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ORIGINAL RESEARCH ARTICLE

Retrospective Comparison of Polymerase Chain Reaction and Culture-Based Identification for Diagnosis of *Microsporum canis* and *Trichophyton Spp*. in Shelter Cats and Dogs

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Abstract

***Introduction*:** Canine and feline dermatophytosis is challenging to manage in shelter settings because of its transmissibility, potential for zoonosis and labour-intensive treatment. An efficient and accurate initial diagnostic plan is essential to confirm infection, prevent outbreaks and ensure public safety. While quantitative polymerase chain reaction (PCR) has gained attention for its rapid turnaround time, studies have shown variable sensitivity. This study aimed to assess the performance of PCR in detecting *Microsporum canis* and *Trichophyton* spp. in shelter cats and dogs when compared to culture-based identification.

***Methods*:** Between 1 June 2015 and 10 January 2023, 125,939 cats and dogs entered 34 sheltering locations in coastal Western Canada. Of these, 246 animals (48 dogs, 198 cats) had both culture and PCR results, and sufficient records for analysis. Toothbrush samples were collected for dermatophyte test medium (DTM)/enhanced sporulation agar (ESA) bi-plates at a central shelter location and submitted for PCR at a referral laboratory before treatment was initiated. The diagnostic accuracy of PCR was evaluated using the reference standard of a 14-day culture result combined with P-scoring, a semi-quantitative method to assess DTM culture colonies.

***Results*:** Culture readings identified 72/246 (29.27%) lesional animals as positive (63 cats [31.81%] and 9 dogs [18.75%]). PCR demonstrated an overall sensitivity of 86.1% and specificity of 94.8% for both animal species combined. PCR for *M. canis* showed a sensitivity of 86.4% and a specificity of 97.8%, while PCR for *Trichophyton* spp. showed a sensitivity of 84.6% and specificity of 97.9%. DTM/ESA culture was highly efficient, with positive results available within 7 days for most cases (59/63 cats [93.6%] and 9/9 dogs [100%]).

***Conclusion*:** The variability in dermatophyte PCR sensitivity across recent studies highlights the risks of reliance on PCR as a sole diagnostic tool for suspect animals, especially when considering the demonstrated efficacy of in-house culture.

Keywords: *ringworm*; *dogs*; *cats*; *shelter medicine*; *diagnostic methods*; *public health*

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Supplementary material
Supplementary material for this article can be accessed here.

Dermatophytosis is a fungal skin infection affecting many species of animals. The most commonly isolated dermatophytes in cats and dogs are *Microsporum canis* (*M. canis*), *Trichophyton* spp. and *Microsporum gypseum* (*M. gypseum*).[1,2] Although dermatophytosis is not a life-threatening disease, it is highly contagious and potentially zoonotic.[3] Unidentified or misdiagnosed cases in animal shelters can lead to outbreaks or community transmission, potentially leading to devastating consequences for the shelter and the affected animals.[2] A system of screening and diagnostic tests is therefore recommended to efficiently identify clinically affected animals at shelter intake and in shelter care to minimise the risk of transmission.[2]

Shelter screening tests for dermatophytosis include thorough history collection, physical examination and Wood's lamp examination.[2] For animals that require further confirmation, the two most common diagnostic tests are culture-based identification and polymerase chain reaction (PCR), a molecular diagnostic technique that amplifies DNA to detect the presence of specific genetic material.[3]

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Culture-based identification involves isolating fungal spores from a clinical sample grown in culture medium for microscopic identification. Commonly used fungal culture systems include dermatophyte test medium (DTM), enhanced sporulation agar (ESA) and modified Sabouraud dextrose agar (SDA). DTM and ESA contain a colour indicator, while ESA and SDA contain additives that inhibit bacteria and saprophytic fungi.[4] A positive result indicates the presence of fungal spores on the coat of the sampled animal, but it does not distinguish between animals acting as mechanical carriers and truly infected animals.[2] A non-validated semi-quantitative method of counting colony-forming units (CFUs), termed "P-scoring", is described in several studies and can be used in shelter settings to better distinguish between these two categories.[1,5–7] Alongside clinical examination and Wood's lamp results, this technique can guide diagnostic and treatment decisions.[5]

The main disadvantage of fungal culture is that it can take up to 21 days to allow for dermatophyte identification or confirm a negative culture, though there is evidence that characteristic culture growth often appears by Day 7.[8–11] In comparison, PCR testing has a higher cost but an estimated turnaround time of only 1–3 days.[12] Dermatophyte PCR testing first became commercially available in Canada in 2014 (IDEXX Reference Laboratories, Inc.; Westbrook, ME, USA), with various additional assays becoming available since.[13,14] In a shelter setting, the shorter turnaround time for PCR testing may alleviate the resource and welfare issues associated with holding animals for extended periods while waiting for results. However, the diagnostic accuracy of dermatophyte PCR assays has been studied in both shelter cats and owned cats and dogs with conflicting results.[15–20] The additional shipping time of samples to an external laboratory must also be considered, whereas DTM cultures can often be conducted and interpreted internally.

Although no single diagnostic test can accurately identify every case of dermatophytosis, culture-based identification is still considered the most reliable diagnostic method.[3] It remains the current reference standard in several studies evaluating the efficacy of PCR in diagnosis and determining treatment success of dermatophytosis in humans and domestic animals.[11,15,16,20–23]

Using data collected over several years, the objective of this study was to assess the sensitivity and specificity of initial dermatophyte PCR assays performed by a referral laboratory for *M. canis* and *Trichophyton* spp. compared to in-house fungal culture results in shelter dogs and cats with suspicious lesions. To the authors' knowledge, this is the first study that evaluates the performance of PCR in the detection of two common dermatophyte species in both shelter cats and dogs.

Methods

Shelter practices and data collection

All study data are from the British Columbia Society for the Prevention of Cruelty to Animals (BC SPCA), a shelter system in coastal Western Canada that operates 34 sheltering locations, each with an annual intake ranging from 139 to 1,952 animals. During the study period (1 June 2015 to 10 January 2023), a total of 125,939 dogs and cats entered BC SPCA shelters. The scope of this study was limited to cats and dogs, as a previous study found that dermatophytosis occurred infrequently in rabbits and guinea pigs entering BC SPCA shelters and because the Dermatophyte PCR Panel used is only validated for cats and dogs.[1,12] The BC SPCA Animal Welfare Committee reviewed the proposed study and granted permission to access shelter data, in accordance with the internal ethics guidelines. Data were retrieved from the shelter software database used across all BC SPCA locations (Shelter Buddy, The Animal Shelter Database System, RSPCA).

All incoming cats and dogs received a standardised physical check by trained staff members at shelter intake. This included recording signalment as well as an examination of the haircoat in room light, followed by screening with a Wood's lamp. If lesions consistent with possible dermatophytosis were identified or an animal had known exposure (such as sharing a highly contaminated environment), the animal was defined as high risk and sampled for dermatophyte culture. A subset of animals cultured was also sampled for PCR testing. Because of cost, not all animals underwent PCR testing, but all high-risk animals were cultured. Selection for PCR testing was based on the presence of lesions (only animals with lesions were selected for PCR) and whether a faster initial result might aid in pathway planning.

Animals receiving both tests were sampled in duplicate by trained shelter staff using a modified Mackenzie toothbrush technique.[3] In most cases, both samples were collected at the same time. If only one sample was initially collected, the second sample was obtained within 3 days, before initiating treatment. Personnel wore personal protective equipment during sampling to minimise cross-contamination. Samples were placed into clean sealable plastic bags; culture samples were sent to the BC SPCA Vancouver Hospital, and PCR samples were sent to IDEXX Veterinary Laboratories via courier or mail. All cultures were plated onto DTM/ESA bi-plates (Sensor Health RW2 Culture System, Cambridge, Ontario) and managed in an incubator (Quincy Lab, Model 12E Incubator, Chicago, IL) kept at 25–30°C and 42–46% humidity for 14 days. Plates were monitored by a small number of trained veterinary personnel.

Plate examination was performed every 1–3 days with results recorded in a written laboratory notebook; formal

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results were recorded in an online spreadsheet at 7 and 14 days. Standard techniques were used to plate, monitor and record cultures, including microscopic identification of all suspect fungal colonies and P-scoring.[1,3] A P-score was obtained for each culture plate by counting the number of CFUs at Day 7 and Day 14 following inoculation.[7] Bi-plates were used for all cultures and CFUs were counted on both sides of the plate (DTM/ESA). Plates with 1–4 CFUs were given a P1 score, plates with 5–9 CFUs were given a P2 score, and plates with over 10 CFUs were given a P3 score. For the purpose of this study, and consistent with a previous study, all P2 and P3 plates, as well P1 plates from animals with subsequent positive follow-up cultures were coded as "positive".[1] Plates with no growth, contaminant growth or P1 growth from animals, who were subsequently negative (fomite carriers), were coded as "negative".

PCR results were reported electronically to the same personnel who managed the plates, and transcribed into the main results spreadsheet used to track culture results. Because *M. canis* was the only *Microsporum* species identified on culture across the study period, PCR results reported as *Microsporum* spp. or *M. canis* were coded as "*M. canis*" for analysis. Results were also reported and coded as *Trichophyton* spp. or negative. Results returned as "insufficient sample" were not included in the study.

Data cleaning

Out of the 125,939 cats and dogs that entered BC SPCA shelters during the study period, 658 cats and dogs met the definition of high risk and had culture samples taken. Among these high-risk animals, there were 308 recorded PCR test results.

The data were cleaned, and 62 animal records were removed for following reasons: record-keeping errors or discrepancies ($n = 30$), if PCR and culture samples were collected more than 3 days apart ($n = 20$), if samples were collected after treatment had started ($n = 5$), if PCR was conducted at another diagnostic laboratory ($n = 1$) or if samples were pooled from litters ($n = 6$). The remaining 246 records were retained for analysis, comprising 48 dogs and 198 cats. These included three non-standard cases: a kitten whose fungal speciation was performed at the referral laboratory (rather than in-house), a kitten who had initial PCR results listing both positive and negative (with laboratory confirmation of an actual negative result), and a kitten whose fungal identification was not recorded, but a littermate's identification was recorded.[11]

Statistical analysis

In this dataset, a 14-day culture result was used as the reference standard to which PCR results were compared.

Analyses were completed using Microsoft Excel (Microsoft Corporation, 2018) for initial data cleaning, followed by descriptive and analytical analysis using Stata (StataCorp, release 17, 2021). Diagnostic calculations were done using the Stata "-DIAGT- version 2.0.5" package (Seed, Statistical Software Components S423401, 2001), which uses exact binomial distribution to calculate confidence intervals (CI).

Pearson chi-square tests were used to compare cases by host species (feline vs. canine) and by age (adult vs. juvenile).

The initial analysis consisted of calculations of sensitivity and specificity for the comparison of PCR to the Day 14 culture readings for all animals for all positive cases of dermatophytosis. Subsequent calculations separated the dataset by target group (species, age) and by dermatophyte species (*M. canis*, *Trichophyton* spp.) to obtain more detailed results.

Sensitivity and specificity were calculated using a 2 × 2 table where the results of the PCR were compared to the Day 14 culture readings.

Results

Overall, culture readings identified 72/246 animals (29.27%) as positive (cats: $n = 63$; 31.81%; dogs: $n = 9$; 18.75%). PCR was positive for dermatophytosis in 71 of the suspected animals (cats: $n = 61$; 30.81%; dogs: $n = 10$; 20.83%), but positive results were sometimes discordant between PCR and culture. There were nine "false negative" PCR results, all of which had a P3 score on culture. No dogs cultured positive for *Trichophyton* spp. during this study period. A chi-square analysis revealed no statistically significant difference between species in their proportion of positive cases as determined by culture (χ^2 (1, $n = 246$) = 3.19; $p = .07$).

Table 1 lists the overall sensitivity and specificity of PCR for all species of dermatophytes combined as well as *M. canis* and *Trichophyton* spp. individually. Table 2 further lists the sensitivity and specificity of PCR for each dermatophyte species in cats and dogs, respectively.

Across both species, more juvenile animals had positive culture results ($n = 51/83$; 61.45%) compared to adult animals ($n = 21/163$; 12.89%; $\chi^2(1, n = 246) = 62.65$; $p < .001$). *M. canis* infection was more frequent in juvenile animals (32 cats and 8 dogs out of 83 juvenile animals; 48.19%) than adult animals (18 cats and 1 dog out of 144 adult animals; 13.19%). *Trichophyton* spp. infection occurred almost exclusively in juveniles (10 cats out of 73 animals; 13.70%) with only 2 cases in adults (2 cats out of 163 animals; 1.23%).

Among culture-positive cats, 59/63 (93.6%) had positive culture results by Day 7, while the remaining 4 culture plates became positive by Day 14. These were 2 juvenile and 2 adult cats that were negative on Day 7 but became positive by Day 14 (culture identified *Trichophyton* spp. in 1 case and *M. canis* in 3 cases by Day 14). All nine culture-positive dogs had finalised culture results by Day 7 (Table 3).

Table 1. Sensitivity and specificity of PCR for diagnosis of *Microsporum canis* and *Trichophyton* spp. in both cats and dogs combined, when compared to DTM/ESA culture results

PCR result	Culture positive	Culture negative	Total	Sensitivity (CI 95%)	Specificity (CI 95%)
Overall					
PCR positive	62	9	71	86.1% (75.9%–93.1%)	94.8% (90.4%–97.6%)
PCR negative	10	165	175		
Total	72	174	246		
***Microsporum canis* in cats and dogs combined**					
PCR positive	51	4	55	86.4% (75.0%–94.0%)	97.8% (94.6%–99.4%)
PCR negative	8	183	191		
Total	59	187	246		
***Trichophyton* spp. in cats and dogs combined**					
PCR positive	11	5	16	84.6% (54.6%–98.1%)	97.9 (95.1%–99.3%)
PCR negative	2	228	230		
Total	13	233	246		

PCR, polymerase chain reaction; DTM, dermatophyte test medium; ESA, enhanced sporulation agar.

Table 2. Sensitivity and specificity of PCR for diagnosis of *Microsporum canis* and *Trichophyton* spp., separated by animal species (cats, dogs), when compared to DTM/ESA culture results

PCR result	Culture positive	Culture negative	Total	Sensitivity (CI 95%)	Specificity (CI 95%)
***Microsporum canis* in cats**					
PCR positive	43	3	46	86.0% (73.3%–94.2%)	98.0% (94.2%–99.6%)
PCR negative	7	145	152		
Total	50	148	198		
***Trichophyton* spp. in cats**					
PCR positive	11	4	15	84.6% (54.6%–98.1%)	97.8% (94.6%–99.4%)
PCR negative	2	181	183		
Total	13	185	198		
***Microsporum canis* in dogs**					
PCR positive	8	1	9	88.9% (51.8%–99.7%)	97.4% (86.5%–99.9%)
PCR negative	1	38	39		
Total	9	39	48		
***Trichophyton* spp. in dogs**					
PCR positive	0	1	1	NA	NA
PCR negative	0	47	47		
Total	0	48	48		

PCR, polymerase chain reaction; DTM, dermatophyte test medium; ESA, enhanced sporulation agar.

Discussion

The overall sensitivity and specificity of PCR were 86.1% and 94.8%. PCR for *M. canis* in both animal species combined had a sensitivity and specificity of 86.4% and 97.8%, respectively, while PCR for *Trichophyton* spp. had a sensitivity and specificity of 84.6% and 97.9%, respectively. Of note, there were nine truly infected animals, as confirmed by P3 score on culture results, which had false-negative PCR results.

Two existing published studies have yielded varying results regarding the accuracy of dermatophyte PCR. One study in shelter cats comparing *Microsporum* PCR to culture for initial diagnosis reported a sensitivity of 100% and specificity of 88.5%.[16] Another study conducted in a referral hospital setting examined the concordance between PCR and culture for both *M. canis* and *Trichophyton* spp. in cats and dogs, reporting a sensitivity of 72.4% and a specificity of 98.7% for the initial diagnosis of dermatophytosis.[19]

Two additional studies have shown a strong concordance between non-commercially available dermatophyte PCR and culture in a small group of patients.[17,18] However, PCR techniques and equipment are not standardised across different laboratories, and the accuracy of

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Table 3. Day 7 and Day 14 fungal culture results in cat and dog samples

	Feline		Canine		Total	Percentage (%)
	Juvenile	Adult	Juvenile	Adult		
Culture on Day 7						
Negative	29	109	5	34	177	72
P1	2	2	0	0	4	2
P2	1	2	0	0	3	1
P3	38	15	8	1	62	25
Total	70	128	13	35	246	100
Culture on Day 14						
Negative	27	107	5	34	173	70
P1	0	3	0	0	3	1
P2	1	0	0	0	1	0
P3	42	18	8	1	69	28
Total	70	128	13	35	246	100

these tests may vary because of the differences in DNA extraction methods, assay conditions and primer design.[24]

Although earlier data suggested a very high PCR sensitivity for the initial diagnosis of *Microsporum*, the findings of the present study and those by Frost et al. suggest that the sensitivity of PCR may be comparatively lower than previously observed.[16,19] While PCR can offer quicker turnaround times than fungal culture, negative results should be carefully interpreted. An overreliance on PCR to rule out dermatophytosis in a shelter environment could have potentially devastating population-level and public health impacts.

The overall specificity in this study was similar to the specificity reported by the laboratory and Frost et al., which were higher than that reported by Jacobson et al., suggesting that there may be less concern over "false positive" PCR results, leading to the unnecessary treatment of animals with suspicious lesions.[16,19]

In this study, the accuracy of PCR testing was evaluated against the reference standard of a 14-day culture result. Although earlier literature recommends monitoring inoculated culture plates for 14–21 days, recent data suggest that a 14-day period is sufficient to appreciate fungal growth in samples taken from untreated cats.[9,11] The pattern of early DTM/ESA culture growth was also observed in this study for both dogs and cats. These results further support that culture can be used to identify most animals with either *M. canis* or *Trichophyton* spp. infections within 7 days, making it a comparably efficient tool for diagnosing dermatophytosis in a shelter setting regardless of dermatophyte species.

M. canis and *Trichophyton* spp. infections were more frequent in juvenile animals in this study. This finding was expected as younger animals are thought to be more susceptible to dermatophytosis and more likely to suffer from more severe infections.[2,11,25,26]

Limitations of the study

Limitations that should be considered when interpreting the findings of this study include the number and selection of cases, sample collection and handling methods, and result interpretation.

The scope of this study was limited by the number and selection of animals. The limited number of *Trichophyton* spp. cases in both animal species and *M. canis* cases in dogs is reflected in the wider CIs in these categories. Additionally, the data were collected from working animal shelters, in which animals were selected based on clinical signs. The animal population in shelters may not be exactly comparable to animals seen in private practice. For example, previous studies have found an increased risk of dermatophytosis in cats and dogs from animal hoarding situations that are relocated to animal shelters.[1,27] This limits the generalisability of sensitivity and specificity data from a research perspective. From a clinical perspective, however, these data are valuable and applicable to those with clinical signs consistent with dermatophytosis.

Another potential limitation relates to the sample collection process. Toothbrush samples were collected by trained shelter staff across the different shelter locations in accordance with protocols developed by a shelter veterinarian. Although the same technique was used to collect samples for both PCR and culture, there could be potential variation in sampling between animals, shelter locations, or if PCR samples were not collected at the same time as culture samples. Nevertheless, in most cases, PCR and culture samples were collected simultaneously, and all samples in this study were collected fewer than 3 days apart and before treatment was implemented.

The variability in the time span between sample collection and plate inoculation is another factor to consider.

Collected samples were sent to a central location for culture and to a referral laboratory for PCR, leading to differing transit times and time to inoculation. This is likely less of a concern for these particular pathogens, as studies have repeatedly demonstrated the environmental resilience of different species of dermatophytes.[28–30] In particular, one study demonstrated the survival of *Trichophyton* spp. spores after exposure to either freezing temperatures for 1 week or to extreme heat after 90 min.[29] As the samples in this study were transported inside two sealed layers, it is unlikely that there would have been big fluctuations in environmental factors that could have affected the viability of the spores.

There was a reliance on culture-based identification with P-scoring as a reference standard. Both culture and PCR have limitations in fully distinguishing truly infected cats from mechanical carriers. In this study, the standardised P-scoring system allowed for more accurate distinction between a true infection (P2, P3) and fomite contamination (P1). Although this system has not been formally validated, it has been described and implemented in published literature, and modified for use in shelter protocols.[1,5,7,11] Although the use of P-scoring can vary between organisations depending on their risk tolerance, the use of consecutive testing helps reduce uncertainty surrounding an individual animal's disease status. However, there is still a possibility that a "positive" result may represent fomite contamination, while a "negative" result may represent true infection. As there is no universal case definition for "positive", "negative", and "carrier", there may be subjective variation in the interpretation of these results.

Implication of results

The use of commercially available PCR to detect dermatophytosis in shelter cats and dogs with suspected lesions offers the advantage of a rapid turnaround time and a high specificity, enabling the identification of positive animals and prompt treatment initiation. However, the observed variations in sensitivity across multiple studies raise concerns about reliance on PCR as a standalone diagnostic tool, particularly when used to determine that a suspect animal is not truly infected. Considering the higher cost of PCR and risk of population-level and public health repercussions, the risks and benefits of testing methods in shelter settings should be assessed carefully.

Further research is needed to evaluate the performance of PCR for the diagnosis of dermatophytosis in cats and dogs, especially for *Trichophyton* spp. and *M. gypseum* cases, and animals undergoing treatment, to optimise the use of PCR testing in clinical practice and improve both animal and human health outcomes.

Conclusion

PCR can provide a rapid diagnosis in cases of dermatophytosis and has a high specificity when used on animals with suspicious lesions. However, relying on PCR as a standalone diagnostic tool may increase the risk of underdiagnosis. In this study, nearly 14% of positive results would have been missed if PCR were used as the only diagnostic tool, potentially increasing the risk of both shelter outbreaks and community exposure. The use of in-house fungal cultures and routine plate monitoring may be more reliable, with positive results available within 7 days of inoculation in most cases. Our findings emphasise the importance of taking a balanced approach when considering dermatophyte diagnostic plans in shelter settings.

Authors' contributions

Wesley Cheung: Conceptualization, methodology, project administration, writing-original draft, writing-review/edits. Alexandre Ellis: Conceptualization, methodology, visualization, writing-review/edits. Amanda Idle: Data curation, investigation, resources, writing-review/edits. Alexandra Protopopova: Formal analysis, visualization, writing- original draft, writing- review/edits. Emilia Gordon: Conceptualization, methodology, data curation, investigation, resources, project administration, supervision, writing- original draft, writing- review/edits

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Conflict of interest and funding

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