SENSITIVITY OF Microsporum canis ISOLATED FROM DOGS TOWARDS ANTIFUNGALS

Gerson Yohanes I Sakan1, Soedarmanto Indarjulianto2*, Alfarisa Nururrozi2, Yanturowno2, and Slamet Raharjo2
1Animal Health Study Program, the State Agricultural Polytechnic of Kupang, Indonesia
2Department of Internal Medicine, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

*Corresponding author: indarjulianto@ugm.ac.id

ABSTRACT

The dermatophytosis treatment in dogs can fail due to dermatophyte resistance to antifungals. The purpose of this study is to determine the antifungal sensitivity of dermatophyte Microsporum canis (M. canis) isolated from dogs with dermatophyte. A total of 17 M. canis dog isolates were tested for sensitivity to 25 μg fluconazole, 25 μg griseofulvin, 15 μg ketoconazole and 30 μg terbinafine, using the disc diffusion method. The M. canis isolates were cultured on Sabouraud’s Dextrose Agar media, placed an antifungal disc, and then incubated at 28 °C for 7 days. The diameter of inhibition zone was measured, then compared with the standard, to determine whether they were sensitive, intermediate or resistant. The results of this study indicate that 16 isolates were sensitive to fluconazole; 17 isolates were sensitive to griseofulvin, ketoconazole and terbinafine; and one isolate was intermediate to fluconazole. Based on these results, it is concluded that all 17 (100%) isolates are sensitive to the antifungals griseofulvin, ketoconazole, and terbinafine, and 16 (94%) isolates are sensitive to fluconazole. Thus, the four antifungals can be selected to treat dermatophytes in dogs.

Key words: antifungal sensitivity, dogs, griseofulvin, Microsporum canis, terbinafine

INTRODUCTION

Dermatophytosis or ringworm is a skin disease (dermatitis) caused by dermatophyte fungi. It often affects animals, including dogs and cats, and can be transmitted to humans (Moriarty et al., 2012; Indarjulianto et al., 2014; Achterman et al., 2015; Indarjulianto et al., 2017; Brosh-Nissimov et al., 2018; Boehm and Mueller, 2019; Yamada et al., 2019). Dermatophytes belong to the class of fungi imperfecti, which have three genera: Microsporum, Trichophyton, and Epidermophyton. These three genera are zoophilic strains and reported dermatophyte agents in dogs of various countries (Bond, 2010; Adimi et al., 2013; Indarjulianto et al., 2014; Moriello et al., 2017; Yamada et al., 2019; Paryuni et al., 2020). Identified dermatophyte species include Microsporum canis (M. canis), M. Gypseum, and Trichophyton mentagrophytes, with M. canis being the most common dermatophyte fungus found in canines. Seker and Dogan (2011) reported that 46.0% of ringworm cases on dogs were caused by the fungus M. canis, 32.4% were caused by T. mentagrophytes, and 10.8% were caused by M. gypseum.

Treatment of dermatophytosis in pets is usually done topically and orally, individually or in combination. One of the goals of topical therapy is to reduce dermatophyte transmission through direct contact with infectious substances derived from the skin and hair of infected animals. To avoid failure and promote fast recovery, the right type and dose of drugs must be administered. Proper dermatophytosis treatment will also reduce the risk of infection, transmission, and zoonotic risks associated with this disease, as well as reduce contamination to the environment. Some of the drugs used in dermatophytosis therapy in dogs and cats include: chlorhexidine, enilconazole, fluconazole, Griseofulvin, Itraconazole (Sporanox), ketoconazole, lufenuron, miconazole, and terbinafine (Bond, 2010; Moriello et al., 2017).

Up to 40% of failures to treat M. canis-derived dermatophytosis are due to antifungal resistance. In recent years, bacterial and fungal resistance to drugs has developed, threatening current therapies for dermatitis or other infections (Gupta et al., 2009; Bueno et al., 2010; Soedarmanto et al., 2011; Vandeputte et al., 2012; Anke et al., 2018; Hsiao et al., 2018). M. canis resistance to antifungals can be minimized if the drugs administered are sensitive to M. canis. Sensitivity to antifungals is crucial for drug selection, as well as for screening the development of antifungal resistance (Yenisehirli et al., 2013). Therefore, information regarding the sensitivity or resistance of M. canis is needed as a basis for selecting a dermatophyte treatment. The purpose of this study is...
to determine the antifungal sensitivity of *M. canis* isolated from dogs.

**MATERIALS AND METHODS**

The sample used in this study consisted of 17 *M. canis* isolates identified from dogs with dermatitis. Other materials used were Sabouraud’s dextrose agar (SDA, Diagnostic Merck, Germany) and 6-mm diameter paper dises each containing 25 μg fluconazole (Oxoid, England), 25 μg griseofulvin (Indofarma, Indonesia), 15 μg ketoconazole (First Medipharma, Indonesia), and 30 μg terbinafine (Interbath, Indonesia). The antifungal sensitivity test of *M. canis* was carried out using the disc diffusion method (Esteban *et al*., 2005; Pakshir *et al*., 2009). *M. canis* isolates were cultured on SDA media and incubated at 28° C for 7 days (Indarjulianto *et al*., 2014). The colonies that grew on SDA media were mixed with 0.5 ml of sterile distilled water, and the concentration of the suspension was determined using a spectrophotometer at 65% transmittance and a wavelength of 530 nm. A total of 0.5 ml *M. canis* suspension was spread on the SDA media surface and 4 antifungal discs were placed on the surface of the media, then incubated at 28° C for 7 days. After the colonies grew, the zone of growth inhibition around the disc was measured and interpreted as sensitive, intermediate or resistant based on references from Pakshir *et al.* (2009).

**RESULTS AND DISCUSSION**

The results of the sensitivity test for 17 *M. canis* isolates against four antifungals can be seen in Table 1 and Figure 1. Interpretation of the growth inhibition zone based on Pakshir *et al.* (2009) can be seen in Table 2. Based on the growth inhibition zone standard, all 17 isolates (100%) of *M. canis* were sensitive to griseofulvin, ketoconazole and terbinafine; 16 isolates (94.11%) were sensitive and one isolate (5.89%) was intermediate to fluconazole.

| Antifungal     | Concentration | Zone diameter (mm)* | Sensitive (%) | Intermediate (%) | Resistant (%) |
|----------------|---------------|---------------------|--------------|-----------------|--------------|
| Fluconazole    | 25 μg         |                     | 16 (94.11%)  | 1 (5.89%)       | 0 (0.0%)     |
| Griseofulvin   | 25 μg         |                     | 17 (100%)    | 0 (0.0%)        | 0 (0.0%)     |
| Ketoconazole   | 15 μg         |                     | 17 (100%)    | 0 (0.0%)        | 0 (0.0%)     |
| Terbinafine    | 30 μg         |                     | 17 (100%)    | 0 (0.0%)        | 0 (0.0%)     |

* Compared to the standard (Pakshir *et al*., 2009)

![Figure 1. Examples of sensitivity test results for *Microsporum canis* isolates (JA/09) to fluconazole (F), griseofulvin (G), ketoconazole (K) and terbinafine (T)](https://example.com/figure1.png)

Table 2. Standard results of *M. canis* sensitivity test to antifungals (sensitive, intermediate and resistant)

| Antifungal     | Concentration | Zone diameter (mm)* | Sensitive | Intermediate | Resistant |
|----------------|---------------|---------------------|-----------|--------------|-----------|
| Fluconazole    | 25 μg         | ≥22                 | 21-15     | ≤14          |
| Griseofulvin   | 25 μg         | ≥10                 | -         | without zone (0) |
| Ketoconazole   | 15 μg         | ≥30                 | 29-23     | ≤22          |
| Terbinafine    | 30 μg         | ≥20                 | 19-12     | ≤11          |

*Pakshir *et al*., 2009
Sensitivity test by disc diffusion is a good model for testing fungal genus as a basis for selecting a suitable antifungal. The test can be applied for routine laboratory diagnosis and to estimate the resistance of dermatophytes to antifungal drugs. Although many guidelines such as the National Committee for Clinical Laboratory Standards (NCCLS) have published sensitivity tests for fungi (M-27A, M-28A), the method of evaluating dermatophyte antifungal activity remains controversial (Pakshir et al., 2009). Several studies have compared the disc diffusion method to the microdilution method. Studies from Karaca and Koc (2004), Pakshir et al. (2009), and Nweze et al. (2010), showed the disc diffusion method generally compares well to the microdilution method. Thus, it can still be used for screening. A second method for testing antifungal sensitivity, disc diffusion on agar media, is also considered simple, easy to use, accurate, inexpensive, and requires no special equipment. The disc diffusion method has also compared well to liquid dilution testing (Fernandes-Torres et al., 2005; Pakshir et al., 2009; Nweze et al., 2010).

The results of the sensitivity test corroborate findings from Adimi et al. (2013), which determine that the potency of griseofulvin, ketoconazole and terbinafine are quite high, while fluconazole has less potential for M. canis treatment in humans in Tehran, Iran. Behnam et al. (2020) reported that among the tested antifungals, terbinafine and griseofulvin are the most effective agents against M. canis isolates from human dermatophyte in Mashhad, Iran. Contrary to the results of this study, Abastabar et al. (2019) reported that luliconazole, efinaconazole and lanoconazole show the highest antifungal activity against M. canis dermatophytosis in human and animal isolates in Iran, France and Turkey. This difference may be due to variations in the types of antifungals that are often used in each country, or the improper use of antifungals in the field. Inaccurate use of antibacterial or antifungal can lead to resistance (Vandeputte et al., 2012; Apsari and Adiguna, 2013; Indarjulianto et al., 2018; Martinez-Rossi et al., 2018).

Differences or variations in antifungal sensitivity results were also reported in dermatophytes other than M. canis. Yenisehirli et al. (2013) report that griseofulvin is an antifungal drug that is less active against T. mentagrophytes isolates. Díaz-Jarabrán et al. (2015) found that fluconazole is less active than clotrimazole, terbinafine, itraconazole and griseofulvin for T. rubrum and T. mentagrophytes. T. mentagrophytes isolate was found to be more sensitive to itraconazole and ketoconazole than terbinafine (Bhatia and Sharma, 2015). According to Singh et al. (2019), T. mentagrophytes was the most sensitive to ketoconazole and voriconazole, followed by itraconazole, amphotericin B, fluconazole, and griseofulvin. The high incidence of T. mentagrophytes resistance against terbinafine was found in 33 of 41 samples (65.9%) and griseofulvin in 20 of 41 (48.8%).

The resistance characteristic of M. canis to the four types of antifungal was not found in this study, but there was one isolate that had intermediate properties against griseofulvin. This result differed from research conducted by Anggarini et al. (2015) in humans, which found resistance to the dermatophytes griseofulvin, ketoconazol, itraconazol, and terbinafine. Although only 1 isolate was found to be intermediate, the possibility of developing resistance through acquisition must be monitored to minimize the risk of resistance. Revie et al. (2018) states that antifungal resistance can occur through mutations or genomic changes. One way to reduce antifungal resistance is to apply antifungal treatment in the field using reasonable guidelines. According to Apsari and Adiguna (2013), strategies to reduce the occurrence of antifungal resistance include an antifungal-control program to avoid widespread use of antifungals, preventing their improper use and dosage. The use of high doses of antifungals, rather than low doses, is another strategy for avoiding antifungal resistance in less susceptible fungi. The absence of antifungal-resistant M. canis isolates indicated that all antifungals in this study can be used as dermatophytosis drugs for dogs.

**CONCLUSION**

Based on the results of the analysis above, it can be concluded that all 17 M. canis isolates from dogs are sensitive to griseofulvin, ketoconazole, and terbinafine antifungals, and 16 (94%) isolates are sensitive to fluconazole. Thus, the four isolates can be selected as dermatophytosis drugs for dogs.

**ACKNOWLEDGEMENT**

We would like to thank the Department of Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada for providing their laboratory facilities and equipment.

**REFERENCES**

Abastabar, M., A. Jedi, J. Guilhot, M. Ilkit, S. Eidi, M.T. Hedayati, T. Shokohi, R. Daie Ghazvini, A. Rezaei-Matehkolaee, F. Katraee, J. Javidnia, B. Ahmadi, and H. Badali. 2019. In vitro activities of 15 antifungal drugs against a large collection of clinical isolates of Microsporum canis. Mycoses. 62(11):1069-1078.

Achterman, R.R., D.L. Moyes, S. Thavaraj, A.R. Smith, K.M. Blair, T.C. White, and J.R. Naglik. 2015. Dermatophytes activate skin keratinocytes via mitogen-activated protein kinase signaling and induce immune responses. Infect. Immun. 83:1705-1714.

Adimi, P., S.J. Hashemi, M. Mahmoudi, H. Mathendi; M.R. Shidfar, M. Ennami, A. Rezaei-Matehkolaee, M. Gramishour, and P.I. Kordbacheh. 2013. In-vitro activity of 10 antifungal agents against 320 dermatophyte strains using microdilution method in Tehran. Iran. J. Pharm. Res. 12:537-545.

Aneka, C.I, D. Ortan, and C. Cafarchia. 2018. Therapy and antifungal susceptibility profile of Microsporum canis. J. Fungi (Basel). 4(3):107, doi:10.3390/jof4030107.

Anggarini, D.R., H. Sukanto, L. Astari, and P.D. Endraswari. 2015. Uji kepekaan griseofulvin, ketoconazol, itraconazol, dan terbinafine terhadap spesies dermatofil dengan metode mikrodilusi. Berkala Ilmu Kesehatan Kulit dan Kelamin. 27(1):55-61.

Apsari, A.Y. and M.S. Adiguna. 2013. Resistensi antijamur dan strategi untuk mengatasi. MDVI. 40(2):89-95.

Behnam, M., H. Zarrinfar, M.J. Najafzadeh, A. Naseri, L. Jarahi, and M.N. Babić. 2020. Low in vitro activity of sertaconazole against
clinical isolates of dermatophyte. Curr. Med. Mycol. 6(1):36-41.
Bhatia, V.K. and P.C. Sharma. 2015. Determination of minimum inhibitory concentrations of itraconazole, terbinafine and ketoconazole against dermatophyte species by broth microdilution method. Indian J. Med. Microbiol. 33(4):533-537.
Boehm, T.M.S.A. and R.S. Mueller. 2019. Dermatophytosis in dogs and cats-an update. Dermatophytose bei Hund und Katze-ein Update. Tierarztl. Prax. Ausg. K. Kleintiere Heimtiere. 47(4):257-268.
Bond, R. 2010. Superficial veterinary mycoses. Clin. Dermatol. 28:226-236.
Brosh-Nissimov, T., R. Ben-Ami, N. Astman, A. Malin, Y. Baruch, and I. Galor. 2018. An outbreak of Microsporum canis infection at a military base associated with stray cat exposure and person-to-person transmission. Mycoses. 61(7):472-476.
Bueno, J.G., C. Martínez, B. Zapatía, G. Sanclermente, M. Gallego, and A.C. Mesa. 2010. In vitro activity of flucon-azole, itraconazole, voriconazole and terbinafine against fungi causing onychomycosis. Clin. Exp. Dermatol. 35:658-663.
Díaz-Jarabrán M.C., P. Díaz-González, J.E. Rodríguez, and A.C. Muñoz. 2015. Evaluation of the in vitro susceptibility pattern of clinical isolates of Trichophyton mentagrophytes and Trichophyton rubrum in Santiago, Chile. Rev. Iberoam. Mic. 32(2):83-87.
Esteban, A., M.L. Abarca, and F.J. Cabanes. 2005. Comparison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. Med. Mycol. 43:61-66.
Fernandes-Torres, B., I. Inza, and J. Guarro. 2005. Evaluation of disk diffusion method for determining eberconazole susceptibility of dermatophytes and influence of culture medium. Antimicrob. Agents Chemother. 49(5):2116-2118.
Gupta, A.K., J.V. Williams, M. Zaman, and J. Singh. 2009. In vitro pharmacodynamic characteristics of griseofulvin against dermatophyte isolates of Trichophyton tonsurans from tinea capitis patients. Med. Mycol. 47(8):796-801.
Hsiao, Y.H., C. Chen, H.S. Han, and R. Kano. 2018. The first report of terbinafine resistant Microsporum canis from a cat. J. Vet. Med. Sci. 80(6):898-900.
Indarjulianto, S., Yanauwartno, H. Purnamaningsih, P. Wikansari, and G.Y.I. Sakan. 2014. Isolation and identification of Microsporum canis from dermatophytosis dogs in Yogyakarta. J. Vet. 15(2):212-216.
Indarjulianto, S., Yanauwartno, S. Widyarini, S. Raharjo, H. Purnamaningsih, A. Nururrozi, N. Harisibowo, and H.A. Jaimudin. 2017. Microsporum canis infection in dermatitis cats. J. Vet. 18(2):207-210.
Indarjulianto S., A. Nururrozi, Y. Yanauwartno, R. Rusmihayati, S. Widyarini, P.W. Sari, and V.J. Bramono. 2018. Perkembangan resistensi Escherichia coli terhadap oksitetrasiklin. J. Sain Vet. 26(1):1-10.
Karaca, N. and A.N. Koç. 2004. In vitro susceptibility testing of dermatophytes: Comparison of disk diffusion and reference broth dilution methods. Diagn. Microbiol. Infect. Dis. 48(4):259-264.
Martínez-Rossi, N.M., T.A. Bitencourt, N.T.A. Peres, E.A.S. Lang, E.V. Gomes, N.R. Quaresimin, M.P. Martins, L. Lopes, and A. Rossi. 2018. Dermatophyte resistance to antifungal drugs: Mechanisms and prospectus. Front. Microbiol. 29(9):1108. doi: 10.3389/fmicb.2018.01108.eCollection 2018.
Moriarty, B., R. Hay, and R. Morris-Jones. 2012. The diagnosis and management of tinea. Brit. Med. J. 345:e1380.
Morielo, K.A., K. Coyer, S. Paterson, and B. Mignon. 2017. Diagnosis and treatment of dermatophytosis in dogs and cats. Vet. Dermatol. 28(3):266-e68.
Nweze, E.I., P.K. Mukherjee, and M.A. Ghannoum. 2010. Agar-based disk diffusion assay for susceptibility testing of dermatophytes. J. Clin. Microbiol. 48(5):3750-3752.
Pakshir, K., L. Bahaeidine, Z. Rezaei, M. Sodaii, and K. Zomarodian. 2009. In vitro activity of six antifungal drugs against clinically important dermatophytes. Jundishapur J. Microbiol. 2(4):156-163.
Paryuni, A.D., S. Indarjulianto, and S. Widyarini. 2020. Dermatophytosis in companion animals: A review. Vet. World. 13(6):1174-1181.
Revie, N.M., K.R. Iyet, N. Robbins, and L.E. Cowen. 2018. Antifungal drug resistance: Evolution, mechanisms and impact. Curr. Opin. Microbiol. 45:70-76.
Seker, E. and N. Dogan. 2011. Isolation of dermatototis from dogs and cats with suspected dermatotitosis in Western Turkey. Prevent. Vet. Med. 98:46-51.
Singh, S.K., D.K.Patwa, R. Tilak, A. Das, and T.B. Singh. 2019. In vitro susceptibility of dermatophytes to oral antifungal drugs and amphotericin B in Uttar Pradesh, India. Indian J. Dermatol. Venereol. Leprol. 85(4):388-392.
Soedarman, I., T. Kanbar, H. Ülbegi, R. Yavuz, E. Gür, T. Akin, A. İpek, and M. Zöchök. 2011. Genetic relatedness of methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolated from a dog and the dog owner. Res. Vet. Sci. 91:e25-e27.
Vandeputte, P., S. Ferrari, and A.T. Coste. 2012. Antifungal resistance and new strategies to control fungal infections. Int. J. Microbiol. doi:10.1155/2012/713687.
Yamada, S., K. Anzawa, and T. Mochizuki. 2019. An epidemiological study of feline and canine dermatophytoses in Japan. Med. Mycol. 67(2):39-44.
Yenişehrli, G., E. Tunçoğlu, A. Yenişehrli, and Y. Buhlt. 2013. In vitro activities of antifungal drugs against dermatophytes isolated in Tokat, Turkey. Int. J. Dermatol. 52(12):1557-1560.