Molecular Detection of Microsporum Canis Infection in Cats in Baghdad Governorate

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ABSTRACT

Microsporum canis is a keratinized skin fungus that causes diseases in all animals, especially in cats. It is able to infected human causes Tinea corporis and Tinea capitis. In this study,the aim was toward detection and confirmation of Microsporum canis infection in cats in Baghdad governorate using polymerase chain reaction (PCR).The diagnosis depend on sequencing of the PCR products obtained by using a universal primer to amplification of the primer DppV which was 895 bp.A total of two hundred and eighty three hair and skin scraping samples were collected from infected cats with and without skin lesions. Preliminarily examined of samples with 10% KOH preparation, and cultured for fungal identification . For the culture; Saboruad dextrose agar was used . Culture results showed that four isolates were positive for M. canis , the best growth of these isolates was confirmed by PCR and DPPV gene then sequencing of this gene. PCR technique showed that one isolate from the total of 283 isolates were assured for the DPPV gene and yielded a band at 895 bp, this finding is regarded as distinctive for the genus Microsporum canis ,which was dumped in the GenBank nucleotide sequence database under accession number OR126262. Results sequencing of this gene in Iraq revealed compatibility with global results in USA and Belgium.

1. Introduction

Dermatophytes are fungi which need the use of keratin for growth (Hasan and AL-Jubori,2015) (Lagowski *et al*., 2019) and (Minnat,2019). These fungi can cause superficial infections in hair, skin, and nails (Mohammed,2011) and (Achterman and White, 2013).One of the most common dermatophyte which infected Cats is *Microsporum canis (M.canis)* (Cambier *et al.*, 2015). *Microsporum canis* is a pathogenic fungus and regarded as one of the zoophilic dermatophytes (Aasi and Al-Aaraji,2018) that are invading keratinized structures of cats causing clinical conditions often marked by itching , circular lesions , multifocal alopecia and Scaling (Mao *et al.*, 2014). It is considered a contagious disease capable to infected humans especially young adults and children by direct contact or transmission via material that is contaminated by fungal (indirect) (Moriarty and Morris-Jones,2012). Direct communication between infected cats is the primary mode of transmission of disease and transmissions also occur by other routes; gloves contaminated and fomites (Mohammed and AL-Jibouri,2015) and (Moriello *et al.*, 2017).Extracellular proteases is one of the main virulence factors of pathogenic fungi. Their proteolytic activities plays an important role in the acquisition of nutrients from the external environment, destroying host barriers and defenses, and disrupting homeostasis in hosts (Satala *et al.*, 2023). *Microsporum canis* secretes many proteases example dipeptidyl-peptidases V (DppV) (Vermout *et al.*, 2008), their role could be involved in fungal adherence and/or invasion . *DPPV* genes of *Microsporum canis* strains detected in both asymptomatic and symptomatic cats (Mathy *et al.*, 2010). The goal of this study was toward detection and confirmation of *Microsporum canis* from feline dermatophytosis cases and to assess the incidence of this species in cats by using PCR And *DPPV* Gene Then Sequencing Of This Gene.

2. Materials And Methods

Two hundred and eighty-three skin scraping and hair sample was collected from cats with various sex, breed and age during the duration from April-2022 to the end of March - 2023 from different regions of Baghdad governorate.

Collection of samples:

Clinically, All cats suffered from cutaneous lesions or just having itching were cleaned with 70% ethyl alcohol to remove any dust or contaminated bacteria and by using the blunt edge of a sterile surgical

blade ,skin scales and crusts were collected by scraping from the edge of actively growing of the lesions then put onto a clean container. While in hair specimen collection, epilating forceps was used to pluck along the base of the hair shaft, then sealed in a sterile container; then labeled with the date of collection, animals name ,age, sex and site of infection, then sent to the mycological testing laboratory. The samples were separated into two parts: one for direct microscopic examination by using KOH 10% and other part for culturing according to (Shalaby *et al.,* 2016).

Diagnosis of *Microsporum canis*

1. Direct examination: sample was treated with 10% potassium hydroxide(KOH) on a clean slide then heated for 5-10 minute and let the slide to cool, and covered by cover slip for identification of fungal elements by using magnification (X40) lens, (Kurade *et al.,* 2006) ;(Shalaby *et al.,* 2016), (Ahmed *et al* ., 2019).

2. Isolation and Identification of *M. canis* by culture : sample was cultivated on Sabouraud dextrose agar (SDA) and incubated at 25°C for up to 2 weeks. The fungal growth was examined by taking part from fungal growth and mixed with one drop of lactophenol cotton blue and covered with a cover slip and examined under Microscope by using X40 lens (Hayyawi, 2012) ; (AL-Tameemi and Khalaf,2013) ;(Shalaby *et al.,* 2016) and (Jameel and Yassein,2021). An oil immersion lens (X100) was used to obtain better clarity for macroconidia of *M.canis* .

3. Confirmation of the best in growth isolate of *M.canis* by using PCR .

- Primers used in the study

Table (1): Sequence of primers that used this study

Primer	Sequence	Primer sequence	Size of Product (bp)
DPPV	F	5′- CAACTGGCAGCACTGGTTTC -3′	895bp
	R	5′ TCAAAGCCGTGGAGCTGTAG-3′	

Table (2): Reaction components of PCR

Component	25 µL (Final volume)
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl

Table (3): Optimum condition of detection .

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45 Sec.	35 cycle
3-	Annealing	53°C	1 min.	
4-	Extension-1	72°C	1 min.	
5-	Extension -2	72°C	5 min.	1 cycle

3. Results And Discussion

Macroscopic characteristic of *M. canis* infection in cats

Clinical examination of cats suffering from *M. canis* was characterized clinically by circular lesions , focal and multifocal alopecia, itching , and Scaling in different area of body cats typically on the face, head, ears, back, abdomen, feet and fore and hind limbs **(Figure 1).** On the other hand, the study show infected hair by dermatophytosis were characterized enlarged and swollen structures with a rough and irregular surface **(Figure 2).**All fungi (*M.canis*) isolated were from male and female cats with skin lesions**.**

Macroscopic Characteristic of Colonies Grown on SDA Agar

Macroscopic characteristic of *M. canis* colonies growth on SDA agar at 25℃ for up to 2 weeks , soft, white and fluffy in the center with golden yellowish or yellow border closely spaced radial grooves also became white all the top with age 3-4 weeks. While reverse colony color (Undersurface view) represented by yellow that dulls to brown and darker with age as in **(Figure 3)**

Microscopic Characteristics of Colonies Grown on SDA at 25 C°.

Mormphology of *Microsporum canis* macroconidia on lacto phenol cotton blue stained preparations showing rough surface with boat like and separated into segments .Microconidia may present or absent along the length of the hyphae pyri form to round as shown in **(Figure 4).**

The total number of *M. canis* isolated from cats 4/283 (1.41%) **.**

Figure (1): Typical lesion of dermatophyte (*Microsporum canis*) infection in cat **.**

Figure (2): Infected hair by dermatophytosis were characterized enlarged and swollen structures with a rough and irregular surface (40X)

Figure (3): Macroscopic characteristic of *M. canis* colony grown on Sabouraud dextrose agar at 25°C for up to 2 weeks (Top view) and (Reverse View)

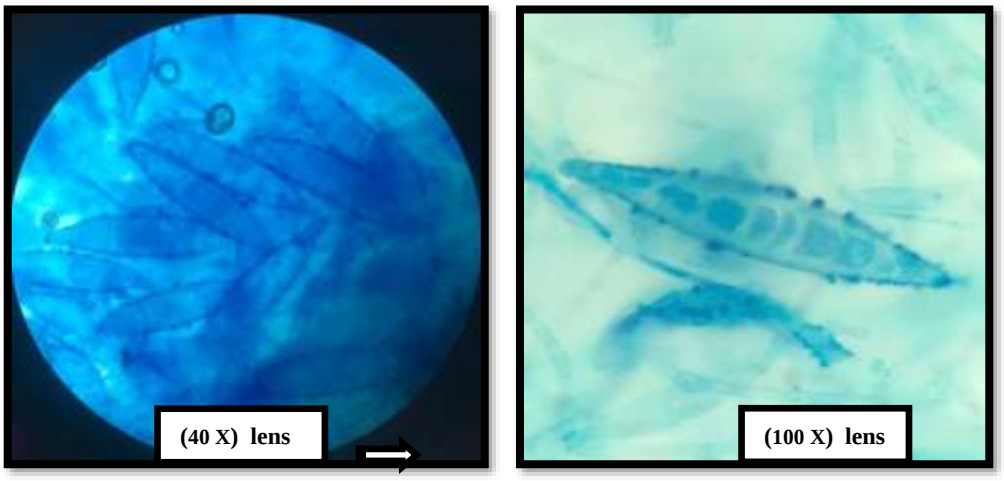

Figure (4): Macroconidia of *M. canis* stained with lactophenol cotton blue under microscope

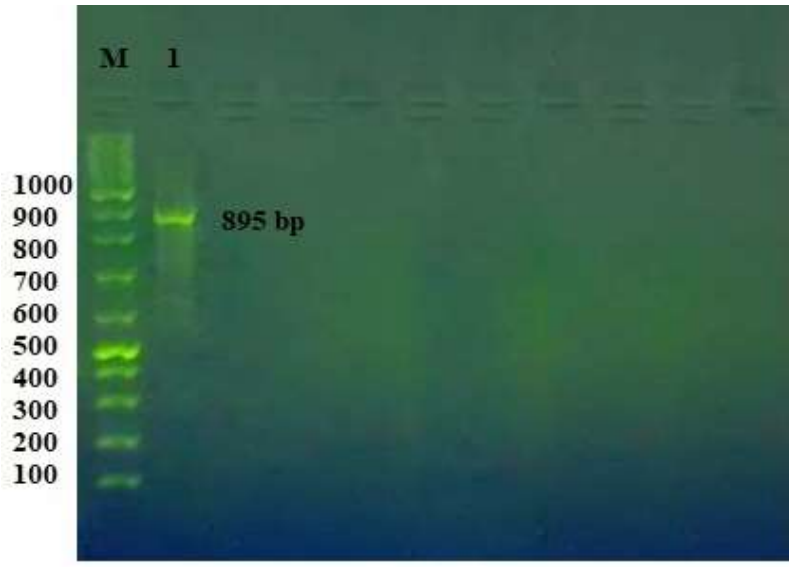

Figure (5) PCR product the band size .1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100)

This isolate of *M.canis* was sent for gene analysis and the results matched the global results represented in the **table (4)** and **figure (6) .**

Table (4): Iraqi *M. Canis* isolate compared with global isolates

	Accession	Country	Source
1.	**ID: OR126262**	**Iraq**	***Microsporum canis***
2.	**ID: DQ286525.1**	**Belgium**	***Microsporum canis***
3.	**ID: XM_002848646.1**	**USA**	***Microsporum canis***

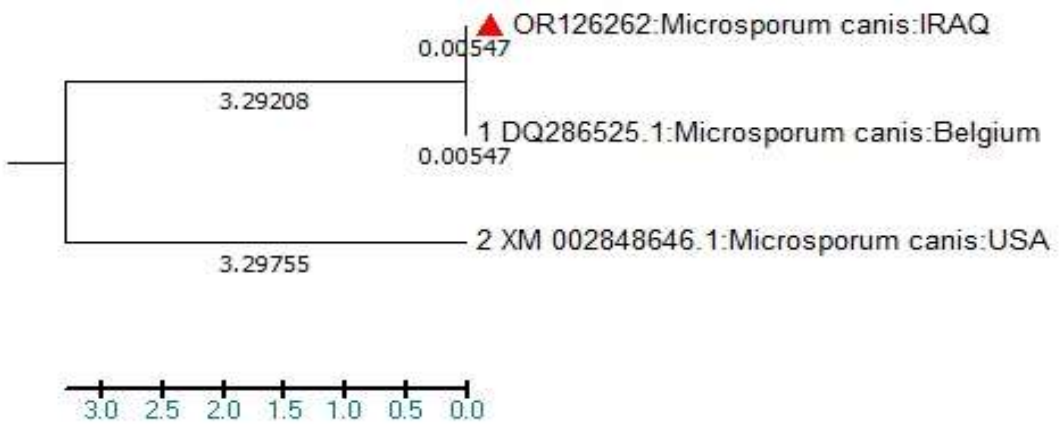

Figure (6) : Neighbor-joining tree of the DPPV gene of Iraqi *M. Canis* isolate compared with global isolates

The fungal skin diseases suffered by small animals, specially cats are caused by *Microsporum canis* (Willemse,2015), which is considered zoophilic dermatophytes. This infection can also cause ringworm in humans body, specially in the nails and head (Chupia *et al.*,2022).Therefore, the goal of this research is to detect of *Microsporum canis* in cats in Baghdad governorate in Iraq, which are lives very close to humans. According to this research, the percentage of the infection with *M. canis* in cats in Baghdad governorate is (1.41%) , it is represented by the presence of four isolates that were positive for *Microsporum canis* out of 283 different isolates , the best in growth of these isolate was confirmed by PCR using primer specific for (*DPPV*) gene was applied for the detection of *Microsporum canis* infection in cats, case based on the amplification of the *DPPV* gene . The PCR product was about 895 base pairs (pb). Sequencing data were analyzed . The analysis found that sequence was *M. canis* . Result of *Microsporum canis* isolation of this study represented by the presence of *Microsporum canis* infection in Baghdad governorate but, the infection rate of this fungus in cats was lower compared to other studies. The current the study reported 1.41 % of ringworm cases were isolated from cats ; this result is not consistent with a result of study conducted by (Copetti *et al.*,2006),which reported the isolation rate was 25.2%. The result of this study was far from (Paixão *et al.*,2011), who reported that *Microsporum canis* was isolated from cats with (28.6%). but the investigation of (Brilhante *et al.*,2003), was higher slightly in which from 38 cats, 14 *Microsporum canis* was isolated with (36.8%). While was higher than from the result of two studies; firstly, (Abou-Eisha *et al.*,2008) recorded that the dermatophytes represented by *Microsporum canis* were (10%) of the examined cats. (Nwiyi and Ottah,2020) reported the isolation rate of *Microsporum canis* in cats was 22%.The differentiation in the rate of isolation dermatophyte *Microsporum canis* from cats between researches can be explained by the presence of virulence factors of dermatophytes spp. isolates more than others and the climate condition that is more suitable in some regions than others when these studies were conducted.These findings explain the variation allegedly occurs due to difference in relative temperature, climate, humidity, pollution of the environment and the rainfall between the geographical areas where the studies were executed (Zenad *et al*.,2015). According to this research, humans who are close to and exposed to cats should be more aware of animal diseases, especially skin diseases such as ringworm. Zookeepers, veterinarians, or people who come into contact with these animals should be aware, as contact with infected animals (both at lesions and non-lesion sites) can cause infection with *Microsporum canis*. Early diagnosis with rapid treatment, reducing the risk of cats carrying the *Microsporum canis* to humans and pets animals and increasing the chance of them recovering from the infection (Chupia *et al.*,2022). Two infected cats studies reported symptoms such as scabs and redness, showing severe itching and found that the symptoms of both cases were caused by *Microsporum canis* , Both patients had a cat in their household. The cats were strong, normal, and healthy cats that did not show any symptoms. Preliminary examinations with Wood's lamp method provided positive results and diagnoses of *Microsporum canis,* so it is possible that both of these cases were caused by *Microsporum canis* infection from healthy cats to humans. There were no lesions, making humans think that the cats were uninfected and reducing the care taken when dealing with the animal, this increased the risk of infection with *Microsporum canis* (Boothe,2012).Many antifungal drugs have been used successfully for *Microsporum canis*. Most commonly used antifungal drugs in veterinary medicine were griseofulvin , itraconazole, ketoconazole , terbinafine, and fluconazole (Chupia *et al.*,2022). Besides the treatment, the prevention is very important; the owner should wash/change the bedding frequently because of the contaminated fomite. Washing hands immediately after contact with cats (with and without skin lesion) every time is necessary.Mechanical removal of infected organic material/hair and surface washing, with a detergent, is the most important step for environmental cleaning/disinfection, disinfectant should be used after cleaning. In Iraq, The incidence of *Microsporum canis*,even if it is low ,must be taken with caution because of it is possibility of some multidrug resistant strains to transmit to humans and pet animals (Abulkareem Abdulshaheed,2009).

Conclusion

Microsporum canis is one of the major dermatophytic diseases in cats so; the study recommended to take high precaution toward cats due to zoonotic nature and easy transmitted of their spore**.**Therefore; in this research focused on the presence of *Microsporum canis* in cats, collecting samples from cats with and without lesions of dermatitis because this pathogen can be found in the hair of cats with and without skin lesions, owners, veterinary staff, and others who come into contact with the animals are at risk of infection if they are not aware or do not take precautions. The zoonotic risk and potential as an etiologic agent for a variety of diseases should be considered and investigated further**.**

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