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Genotyping of *Microsporum canis* Isolated from Dogs with Clinical Dermatophytosis by ERIC-PCR

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ABSTRACT

Microsporum canis is a zoonotic dermatophyte responsible for skin and hair infections in cats, dogs, and humans. Previous reports showed that, primary reservoirs include pet shops and commercial breeding facilities, while stray and domesticated cats with outdoor access are additional sources of infection. In dogs, pet stores remain the predominant reservoir. Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) has greater power in revealing the genetic diversity of isolates, due to the use of longer primers and higher annealing temperatures, and separating isolates into completely distinct genetic groups. In this study, genotypes of *M. canis*, which were isolated from dogs with clinical dermatophytosis by ERIC-PCR, were investigated. Skin and hair samples were collected from dogs with clinical dermatophytosis at veterinary clinics in Tehran. Species samples were cultured and confirmed by PCR. ERIC-PCR fingerprinting was performed with ERIC-R and ERIC-F primers, generating unique banding patterns of 33 *M. canis* isolates. Binary scoring and phylogenetic analysis using NTSYSpc software revealed six distinct genotypes based on band variation ranging from 100 to 3,000 base pairs. These findings suggest notable intra-species genetic variability and provide insights into potential genotype–phenotype relationships relevant to clinical manifestations and treatment strategies. Identifying *M. canis* genotypes, which lead to more severe clinical presentations, may help clinicians to estimate recovery timelines. Nevertheless, the study findings suggest that variation in *M. canis* virulence may be genetically determined to a certain extent. To uncover the genetic underpinnings of *M. canis* virulence, further investigation using genome-wide sequencing techniques is warranted. In conclusion, we characterized Iranian *M. canis* isolates by ERIC-PCR for the first time. The resulting genetic profiles and phylogenetic data provide insights into intra-species diversity, potential genotype-phenotype associations, and regional transmission dynamics.

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Introduction

Microsporum canis is a dermatophyte that causes various forms of skin and hair infections, particularly in cats and dogs (Zhou *et al*., 2025; Goln *et al*., 2025). It also poses a zoonotic threat to humans and dogs (Yamada *et al*., 2022; Yamada *et al*., 2019). Primary reservoirs include pet shops and commercial breeding facilities, while stray and domesticated dogs with outdoor access are additional sources of infection. In dogs, pet stores remain the predominant reservoir (Segal *et al*., 2021; Nametov *et al*., 2025). Despite its widespread occurrence, the epidemiological patterns and transmission risks to humans remain poorly understood (Wang *et al*., 2023; Zhou *et al*., 2023b). Advancements in molecular biology have enabled intra-species genotyping to analyze transmission dynamics and pathogenic variations. Techniques such as microsatellite DNA analysis

and Random Amplified Polymorphic DNA (RAPD) have been evaluated to detect genetic polymorphisms and track infection routes. When combined with conventional epidemiological tools, molecular typing has significantly advanced understandings of adaptation, transmission, and virulence in fungal species (Persinoti *et al*., 2018; Ratemo *et al*., 2023; Yamada *et al*., 2022). Genotyping may also help assess the relationship between clinical manifestations and genetic variabilities, offering deeper insights into pathogenesis and informing diagnostic approaches (Zhou *et al*., 2024; Wang *et al*., 2023). Although previous studies have revealed variations in *M. canis* virulence among genotypes, a clear correlation between genotype and clinical presentation has not been established (Yamada *et al*., 2022). Recent findings by Zhou *et al.* (2023) indicate that the *M. canis* complex includes three closely related species: zoophilic *M. canis* and two anthropophilic species, *M. audouinii* and *M. ferrugineum* .Understanding the evolutionary relationships among species requires phylogenetic analysis, population-structure study, ideogram distribution, and assessment of host specificity. Evidence suggests that virulence variation in *M. canis* may be genetically determined (Yamada *et al*., 2022; Pasquetti *et al*., 2017). Dermatophytic lesions in dogs are seen on the face, feet, and/or tail. Studies have shown that dogs infected with *M. canis* develop alopecia (1-3 mm) in the infected area, and permanent alopecia occurs if the inflammatory reaction persists for a long time. Lesions caused by dermatophytes can range from mild to severe, depending on several factors, including the infecting dermatophyte species, virulence factors, sites of infection, secondary infections, and environmental conditions (Paryuni *et al*., 2020). Recently, researchers proposed a typing method based on enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), which was shown to be a good test for genetic discrimination of strains, with high resolution, repeatability and typeability (Elaine *et al*., 2014). The indicators studied in ERIC-PCR primers were higher than those of ISSR and RAPD primers due to the use of longer primers and higher annealing temperatures. The findings show that despite common characteristics such as dominance, simplicity, and high speed of action in all three primers, and to separate isolates into completely separate genetic groups, ERIC primers have greater power in revealing the genetic diversity of isolates (Alsultan *et al*., 2022). ERIC-PCR, a fingerprinting method based on extragenic repetitive sequences originally identified in *Escherichia coli* and *Salmonella enterica*, has proven valuable in distinguishing bacterial and fungal strains (Telli *et al*., 2022). This study aimed to characterize Iranian *M. canis* isolates by using ERIC-PCR for the first time. The resulting genetic profiles and phylogenetic data provide insights into intra-species diversity, potential genotype-phenotype associations, and regional transmission dynamics.

Material and Methods

Sample collection

Skin and hair samples were collected from the diagnosed dogs with dermatophytosis at veterinary clinics in Tehran. Before sampling, lesion areas were disinfected with 70% ethanol. Samples were placed in sterile petri dishes and transported to a research laboratory for analysis.

Laboratory diagnosis of *M. canis*

To identify dermatophyte species, several culture media were used: basic (Sabouraud Dextrose Agar, SDA), selective DTM (Dermatophyte Test Medium) and differential (Sabouraud Dextrose Agar with rice extract). Samples were inoculated as point cultures and incubated at 25 °C and 27 °C to promote colony growth.

Molecular identification

Frozen mycelial mats were grounded in a cold mortar with liquid nitrogen. Genomic DNA was extracted using the SinaClon commercial kit (Tehran, Iran), following the manufacturer's protocol. To confirm the presence of *M. canis*, PCR amplification, with the ITS1 region of ribosomal DNA targeting, was performed using species-specific primers described by Khalili *et al*. (2018). The forward primer sequence was 5′-GTGTGATGGACGACCGTCCCCCCT, while the reverse primer sequence was 5′-ATAATACATGGTGCGTTAGGCCAGCCTG. The corresponding NCBI reference sequence was NW_003299166.1. DNA samples were electrophoresed on a 2% agarose gel prepared with 0.5X. TBE buffer was used for visualization.

ERIC-PCR amplification

The enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) was performed to genetically characterize the isolates. To ensure reproducibility and validate the resulting banding patterns, the ERIC-PCR assay was performed in triplicate for each isolate. The primers, which were utilized for amplification, included ERIC-1R (5'-ATGTAAGCTCCTGGGGATTCAC) and ERIC-1F (5'-AAGTAAGTGACTGGGGTGAGCG), as described by Elaine *et al*. (2014). The amplification was carried out in a total reaction volume of 50 µL. Each reaction mixture consisted of 5 µL of 10X PCR buffer, 4 µL of $MgCl_2$, 1.75 µL of dNTP mix, and 0.6 µL of *Taq* DNA polymerase. Additionally, 3 µL of DNA template and of the forward/reverse primer mixture were incorporated, and the final volume was adjusted by adding 29.65 µL of double-distilled water (ddH_2O) sterile. Thermocycling profile was initiated with an initial denaturation step at 94°C. This was followed by amplification cycles comprising denaturation at 94°C for 30 seconds, primer annealing at 52°C for 1 minute, and an extension step at 72°C for 1 minute. The reaction concluded with a final extension step at 72°C to ensure the complete synthesis of the amplicons.

Gel electrophoresis and band analysis

The PCR products were separated via electrophoresis on a 2% agarose gel at 100 V for one hour. The gels were subsequently stained with ethidium bromide and destained with distilled water. A 100-3000 bp DNA ladder (Ladder 100bp Plus, ready-to-use) was used as a molecular weight marker. The resulting band patterns were captured and documented using a gel imaging system. The presence or absence of bands was first analyzed visually, followed by numerical tabulation into a binary matrix for further evaluation.

Dendrogram construction

Binary banding data were used to calculate genetic similarity using Jaccard's, Dice's, and Simple Matching coefficients. Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) in NTSYSpc ver. 2.02e. Cophenetic correlation coefficients were computed to assess clustering accuracy and identify optimal grouping among isolates.

Results

ERIC-PCR Genotyping

DNA extraction products were subjected to 2% agarose gel electrophoresis alongside a standard size marker. Observation of a distinct 176 bp band in 33 samples indicated successful identification of *M. canis* isolates. Genomic DNA fingerprinting was performed using the ERIC-PCR technique with the primer pair ERIC-F and ERIC-R. Banding profiles were scored as binary patterns, reflecting presence or absence of specific fragments. These patterns were entered into a similarity matrix, and phylogenetic analysis was conducted using NTSYS software. The ERIC-PCR generated bands ranging in size from 100 to 3000 base pairs. Thirty-three isolates were assigned numerical identifiers (1-33), corresponding to their banding profiles (Fig. 1).

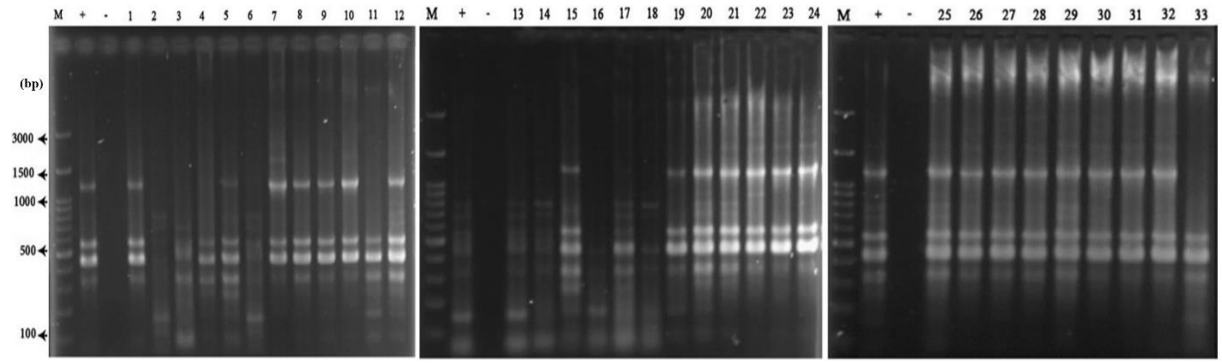

Fig. 1. Electrophoretic patterns of ERIC-PCR products of 33 *M. canis* strains in the 2% agarose: Lane (M)= Molecular weight marker, Lane (+) = Positive control/ PCR products of standard *M.canis* strains; Lane (–) = Negative control (distilled water); Lanes 1-33= PCR products of *M. canis strains*; bp= Base pairs

Based on genetic similarity among the isolates, six distinct genotypes were identified (Fig. 2). These findings demonstrate both conserved and variable regions in the *M. canis* genome, highlighting intra-species diversity among Iranian canine samples.

Discussion

Before the advent of molecular biology techniques, researchers encountered considerable limitations in investigating disease transmission, identifying sources of infection, and understanding patterns of spread (Eybpoosh *et al*., 2017; Yamada *et al*., 2022; Zhan *et al*., 2018; Zare-Bidaki *et al*., 2022). Early approaches relied on phenotypic methods, including genotyping, bio-typing, colistin typing, analysis of antibiotic resistance, and plasmid profiling (Vite-Garin *et al*., 2024; Moriello *et al*., 2017; Persinoti *et al*., 2018). Accordingly, it is anticipated that *M. canis* comprises distinct genotypes which are associated with varying degrees of disease severity, enabling phylogenetic tree construction using diverse molecular fingerprinting techniques (karamirobati *et al*., 2018; Watanabe *et al*., 2017; Zawrotniak *et al*., 2019).

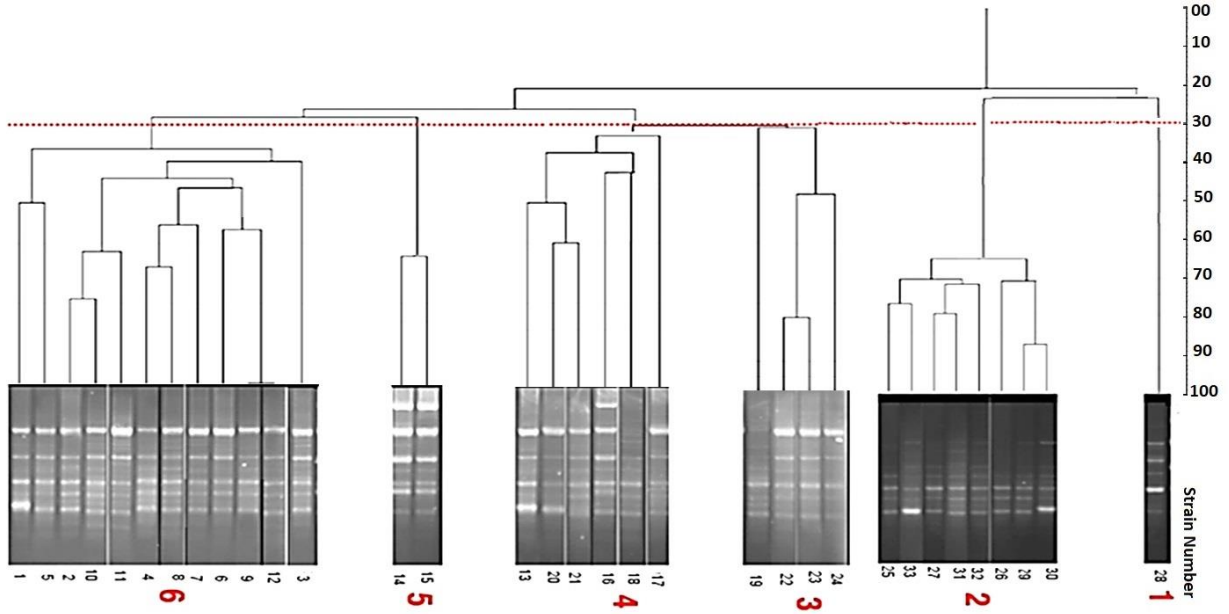

Fig. 2. Dendrogram obtained from the ERIC-PCR assay for *Microsporum canis* strains.

In the present study, the ERIC-PCR technique was employed for the first time to fingerprint 33 isolates of *M. canis*, resulting in the identification of six genotypes within this dermatophyte species. *M. canis* is a zoophilic dermatophyte with widespread occurrences, primarily infecting domestic cats and dogs, and has recently shown increased pathogenicity in humans (Sierra-Maeda *et al*., 2024; Segal *et al*., 2021). Yamada and colleagues (2019) reported that anthropophilic dermatophyte species exhibit higher levels of kinases and transcription factors than their zoophilic counterparts, reflecting evolutionary adaptations and providing deeper insights into the pathogenic mechanisms of *M. canis*.

Multi-locus microsatellite typing (MLMT) was conducted on 93 strains of *M. canis* isolated from 2012 to 2017, including samples from 75 cats, 8 dogs, and 10 pet owners. This process revealed 22 genotypes, 11 of them previously documented in Japan and 11 novel genotypes. Among the identified genotypes, genotype P was the most prevalent which comprises 37 of 93 isolates (39.8%), followed by genotype A with 11 strains (11.8%). All novel genotypes were found exclusively in feline samples. MLMT genotyping has proven effectiveness of tracing *M. canis* transmission and prevalence pathways throughout Japan. Despite low intraspecific variation in *M. canis*, available molecular markers for strain identification remain limited (Yamada *et al*., 2022). MLMT is regarded as the most sensitive technique for molecular typing that have been applied in identifying pathogenic strains

(Pasquetti *et al*., 2013; Alanio *et al*., 2017) and tracing infection sources (Yamada *et al.*, 2022). This methodology holds particular utility in evaluating the molecular epidemiology of *M. canis*, especially in human isolates from Japan (Vite-Garin *et al*., 2024; Yamada *et al*., 2022).

Zhou *et al.* (2023) employed phylogenetic, population-structure analyses, multi-species comparisons, MAT idiomorphic distributions, sexual-reproduction assessments, and examination of macro-morphological and physicochemical characteristics to identify 12 genotypes. Additionally, ERIC-PCR has demonstrated value and efficiency in the molecular typing of Salmonella species (Souza *et al.*, 2015). ERIC-PCR has greater power in revealing the genetic diversity of isolates, due to the use of longer primers and higher annealing temperatures, and to separate isolates into completely distinct genetic groups. (Alsultan *et al*., 2022). Godoy and colleagues (2004) conducted a retrospective study over 6 years in Brazil, finding that *Fusarium solani* is the most common species causing fungal keratitis. The genetic diversity of 44 isolates from 39 patients was evaluated using PCR fingerprinting by ERIC and RFLP. Khodagholi and colleagues (2013) studied the phenotypic and genotypic types of pathogenic isolates of *Fusarium solani*, which is considered one of the most important fungal diseases of beans in the world and Iran. Sampling was carried out from 11 regions of the province and 30 isolates were identified as *Fusarium solani* species, using RAPD and ERIC-PCR primers on extracted DNA. Mishra and colleagues (2015) suggest that BOX-PCR and ERIC-PCR could be suitable, rapid, reproducible, and useful techniques for the discrimination of *F. oxysporum* to better manage guava wilt disease.

Conclusions

Previous research indicates that more severe inflammatory dermatophytes tend to resolve more quickly, likely reflecting the efficiency of the host's immune response. Identifying *M. canis* genotypes associated with more severe clinical presentations may help clinicians estimate recovery timelines. Nevertheless, the study findings suggest that variation in *M. canis* virulence may be genetically determined to a certain extent. To uncover the genetic underpinnings of *M. canis* virulence, further investigation using genome-wide sequencing techniques is warranted. Therefore, conducting a genotyping study of *M. canis* strains obtained from clinical samples of other animals and humans from Iran is strongly recommended.

Ethical approval

The study was conducted by determining the molecular genotyping identity of *Microsporum canis* which were isolated from dogs with clinical dermatophytosis by ERIC-PCR, Islamic Azad University (Baft Branch), and was approved under ethical code 162811486.

Author contributions

AK: Study conception and design, manuscript drafting, reviewing and editing, and schematics preparation; **BK**: Study conception and design, material preparation, data collection, data analysis, manuscript drafting, schematics preparation, and project supervision; **SB**: Manuscript drafting, project administration, and funding acquisition. Also, all authors read and approved the final manuscript**.**

Data availability

The data will be not made available on request.

Conflict of interests

The authors declare no conflict of interest.

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