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Research Article

ISOLATION AND IDENTIFICATION OF MICROSPORUM CANIS IN 3 LION'S CUBSAT BAGHDAD ZOO BY LABORATORY TESTS AND PCR

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ABSTRACT

Microsporum canis is a cosmopolitan zoophilic dermatophyte usually acquired from infected dogs or cats. Most prevalent in children, it has been implicated in infections of the scalp and skin and occasionally nails. In this study *M. canis* was recovered from 3 lion's cubs at Baghdad zoo (Al Zwraa Park) in March 2015. The fungal characterization was performed with classical mycological analysis by direct microscopic examination with 20% KOH and by fungal culture on Sabouraud's dextrose agar. The results confirmed by genotypical analysis using the ITS1 – ITS4 primer in PCR. DNA amplification of all isolates showed one distinct band with molecular weight of 740 bpamplified fragment on electrophoresis in agarose gel.

INTRODUCTION

Dermatophytes are a uniquely pathogenic group of fungi that cause most common fungal infections globally (Rivera, 1985). These fungi are widespread in nature and its classification depends on the habitat and their presence in various ecology niches. They are classified into zoophilic dermatophytes, geophilic and anthrophilic (Chabassea and Piheta, 2008). The three anamorphs (asexual, conidial or imperfect state) ofgenera *Epidermophyton, Microsporum* and *Trichophyton* are recognized as belonging to the family *Arthrodermataceae* of order *Onygenales* (Padhye and Summerball, 2005). *Microsporum canis*, zoophilic dermatophyte, is responsible for most casesof tinea capitis in children and tinea corporis in adults. The geographic spread of this infection is worldwide, because it is transmitted by cats (the principal reservoir of this fungus) or dogs (Weitzman and Summerbell, 1995). Dermatophytes growing periods cover approximately two weeks in suitable media and identification are made with conventional methods as typical macroscopic and microscopic appearance. However, successful results are not always obtained with the phenotypic features, and thus, diagnostic problems and delay in diagnosis and treatment may arise. For this reason, the methods based on nucleic acid amplification have been necessary (Ray and William, 1992). Analysis of the internal transcribed spacer (ITS) regions of ribosomal DNA is a simple and reproducible molecular tool for identification of dermatophyte species (Shehata *et al*., 2008). According to several authors, dermatophytosis occurs widely as in domestic animals (Rotsten *et al*., 1999; Kesdangsakonwut *et al*., 2006; John and Edward, 2007; Memarian *et al*., 2012). The aim of the present study was isolation and identification of *Microsporum canis* recovered from lion's cubs by conventional laboratory methods and confirms the identification by genotypical method using Polymerase Chain Reaction (PCR) technique.

MATERIAL AND METHODS

The study was carried out in Baghdad zoo (Al Zwraa Park) in March 2015. A total of three lion's cubs (*Panthera leo)* in 4 months old were selected based upon their skin lesions, all of them have disseminated round skin lesions with alopecia with different size and location on face, neck, extremities and on both sides of the abdomen (Figure 1).

Figure 1 Shows skin lesions on lion's cubs

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Specimens' collection: Clinical specimens were obtained from the animals'skin by scraping epidermal scales from the edge of lesion with a surgical blunt blade, and swab sample were taken after sterilization by alcohol 90%, and then some samples to direct microscopic examinations of the epidermal scales and hair, using 20% KOH were taken. The clinical specimens were cultured on Sabouraud's dextrose agar (SDA), with chloramphenicol (0.05 mg/ml), and incubated at 28°C for 28 days.

Classic mycological examination: Identification of the growth have been depended on the following: Colony observation (colour, consistency and topography), Colony reverse (colour, significant pigment), Microscopic morphology: (microconidia and macroconidia: their size, shape, arrangement and hyphal structures). These colonial and microscopic features on selected media was performed according to (Rippon, 1985), and (St-Gemain and Summerbell, 2011).

Genotypical analysis: Genomic DNA of culture was extracted by using EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit as the instruction of the company (BIO BASIC INC., Canada). PCR amplification of the ITS 1 and ITS 2 ribosomal regions was achieved with the primers ITS1 and ITS4 (Table 1):

Table 1 the primers with their sequences and product size

Primer		Sequence (5' – 3')	Product size
ITS1 & ITS4	F	TCCGTAGGTGAACCTGCGG	740 bp
	R	TCCTCCGCTTATTGATATGC	

PCR master mix reaction was prepared by using(AccuPower PCR PreMix Kit)and this master mix was done according to the company instructions(Bioneer, Korea). The PCR cycling conditions were as in Table 2:

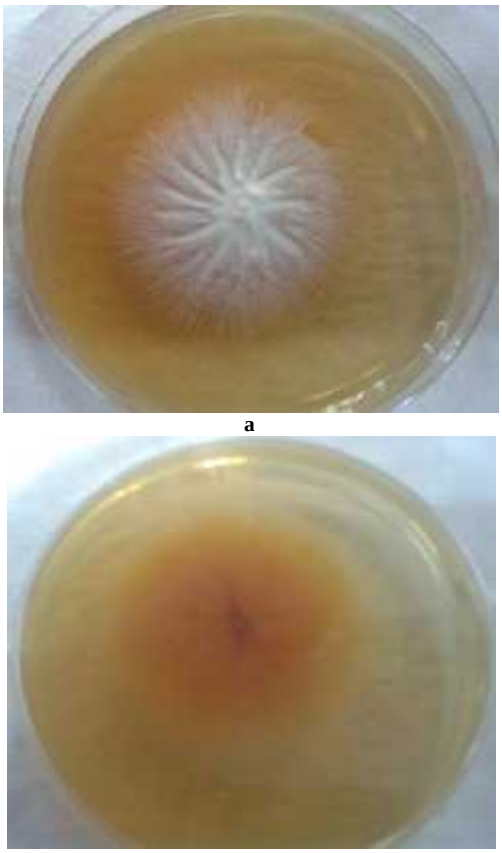
Table 2 PCR thermocycler condition of *Primers*

Thermocycling	ITS1, ITS4
Initial denaturation	95°C/1min.
PCR Amplification cycle	
Denaturation	95°C/1min.
Annealing	55°C/1min.
Extension	72°C/1min.
Final extension	72°C/10min.
Repeated cycle	35 cycle

RESULTS AND DISCUSSION

The direct microscopic examinations of the epidermal scales and hair, using 20% KOH were negative for mites, while showed numerous septate branching hyphae, with ectothrix observed in the hairs. The mycological examination of all samples confirmed the identification of *Microsporum canis.* SDA culture yield colonies having a white to yellow color with a yellow to orange reverse (Fig. 3a &b). Microscopically with tease mount preparation stained with lactophenol cotton blue (LPCB) showed abundant macroconidia that are thick-walled with many septa. Macroconidia are often hooked or curved at ends (Fig. 4).

In current study *Microsporum canis* was isolated from healthy lion's cubs, although (Scott *et al.*, 1995) mentioned that dermatophyte infections are usually seen in either young or old animals or in animals with systemic or immunosuppressive disease.



a

b

Figure 3 a Colonial morphology of *Microsporum canis* on Sabouraud's dextrose agar for 8 days at 28°C. **b**: the reverse.

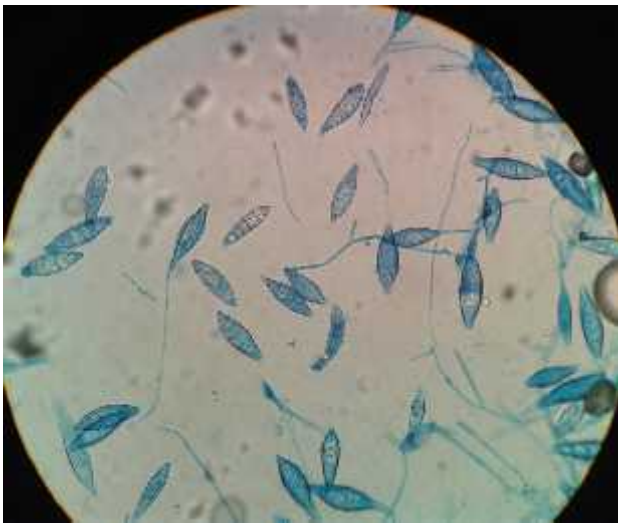


Figure 4 Microscopic morphology of a*Microsporum canis* colony (cultured on SDA) mounted with lactophenol Cotton Blue Stain, macroconidia spindle- shape multicellular 6 - 12 cells (40X).

Microsporum canis identification using ITS-based PCR

To determine the agreement of PCR-based methods with culture-based techniques, PCR-based performed identification using PCR with the ITS1/ITS4 primer set. This primer set amplified the *ITS I, 5.8S*, and *ITS II* regions of the ribosomal DNA in all strains tested, resulting in amplified products of approximately 740 bp in the *M. canis*, and this proves that all these strains are genetically similar (Fig.4).

Jackson *et al*., (1999) used the internal transcribed spacer (ITS) region of ribosomal DNA as a target for PCR amplification using the ITS1 and ITS4 primers, for identification of 17 dermatophyte species. This method produced unique fragment patterns for most dermatophytic species studied.

Subsequent of these products identified the studied strains in complete agreement with the culture-based identification, but no strain variation was detected in any of the studied species by using this method. The results are in agreement with those reported by (Jackson *et al*., 1999), who found that PCR-RFLP of the ITS region was a useful molecular tool for the identification of dermatophytes to the species level. Also the results agree (Shehata *et al*., 2008) that employed a two-step method: PCR amplification, using ITS1 and ITS4 as primers.

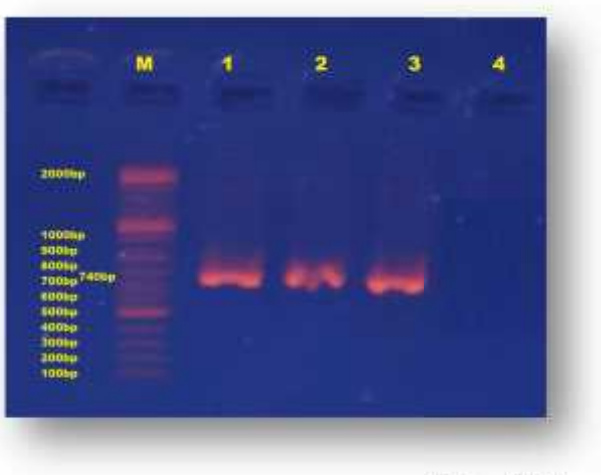


Figure 4 Agarose gel electrophoresis showed amplification of 740 bp fragments of ITS I 5.8S, ITS II regions of the three phenotypically identified species. Lanes: M showed molecular weight PCR marker, 1 M. canis First cub, 2 M. canis Second cub, 3 M canis Third cub, 4 negative control.

The Internal Transcribed Spacer (ITS) regions of the fungal ribosomal DNA (rDNA) were frequently used for species identification because it is faster, accurate species determination, specific and are less feasible to be affected by exterior effects such as temperature changes and chemotherapy (Girgis *et al*., 2006; Kong *et al*., 2008). Ellis *et al*. (2007), Aala *et al*. (2012) and Samuel *et al*. (2013) used both conventional and molecular methods to identified dermatophytes species. They revealed that the conventional methods are generally prolonged and may be indecisive. However, molecular studies based on Internal Transcribed Spacer (ITS) sequencing provide a very accurate result.

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