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RESEARCH ARTICLE

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Molecular Characterization of *Demodex Canis* (Acarina: Demodicidae) in Domestic Dogs (*Canis Familiaris*)[#]

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ABSTRACT

Canine demodicosis (CD) is an inflammatory skin disease caused by excessive proliferation of *Demodex* mites (Acarina: Demodicidae) former in the dogs' follicles latter in the sebaceous glands. To date *Demodex canis*, *D. injai*, and *D. cornei* are the three *Demodex* species that recognized in the domestic dog *Canis familiaris*. There are reports of morphological identification of *Demodex* spp. from pet dogs in Turkey. Nevertheless there had been no reports of characterizing the *Demodex* mite from pet dogs in Turkey using well-defined mitochondrial DNA gene sequences. The aim of the present study is to reveal first molecular characterization data on *D. canis* infesting domestic dogs (*Canis familiaris*) in Turkey. *Demodex* mites were obtained from skin scrapings of dogs with CD and identified microscopically as *D. canis*. The mitochondrial DNA 12S (mtDNA 12S) rRNA gene amplified, sequenced and compared with available *Demodex* sequences in Genbank using BLAST analyses. *D. canis* isolate (DP-Samsun, MH374631) obtained in the study matched 100% (474/474) with previously reported gene sequences for the 12S rRNA of mtDNA in *D. canis* (KX264486, isolate Demo.can2) from dogs in China. Pairwise comparison between the 12S rRNA sequences of the *D. canis* from Turkey (MH374631) and other mtDNA rRNA gene of *D. canis* isolates from China (KX264486-88) presented differences ranging from 0.0 to 0.6 %. In conclusion, within the present study, we provided the molecular characterization of *D. canis* in domestic dogs for the first time by sequencing of the partial mtDNA in Turkey.
Keywords: *Demodex canis*, Dog, Molecular characterization, PCR, Sequencing.

Evcil Köpeklerde (*Canis Familiaris*) *Demodex Canis*'in (Acarina: Demodicidae) Moleküler Karakterizasyonu

ÖZ

Kanin demodikozis (KD) *Demodex* akarlarının (Acarina: Demodicidae) kıl köklerinde ve yağ bezlerinde aşırı çoğalmasının neden olduğu inflamatuar bir deri hastalığıdır. Bugüne kadar evcil köpeklerde *Canis familiaris* bildirilen üç *Demodex* türü *D. canis*, *D. injai* ve *D. cornei*'dir. Türkiye'de pet köpeklerinde *Demodex* spp.'nin morfolojik identifikasyonu ile ilgili raporlar vardır. Buna rağmen Türkiye'de evcil köpeklerde *Demodex* akarlarının iyi tanımlanmış mitokondriyal DNA gen sekanslarının moleküler karakterizasyonu ile ilgili raporlar yoktur. Bu çalışmanın amacı Türkiye'de evcil köpeklerde (*Canis familiaris*) *D. canis* üzerine ilk moleküler karakterizasyon verilerini ortaya çıkarmaktır. *Demodex* akarları KD'li köpeklerin deri kazıntılarından elde edildi ve mikroskobik incelemeler ile *D. canis* olarak teşhis edildi. Mitokondriyal DNA'nın 12S rRNA geni çoğaltıldı, sekanslandı ve Genbank'ta BLAST analizleriyle mevcut bilinen *Demodex* sekansları ile karşılaştırıldı. Araştırmada elde edilen *D. canis* izolatı (DP-Samsun, MH374631) Çin'de daha önce köpeklerde rapor edilen *D. canis* 12S rRNA gen sekansı (KX264486, Demo.can2) ile % 100 (474/474) olarak eşleşti. Türkiye'den *D. canis*'in 12S rRNA dizisi (MH374631) ile Çin'den rapor edilmiş *D. canis* izolatlarının (KX264486-88) 12S rRNA sekansları arasındaki ikili hizalama analizleri % 0,0 ile % 0,6 arasında değişen farklılıkları ortaya çıkarmıştır. Sonuç olarak bu araştırma ile Türkiye'de ilk kez evcil köpeklerde *D. canis* türünün moleküler karakterizasyonu mitokondriyal DNA sekansları kullanarak sağlandı.
Anahtar Kelimeler: *Demodex canis*, DNA dizileme, köpek, moleküler karakterizasyon, PZR.

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INTRODUCTION

Canine demodicosis (CD) is an inflammatory skin disease caused by excessive proliferation of *Demodex* mites (Acarina: Demodicidae) in the hair follicles and sebaceous glands. There are three recognized canine *Demodex* mites and these species are *D. canis*, *D. injai*, and *D. cornei* in domestic dog *Canis lupus familiaris* (L.). *Demodex canis* is the main causative agent of CD (Scott et al. 2001, Izdebska and Rolbiecki 2018).

Despite CD is a well-known challenging problem in veterinary medicine, little is known about the phylogenetic relationships of this demodecid (Silbermayr et al. 2015). Mitochondrial genes have been successfully applied in order to carry out phylogenetic and taxonomic works in various taxa of mites (Simon et al. 1994). The rapid advances in molecular techniques support a usefull technical achievement for the molecular characterization of *Demodex* at a genetic level (Hu et al. 2017). The mitochondrial (mtDNA) 16S has been used for molecular characterization of the Demodecid mite (Frank et al. 2013, Zhao et al. 2013, Silbermayr et al. 2015, Sastre et al. 2016) and mt-cox1 (De et al. 2012, Zhao et al. 2014). Moreover, mitochondrial 12S was recently found more applicable than other DNA barcoding gene (cox1 and 16S) for molecular characterization of Demodecid mites at genus or species level (Hu et al. 2017).

In Turkey, there are reports of morphological identification of *Demodex* spp. from pet dogs (Deger et al. 1994, Beyazıt et al. 2010, Maden et al. 2012, Pekmezci et al. 2014). To date there had been no reports of characterizing the *Demodex* mite from pet dogs in Turkey using well-defined mtDNA gene sequences.

The aim of the present study is to describe first molecular characterization of *D. canis* in domestic dogs (*Canis familiaris*) in Turkey.

MATERIAL and METHODS

Parasitological examinations, DNA extraction and PCR Analysis

Demodex mites were obtained from skin scrapings of dogs which brought to the Veterinary Teaching and Animal Hospital, of the Faculty of Veterinary Medicine, University of Ondokuz Mayis, in April 2017 to December 2017 diagnosed as CD in Turkey. *Demodex* mites were primarily identified microscopically and classified as *D. canis*. The parasites were collected by using a micropipette and added to 50 µl of ethanol. Genomic DNA (gDNA) was extracted from polled mites using a commercial kit (GeneJet, Thermo Scientific). The mitochondrial 12S rRNA gene was selected to molecular identification of *Demodex* species. The mtDNA 12S was amplified using 12S-F (CTACTTTGTTACGACTTATTTTA) and 12S-R (GCCAGCAGTTTCGGTTA) (Hu et al. 2017). The PCR conditions followed the protocol described by Cheng et al (2015). PCR amplicons were visualized on 1% agarose gel by UV transillumination.

DNA sequencing and molecular analysis

DNA sequencing was performed by Macrogen Inc. (Amsterdam, NL) for mtDNA 12S gene. Sequences quality was checked using Geneious R11 (Biomatters Ltd) (Kearse et al. 2012). Later, obtained sequences were confirmed by both comparisons, assembled and edited with using Geneious R11 (Biomatters Ltd) (Kearse et al. 2012). The consensus sequences were compared with those previously published data for molecular identification by using the BLAST within the GenBank database (Altschul et al. 1990) and aligned with those previously characterized sequences of *D. canis* using ClustalW in Mega 7.0 multiple sequence alignments (Thompson et al. 1994). Nucleotide composition was calculated using Bioedit (Hall 1999). Genetic distances were calculated using the Kimura two-parameter model with pairwise deletion in Mega 7.0 (Kumar et al. 2016). Molecular analyses were carried out comparatively according to sequences obtained in previous studies in Genbank (Hu et al. 2017).

RESULTS

In the present study, *Demodex canis* was microscopically identified from dogs (Figure 1). The amplification of the mtDNA 12S RNA gene produced a fragment of approximately 500 bp. The mtDNA PCR product was subjected to direct sequencing giving products 474 bp long. The average percentage of nucleotide composition for the partial fragment was: 30.17 % (A), 6.12 % (C), 17.09 % (G), and 46.62 % (T). The G+C content were 23.21%. The consensus nucleotide sequence was submitted in the GenBank database under the accession number MH374631. *Demodex canis* (isolate DP-Samsun, MH374631) from Turkey matched 100% (474/474) previously reported gene sequences for the mtDNA 12S rRNA in *D. canis* (KX264486, isolate Demo.can2) from dogs in China (Hu et al. 2017). Moreover, *D. canis* isolate DP-Samsun from Turkey (MH374631) showed 99.2 to 99.8% identities with *D. canis* (isolate Demo.can3, KX264487 and Demo.can4, KX264488) from China according to 12S region (Hu et al. 2017). We herein report that our sequence (MH374631) differed by three

polymorphic sites (alignment positions 28, 33 and 257) with the sequences of *D. canis* isolate Demo.can3 (KX264487) and one nucleotide (alignment position 210) in the mtDNA 12S rRNA sequence of *D. canis* isolate Demo.can4 (KX264488) from the China (Hu et al. 2007). Pairwise comparison between the mtDNA 12S rRNA sequences of the *D. canis* from Turkey (MH374631) and other *D. canis* isolates from China (KX264486-88) presented differences ranging from 0.0 to 0.6 %.

Figure 1: Microscopic image of *Demodex canis* mite (original).

DISCUSSION

For a long while classification of *Demodex* mites has relied on hosts and morphological characteristics. Interestingly, the morphological characteristics of *Demodex* mites are prone to the influence of environment which may lead to different classifications of phenotypes. Additionally, different *Demodex* species might coexist in the host and cause difficulty in species differentiation (Zhao et al. 2013). Successfully, in the last decade DNA barcoding techniques support a useful achievement for the molecular characterization of *Demodex* species in animals (Zhao et al. 2014). The mitochondrial genes have a maternal inheritance, rapid evolution and various evolution rates for different regions have been most entirely provided in molecular characterization among the arthropod species (De et al. 2012, Frank et al. 2013, Zhao et al. 2013, 2014, Silbermayr et al. 2015; Sastre et al. 2016). Recently, the mtDNA 12S is proved to be used more suitable than the other DNA barcoding gene for molecular characterization of Demodecid mites (Hu et al. 2017). Therefore, molecular identification and characterization of *D. canis* has also proved by molecular evidence inferred from a mitochondrial 12S marker used in the current study. In Turkey, *Demodex* species was only morphologically reported from different companion animals (Deger et al. 1994, Beyazıt et al. 2010, Maden et al. 2012, Pekmezci et al. 2014). However, to date, there is no molecular identification of *Demodex* species in Turkey. Furthermore, within the present study, we also provide the first molecular evidence of *D. canis* in domestic dogs.

In conclusion, present study confirms that mitochondrial 12S partial sequence is a useful tool to discriminate *D. canis* isolate from domestic dog in Turkey as previously reported by Hu et al (2017). Therefore, mitochondrial 12S-based identification in Demodicidae should also be used in the other domestic and livestock animals in Turkey.

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