1.00 |
| Fusarium | 1.00 |
| Alternaria | 1.00 |
| Curvularia | 2.90 |
| Microsporum | 5.90 |
| Aspergillus | 5.90 |
| Trichophyton | 10.70 |
| Stray Dog's Skin | |
| Trichoderma | 1.00 |
| Bipolaris | 1.00 |
| Epidermphyton | 3.00 |
| Alternaria | 4.00 |
| Absidia | 5.00 |
| Microsporum | 6.00 |
| Curvularia | 6.00 |
| Aspergillus | 10.00 |
| Trichophyton | 65.00 |

Other dermatophytes that were isolated from dog's skin were *Absidia spp.* (5%), *Alternaria spp.* (4%), *Epidermaphyton* (3%), and 1% for both *Bipolaris spp.* and *Trichoderma*. In general, there are various types of dermatophytes that present on a stray dog specimen located on their skin, nails, and fur. The most common type of dermatophyte that affects a dog is *Trichophyton spp.*, which appeared in 198 out of 306 samples (64.7%), and

the least common was *Ulocladium spp.*, which appeared in only 1 out of 306 samples (0.3%). A summary of results for isolated dermatophytes from stray dogs from Selangor are represented in Table 2.

Table 2

The Type of Dermatophytes that Affect Dogs in Selangor

| Type of Dermatophytes | % |
|-----------------------|--------|
| Ulocladium | 1.00 |
| Epidermphyton | 3.00 |
| Bipolaris | 3.00 |
| Trichoderma | 5.00 |
| Fusarium | 6.00 |
| Alternaria | 8.00 |
| Absidia | 9.00 |
| Curvularia | 17.00 |
| Microsporum | 22.00 |
| Aspergillus | 31.00 |
| Trichophyton | 198.00 |

DISCUSSION

Several studies on various types of dermatophytes in dogs found that some dermatophytes are the cause of ringworm, namely *Microsporum* and *Trichophyton* dermatophytes. The majority of dermatophytes found in dogs were *Microsporum canis* (70%), while the others were *Microsporum gypseum* as much as 20% and *Trichophyton spp. mentagrofite* as much as 10%. *Trichophyton* is a genus of fungi that are parasitic in nature and can cause tinea diseases including itching in the groin, water fleas, ringworm, and similar infections of the nails, beard, skin, and scalp (K. Moriello, 2019). According to Debnath, Mitra, Kumar, & Samanta (2016) the most common type of dermatophytes in Eastern India are *Microsporum canis* (43.55%), *M. gypseum* (36.69%), and *Trichophyton mentagrophyte* (19.79%).

Animal dermatophytes are communicable to humans (Bennett, Dolin, & Blaser, 2014). Fungal infections in humans because of human or animal contact are relatively uncommon, some of the most common encountered diseases of the integument are dermatomycoses. Human or animal contact may be the source of occasional candida infections, all types of tinea infections, and some other types of superficial or deep fungal infections (Baumgardner, 2017). Asymptomatic animal carriers of *Microsporum canis* are considered to be important factors in epidemiology. This is because 50% of people in direct contact with infected dogs

or cats or arthroconidia found in the environment can contract the disease (Pin, 2017).

Some diseases that have the potential to occur in humans are tinea capitis, tinea corporis, and tinea imbricate. Tinea capitis is caused by *Trichophyton schoenleinii*. Tinea corporis and tinea imbricate are caused by *Trichophyton concentricum* (K. Moriello, 2019). According to Khaled, Golah, Khalel, Alharbi, & Mothana, (2015), the major types of tinea are tinea capitis and tinea corporis. *Microsporum canis*, *Microsporum audouinii*, *Trichophyton violaceum*, *Trichophyton verrucosum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes spp.*, *Trichophyton schoenleinii*, *T. concentricum* and *Epidermaphyton floccosum* are responsible for dermatophytosis. *M. canis* and *T. violaceum* were the most prevalent among the dermatophyte's species in Riyadh City, Saudi Arabia.

Another study conducted in Istanbul, Turkey showed *Microsporum canis* as the most frequently isolated species followed by *Trichophyton spp.*, *M. gypseum*, *T. mentagrophytes*, *M. nanum*, and *Microsporum spp* (Sığircı et al., 2019). *Microsporum canis* is the most common pathogen causing dermatophytosis in animals, whereas *Microsporum gypseum* and *T. mentagrophytes* are other dermatophytes associated with this infection. These infections are more common during the rainy season and in contact with human patients or pet owners (Murmu et al., 2015).

A study conducted by da Cunha et al (2019) showed that among symptomatic animals, female dogs were the largest contributor to the prevalence of *M. canis*, namely 77.77% and up to five months of age as much as 77.90%. This suggests that younger animals have a higher susceptibility to contracting infections due to immature immune systems. Regarding sex variable, there are several other studies that show different results that there is no correlation between sex and infection, although some authors report that male dogs have more frequent dermatophyte infections (Mancianti, Nardoni, Cecchi, Corazza, & Taccini, 2002; Seker & Dogan, 2011). Regarding to the age variable, some similar epidemiological data on dermatophytosis in relation to animal age had different results, but researchers agree that young dogs and cats (especially those less than one year old) are susceptible to the development of dermatophytosis (Mancianti, Nardoni, Cecchi, Corazza, & Taccini, 2002). Seker & Dogan (2011) also stated that animal age is a risk factor that has a significant relationship to the incidence of dermatophytosis in animals. The dermatophytosis

isolation rate was significantly higher in younger dogs ($P < 0.05$). This is related to immunological immaturity, biochemical exchange of the skin, deficiency of fungistatic sebum or linoleic acid, being in the anagen phase of the hair, or its physiological situation.

Regarding the seasonal or weather variable, this study showed a relatively high isolation rate of dermatophytes in spring and winter, although, there was no correlation between seasons and prevalence of dermatophyte incidence. The results of this study are in line with other studies, which also reported the same thing that there was no conclusive evidence of seasonal variation in the incidence of dog infection, although there were some results that reported a seasonal distribution of the incidence of dermatophyte infection in dogs (Mancianti, Nardoni, Cecchi, Corazza, & Taccini, 2002; Seker & Dogan, 2011).

As mentioned earlier, dermatophyte infections are the most common human fungal infections transmitted from animals, and tinea capitis is the predominant disease of these infections. Domestic animals can transmit dermatologic fungal infections, especially tinea corporis and pedis, by direct contact. Zoophilic *T. interdigitale* may cause the rare acute ulcerative type of tinea pedis described earlier (Canavan & Elewski, 2015). *Malassezia pachydermatis* fungemia has occurred as outbreaks in neonatal intensive care units, including one case in which the employee acquired the strain from a household dog (Reiss, Shadomy, & Lyon, 2011). In general, tinea capitis due to zoophilic and geophilic species is a rare infection compared to anthropophilic species. However, they tend to cause more severe inflammatory disease than anthropophilic species. The dominant zoophilic tinea capitis species in North America is *M. canis*. Kerion can be caused by zoophilic *Trichophyton verrucosum* or *T. mentagrophytes* (Hay, 2017).

Anthropophilic dermatophytes isolated in pediatric patients include *T. tonsurans* (associated with wrestling mats) and *Microsporum audouinii* (more common in kindergartens and schools). Zoophilic dermatophytes are the most common types of pathogens found in children and adolescents. This breed is generally sourced from domesticated animals, although there are some from livestock (very rare). Anthropophilic dermatophytes are more common in Germany than Austria. This is because immigration and urban density in Germany is greater than that in Austria. (Hayette & Sacheli, 2015).

According to Gupta et al (2016), the general action to reduce the risk of transmission of fungal organisms is to maintain general hygiene for pets, pet owners, and the environment where both of them (pets and pet owner) live. In addition to paying attention to general hygiene, tinea capitis sufferers are also advised to remove or wash all the combs, brushes, and headgear they use. Prevention of recurrence of onychomycosis after clinical cure can be assisted by periodic application of topical antifungals for plantar and inter-digital nails or feet, decontamination of footwear, close family care, and avoidance of public swimming pools.

Research Limitations

Limitations in this study include time constraints on the collection of data, and the high-cost required to perform molecular analysis of the data.

CONCLUSION

In conclusion, this study was performed to find the most common type of dermatophytes present in the stray dog population in Selangor, Malaysia, and conduct an analysis to identify the dermatophytes' burden in stray dogs. This study can assist investigators in understanding the prevalence of dermatophytes burden in stray dogs and help prevent further complication, such as the spread of illness, especially zoonotic infection

CONFLICT OF INTEREST

There is no conflict of interest

AUTHOR CONTRIBUTION

All authors declare that they are participating actively in research and article writing and are partly responsible for the content of the writing, including in the preparation and writing of concepts, designs, analysis, or revision of the article. PR: conceptualization, methodology, software, data curation, writing- original draft preparation. KP: visualization, investigation, supervision. KS: software, validation, writing-reviewing and editing.

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