ISOLATION AND MOLECULAR DETECTION OF DERMATOPHYTES FROM HORSES IN THE GOVERNORATE OF WASIT, IRAQ

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ABSTRACT

Dermatophytosis causes superficial fungal infection that poses public health problems to man and animals and economically significant causes of numerous dermatomes that have been observed in equine species, therefore the aim of this study to investigate the distribution of dermatophytes species in horses and comparing the traditional methods with PCR techniques for detection of dermatophytes in the governorate of Wasit, Iraq. A total of 50 horses samples were collected from skin scrapings suggestive of dermatophytosis from diverse ages, sexes and areas, during the period of June to August 2018. The results showed that 32 (64%) out of 50 horses sampled were positive for dermatophytes by direct microscopic, while cultures only 26 (52%) out of 50 horses sampled were positive results for dermatophytes and results shown all culture positive samples were also positive in direct microscopic. In this study which exposed most frequent dermatophytes isolates were Trichophyton and Microsporum species in cultures and five species were recovered, T. equinum was the most common species 9 (34.6%) out of 26 positive culture, followed by T. mentagrophytes 7 (26.9%), M. equinum 5 (19.3%), T. verrucosum 3 (11.5%), M. gypseum 2 (7.7%) and 6 isolated of non-dermatophytes were also detected. This study showed all culture positive samples were also positive in PCR technique. While six isolates were positive by direct microscopic show negative result by PCR technique. This indicates the infected of the skin with other types of non-dermatophytes. In conclusion that Trichophyton and Microsporum species were most frequently occurring dermatophytes in horses and PCR targeting the (Chs1) gene may be considered confirmed correct identification and screening test for detection of dermatophytes in horses.

Keywords: Dermatophytes, Skin scrapings, Horses, Molecular detection, Wasit University, Iraq

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INTRODUCTION

Dermatophytes which is a highly contagious infection of the skin that affects horses and other animals of all ages and breeds¹. Dermatophytosis causes superficial infection that poses public health problems to man and animals² and can be disfiguring and recurrent and generally need long term treatment with antifungal agents³. Dermatophytosis is one of the mainly frequent and efficiently imperative causes of a number of dermatomes that have been shown in equine species⁴. It is caused by 2 main genera of fungi Trichophyton and
There are about 40 diverse species of dermatophytes categorized their ability to digest keratin and separated three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. These genera are reported to recurrently cause dermatophytosis in horses. The aim of this study was to investigate the occurrence and species distribution of dermatophytes from horses and comparing the traditional methods with PCR techniques for identification of dermatophytes in the governorate of Wasit, Iraq.

**MATERIALS AND METHODS**

**Study Area and Sample Collection**

Full amount of 50 horses samples with cutaneous lesions indicative of dermatophytosis composed from (27 males and 23 females) and ages (1 year to 15 years), and study areas included: Al-Kut (5 horses), Al-Mufqiya (7 horses), Al-Sheikh Saad (9 horses), Al-Bashair (16 horses) and Hay city (13 horses) in Wasit province, Iraq during the period of June to August 2018. Skin scrapings collection from the borders of the lesions consistent with method of 8. These samples were kept in sterile envelopes in separate polyethylene bags, and transported as dry packet to the microbiology lab of veterinary medicine, Wasit University for diagnosis of dermatophytes.

**Conventional Identification**

All samples collected were divided into two portions. The first portion was examined microscopically in (20% KOH) for the incidence of fungal fundamentals. The second portion was cultured on Sabouraud dextrase agar supplement with chloramphenicol (50 mg/l) and/or cyclohexemide (500 mg/l) at 27°C for up to 4 weeks. The diagnosis depends on the appearance of colony on the agar plates and microscopic examination after staining with lactophenol cotton blue and presentation using ×10 and ×40 magnification) with the help of the fungal color atlas 10,11.

**Molecular Identification**

**DNA isolation from culture**

Fungal growth from the SDA cultures were subculture by potatoes dextrose agar (PDA) to make easy sporulation then leave at room temperature for (1–4) weeks followed by complete extraction was used QIAamp DNA mini kit (Qiagen, Germany) and provided by the manufacturer procedure. The samples of DNA extraction were stored at (-20° C) until use.

**Primers design**

The specific of oligonucleotide primers sequences to detect DNA fragment program chitin synthase1 (*CHS1*) gene of dermatophytes, This primers sequences were taken from 13,14 and synthesized in (Alpha DNA, Canada) was used PCR assay to identify the dermatophytes species as shown in (Table1)

**PCR reactions and amplification**

The specific primers sequences were used to detect the presence of chitin synthase1 (*CHS1*) gene of dermatophytes species fungi, the primers (Pan-Derm1 and Pan-Derm2) were diluted in relation to manufacturer information. The PCR reaction as shown in table no. 2 and The PCR thermo cycler program as shown in table no. 3 were performed according 13, 14. All PCR amplification reactions were used cleaver scientific thermal cycler TC32/80. The specific PCR product of approximately 366 bp was detected by electrophoresis on a 2% agarose gel containing ethidium bromide. The gel was exposed to UV using UV light transilluminator and then photographs using digital camera (Sony-Japan).
Table 1: Primers sequences with their relevant product size

<table>
<thead>
<tr>
<th>Gene Target</th>
<th>Primer name</th>
<th>Primer Sequence</th>
<th>Product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS gene</td>
<td>PanDerm1 F</td>
<td>5’GAAGAAGATTGTCGTTTGCATCGTCTC3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PanDerm2 R</td>
<td>5’CTCGAGGTCAAAAGCAGCCAGAG3’</td>
<td>366</td>
</tr>
</tbody>
</table>

Table 2: Composition of PCR reaction mixture used for amplification of (CHS1) gene.

<table>
<thead>
<tr>
<th>Gene target</th>
<th>Components</th>
<th>Volume /μl</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS1 gene</td>
<td>Green Master Mix(Promega, USA)2x</td>
<td>12.5 μl</td>
<td>1x</td>
</tr>
<tr>
<td></td>
<td>Forward primer, 10 μM</td>
<td>2 μl</td>
<td>2μM</td>
</tr>
<tr>
<td></td>
<td>Reverse primer, 10 μM</td>
<td>2 μl</td>
<td>2μM</td>
</tr>
<tr>
<td></td>
<td>DNA template</td>
<td>8 μl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(DNAse) Free distilled water</td>
<td>0.5 μl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total volume</td>
<td>25 μl</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The PCR thermo cycler program for (CHS1) gene

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>No. cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>40 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>60°C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>7 min</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Conventional Identification

Microscopy and Culture

All samples of cutaneous lesions characteristic for dermatophytes were subjected to direct microscopic inspection using (20 % KOH) which resulted in 32 (64%) out of 50 horses sampled were positive for dermatophytes, which showed numerous round micro conidia clustered on branched conidiophores and coiled hyphae. While cultures for dermatophytes agent on Sabouraud dextrose agar and potatoes dextrose agar, only 26 (52%) out of 50 horses sampled were positive results, which detected based on colonies characteristic in the agar plates and stained with lactophenol cotton blue was used for microscopic examination (Figure 1). All positive culture were also positive in direct microscopic. The isolates culture included, Trichophyton and Microsporum species were the major dermatophytes isolated in this study. Consisting of five species were recovered, T. equinium was the most common species 9 (34.6%) out of 26
positive culture, followed by *T. mentagrophytes* 7 (26.9%), *M. equinum* 5(19.3%), *T. verrucosum* 3(11.5%), *M. gypseum* 2(7.7%) as shown in (Figure 2). and 6 isolated of nondermatophytes were also detected.

**Molecular Identification**

PCR detected of fungal in all 26(52%) samples shown to be positive in culture, and it gave accurate identifications for all the species in culture, were used set et Panderm primers copied from the chitin synthase1 (Chs1) gene, which diagnosis of all dermatophytes with a PCR product size of approximate 366 bp, while show negative results for the attendance of this gene as indicate by the absence of the PCR products in their significant lanes as shown in (Figure 3).

![Figure 1](image1.png)

*Figure 1:* *Trichophyton mentagrophytes* was grow on SDA A- Shown white to creamy powdery colony B- Shown yellowish reverse colony, C- Microscopic examination of it was staining with lactophenol cotton blue shown Spiral hyphae and microconidia were numerous, unicellular, round to pyriform and found in grape like clusters (X40).

![Figure 2](image2.png)

*Figure 2:* Distribution of dermatophytes species isolated by culture on SDA

![Figure 3](image3.png)

*Figure 3:* Gel electrophoresis (2% agarose, 7v/cm, 1.5hrs ) of the PCR products, shown: lane (1-5): Positive sample for dermatophytes spp. (Chs1) gene 366bp); lane 6 Negative sample; lane 7: Negative control; (MW): One hundred base pairs DNA ladder.
DISCUSSION

Dermatophytosis can play an effective role in control of infection outbreak by establish the basis of infection. The current study total 50 samples clinically examined horses suspected dermatophytes lesions and identification, were showed 32 (64%) out of 50 horses sampled were positive for dermatophytes by direct fungal examination, While cultures on Sabouraud dextrose agar detected only 26 (52%) out of 50 horses sampled were positive results for dermatophytes, All positive culture samples were also positive in microscopic. These results corresponded with these mentioned by13,15 it was found that, direct microscopy was more sensitive than culture for detected dermatophytes. This result also in agreement with the those published by16,17 also well documented found that, microscopic examination was capable of detected fungal hyphae in samples but cannot recognize the accurate species, while culture will allow recognition of the causative organisms but requests a long time (more than 2 weeks) for the dermatophytes fungi to grow and high false-negative rate. This observation was consistent with stated by Hall et al. 18 In the present study revealed, Trichophyton and Microsporum species were the most important dermatophytes isolated, this results was in consistent with19,4 found that dermatophytes in horses were major caused by Trichophyton and Microsporum species. Other study which consistent with our result indicate that Trichophyton and Microsporum genera major dermatophytes diagnosis in horses20. In this study, it has been found that the five species of dermatophytes were isolated from horse included, T. equinum was the most common species at 9 (34.6%) out of 26 positive culture, followed by T. mentagrophytes7(26.9%), M. equinum 5(19.3%), T. verrucosum 3(11.5%), M. gypseum 2(7.7%) and non dermatophytes were also detected. This finding agrees with those published by21,22,3 who observed that T. equinum and T. equinum var autotrophicum were the most frequently isolated dermatophytes species from horses. Other studies which consistent with present result concerning the etiology agent of equine ringworm all over the world as reported by23.6. Present study showed, The process of identification of dermatophytes and non dermatophytes, somewhat difficult using the conventional methods (direct examination and culturing) because needs more time and mycological experience. Therefore, In the current study, was used two specific primers based on the sequence of Chs1 gene as conserved primers for all dermatophytes and careful more stable and accurate compared to morphology characteristics24. In the present results, showed 26 (52%) out of 50 horses sampled were positive results by molecular method, it gave right identifications for all grown in culture for dermatophytes species. While six isolates were given a negative result by means of a molecular detection, which was positive by direct examination. This indicates the infected of the skin with other types of non dermatophytes. The current results suggests other agents and factors causes skin lesions such as Poor nutrition, management and infectious diseases which produce cutaneous lesions related to dermatophytosis and may relation for a few of the skin lesions observed25. In the present study indicated that integration of PCR technique in routine laboratory process of skin scrapings improved detection rate of dermatophytes compared to culture and direct microscopic examination. This result agreement with the findings of other studies26,27.

CONCLUSION

In the present study showed that Trichophyton and Microsporum species were the most frequently occurring dermatophytes in horses and PCR targeting the (Chs1) gene possibly considered confirmed correct
identification and screening test for detection of dermatophytes in horses suspected of dermatophytosis and appropriate antifungal treatment, thus reducing the risk of resistance development and morbidity.

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ETHICAL CLEARANCE
The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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