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Dermatophytosis in cats: A comprehensive study on diagnostic methods and their accuracy

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Abstract

Background: Dermatophytosis is a contagious fungal infection that affects mainly cats. It poses significant challenges in veterinary medicine due to its zoonotic potential and impact on animal and public health. Rapid and reliable diagnosis is crucial for preventing the spread of the disease, guiding treatment decisions, and monitoring disease control efforts. Although there are several studies on diagnostic methods in feline dermatophytosis, the comparison between them from the same sample lacks data. The absence of a universally accepted gold standard diagnostic method highlights the need for a multifaceted approach to diagnosing feline dermatophytosis.

Aim: This study aims to assess the accuracy and efficacy of different diagnostic techniques comprehensively.

Methods: For this, 48 samples of cats were analyzed by dermoscopy, direct hair examination, fungal culture using various media (Mycosel, Sabouraud, and Dermatophyte Test Medium), and polymerase chain reaction (PCR).

Results: Direct examination and dermoscopy yielded unsatisfactory results. Mycosel and Sabouraud were suboptimal. DTM demonstrated superior selectivity, making it the most reliable among traditional methods. PCR was the top performer, exhibiting singular sensitivity, specificity, and accuracy.

Conclusion: The study suggests that PCR may be the preferred choice for diagnosing feline dermatophytosis in clinical practice, especially when rapid and accurate results are essential.

Keywords: Dermoscopy direct examination, Fungal culture, PCR.

Introduction

Dermatophytosis is a disease due to superficial fungal and zoonotic of great importance for felines. This disease is easily contracted by direct contact with infective arthrospores and may present miscellaneous clinical presentations, making the diagnostic tools important in detection (Moriello, 2014). The absence of a universally accepted gold standard diagnostic highlights the need for a multifaceted approach to diagnosing dermatophytosis in this animal species.

Materials and Methods

Forty-eight cats were selected based on sample size calculation: 24 cats were needed for both a symptomatic (SF) and asymptomatic (AF) group to ensure reliable results. The cats underwent a dermatological examination, assessed the entire body, and examined the skin by lifting the hair in the opposite direction of its growth. Wood's lamp (Estek[®], Rua Tenente-Coronel Soares Neiva, 412, Vila Aricanduva, São Paulo, SP, Brasil) assessment was utilized to pinpoint lesions and evaluate luminescence, categorizing cats into

symptomatic and asymptomatic groups based on lesion presence and luminescence intensity.

Dermoscopy was conducted with minimal restraint using an electronic microscope (Imports[®], type M 1,000×), with recorded images later subjected to evaluation. The time used to collect images and samples was allowed by the animal, valuing comfort and well-being. Sample collection involved disposable sterile brushes (Kolplast[®] Estr Municipal Benedito de Souza, 418, Itupeva, SP), with samples obtained from the margins of recent lesions in the SF group and over the entire body in the AF group. The collected hair was subdivided into five sub-samples (A, B, C, D, and E). The direct examination involved transferring Sample A from each cat, in both groups, to a glass slide immersed in 10% Potassium Hydroxide (KOH) for 20 minutes, followed by a 400× examination. Fungal culture samples (B, C, and D) were inoculated onto plates containing Mycosel, Sabouraud (own manufacturing), and dermatophyte test medium (DTM) (Conclue[®], Ouro Fino Saúde Animal, Cravinhos, São Paulo) media, respectively, with samples showing growth examined microscopically to identify fungal elements.

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Samples E underwent DNA Red Extract-N-Amp Plant (SIGMA, Eschenstr. 5 82024 Taufkirchen, Germany) and polymerase chain reaction (PCR) using specific dermatophyte primers (Dąbrowska et al., 2014) and PCR results analyzed via agarose gel electrophoresis.

Ethical approval

The study was approved by the Committee on Ethics in the Use of Animals (CEUA) under protocol number CEUA - 315/2022. All owners of the animals selected for this study signed informed consent forms.

Results and Discussion

As shown in Table 1, no affected hairs were found in the direct examination in the SF group. In dermoscopy, “comma hairs” were found in only 9% of the animals. In fungal cultures, out of the 23 cultures on Mycosel medium, 15 (65%) isolated some dermatophyte. This result was also obtained in 12 (52%) cultures on the Sabouraud medium. In both media, contaminating fungal growth was observed in all plates. All individuals in the SF group would have tested positive for dermatophytosis on the DTM medium if only the color change of the medium was evaluated

simultaneously with the growth of a white “cloud”-shaped colony. However, at the end of 28 days, macroconidia of *Microsporum canis* were observed in only 16 (69%) of the 23 microscopic examinations. Only one DTM tube showed contaminating fungal growth. In the AF group, all plates and tubes of culture media showed contaminating fungal growth.

All results from individuals in the SF group were positive in PCR. Considering these results, the values of accuracy, sensitivity, and specificity of the tests were presented in Table 2.

In 2017, the World Association for Veterinary Dermatology recognized the absence of a gold standard for feline dermatophytosis (Moriello et al., 2017). This shift in perspective signifies the reconsideration of previously accepted clinical protocols.

This study employed the Wood’s Lamp technique to identify lesions and aid in selecting animals for the research. Individuals with ulcerated and exudative dermatological complaints were excluded. Studies have shown that the sensitivity of direct examination varies, and it has been described as unsatisfactory (Moriello et al., 2017). An essential factor highlighted elsewhere

Table 1. Results of the methods: direct examination, dermoscopy, cultures, and PCR of the SF Group.

Indivíduo	Exame direto	Dermoscopia	Mycosel	Sabouraud	DTM	PCR
1	Negative	Negative	Negative	Positive	Positive	Positive
2	Negative	Negative	Negative	Negative	Negative	Positive
3	Negative	Negative	Negative	Positive	Positive	Positive
4	Negative	Negative	Positive	Positive	Positive	Positive
5	Negative	Negative	Negative	Positive	Negative	Positive
6	Negative	Positive	Positive	Negative	Positive	Positive
7	Negative	Negative	Positive	Positive	Positive	Positive
8	X	x	x	x	x	x
9	Negative	Negative	Negative	Negative	Positive	Positive
10	Negative	Negative	Positive	Positive	Negative	Positive
11	Negative	Negative	Positive	Negative	Positive	Positive
12	Negative	Negative	Positive	Negative	Negative	Positive
13	Negative	Negative	Negative	Positive	Positive	Positive
14	Negative	Negative	Positive	Positive	Positive	Positive
15	Negative	Negative	Positive	Positive	Positive	Positive
16	Negative	Negative	Negative	Negative	Negative	Positive
17	Negative	Negative	Positive	Negative	Positive	Positive
18	Negative	Negative	Positive	Positive	Positive	Positive
19	Negative	Negative	Positive	Negative	Positive	Positive
20	Negative	Negative	Negative	Negative	Positive	Positive
21	Negative	Negative	Positive	Negative	Negative	Positive
22	Negative	Negative	Positive	Negative	Positive	Positive
23	Negative	Positive	Positive	Positive	Positive	Positive
24	Negative	Negative	Positive	Positive	Negative	Positive

Table 2. Sensitivity and specificity analysis, positive predictive value, negative predictive value, and accuracy.

Sensibilidade		Especificidade	VPP	VPN	Acurácia
Exame direto	-	100%	-	51%	51%
Dermoscopia	8%	100%	100%	53%	55%
Mycosel	65%	100%	100%	75%	82%
Saubouraud	52%	100%	100%	68%	76%
DTM	69%	100%	100%	77%	85%
PCR	100%	100%	100%	100%	100%

(Căpitan *et al.*, 2018) is the examiner's experience. Due to the lack of positive results, it was not possible to calculate the sensitivity and positive predictive value (PPV) of the technique. Despite 100% specificity, the negative predictive value (NPV) was 51%, and the accuracy was 51%, representing the lowest values among all the tests.

Dermoscopy was employed in this study as a rapid and cost-effective method (Dong *et al.*, 2016). However, extensive physical restraint would have been required to obtain more precise imaging, contrary to the findings of other researchers (Scarpella *et al.*, 2015). The level of physical restraint needed was time-consuming and substantial compared to alternative techniques, and no literature supported the use of chemical restraint. The significant magnification of the camera, coupled with the subject's constant movement, posed challenges in image analysis. Areas of alopecia, skin scaling, and damaged hairs were observed in all SF groups, consistent with previous studies (Malvey, 2007; Scarpella *et al.*, 2015). However, the "comma hairs," found in only two SF group cats, were considered unequivocally positive (Malvey, 2007). Veterinary medicine lacks standardization for interpreting "comma hairs." In human medicine, they are associated with dermatophytosis. This lack of standardization raises concerns about potential false positives when using "comma hairs" as a diagnostic criterion. Nonetheless, the absence of these hairs in the AF group led to a specificity of 100%, resulting in a high PPV (100%). However, the technique's sensitivity in this study was low at 8% due to its reliance on "comma hairs" as a diagnostic determinant, yielding an NPV of 75%.

In this study, all fungal culture media in the SF group exhibited fungal growth, with both Mycosel and Sabouraud media showing multiple colonies on all plates after 4 weeks: contaminating fungi were observed even when dermatophytes were present. This rapid growth of non-pathogenic fungi may compete with dermatophytes on the plate, potentially leading to false negatives. Both media demonstrated similar performance, with the Mycosel medium achieving a sensitivity of 65%, an NPV of 75%, and an accuracy of 82%, surpassing that of the Sabouraud medium. These values aligned with findings from other researchers (Kaufmann *et al.*, 2016). Due to the absence of

false positives, both techniques exhibited maximum specificity and PPV of 100%, ensuring the reliability of positive results.

DTM media in the SF group exhibited a color change between 3 and 6 days, with whitish cottony colonies growing over the 4-week observation period. However, microscopic confirmation of *M. canis* presence was possible in only 69%. Therefore, microscopy evaluation remains crucial, as recommended (Kaufmann *et al.*, 2016). The lack of complete fungus growth in seven tubes after 28 days led to their classification as negative. One probable reason for unsatisfactory growth is the limited space for fungal expansion within the culture medium tubes, which have a width of 2.5 cm, significantly smaller than the plates used with Mycosel and Sabouraud media. This space limitation and potential nutrient limitation can impede fungal growth and macroconidia development, as previously observed for dermatophytes in DTM (Moriello *et al.*, 2010). In the AF group, there was some color variation in 66% of the tubes, but the dark red color seen in the SF group was not consistently achieved. This phenomenon indicates that contaminating fungi can induce the pH change responsible for color conversion (Moriello *et al.*, 2017). Historically, the color change in DTM was considered evidence of dermatophyte presence. This was not confirmed microscopically, with no dermatophytes found in these samples. DTM presented the highest values among traditional fungal cultures.

The PCR technique for dermatophytosis was developed and published in 2014 (Dąbrowska *et al.*, 2014). Using the "Pan-dermatophyte" primer did not allow the identification of the dermatophyte species. Although a positive diagnosis for dermatophytosis is already sufficient to initiate treatment, it is not specific to each dermatophyte species. Thus, reliability in the result and speed are more valuable factors for the clinical veterinarian than species differentiation. There were no false positives or negatives for PCR in this study, demonstrating 100% sensitivity, PPV, NPV, and accuracy. The test was also one of the fastest, providing results in less than 8 hours, comparable to dermoscopy and direct examination regarding result readiness.

Conclusion

This study evaluated various diagnostic techniques for dermatophytosis in feline patients with clinical signs of active infection. While direct examination and dermoscopy performed unsatisfactorily, traditional fungal culture media, including Mycosel and Sabouraud, provided better but still less than ideal results. Notably, the DTM showed superior selectivity, making it the most reliable among the traditional methods. However, the real standout was PCR, which demonstrated singular sensitivity, specificity, and accuracy, outperforming all other techniques. Due to its speed and reliability, these findings suggest that PCR may be the preferred choice for diagnosing feline dermatophytosis in animals with classical signs of the disease, especially when rapid and accurate results are crucial. However, the use of complementary examinations is often needed for a more accurate diagnosis.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors contributions

All authors contributed to this study. All authors read and approved the final manuscript.

Data availability

All data supporting the findings of this study are available within the manuscript.

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