Comparison of three staining techniques for rapid diagnosis of feline dermatophytosis[#]

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Abstract

Feline dermatophytosis is a commonly encountered fungal skin disease in cats. Skin scrapings from cats with varying degrees of clinical signs were collected and subjected to microscopic and cultural examination. The clinical samples were stained with three different stains, lactophenol cotton blue (LPCB), Chicago sky blue (CSB) and Calcofluor white (CW) and were subjected to microscopic examination for fungal spores. The results of this microscopic examination were analysed and compared with growth of dermatophytes in Dermatophyte test medium (DTM). Based on cultural isolation, dermatophytosis was confirmed in 36 out of 82 cases tested. Comparison of staining techniques revealed that CW staining had better sensitivity (97.22 per cent), specificity (65.22 per cent) and overall agreement with cultures in DTM. Occurrence was found to be higher in kittens less than six months of age and long-haired breeds.

Keywords: *Feline dermatophytosis, lactophenol cotton blue, chicago sky blue, calcofluor white, dermatophyte morphology*

Dermatophytosis is caused by keratolytic fungi belonging to the genera *Trichophyton, Epidermophyton, Microsporum, Lophophyton, Nannizzia, Arthroderm* and *Paraphyton*. The prevalence of dermatophytes was higher in cats and dogs when compared against other species in India (Debnath *et al.,* 2016). Cats are considered to be reservoir host for some of them such as *Microsporum canis* (Tunsagool *et al.*, 2021*)*. Staining of skin scrapings for initial screening of dermatophytes will facilitate improved diagnosis and hence better treatment of dermatophytes. The

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study was conducted to evaluate three staining methods for visualisation of fungal spores from skin scrapings and thus help in initial screening and rapid diagnosis of dermatophytosis among cats.

A total of 82 cats presented to the Teaching Veterinary Clinical Complex, Pookode and Peripheral Veterinary clinic, Kakkavayal during the period from September, 2021 to December, 2022 with dermatological signs suggestive of dermatophytosis were selected for the study. Skin scrapings and hair plucking were aseptically collected and subjected to direct microscopy as well as cultural examination.

Comparative efficacy of three staining techniques using Lactophenol cotton blue (LPCB), Chicago sky blue (CSB) and Calcofluor white (CW) stains were assessed. Skin scrapings were evenly mixed with 10 per cent potassium hydroxide solution on a clean glass slide, heated for few minutes and cooled. A drop of the stain was then added to this mixture and a coverslip was placed. The mixture was allowed to react for one minute. The procedure was repeated for all three stains. The prepared slide was subsequently observed under 10x and 40x objective immediately after 5 min, 15 min and 30 min respectively. Light microscope was used for LPCB and CSB and a fluorescence microscope (Olympus CX 51™) with violet blue light source was used for CW stains. The clinical samples were also inoculated on the surface of DTM and incubated at room temperature for a maximum of four weeks.

Of the 82 samples tested, 59 (71.95 per cent) samples were positive using lactophenol cotton blue stain (LPCB). A total of 57 (69.51 per cent) and 51 (62.19 per cent) samples were found positive for fungal spores upon staining with Chicago sky blue (CSB) and calcofluor white (CW) stain respectively (Fig 1). Prakash *et al.* (2023) reported that, CSB was a more effective stain than normal potassium mount for diagnosing superficial fungal infections.

Out of the 82 samples cultured in DTM, 36 isolates were identified as dermatophytes based on colour change in DTM and microscopic examination. Non dermatophytic fungi either fails to grow in DTM or will not be able change the colour of the medium to red (Rich *et al.*, 2003). Ashwini *et al.* (2020), reported two cases of feline dermatophytosis diagnosed by cultural isolation in DTM. Results of staining techniques were compared with culture in DTM as a gold standard using Kappa statistics.

Statistical analysis revealed that CW stain showed significant agreement with culture results with maximum sensitivity (97.22), specificity (65.22), positive predictive value (68.6), negative predictive value (96.8) and accuracy (45.12). This finding is in accordance with Dass *et al.* (2015), who stated CW white as an excellent way of finding clinically suspected mycotic agents with high sensitivity and negative predictive value. The higher sensitivity of CW could be due to ease of identifying fluorescence in dark background produced by fungal elements. Robert and Pihet (2008), stated that CW stain had better sensitivity and
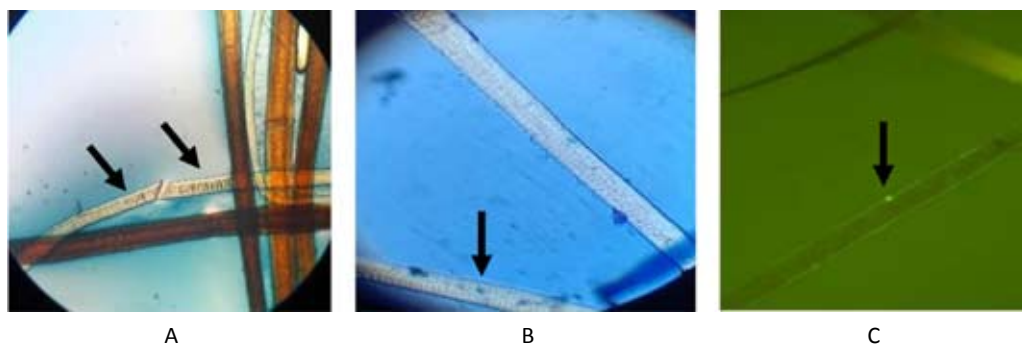
Fig 1. Direct staining of skin scrapings

A: Lactophenol Cotton Blue B: Chicago Sky blue C: Calcofluor White

specificity when compared against many other stains for the detection of dermatophytes from clinical samples but had the disadvantage of requiring a fluorescence microscope.

The age group of affected cats were compared using chi square test for multiple proportion and as the p-value was found to be significant, pair wise comparison was done by using z-test for two independent proportions. Occurrence of dermatophytes was found to be highest in the age group below six months of age, with 82.25 per cent of affected cats being less than six months of age. Similarly, an increased rate of *M. canis* infection in animals younger than one year was evident from the studies of Cafarchia *et al.* (2004). Gross *et al.* (2008) observed more dermatophytosis in kitten and queens due to stress and poorly developed immunity. Similar observations were also reported in canine cases by Prakash *et al.* (2022). Out of 36 positive cases, 28 (77.77 %) were long haired breeds. Analysis revealed significantly higher number of long haired breeds (Z value = 4.772, p<001) being infected. Moriello (2004) inferred the high clinical prevalence of dermatophytosis in long haired cats because longer hairs trapped more spores, creating a hot spot for fungal invasion. Clinical signs and lesions varied greatly among all the affected cats, with alopecia and pruritus being predominant signs. Similar clinical manifestation was reported by Moriello and Newbury (2006). Tarra *et al.* (2022) reported that majority of the animals affected with dermatophytosis were presented with variable clinical signs and distribution of lesions.

Summary

Dermatophytosis is a common condition in cats and is presented with varying clinical signs. Prompt and efficient treatment for the disease can be ensured by having a rapid and valuable diagnostic test like microscopy of skin and hair samples to detect fungal spores. In the present study, Calcofluor white staining of skin scrapings showed better agreement with cultural isolation due to the high contrast provided by the dark background in a fluorescence microscope. It was observed that 43.9 per cent of cats with suggestive clinical signs tested positive for dermatophytes and out of 36 isolates 29 were *M. canis* and seven were *M. gypseum.* The occurrence of dermatophytosis was more frequent among kittens below six months of age and long-haired breeds.

Acknowledgement

Conflict of interest

The authors declare that they have no conflict of interest.

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