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Molecular Diagnosis as an Alternative Method for Dermatophytes Identification in Cattle Herds

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Abstract:
Dermatophytes are filamentous fungi that cause infections in keratinised animal tissues as well as human including hair, skin, and nails. Dermatophytosis or ringworm is a highly contagious disease caused by fungi by fungi from three genera: Trichophyton, Epidermophyton (Deuteromycetes) and Microsporum. Its zoonotic potential causes economic losses in cattle farms. This study assessed the prevalence of calves' dermatophytosis in north-western of Iraq using PCR technique compared with traditional method. Fifty affected calves were used in this study during the period from September 2019 to March 2020. The affected calves were distributed in seven herds from different areas in Nineveh province /Mosul-Iraq. Skin scraping and hair samples were collected from these affected calves. The samples were mixed with 10-15% KOH and cultured on Sabouraud dextrose agar with gentamicin and chloramphenicol. The isolates of dermatophytes were identified based on their colony morphology while morphological characterization was done using direct microscopy. Fungal isolates were confirmed by internal transcribed spacer (ITS)-PCR method. Thirteen isolates of the genus Microsporium spp. (*Microsporium canis*) with a percentage of 26% and one isolate of the genus Epidermophyton spp. (*Epidermophyton floccosum*) with a percentage of 2% was detected. PCR method offers stellar performance in all tested samples. In fact detection of dermatophytes were increased the species-specific based on PCR technique compared with culture. We concluded from this study that applying ITS-PCR technique confirmed as a reliable method for the identification of dermatophytes because it is a rapid and sensitive diagnostic assay for dermatophytosis compared to the conventional methods.

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INTRODUCTION

Bacteria, virus, parasites, fungi, and algae are the common causes of dermatitis (Achterman *et al.*, 2011; Aghamirian and Ghiasian, 2011; Aly, 1996). However, dermatitis in humans and in animals are commonly caused by fungi, includes dermatophytes (Agnetti *et al.*, 2014). It is an important public health trouble and a global zoonotic transmission (Gupta *et al.*, 2025), causes direct effects on the quality of skin, fur and wool of animals resulting in heavy economic loss (Achterman *et al.*, 2011; Bergmans *et al.*, 2010). In fact in tropical and subtropical regions dermatophytes impact more than 20%- 25% of people and animals (Bergman *et al.*, 2013). Dermatophytosis (tinea) is an important cosmopolitan mycotic disease that occurs in sporadic and in epidemic forms (Aghamirian and Ghiasian, 2011; Pal, 2018).

Dermatophytosis is a fungal infection of keratinized layers of the epidermis and hair (Moskaluk and VandeWoude, 2022) may range from superficial and mild nearly subclinical, to a little scaling area to comprehensive areas of scarring and alopecia. The comprehensive areas are characterized by a highly inflammatory reaction (Kruithoff *et al.*, 2024).

In many countries and all around, dermatophytosis conceders as is an endemic infection affecting cattle, camel, goat, buffalo, sheep, laboratory animals, dogs and cats in addition to humans (Gupta *et al.*, 2025; Shihab *et al.*, 2023). Depending on the host's strain, immune status, virulence, and many environmental factors, this disease is quite transmissible (Dubljanin *et al.*, 2024; Jartarkar *et al.*, 2021; Sardana *et al.*, 2021).

However, in affected cattle economic losses could be due to loss of animal weight, decimated meat and milk, contagiousness among herds, difficulty to perform the control measures and treatment costs (Ellis *et al.*, 2007).

Identification of dermatophytosis constitute the direct microscopic examination and then *in vitro* detection of isolates (Gnat *et al.*, 2019), from the colony morphology; and other microscopic structures involving the manifestation of microconidia, macroconidia in addition to biochemical distinguishing like urease production (Gnat *et al.*, 2021). In fact identification of dermatophytes in cattle using conventional methods has many disadvantages such as time-consuming, arduous and need the mycological diagnostician experience (Gnat *et al.*, 2020; Gnat *et al.*, 2021).

In the field of fungal identification techniques the amelioration is in progress to give new scope for clinical application in hospitals, veterinary clinics and specialized laboratories. To vanquish the disadvantages of culture, molecular technique based on PCR has been applied recently for dermatophyte identification. It is one of the most important biological diagnostic techniques (Aho-Laukkanen *et al.*, 2024). The genomes of dermatophytes are haploid and have a low amount of repetitive DNA and range from 2.25 Mb to 24.1 Mb (Gupta and Cooper, 2008; Martinez *et al.*, 2012).

Dermatophyte conidia (spores) have a single nucleus, but hyphae are typically multinucleated cells with distinct nuclei that are genetically distinct (Jacobson *et al.*, 2018). In this study we detected dermatophytes based on the PCR technique to improve efficacious control measures compared with the conventional methods.

MATERIALS AND METHODS

Samples

For confirmation of dermatophytes infection, skin scraping and hair samples (Figure 1) were collected from fifty affected calves during the period from September 2019 to March 2020. The affected calves were distributed in seven herds from different area in Nineveh province /Mosul-Iraq. These calves exhibited lesions on the neck, head, abdomen, limbs and in some cases whole body is affected. 70% ethanol was used to cleaning the skin lesion of the affected calf suspected with dermatophytes. Fifty hair and skin scraping samples that collected from the margins were clinically examined (Leck,

1999). Hairs and scraping were mixed with 10-15% KOH.

Case history and clinical signs were recorded. The samples transported to microbiology and PCR laboratories of college of veterinary medicine at Mosul University.

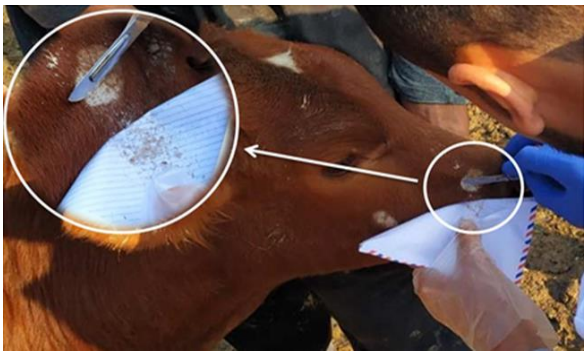

Fig. 1. Pathognomonic lesion of ringworm infection, skin scrapping method.

Mycological examination

All fifty samples were cultured on Sabouraud's dextrose agar (SDA) (65 g) with chloramphenicol and gentamycin. Then plates were incubated at 20 - 25 °C for 5 - 10 days. Negative plates were not discarded before 4 weeks. Sub-culturing on SDA plates, were utilized to purify all the positive cultures which incubated at 20-25 °C for 3-5 days. For macro and micro-morphological distinctive, these purified cultures were examined. The gross morphological examination of the isolated dermatophytes were diagnosed based on the cultural characteristics of the developing colonies, such as surface color, size, texture, the colony surface from front as well as back of the dish (Reverse).

Slides were prepared from each colony using tape method (Ellis *et al.*, 2007) with the help of fungal identification key. The slides were examined under microscope at magnification power (10X and 40X) and microscopic characteristics such as the shape of large conidia, its size and fungal hyphae were identified (Echevarría and Iqbal, 2021; Lund *et al.*, 2014).

PCR technique

According to manufacturer instructions (gSYNC™ Geneaid extraction kit): the DNA extraction was performed directly on tissue samples. For molecular diagnosis, ITS region sequencing was done using universal primers ITS1 and ITS4. Two pairs of primers: (BIONEER Co.) (Korea) were used for detecting the targeted fungus. PCR reaction was done with final PCR buffer using total volume 25µl represented in (Table 1). The amplification was achieved depending on the instructions as in (Table 2).

All PCR products were analyzed by gel electrophoresis 2% agarose, comprise 0.2µl ethidium bromide in TBE buffer (Biometra, Germany). UV transilluminator were used to visualize DNA bands while computer software were used to analysis the data.

Table 1. Final PCR buffer content.

Content	Total Volume (25 µl)
Forward Primer (10 Picomol\µl)	1µl
Reverse Primer (10 Picomol\µl)	1µl
Template DNA 250 ng\µl	5µl
D.D.W.	6.5µl
$MgCl_2$	1.5 µl
Mstermix 2.5X	10µl

Table 2. Amplification program.

Cycle	Temperature	Time	Stage
1	95 ºC	5 min.	Initial DNA denaturation
	95 ºC	20 sec.	DNA denaturation
30	57 ºC	30 sec.	Primer annealing
	72 ºC	30 sec.	Primer extension
1	72 ºC	5 min.	Final extension
1	-------	∞	Cooling

RESULTS AND DISCUSSION

In current study, clinical examination of the calves exhibited head, neck, abdomen, limbs and in some cases whole body, lesions. These lesions manifested as circular grayish-white crusts with alopecic in some area that remained after removal of crusts. The infection variegated from mild to severe. These clinical signs are identical to previously findings of many researchers (Ellis *et al.*, 2007; Gugnani, 2022; Pal, 2017).

The study findings were investigated by cultural isolation on mycological media and by direct microscopy of dermatophyte in skin lesions. However, dermatophyte isolation and identification from clinical samples in a culture were done on Sabouraud dextrose agar (SDA) medium (Gugnani, 2022; Papini *et al.*, 2009). Dermatophyte species were identified by the colony morphology as described previously (Leck, 1999; Patel *et al.*, 2016). Our study findings showed that 28% of samples were positive on fungal cultures, while previous study confirmed that the growth of dermatophyte was positive in 38 (76%) for cows (Mohammed, 2011). The contradiction in incidence of Dermatophyte fungi among regions may be due to breed of the cattle, production and climate (Alhasan *et al.*, 2022; Guo *et al.*, 2020) .

The culture distinguished colonial appearance revealed rapid growth, surface white and silky with bright yellow periphery. Colonies texture was a friable and reverse side of the colonial appearance was bright yellow or orange. In fact morphological characteristics of samples revealed colonies after 14 days of culture. Most frequent dermatophytes isolated were *Microsporum canis* (26%).

This species' colonies range in colour from white to cream, with the reverse pigment being golden-yellow to brownish-yellow. Typically, the terrain is flat, spreading, and has radial grooves. Its texture ranges from cottony to woolly (Figure 2 and 3).

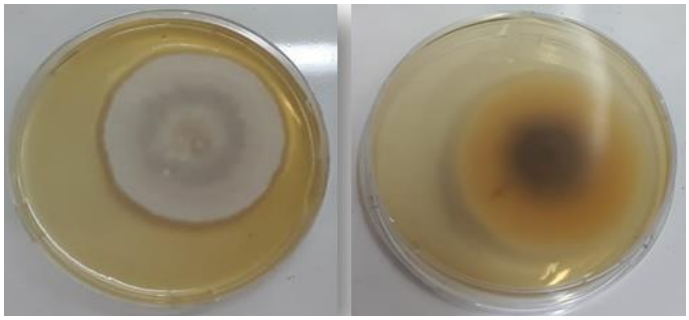

Fig. 2. Growth colony morphology and macroscopic characteristics of isolated fungal genera reveals the positive *M. canis* colony on (SDA) at 14 days.

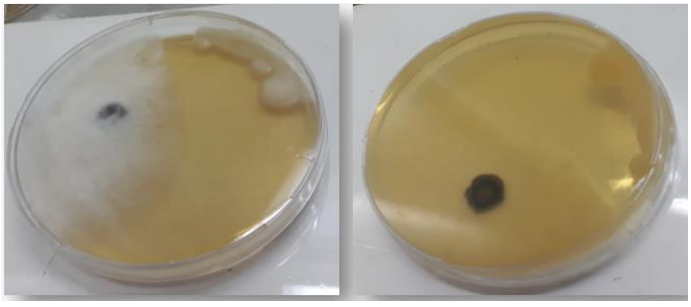

Fig. 3. Growth colony morphology and macroscopic characteristics of isolated fungal genera reveals the positive *E. floccosum* colony on (SDA) at 14 days.

This detection was identical to the previous findings who confirmed that infection of cattle with ringworm in Wasit represented (50%)of skin lesions and the common *dermatophytes spp* was identified as *Microsporum canis* (25%) (Alhasan *et al.*, 2022; Gräser *et al.*, 2000).

The main disadvantage of culture method is the low sensitivity which may be due to non-dermatophyte molds growth in the culture (Kidd and Weldhagen, 2022; Lavorato *et al.*, 2017) and the presence of non-viable fungal material in treated calves samples (Radostits and Done, 2007; Spergser *et al.*, 2024). Other reasons are the low specificity, efficacy and time-consuming.

Furthermore, because pleomorphism frequently occurs, diagnosing the species growing in culture requires knowledge in diagnostics (Moskaluk and VandeWoude, 2022; Warghade and Mudey, 2024).

Detection of dermatophytes by using microscope has been performed in this study. The chitin in fungal cell walls was targeted by the lactophenol cotton blue stain, which improves the visibility of fungal structures.. This stain eliminates the fungus, lowering the possibility of contamination from sample handling (Leck, 1999). Microscopic examination regarding present study isolated *Microsporum canis*, and *M. floccsum* from cattle with dermatophytosis.

In fact two types of fungi, thirteen isolates of the genus *Microsporium spp*. with a percentage of 26% and one isolate of the genus *Epidermophyton spp*. with a percentage of 2% was detected (Table 3).

Table 3. Types of dermatophytes isolated from infected calves under study.

Fungal species	No. of isolates	%age
Microsporium canis	13	26
Epidermophyton floccosum	1	2

Meanwhile under light microscope, specimen's direct examination demonstrated abundant macrocondia. They are spindle- shaped and mature spores. Slender- branched hyphae are also distinguished. Cells numbers are 5-14. Few of microcondia clearly observed (Figure 4). On the surface of hair shaft, arthrospores revealed as chains (Figure 5).

On microscopic examination, the septate, hyaline, filamentous mould M. canis is capable of producing spindle-shaped macroconidia and microconidia, this is identical with findings of previous investigation (Khosravi and Mahmoudi, 2003; Samanta, 2015) who confirmed that one of the most isolated dermatophyte from calves was *M canis*.

The animals could be infected with the most prevalent dermatophyte which is *M canis* that has keratinase. This author concluded that such strain with high keratinase efficacy *in vitro* causes acute infection, suggested a strong interconnection between the symptoms progression and high keratinase activity (Viani *et al.*, 2001).

The other fungi demonstrated in this study was *E. floccosum* (2%), which occasionally reported in animals. *E. floccosum* produces clustered macroconidia and not produce microconidia. However, this organism is limited to the skin. The presence of fungi can be detected with high sensitivity via microscopic analysis. But it is unable to identify certain species or distinguish between living and dead cells (Moskaluk and VandeWoude, 2022).

Dermatophytes are very similar to each other, so different techniques are necessary for accurate diagnosis and identification. Culture and microscopic examination cannot be repeated and are usually time- consuming and the accurate identification is very low (Gnat *et al.*, 2021; Islam *et al.*, 2018). However, molecular techniques have been investigated as clinical diagnosis recently. Molecular biologic assays constitute the PCR technique for the dermatophytes identification and confirmation of culture samples results (Aho-Laukkanen *et al.*, 2024; Tartor *et al.*, 2019).

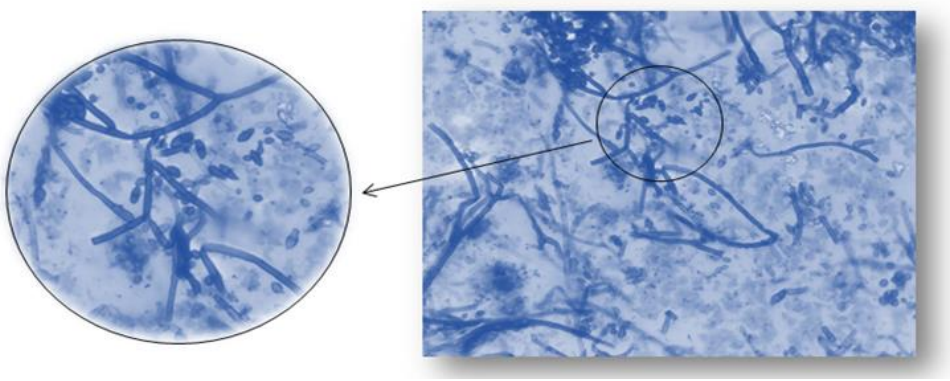

Fig. 4. hyphae of *M. canis* isolate appeared branched - septate, Macroconidia, Microconidia and chlamydospores at magnification 100 X.

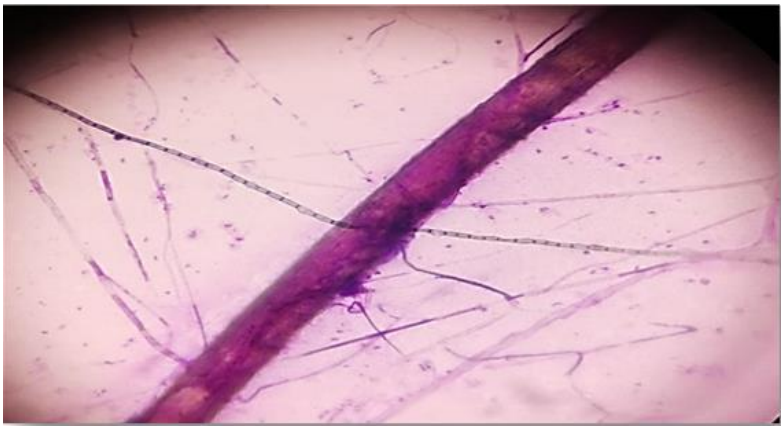

Fig. 5. Direct microscopic examinations of infected hair at magnification 100 X.

PCR methods were performed for dermatophyte detection in 50 samples. The results of this study confirmed that PCR detected *M. canis* and *E. floccosum* correctly in culture-positive samples with an amplicon size of 540 bp (Figure 6). The most considerably dermatophyte isolated was *M. canis* suggests that in this study area, *M. canis* is endemic.

The internal transcriber spacer (ITS) region is typically the focus of conventional PCRs for dermatophyte detection and identification since it may identify several isolates down to the species level (Turin *et al.*, 2000).

To make it simpler to detect dermatophytes, primers have been created that target conserved areas of the ITS that are unique to these organisms (Bergmans *et al.*, 2010).

The technique used for DNA extraction has a significant impact on PCR accuracy; using these methods for fungal detection necessitates specific extraction strategies that can break down the fungal cell. (Gnat *et al.*, 2021; Karakousis *et al.*, 2006; Manian *et al.*, 2001).

The additional procedures may include heat, mechanical or chemical disruptions like beads or extra lysis buffers, or freeze/thaw cycles (Gupta, 2019; Manian *et al.*, 2001).

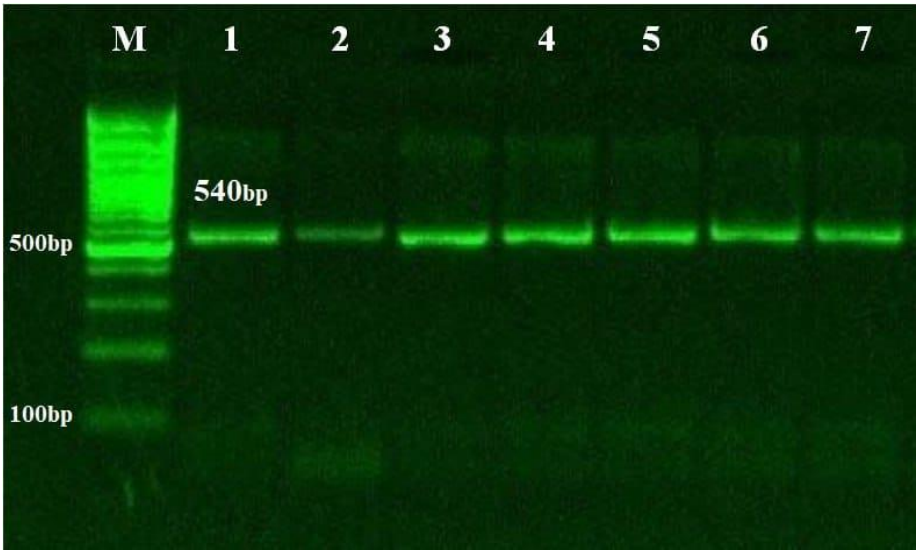

Fig. 6. PCR amplification products of TSI**.** M: Marker (100 – 1000 bp), samples 1 - 5: positive at molecular weight 540 bp. +ve, -ve: positive and negative control respectively.

In our study the PCR technique is proven to be reliable to detect the presence of dermatophytes in samples. In fact, the use of qualitative (conventional) PCR as a diagnostic test to find dermatophytes has increased (Aho-Laukkanen *et al.*, 2024). PCR may identify fungal DNA even if the culture is negative, making it a more sensitive method than culture (Khot and Fredricks, 2009). Like microscopy, it is unable to differentiate between fungal cells that are alive and those that are dead (Chen *et al.*, 2022). However, because of highly sensitive, more accurate results and rapid molecular identification of dermatophytes, these techniques must be used for routine use (Jacobson *et al.*, 2018; Turin *et al.*, 2000). However, this is the first study to identify dermatophyte species directly from calves hair and skin samples based on ITS -PCR assay in Mosul city.

CONCLUSION

The findings of present study detect the *M. canis* in the skin lesions of calves. *M. canis is a dermatophyte of clinical significance* as it represents the most common zoonotic dermatophyte in human and animals. This agent causes *tinea capitis* and *tinea corporis* in humans. Our study improved that the clinical application of PCR method can be used with rapid and reliable detection to detect dermatophyte in clinical specimens of cattle in comparison to the culture and direct microscopy methods.

CONFLICT OF INTEREST

The authors hereby state that they do not have any conflict of interest to declare.

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