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Research Article

MYCOTIC DERMATITIS IN DOGS : A STUDY OF 56 DOGS

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ABSTRACT

The aim of the present study was to identify the fungi associated with canine mycotic dermatitis. Clinical samples (hair plucks, skin scrapings) were collected from 181 dogs with skin lesions suggestive of dermatophytosis. The samples were subjected to direct examination by standard potassium hydroxide (KOH) mount technique and then inoculated into both Sabouraud dextrose agar (SDA) and dermatophyte test medium (DTM). Each of the fungal isolate was identified based upon its colony characteristics and hyphal and conidial cells. Of the181 dogs screened, 56 (30.94%) were diagnosed with fungal dermatitis. Fungal elements were detected in 76.78% samples by direct microscopic examination using 10% KOH solution and recovered from SDA and DTM in 94.64% and 91.07% of the cases, respectively. Among dermatophytes, *Microsporum canis*, *Microsporum gypseum* and *Trichophyton spp*. were isolated from 37.5%, 16.07% and 19.65% dogs, respectively. Besides cultural study, scanning electron microscopy of *M.gypseum* was carried out. Among the non dermatophytes, *Aspergillus spp*. (8.93%) and *Penicillium spp.* (3.57%) were isolated. *Malassezia spp.* was isolated in 14.28 % cases. Microscopic examination was found to be simple and rapid method to detect dermatophytes on hair or scales. However, fungal culture proved to be the gold standard for the detection of dermatophytes.

Key words: Canine, dermatophytes, mycotic dermatitis, scanning electron microscopy

Canine mycotic dermatitis is mainly caused by dermatophytes which grow best in warm and humid environment and therefore, more common in tropical and subtropical region of the world (Pal, 2011). Canines are known to serve as reservoirs of the zoophilic dermatophytes and these infections have important zoonotic implication (Iorio *et al.,* 2007). Canine dermatophytosis is the most frequently encountered fungal pathology in canine medicine. The disease is characterized by a superficial skin infection confined to keratinized epithelium. Fungi produce keratinases and others enzymes capable to digest the keratin protein complex, allowing the dermatophyte to burrow deeper into the stratum corneum in the host and therefore to elicit an inflammatory reaction (Levy *et al.,* 2006). *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes* play major roles in causing dermatophytosis of dogs (Szemeredi and Szenci, 2002). In addition, non dermatophytes also contribute to fungal dermatitis in canines. Mycotic dermatitis can be a diagnostic challenge in companion animals. The present communication reports some of the causative agents of mycotic dermatitis in canines. Further identification of *M. gypseum* through cultural, light and electron microscopy is presented.

MATERIALS AND METHODS

A total of 181 dogs brought to Teaching Veterinary Clinical Complex (TVCC), Pantnagar, India with history of dermatitis from May 2012 to April 2013 were screened for fungal involvement as an etiological agent.

Collection of Sample: Skin scrapings were examined for the identification of mycotic organisms. For direct microscopic examination, the hair were plucked with forceps and scales and crusts were removed with blunt scalpel (Hungerford *et al.,* 1998). For cultural studies, the skin scrapings were collected from the infected areas that were first gently cleaned with a moist surgical swab followed by gauze dipped in 70 % ethyl alcohol to remove surface contaminants and allowed to air dry. Hairs were plucked from the margins of lesion using sterile forceps (Muller, 2001). The scrapings were taken from active border areas of the lesions with a sterile scalpel blade and placed in sterilized vials.

Direct Microscopic Examination

Direct microscopic examination of skin scraping (fine scales) and hair specimens was done in 10% potassium hydroxide (KOH) wet mount. The preparation was allowed to stand for 30 min at room temperature and a cover slip was placed over it, and pressed down gently to achieve a preparation as thin as possible. Alternatively, the slide was gently heated for 15-20 seconds. The slide was then examined under low and high power magnification for the presence of fungal elements as long branching septated hyphae, arthrospores and conidia. Part of the sample was also collected on to glass slide and stained with lactophenol cotton blue stain for staining the fungal elements blue in color for better visibility (Scott *et al.*, 2001).

Cultural Studies

Isolation of fungus was done using two different sets of media; one Sabouraud dextrose agar (SDA; Himedia®) with chloramphenicol and cycloheximide to inhibit the growth of saprophytic fungi and bacteria, respectively (George, 1954) and Dermatophyte test agar medium (DTM; Himedia®) to screen the dermatophytes. Skin scrapings collected aseptically using sterile forceps were transferred on to the media for further cultural studies and the isolation of fungi.

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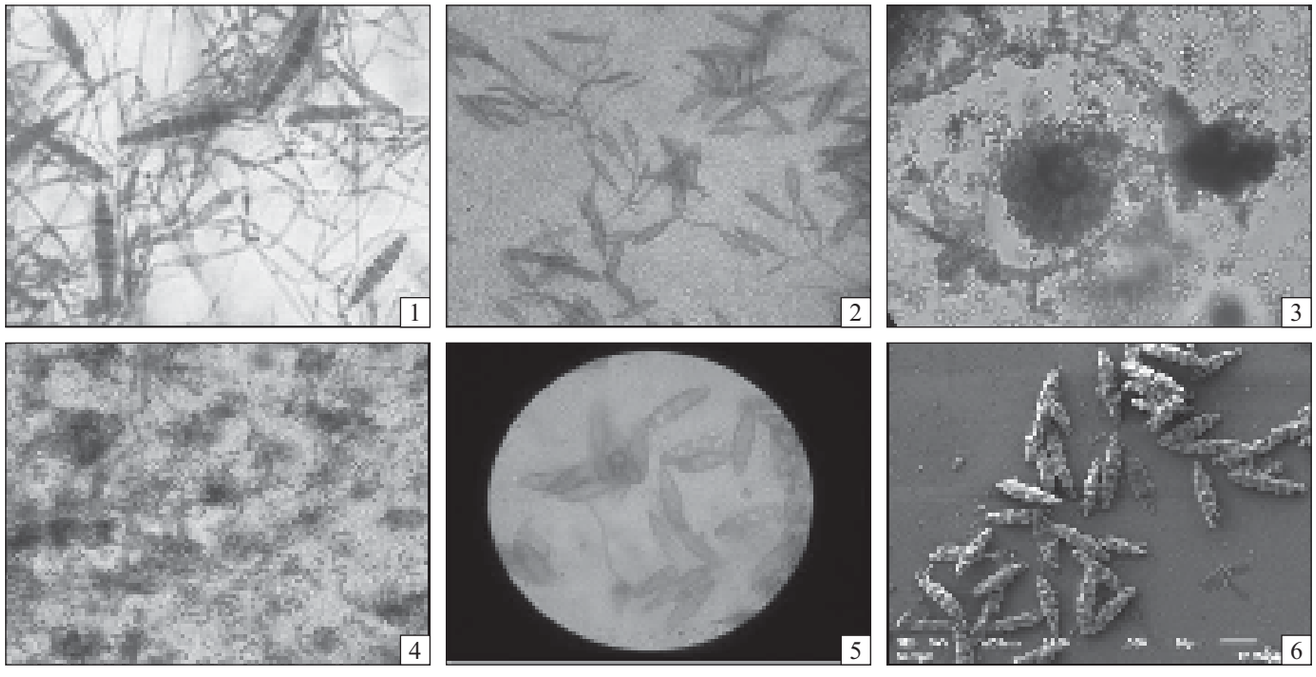

Fig. 1 Well-developed macroconidia and stalked microconidia of *Microsporum canis*; Fig. 2 Thin walled Macroconidia of *M. gypseum*; Fig. 3 Sporangium, spores and hyphae of *Aspergillus spp.* in culture; Fig. 4 *Penicillium spp.* with conidiophores, spores and hyphae in culture; Fig. 5 *M. gypseum* in culture with multicellular thin walled macroconidia with rounded ends; Fig. 6 Scanning electron microscopic image of *M. gypseum* in culture with numerous multicellular macroconidia.

The culture plates and tubes were incubated aerobically at 28°C and the culture growth was observed every two days and the tubes/plates were discarded only after six weeks in the absence of growth. The growth obtained was identified as described by Scott *et al.* (2001). Clear cellophane tape was touched to the surface of the fungal colony and the tape was then adhered to a glass slide already containing a drop of lactophenol cotton blue. The preliminary identification of dermatophytes was based on the macroscopic appearance of colonies and microscopic features of macro conidia and micro conidia (De hoog *et al.,* 2000).

Scanning electron microscopy (SEM) of *Microsporum gypseum* culture was done at Electron Microscopy Lab, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar.

RESULTS AND DISCUSSION

Out of total 181 dogs screened, 56 (30.94%) were diagnosed with mycotic dermatitis. Fungal elements were detected in 43 (76.78%) samples by direct microscopic examination using 10% KOH. Fungi were recovered from SDA and DTM in 53 (94.64%) and 51 (91.07%) of the cases, respectively. The large number of fungal dermatitis cases recorded in the present study might be due to warm and humid climate of the area that favours the fungal growth. Direct microscopy had a higher predictive value for the diagnostic evaluation. These findings corroborated with the findings by Colombo *et al.* (2010) in which direct microscopic examination of skin scrapings for spores and/or hyphae yielded positive results in 29 (78.4%) dogs for dermatophytosis. Tel and Akan (2008) reported higher isolation rates on DTM than SDA which was contrary to our study.

Among dermatophytes, *M. canis* (Fig. 1) and *M. gypseum* (Fig. 2) were isolated in 37.50% and 16.07% cases, respectively, followed by *Trichophyton spp*. (19.65%). Among the non dermatophytes, *Aspergillus spp*. (8.93%) and *Penicillium spp.* (3.57%) were isolated as shown in Fig. 3 and Fig. 4, respectively. *Malassezia spp.* was also identified in 14.28% cases (Table 1). The most common dermatophyte foundto infect dogs was *M. canis* which was in accordance with the study by Torres–Guerrero *et al.* (2016) where *M. canis* was isolated in 72.70% of the dogs with suspected dermatophytosis followed by *M. gypseum* (9.10%) and *T. mentagrophytes* (6.10%). In corroboration with our findings, earlier reports are also available from India and abroad regarding isolation of *M. canis* as the predominant species of dermatophyte in dogs (Beigh *et al*., 2014; Murmu *et al.,* 2015). Order of percentage isolation of fungus in present study is also in accordance with studies conducted by Szemeredi and Szenci (2002) and Brilhante *et al.* (2003), i.e *M. canis* at top in order of prevalence followed by *M. gypseum* and *T. mentagrophytes* in dogs. On the contrary, Álvarez and Caicedo (2001) reported 55.90% isolation of *M. gypseum* which was much higher than in present study. Further, Sidhu *et al.* (1993) reported a higher incidence of *Aspergillus spp.* in dermatomycosis which are in line with the findings of this study. Prado *et al*. (2008)

Table 1

Frequency distribution of the fungi isolated in dermatotic dogs

Fungal isolates	No. of cases	Percentage
Microsporum canis	21	37.50
Microsporum gypseum	9	16.07
Trichophyton spp.	11	19.65
Aspergillus spp.	5	8.93
Penicillium spp.	2	3.57
Malassezia spp.	8	14.28
Total	56	100

reported that the dermatophytes and *Malassezia pachydermatis* were the common pathogenic fungi isolated in dogs and the same was observed in our study. False positive and false negative results are possible with fungal cultures. Cultures may be negative when microscopic examination of hair is positive.

M. gypseum, was studied by cultural methods and scanning electron microscopy (SEM). On SDA, it produced colonies with a flat to granular texture and a buff to cinnamon brown color. The undersurface pigmentation was pale yellow. Microscopic morphology included macroconidia which contained upto six cells with relatively thin walls (Fig. 5). The similar cultural characteristics of *M. gypseum* were studied by Ghannoum and Isham (2009). The SEM image of *M. gypseum* showed numerous multicellular macroconidia with rounded ends (Fig. 6). *M. gyp*seum has been frequently associated with canine dermatophytosis. Its presence is in general attributed to its geophilic character, besides external and internal factors that can alter the normal microbiota of normal animal's skin and thus sets up the infection process (Levy *et al.,* 2006). Species identification of the fungi is important from epidemiological point of view and reducing the spread of fungal infections in animals and humans.

Direct microscopy is rapid method for diagnosis of mycotic dermatitis but cultural examination is gold standard for fungal identification. More of such studies are warranted for the correlation of direct microscopy with cultural methods, so that a quick diagnosis can be made.

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