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Molecular Detection of Dermatophytes Associated with Dermatitis in Dogs of Puducherry, India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NM, KT, HRK and SKM collected the samples. The author BSN isolated the dermatophyte, designed the study, wrote the protocol, the manuscript, and study analyses, and managed the literature searches. Authors JV and VMVS helped in the isolation and identification. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Dermatophytosis (ringworm) is a disease of global significance caused by pathogenic fungi called dermatophytes in animals and humans. Dermatophytes are a group of septate fungi that, invade superficial keratinized structures such as skin, hair, and claws. The most common infection in dogs

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and cats is caused by the genera Microsporum (M.), or Trichophyton (T.). Nowadays the dermatophytes of pet dogs and cats could be a potential source of zoonotic infections causing a serious public health problem. The dermatophytosis is generally chronic, and its control requires proper identification of aetiological agents and its prevalence to prescribe specific treatment. The current study was conducted to identify the causative agent of skin disease from dogs in Puducherry (India). The suspected hair samples collected from dogs having skin infections were examined under a microscope revealing the presence of arthrospores. Traditional diagnostics of ringworms are based on the morphological identification of cultured fungi and are time-consuming. Identification of fungi in dermatological samples using PCR is reliable and provides better results in comparison with cultures. The ITS regions were amplified by PCR. This study is useful to identify the most common pathogenic dermatophytes affecting dogs.

Keywords: Dermatophyte; Microsporum; Trichophyton; ITS.

1. INTRODUCTION

"Dermatophytes are a group of keratinophilic fungi and are found all over the world. According to the latest classification, there are over 50 species of these fungi, which belong to the genera Trichophyton, Microsporum, Epidermophyton, Nannizzia, Arthroderma, Lophophyton, and Paraphyton" [1]. "Regardless of their preferred hosts, all species of dermatophytes can digest keratinous materials found in the outer layer of skin and nails, hair, hoof, horn, claw, and feathers in humans and animals" [2]. "The two main genera of fungi that cause dermatophytosis in animals, particularly in dogs and cats are Microsporum spp. and Trichophyton spp. *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton equinum*, *Trichophyton verrucosum*, and *Microsporum nanum* species of fungi have significant roles in veterinary medicine" [3]. "The annual dermatophytosis cases increased in humans and animals, particularly in dogs and cats" [4]. "Research indicates that in dogs, dermatophyte infection causes lesions on the face, legs, and/or tail" [5]. "The severity of lesions can be mild to severe, influenced by several factors, including the specific dermatophyte species, virulence factors, area of infection, secondary infections, and environmental conditions" [6]. "The accurate diagnosis of dermatophytosis and identification of causative agent is important to know the source of infection, appropriate treatment, and also a better understanding of the epidemiological trend" [7]. "The current standard methods for the detection of dermatophytosis in animals and humans rely on conventional microscopic detection of fungal elements such as arthroconidia and/or hyphae, in KOH preparation of clinical specimens" [8]. "Direct microscopy is useful to confirm the infection but it has limitations. These include difficulty in distinguishing between dermatophytic and non-dermatophytic elements, inability to identify specific causative agents at the genus or species level, and a high number of false-negative results. Culture on selective media is associated with poor sensitivity, primarily because of the growth of various fungal or bacterial contaminants and also the presence of nonviable fungal elements in infected materials" [9,10]. "PCR-based techniques, compared to traditional approaches, generally have higher sensitivity and specificity" [11,12]. "PCR methods that target the internal transcribed spacer (ITS) regions, Chitin synthase (CHS), Topoisomerase II, and beta-tubulin genes directly detect dermatophytes" [8]. Conventional PCR has advantages such as simplicity to perform and cost-effectiveness compared to classical methods. No research was conducted for the identification of causative agents in canine dermatophytosis in the Puducherry region. Therefore, the current study was carried out to identify the dermatophyte by polymerase chain reaction.

2. MATERIALS AND METHODS

2.1 Sample Collection

The hair samples suspected to be infected with dermatophytosis were collected from the various dog breeds exhibiting lesions indicative of the condition (Figs. 1,2). These samples were obtained from dogs brought to the Veterinary Hospital, Department of Animal Husbandry and Animal Welfare, Puducherry. Hair samples suspected of being infected were extracted from lesions using sterile forceps in an aseptic manner, with preference given to the basal part of the hairs for diagnostic purposes. The samples were tagged after being collected and transported to the lab in a sealed container.

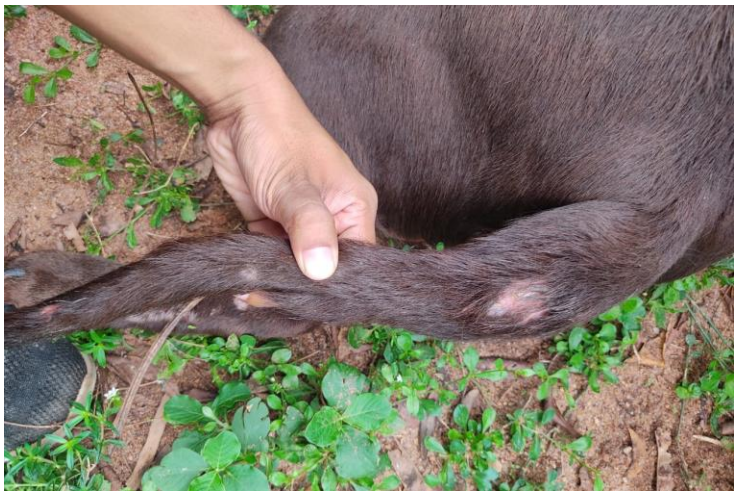

Fig. 1. Patchy ringworm lesion on the tail of a female dog

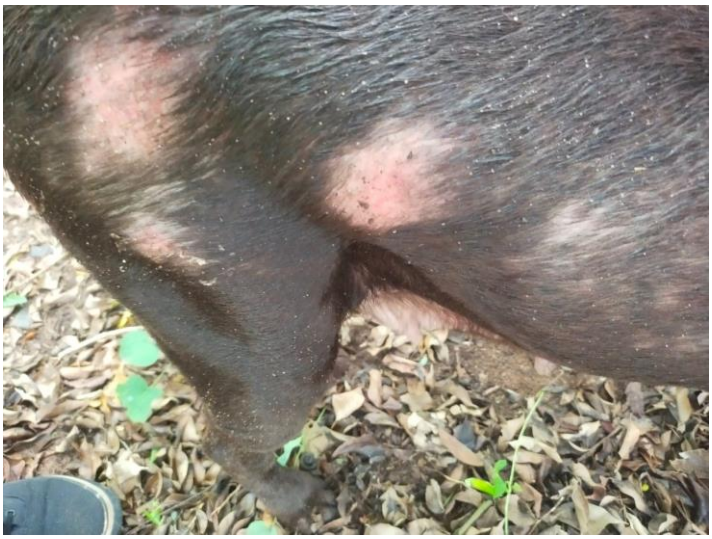

Fig. 2. Patchy hair loss on the hind leg of a female dog

2.2 Direct Microscopical Examination

For direct microscopic examination, the collected hair samples were treated with lactophenol cotton blue. A small amount of stain is applied to a slide, onto which the hair sample was placed. A cover slip was then placed over the sample, and it was examined under low power magnification to detect the presence of arthrospores.

2.3 Isolation and Identification

The dermatophyte test medium (D.T.M) (HI media) was used for isolating dermatophytes. This media contains antifungal and antibiotics such as cycloheximide, chloramphenicol, and gentamicin which inhibit the growth of contaminant fungi and bacteria. The suspected hair sample point was inoculated onto the D.T.M media and plates were incubated at 25ºC for up to 1 week plates were regularly checked for the appearance of fungal growth.

2.4 Polymerase Chain Reaction

The DNA was extracted from suspected hair samples using a commercial kit (Qiagen, Hilden, Germany). ITS rDNA region was amplified using fungal primers Derm-ITS FP (5'-TCCGTAGGTGAACCTGCGG-3') and Derm-ITS RP (5'-TCCTCCGCTTATTGATATGC-3') (10). The PCR amplification was carried out for 35 cycles consisting of 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 45 s, extension at 72°C for 1 min, and a final extension cycle of 10 min at

72°C. The amplified PCR products were loaded on a 1.5% agarose gel for electrophoresis and the gels were visualized using a UV transilluminator.

3. RESULTS AND DISCUSSION

In this study from collected hair samples, all hair samples were positive for the presence of arthrospores (Fig. 3). Samples that are inoculated onto D.T.M. show yellow to red color change in media this indicates positive for the growth of dermatophytes that show' white aerial hyphae and red color around the fungal growth (Plate. 1).

The positive fungal cultures were identified by the conidial structure produced and the presence of septate hyphae (Fig. 4).

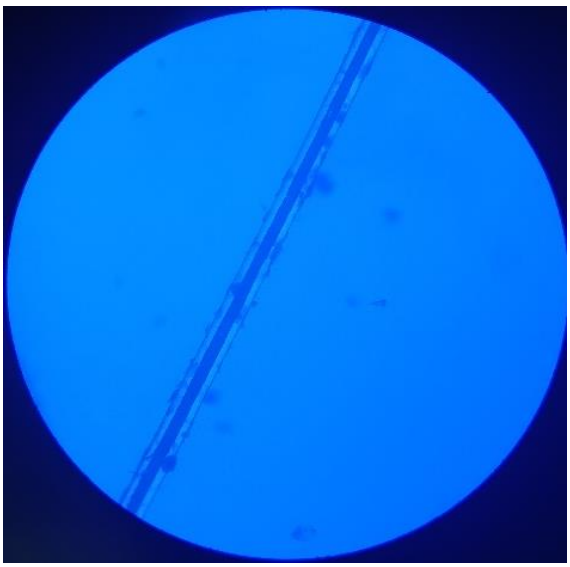

Fig. 3. Direct microscopic examination of a hair sample showing the presence of arthrospores

Plate 1. The hair sample inoculated on D.T.M showed growth of dermatophyte, characterized by white aerial hyphae and a red coloration around the fungal growth

Fig. 4. Macroconidia with septate hyphae of Microsporum canis after staining with Lactophenol Cotton blue stain 40X

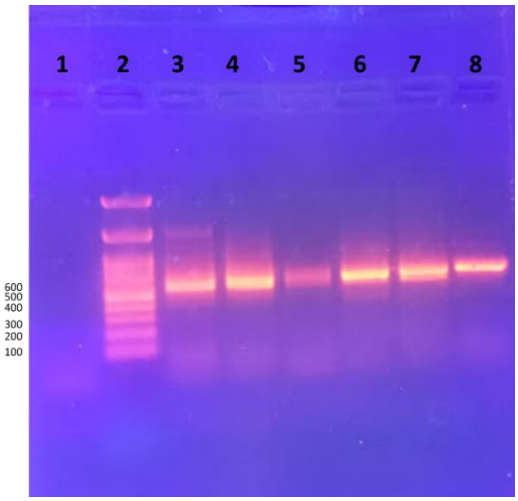

Fig. 5. Agarose gel electrophoresis shows the results of PCR amplified product size 600bp for the ITS gene. Lane 1: Negative control, Lane 2: 100bp ladder, Lane 3,4,5,6,7,8: field samples

In this study, only two samples were subjected to dermatophyte isolation, and two samples were found positive for *M. canis,* and the remaining samples were subjected to PCR for confirmation. The DNA extracted from hair samples was subjected to PCR by targeting the ITS region and was found positive for dermatophyte with an amplicon size of 600 bp product (Fig. 5). In this study, out of 20 collected samples, 17 (85%) samples were positive for dermatophytes by PCR. In this study, samples were tentatively confirmed as positive for dermatophytes based on PCR, but further sequencing for species level was not conducted. By using the same primers, in the study conducted in Iran, the most abundant dermatophyte species detected by PCR-sequencing were *Trichophyton mentagrophytes* (20%), followed by *Trichophyton tonsurans* (10%), *Trichophyton rubrum* (6.7%), *T. interdigital* (6.7%), *Arthroderma otae,* and *Arthroderma vanbreuseghemii,* (3.3%) for each one [13]. Similarly, by using the same primers, the other studies confirm that *M. canis* is the predominant dermatophyte isolated from cats and dogs with dermatophytes with all strains identified as *M. canis* based on sequencing of ITS1-5.8S-ITS2 region [14].

In Yogyakarta, Indonesia 34% of dogs have been diagnosed with dermatophytosis [15]. In Eastern India, 253 out of 1209 dog samples (20.93%) and 109 out of 292 cat samples (37.33%) tested

positive for dermatophyte spores [16]. An early diagnosis and successful treatment of dermatophytosis in a kitten in Puducherry [17]. The zoonotic *M. canis* was the most prevalent in dogs, cats, and humans accounting for 60.0% compared to other species [18]. The various studies concluded that *Microsporum canis* is the primary species causing pets, responsible for 81.8%-97% of cases [19,20,21]. A study in Baku revealed that 108 of 193 dogs and cats were positive for dermatophytosis [22]. The use of the one-step PCR method for the identification of *Microsporum canis* and *Trichophyton mentagrophytes* in pets was also reported [23]. Detection rates for dermatophytes were highest in cattle samples (96.4%-98.2%;) and the lowest in cat and dog samples (60.9%-78.3%) when using direct microscopy, culture, and nested PCR [24]. Few molecular studies conducted to identify the etiological agents of dermatophytes in animals [25,26,27,28,29,30]. DNA-based diagnostic techniques, including sequence analysis of specific gene regions, offer greater reliability than traditional methods for identifying dermatophytes.

4. CONCLUSION

In this study, the early diagnosis of dermatophytosis is useful for early treatment and preventing the disease-causing severity. This study also revealed high dermatophytosis in dogs of Puducherry. Overall, the diagnostic molecular mycological field has undergone significant progress over recent years. Rapid diagnostic tests with objective and reproducible species identification important for treatment purposes and infection control are now an option for the clinical microbiological laboratory. Molecular diagnostic methods are becoming most accessible with high sensitivity, and specificity, and less time-consuming than the conventional methods. The advanced molecular techniques employed in the diagnosis of dermatophytosis are conventional PCR or real-time PCR for DNA extracted from clinical specimens.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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