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Could polymerase chain reaction be an alternative diagnostic method for dermatophytes?

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ABSTRACT

Dermatophytosis are cutaneous mycoses caused by *Microsporum* spp., *Trichophyton* spp. and *Epidermophyton* spp. dermatophytes. Dermatophytosis resembles other skin diseases due to its various clinical manifestations such as multifocal alopecia, circular lesions, scaling, crusting, papular and pustular lesions, follicular obstruction, erythema, hyperpigmentation, miliary dermatitis and dystrophic nail growth, and its diagnosis is based on the use of many different methods. Generally used methods; clinical appearance, microscopic examination, examination with Wood's lamp and mycological culture but these methods have some disadvantages. Microscopic examination requiring expertise, fast and cost-effective method, but in some cases, microbiologists encounter specimens that are microscopically negative but give positive results in mycological culture. Examination with Wood's lamp can only be used for the diagnosis of *M. canis* and its specificity is low. On the other hand mycological cultures require a long time (3-6 weeks) to give definitive results, and their sensitivity may decrease due to common contaminant growth. Considering these reasons, new Polymerase Chain Reaction (PCR) based methods have been developed for the diagnosis of dermatophyte agents. Compared to other molecular methods, the PCR method is easy, fast and applicable for the identification of dermatophyte species that do not show typical morphological features. Although PCR-based diagnostic methods are widely used in humans in the diagnosis of dermatophytosis, its usefulness in dogs and cats has also been confirmed. As a result, the PCR method used in the diagnosis of dermatophytosis; it is emphasized that it can be used in the diagnosis of dermatophytosis due to the ease of obtaining samples, providing faster results compared to mycological culture, and not requiring expertise, and it is emphasized that new and different methods should be used in the diagnosis of diseases. In this study, it was aimed to demonstrate the effectiveness of the PCR method and its applicability in clinical practice, as well as mycological culture, which is frequently used in the diagnosis of dermatophytosis.

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Introduction

Dermatophytosis are cutaneous mycoses caused by *Microsporum* spp., *Trichophyton* spp. and *Epidermophyton* spp. dermatophytes (Cafarchia et al., 2013; Katiraee et al., 2021). Infection is also described as "ringworm" (Tel and Akan, 2008). Dermatophytes are common all over the world and cause infections in various animals (Katiraee et al., 2021). Although there is no difference in terms of age, sex or race in getting the infection, it is generally more common in animals with low immunity (Tel and Akan, 2008). Among the factors affecting the incidence of dermatophytes are humidity and temperature, which vary depending on

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location and season (Cafarchia et al., 2004; Boehm and Mueller, 2019). Dermatophytes, which are classified as geophilic, zoophilic and anthropic according to their primary habitat (Or et al., 1999), also have zoonotic features (Tel and Akan, 2008). While geophilic dermatophytes are associated with the decomposition process of the surrounding keratinous structures such as hair, feathers, hooves and horns; zoophilic and anthropophilic dermatophytes are superficial mycoses that infect the stratum corneum layer, hair, nails and paws on the host (Baldo et al., 2012). About 20 different species have been identified that cause dermatophytosis in cats and dogs, (Tel and Akan, 2008), but among these agents, the most common species that cause dermatophytosis in cats and dogs are *Microsporum canis*, *Microsporum gyps*eum and *Trichophyton mentagrophytes* (Moriello et al., 2017). According to one study, infections caused by *M. canis* account for approximately 80% of dermatophytoses in cats and dogs (Cafarchia et al., 2013), while according to another study, *M. canis* is the cause of more than 90% of dermatophyte infections in cats (Paryuni et al., 2020).

Dermatophytosis resembles other skin diseases due to its various clinical manifestations such as multifocal alopecia, circular lesions, scaling, crusting, papular and pustular lesions, follicular obstruction, erythema, hyperpigmentation, miliary dermatitis and dystrophic nail growth (Tel and Akan, 2008; Kaya et al., 2022), and its diagnosis is based on the use of many different methods (Cafarchia et al., 2013). Although generally used methods of dermatological clinic examination include microscopic examination, examination with Wood's lamp and mycological culture (Verrier et al., 2019), have some disadvantages.

Rapid and accurate diagnosis is very important for the successful treatment of dermatophytes (Gräser et al., 2012). The validity of diagnoses made only in the light of clinical findings is low (Faergmann and Baran, 2003). For this reason, the diagnosis should be supported by laboratory tests.

Microscopic examination requiring expertise, fast and cost-effective method, but in some cases, microbiologists encounter specimens that are negative microscopically but give positive results in mycological culture (Piri et al., 2018). Examination with Wood's lamp can only be used for the diagnosis of *M. canis* and its specificity is low (Larry and Francis, 2015; Marsella R., 2022). On the other hand mycological cultures require a long time (3-6 weeks) to give definitive results, and their sensitivity may decrease due to common contaminant growth (Cafarchia et al., 2013; Verrier et al., 2019). In addition, the identification process on the basis of species becomes even more complicated, since some dermatophytes do not show any characteristic features as a result of the first isolation (Gräser et al., 2012; Piri et al., 2018).

Considering these reasons, new Polymerase Chain Reaction (PCR) based methods have been developed for the diagnosis of dermatophyte agents. Among these methods, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis (RAPD), PCR amplification using non-transcribed spacer (NTS) primers and internal transcribed spacer (ITS) primers, nested-PCR, PCR-restriction fragment length polymorphism (RFLP) analysis, arbitrary primer PCR and ITS region sequencing, have been used for the identification of dermatophyte species and strains (Liu et al., 2014). Compared to other molecular methods, the PCR method is easy, fast and applicable for the identification of dermatophyte species that do not show typical morphological features (Faggi et al., 2001). Although PCR-based diagnostic methods are widely used in humans in the diagnosis of dermatophytosis (Moriello and Leutenegger, 2018), its usefulness in dogs and cats has also been confirmed (Katiraee et al., 2021). In studies comparing microscopic examination, mycological culture and PCR methods, it has been observed that the PCR method can diagnose samples with positive results with conventional methods (Faggi et al., 2001; Dabrowska et al., 2014; Moriello and Leutenegger, 2018; Piri ve ark., 2018; Verrier et al., 2019). To date, many PCR methods have been developed for the diagnosis of dermatophytes. These PCR methods have advantages and disadvantages compared to the methods used for the diagnosis of dermatophytes.

In this study, it was aimed to demonstrate the effectiveness of the PCR method and its applicability in clinical practice, as well as mycological culture, which is frequently used in the diagnosis of dermatophytosis.

Sensitivity and specificity

In many studies in which samples taken from cats and dogs for the diagnosis of dermatophytosis were examined using microscopic examination, mycological culture and PCR methods, it was seen that PCR methods gave reliable results (Tel and Akan, 2008; Moriello and Leutenegger, 2018; Piri ve ark., 2018; Verrier ve ark., 2019). In the study in which 268 samples (97 dogs, 57 cats) were examined by microscopy and mycological culture and 40 samples were isolated, it was observed that all isolated dermatophyte agents were positive as a result of the detection of 3390 bp bands by PCR method (Tel and Akan, 2008). In a study where qPCR was used to

measure clinical utility, confirm dermatophytosis and evaluate mycological recovery in cats with suspected dermatophytes, qPCR and mycological culture results were consistent in 49 of 52 cats. qPCR was able to correctly identify 47 of 50 cases with infection and 2 of 2 cases without infection. In addition, 2 negative mycological cultures were defined as mycological recovery in the study. qPCR correctly defined 30 patients for *Microsporum* spp. and 39 patients for *Microsporum canis*. in 46 patients diagnosed with mycological recovery by mycological culture (Moriello and Leutenegger, 2018). Using a newly developed PCR method (pan-dermatophyte nested-PCR), in a study conducted on 140 samples taken from cattle, sheep, goats, cats, dogs and horses with clinically suspected dermatophytosis, the positivity rates of the samples were 90% with nested-PCR and %85.7 with microscopy and 75% with mycological culture. For this reason, it was concluded that the newly developed nested-PCR method is a fast, sensitive and specific method for the detection of dermatophytes in suitable clinical samples (Piri et al., 2018). In another developed method (PCR assay based on terminal restriction fragment length polymorphism (TRFLP)), the detection rate of agents was found to be 97.1% with PCR-TRFLP from samples that were found positive by both microscopic examination and culture. The PCR method did not give positive results in any of the samples that were detected negative with the classical methods used in this study (Verrier et al., 2019). While the one-step PCR method showed high accuracy in samples obtained from dogs, the accuracy rate seen in cats was lower. However, it was determined that the samples taken from cats with the nested-PCR method showed higher accuracy. The specificity determined by the nested-PCR method in dogs and cats was 94.1%, 94.4%, and the sensitivity was 100% and 94.9%, respectively. At the same time, the nested-PCR method can distinguish *Microsporum canis* from *Microsporum gypseum*, *Trichophyton interdigitale* and *Trichophyton terrestre*, which is not possible in the single-step PCR method (Cafarchia et al., 2013).

False positivity

It has been thought that false positive results seen in the samples examined using the PCR method may be due to false negative microscopic examination and culture results, the presence of non-viable DNA fragments on the hair cover during treatment, contamination of the skin, fomite carrier or environmental fungal spores (Cafarchia et al., 2013; Moriello and Leutenegger, 2018). Positive PCR results seen in animals, although there are no clinical signs, are thought to be related to early diagnosis (Moriello et al., 2017; Moriello and Leutenegger, 2018). In order to avoid false positive results, it is recommended to repeat the PCR test by cleaning the environment and the animal (bath) before starting any treatment (Moriello and Leutenegger, 2018).

Jacobson et al. (2018a) determine that in samples from 132 cats, PCR detected positive in correlation with all samples found positive by mycological culture. However, 12 samples with negative mycological culture results were evaluated as positive. In this study, mycological culture was accepted as the gold standard, and mycological culture and PCR were repeated for 9 out of 12 samples. Mycological culture was positive in 2 out of 9 samples, and it was thought that the first mycological culture gave false negative results and PCR results gave true positive results. Results for the remaining 7 samples did not change and PCR was associated with a false positive result. However, 5 out of 7 cats had a history of past exposure to dermatophytosis, and positive PCR results for samples from these cats may have revealed the presence of fungal agents that could not be detected by culture.

False negativity

In a study in which 52 samples from cats were analyzed using the qPCR technique, the results of 3 samples were observed to be negative. All of these samples had clinical lesions. By cytology and mycological culture, 1 of these patients was confirmed as M. canis and 2 as *Trichophyton* spp. (Moriello and Leutenegger, 2018). It is thought that the amount of hair and crust in the sample taken from the lesions is important when using the qPCR technique, and false negative results may be caused by the inadequate sampling technique (Moriello et al., 2017; Moriello and Leutenegger, 2018). In addition, it was thought that due to the self-grooming feature of cats, fungal DNAs could be found less on the hair cover and their detection by PCR could be detected at a lower rate compared to dogs (Cafarchia et al., 2013).

Diagnostic expenses and relationship with treatment

Although it is thought that the biggest disadvantage of the PCR method when compared to classical methods is its high cost (Faggi et al., 2001), it has been reported that the cost is now approaching classical methods thorugh the developing technology and developed PCR methods (Piri et al., 2018). In addition, it can provide significant savings, especially as negative results can be detected in a short time and thus unnecessary medication, care and accommodation costs can be reduced (Jacobson et al., 2018b). Because of these savings, the cost difference of the PCR method

compared to mycological culture is reduced.

Despite the culture results can be detected as negative in the samples taken from the treated cats and dogs, the PCR method is not affected by the systematic treatment and thus enables the monitoring of the efficacy of the treatment (Cafarchia et al., 2013; Moriello and Leutenegger, 2018). In a study, 3 samples from treated animals were negative in mycological culture but positive by the nested-PCR method. These results suggested that PCR can be used as a routine method for monitoring treatment (Cafarchia et al., 2013). Although the negative PCR results seen during the treatment are reliable, the positive PCR results do not show that there is no improvement due to the false positive reasons we have listed above (Moriello et al., 2017; Jacobson et al., 2018b; Moriello and Leutenegger, 2018).

Antifungal Resistance

In countries where dermatophytosis needs to be confirmed by culture before starting antifungal treatment, the need to take samples again due to false negative culture results causes both loss of time, financial loss and discomfort for the patient and the patient's relatives (Piri et al., 2018).

Today, the emergence of antifungal resistant clinical isolates may lead to failure in dermatophyte treatment. Especially the treatments created as a result of misdiagnosis contribute to the spread of antifungal resistance (Kaya et al., 2022). It is important to determine the species before starting the treatment, as it will prevent antifungal resistance that may occur with incorrect and incomplete treatment (Katiraee et al., 2021). Although the identification of dermatophytes by culture is difficult and the specificity is low, PCR has been found to give more successful results (Piri et al., 2018).

Conclusion

Comparison with other methods, the advantages of the PCR method are that it gives reliable results, the potential to guide early diagnosis, and its ability to determine the effectiveness of treatment. It can also prevent antifungal resistance through species identification and the use of specific antifungals. The disadvantages are that non-living DNA fragments give false positive results, false negative results are seen due to the inadequacy of the sampling technique, and its cost is still high compared to other methods.

As a result, the PCR method can be used in the diagnosis of dermatophytosis; due to the ease of samples collection, providing faster results compared to mycological culture, and not requiring expertise, and it is emphasized that new and different methods should be used in the diagnosis of diseases because of alternative techniques are improving recently.

References

Baldo, A., Monod, M., Mathy, A., Cambier, L., Bagut, E.T., Defaweux, V., Symoens, F., Antoine, N., & Mignon, B. (2012), Mechanisms of skin adherence and invasion by dermatophytes. *Mycoses, 55.* 218-223.

Boehm, T., & Mueller, R. S. (2019). Dermatophytosis in dogs and cats - an update. Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere, 47(4), 257-268.

Cafarchia, C., Romito, D., Sasanelli, M., Lia, R., Capelli, G., & Otranto, D. (2004), The epidemiology of canine and feline dermatophytoses in southern Italy. *Mycoses, 47.* 508-513.

Cafarchia, C., Gasser, R. B., Figueredo, L. A., Weigl, S., Danesi, P., Capelli, G., & Otranto, D. (2013). An improved molecular diagnostic assay for canine and feline dermatophytosis. *Medical Mycology, 51*(2), 136-143

Dąbrowska, I., Dworecka-Kaszak, B., & Brillowska-Dąbrowska, A. (2014). The use of a one-step PCR method for the identification of *Microsporum canis* and *Trichophyton mentagrophytes* infection of pets. *Acta biochimica Polonica, 61*(2), 375-378.

Faergemann J., Baran R. (2003). Epidemiology, clinical presentation and diagnosis of onychomycosis. British *Journal of Dermatology; 149*, Suppl 65:1-4.

Faggi, E., Pini, G., Campisi, E., Bertellini, C., Difonzo, E., & Mancianti, F. (2001). Application of PCR to distinguish common species of dermatophytes. *Journal of Clinical Microbiology, 39*(9), 3382-3385.

Gräser, Y., Czaika, V., & Ohst, T. (2012). Diagnostic PCR of dermatophytes--an overview. *Journal of the German Society of Dermatology, 10*(10), 721-726.

Jacobson, L. S., McIntyre, L., & Mykusz, J. (2018). Comparison of real-time PCR with fungal culture for the diagnosis of *Microsporum canis* dermatophytosis in shelter cats: a field study. *Journal of Feline Medicine and Surgery, 20*(2), 103-107.

Jacobson, L. S., McIntyre, L., & Mykusz, J. (2018). Assessment of real-time PCR cycle threshold values in *Microsporum canis* culture-positive and culture-negative cats in an animal shelter: a field study. *Journal of Feline Medicine and Surgery, 20*(2), 108-113.

Katiraee, F., Kouchak Kosari,Y., Soltani, M., Shokri, H. & Hassan Minooieanhaghighi , M. (2021). Molecular identification and antifungal susceptibility patterns of dermatophytes isolated from companion animals with clinical symptoms of dermatophytosis. *Journal of Veterinary Research, 65*(2) 175-182.

Kaya Y., Dokuzeylül B., Bakırel U.,& Or M. E., (2022). Antifungal resistance and clinical significance in small animals, German *Journal of Veterinary Research, 2*(2), 28-38.

Liu, G., He, C., & Zhang, H. (2014). Identification and characterization of dermatophyte species and strains with PCR amplification. Experimental and therapeutic medicine, 8(2), 545–550.

Marsella R. (2021). *Clinical approach to Feline Dermatologic Diseases*, 1st edition, Edra

Moriello, K. A., Coyner, K., Paterson, S., & Mignon, B. (2017). Diagnosis and treatment of dermatophytosis in dogs and cats.: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Veterinary dermatology, 28(3), 266–e68.

Moriello, K. A., & Leutenegger, C. M. (2018). Use of a commercial qPCR assay in 52 high risk shelter cats for disease identification of dermatophytosis and mycological cure. *Veterinary dermatology, 29*(1), 66-e26.

Or E., Kaymaz A.K., Dodurka T., Tan H., Özgür N. Y. (1999). Zonotic *Microsporum canis* Infection. Turkish Journal of Veterinary & Animal Sciences, 23:3, Article 14. Available at: https://journals.tubitak.gov.tr/veterinary/vol23/iss3/14

Paryuni, A. D., Indarjulianto, S., & Widyarini, S. (2020). Dermatophytosis in companion animals: A review. Veterinary world, 13(6), 1174–1181.

Piri, F., Zarei M. A., Ronagh, A., Ahmadi, B., Makimura, K., & Rezaei-Matehkolaei, A. (2018). Assessment of a pan-dermatophyte nested-PCR compared with conventional methods for direct detection and identification of dermatophytosis agents in animals. Mycoses, 61(11), 837–844.

Tel, O. Y., Akan, M. (2008). Kedi ve köpeklerden dermatofitlerin izolasyonu. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 55*, 167-171

Tilley L. P., Smith F. W. K., (2015). Blackwell's Five-Minute Veterinary Consult: Canine and Feline, 5th edition, Wiley-Blackwell

Verrier, J., Jeanneau-Imparato, L., Guillet, C., Bourdeau, P., & Bouchara, J. P. (2019). PCR-terminal restriction fragment length polymorphism for direct detection and identification of dermatophytes in veterinary mycology. Medical mycology, 57(4), 447–456.