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ISOLATION AND IDENTIFICATION OF *TRICHOPHYTON VERRUCOSUM* IN CATTLE OF DIYALA GOVERNMENT

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ABSTRACT

Skin fungi are widespread in cows and cause health problems for humans and pose an economic and public health risk. The aim of this study is to isolate and diagnose the fungal causes that cause ringworm in cows. They were studied using direct screening methods, culturing the samples on SDA and characterizing them, and PCR technology. One hundred samples were collected from cows (male and female, with age ranged between less than year to four years) in Diyala Governorate. The study was from the October of 2022 to the July of 2023, and the results showed 14% infected cow by different dermatophytes causes included a high percentage of the causative factor for infection with the fungus *T. verrucosum* percentage7(50%). The result was Dermatophytes accession species number (OR362821.1).

KEYWORDS: Dermatophytosis, *T. verrucosum*, Genetic tree, ringworm infection, cattle.

INTRODUCTION

Dermatophytosis is a skin fungal infections in animal and human, caused by dermatophytes affecting the skin, hair, hooves, horns and that invade and feed on keratinized tissue like skin, hair and nails, causing an infection, (Gupta *et al.*, 2005; Jha *et al*., 2019; Qadisiyah and Alaa, 2023). Transmission ringworm in Animals: can contract diseases directly from sick animals or indirectly from diseased environments, inadequate nutrition, livestock poor management, and a lack of clean conditions that foster the spread of fungi (Agnetti *et al*., 2014).

Trichophyton, Epidermophyton, and Microsporum are the three types of dermatophytes that typically infect animals they can also be classified according to the mode of transmission as anthropophilic (from humans), zoophilic (from animals), and geophilic (from soil) (Dolenc-Voljč & Gasparic, 2017). Dermatophytosis is most frequently identified in calves and is characterized by nonpruritic periocular lesions, though generalized skin disease can also occur, bulls get lesions in the dewlap and intermaxillary skin, whereas cows and calves acquire lesions on the chest and limbs, the typical appearance of lesions is discrete, scaling areas of hair loss with the creation of a gray-white crust (Gnat *et al*., 2019; Khurana *et al*., 2019; Karen, 2022).

The prevalence rate of bovine ringworm in various regions of Iraq 21.2 %, Mosul, 26.5%, Baghdad, 68 % Diyala, 90 %, (Hussein *et al*., 1989; Arslan *et al*., 1998; AL-Samarrae, 2009; Jameel, 2015) respectively, while rate of infections in neighboring countries as, Jordan 30.6 % (Al-Ani *et al*., 2002), Turkey 33.33 % (Sever *et al*., 2017).

MATERIALS AND METHOD

Sampling: One hundred samples were collected from bovine in different ages and genders suffered from skin lesion, these samples cultured on Sabouraud dextrose agar SDA, at a temperature of 35±2°C in an incubator for a period of one to two weeks. (Kwon-Chung & Bennett, 1992).

Detection of Gene ITS by Using PCR according to Bio world water sample
DNA/RNA Extraction Kit for Microorganism, 0.22um Manufactured by USA

Table 1: The primers used for 18s rRNA amplification and expected amplicon size.

Primer)	Sequence (5'-3')	Product Size pb	Source
18S Rrna	F 5- GGAAGGGRTGTATTTATTAG -3 R 5-TCCTCTAAATGACCAAGTTTG-3	1500Pb	In this study

RESULTS

According to clinical examination of cow suffering from skin lesion was characterized clinically by a thick, circular or irregular crust that is grayish-white in color apparent above the skin. The lesion ranged from single to malty focal crusts. This crust, distributed in different area of body, typically on the head (face, around eye, ears), neck, tail, fore and hind limbs, we can notice the lesion higher in head while low appearance in tail (Figure 1).

Figure 1: Lesion of dermatophyte (*T.verrucosum*) in head, neck, and other area of body.

The results of direct examination revealed 48positive cases (48%) out of the total number of the tested samples of cow (100 samples),while only 14positive sample(14%) found at culture on SDA, included highest percentage infection with ***Trichophyton verrucosum*** and then other fungal causes, The morphological definition of ***T. verrucosum***: Feature culture colonies are small, button- or disk-shaped, slow-growing on SDA (7 days to 4 weeks, 35±2°C), and is white, soft, and fluffy in the center, with a suede-like to velvety surface, an elevated center, and a flat periphery with some buried development. In (Figure 2), reverse pigment can range from being unpigmented to yellow.

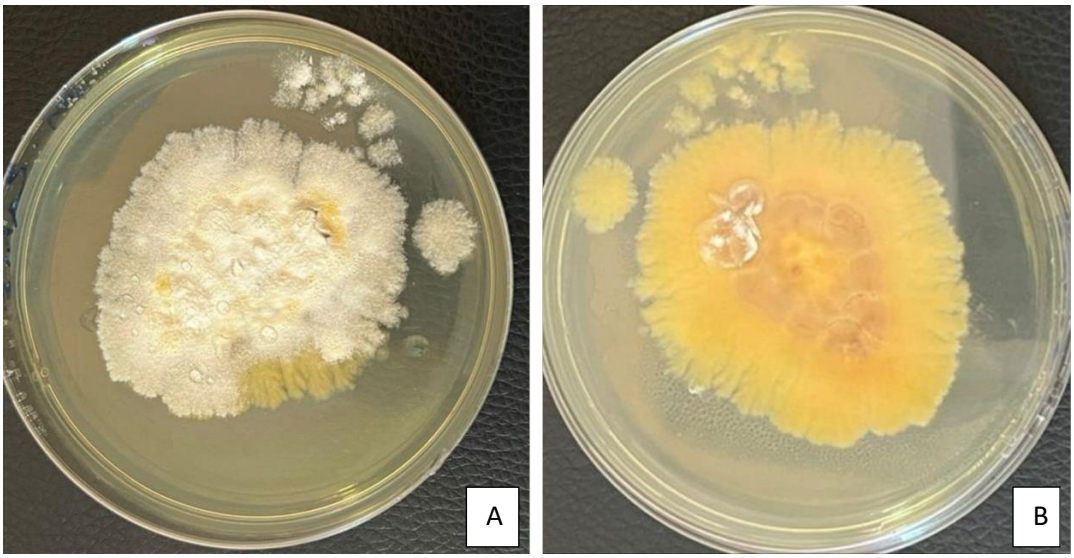

Figure 2: *Trichophyton verrucosum:* A. macroscopic top view grown on SDA at 35±2°C and pH 5.6 for 10 days of incubation, B. reversed view.

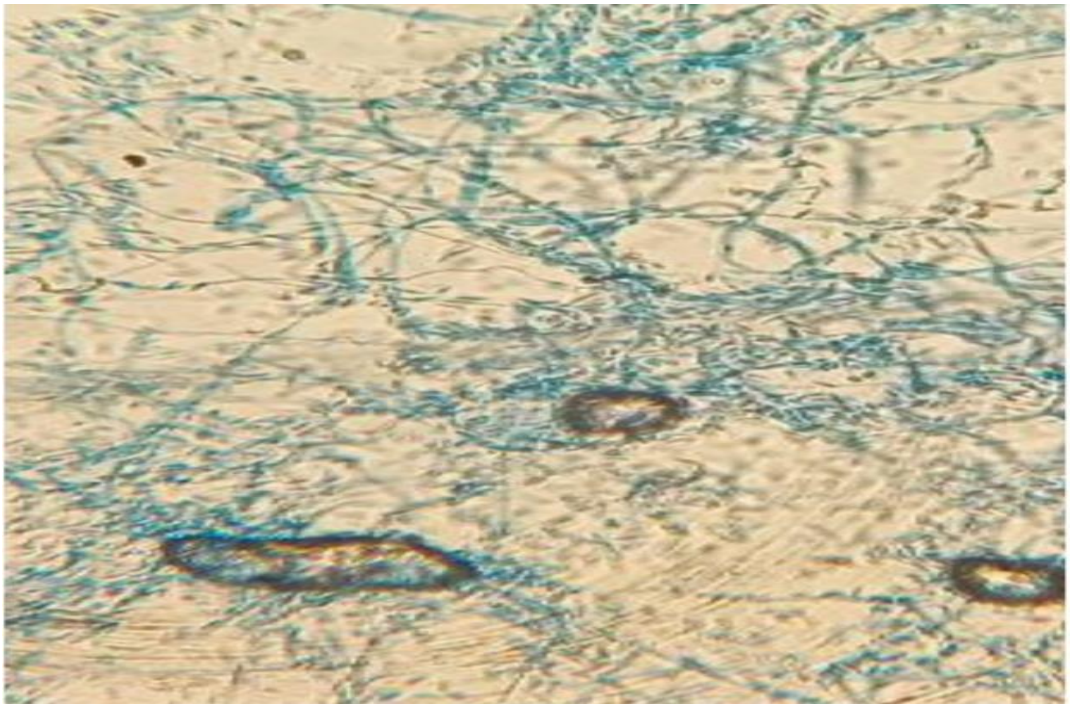

Figure: 3: *T. verrucosum*. microscopic appearance stained with Lactophenol cotton blue(40 X) showing broad, irregular hyphae and "chains of pearls"-like Chlamydospore chains as its primary microscopic characteristics.

Molecular identification: The isolate was diagnosed by PCR. It showed positive in the reaction and was diagnosed with a PCR product size of 1500pb, Amplification reactions of a fragment of the ITS1region.

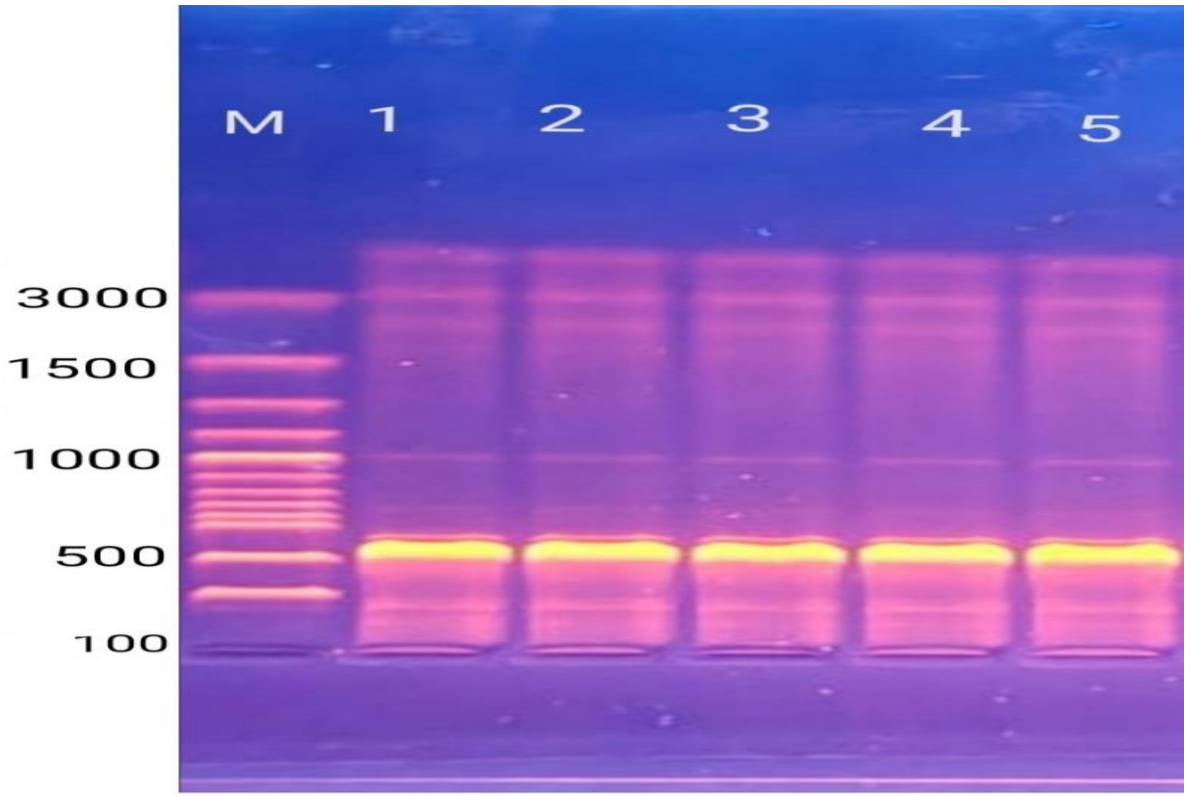

Figure 4: PCR products: the size of the selected bands of 18S rRNA production (1500bp), Amplification reactions of a fragment of ITS region, from the fungal isolates, band size 100-3000 bp, Electrophoresis on a 1.5% agarose gel, at a voltage of 100, for a one hour of time with UV visualization for isolates of dermatophytes of (1500 bp) Positive for isolation dermatophytes.

Iraq's local isolation: At the NCBI was sampled for PCR results that were found to be positive using primers for sequencing. Bank Database No. 1 sequences from the NCBI gene were utilized (OR362821.1). BLAST-NCBI was used to evaluate these sequences an application to find closely comparable sequences that are listed in Gen Bank.

Genetic tree and fungus symmetry table of *T. verrucosum*.: The results of genetic tree analysis showed that the Iraqi isolate of the fungus *T. **verrucosum***. (OR362821.1), Same a close relationship with different international isolates of the same fungus, as the percentage of similarity between them and the following isolates Germany (KY623476.1), Iran (MN808788.1), Egypt (MT261759.1), Iran (MN808787.1), South Korea (OR058558.1) was (100%).

Table 2: Fungus symmetry table of *T. verrucosum*.

ACCESSION	Gene	Country	Source	Compat.
KY623476.1	18S ribosomal RNA	Germany	*Tichophyton Verrucosum*	*100%*
MN808788.1	18S ribosomal RNA	Iran	*Tichophyton Verrucosum*	*100%*
OR362821.1	18S ribosomal RNA	*Iraq*	*Tichophyton Verrucosum*	*100%*
MT261759.1	18S ribosomal RNA	*Egypt*	*Tichophyton Verrucosum*	*100%*
MN808787.1	18S ribosomal RNA	*Iran*	*Tichophyton Verrucosum*	*100%*
OR058558.1	18S ribosomal RNA	*South Korea*	*Tichophyton Verrucosum*	*100%*

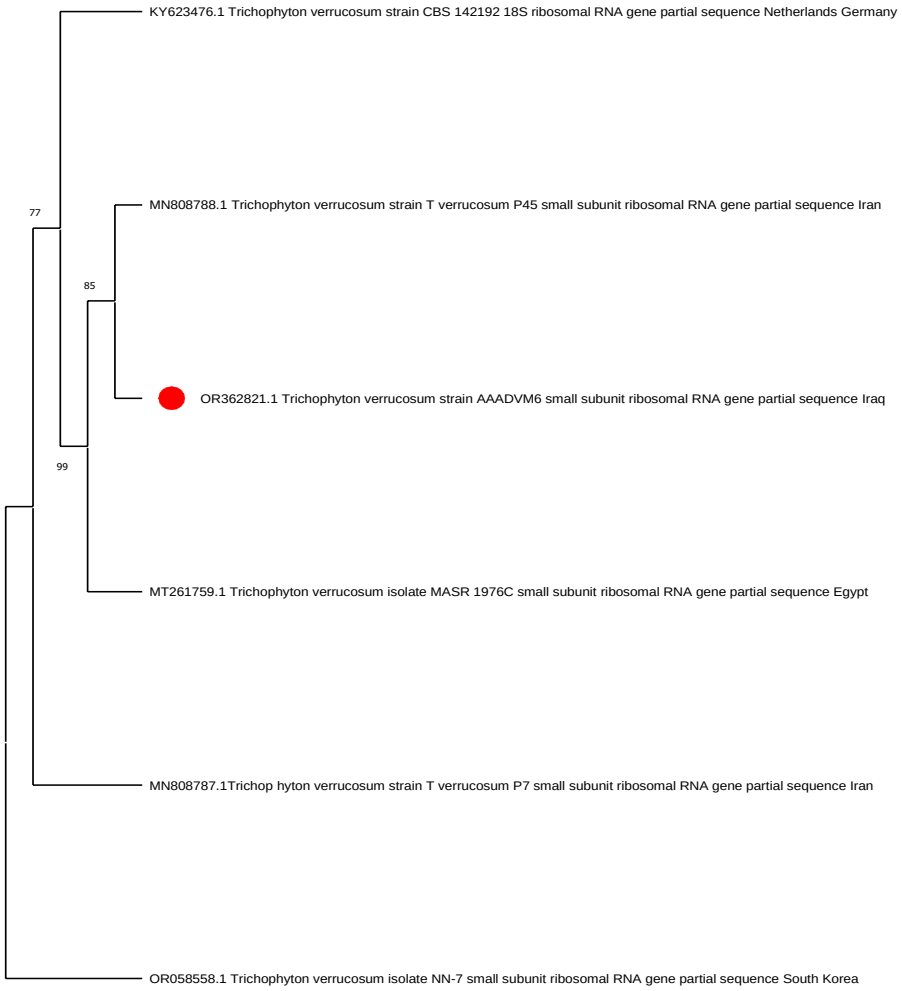

Figure 5: Genetic tree of Trichophyton verrucosum.

DISCUSSION

Dermatophyte infections are not fatal, but because of their chronic nature and resistance to treatment, they have a major morbidity and economic impact on community, Adnan *et al* (2014).

In this study, the highest percentage of fungal pathogens isolated was *T. verrucosum* (50%). Similar results were reported by Guo *etal* (2020), which found 61.1% infected cattle in current study T. *verrucosum* was the most frequently isolated fungus than others, making it principal fungal causes responsible for cow dermatophytes. This can because the cattle's feed or litter contains these fungus. As a result, when it is came with feed, it causes the fungi to stick to the lesion and become infected Singh and Kushwaha. (2010) revealed, *T. verrucosum* can remain viable in the environment for five to seven years (which raises the possibility that residences, contaminated practices, and even soil could be sources of infection for animals. Dalis *et al* (2019) report, the rate of infection in cattle were highest of *T.verrucosum*, then *T. mentagrophytes* and at last, *M.canis* respectively, this similar with result of current study.

The lower sensitivity of traditional methods for dermatophyte detection were microscopic inspection and culture, which only able to identify the presence of fungi in the samples or not; currently, the most frequent detection method used to identify fungal species is PCR technology in conjunction with DNA sequence analysis technology. The internal transcribed spacer (ITS) is often used in species taxonomic examinations because it is comparatively kept in long-term

evolution and has major evolutionary changes across other species (Dalis., *etal*., 2014; De Chaumont *etal*., 2014; Guo *etal*., 2020). Using the DNA ITS1 Sequence database, it was demonstrated that the basic sequences of *T. verrucosum.*602bp (OR362821.1). The result appeared for the genetic tree of pathogens T. *verrucosum*, which recorded in the gene bank and was similar at 100% to the strains that appeared in the countries of the world, such as different international isolates of the same fungus, as the percentage of similarity between them and the following isolates, Germany (KY623476.1), Iran (MN808788.1), Egypt (MT261759.1), e Iran (MN808787.1), south Korea (OR058558.1).

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