Kafkas Univ Vet Fak Derg
20 (1): 161-164, 2014
DOI: 10.9775/kvfd.2013.9615

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

SHORT COMMUNICATION

Zoonotic *Trichuris trichiura* Infections in Non-Human Primates at Samsun Zoo, Turkey: First Molecular Characterization

Cenk Soner BÖLÜKBAŞ [1] Gökmen Zafer PEKMEZCİ [2] Ali Tümay GÜRLER [1]
Mustafa AÇICI [1] Şinasi UMUR [1]

[1] Department of Parasitology, Faculty of Veterinary Medicine, Ondokuz Mayis University, TR-55139 Kurupelit, Samsun - TURKEY
[2] Department of Preclinical Sciences, Faculty of Veterinary Medicine, Ondokuz Mayis University, TR-55139 Kurupelit, Samsun - TURKEY

Makale Kodu (Article Code): KVFD-2013-9615

Summary

Trichuris trichiura were collected from rhesus macaque *(Macaca mulatta)* and Hamadryas baboon *(Papio hamadryas)* in Samsun Zoo, Turkey. DNA was isolated from individual worm in baboon for molecular characterization. The ITS region was amplified and sequencing in both directions using NC5-NC2 primers. Pairwise comparison between ITS region of the *T. trichiura* isolates from Turkey (KC877992) and other isolates China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553) showed differences ranging from 0.1% to 1.2%. With the present study, *T. trichiura* from Turkey were characterized for the first time by sequencing of the ITS.

Keywords: *Trichuris trichiura, Macaca mulatta, Papio hamadryas, Molecular Characterization, Samsun, Turkey*

Samsun Hayvanat Bahçesi'ndeki Primatlarda Zoonotik *Trichuris trichiura* Enfeksiyonu: İlk Moleküler Karakterizasyon

Özet

Samsun Hayvanat Bahçesi'ndeki rehesus maymunu *(Macaca mulatta)* ve babun'dan *(Papio hamadryas) Trichuris trichiura* toplanmıştır. Moleküler karakterizasyon için *Papio hamadryas*'dan toplanan ergin parazitin DNA'sı izole edildi. Ribosomal DNA'nın ITS gen bölgesi çoğaltıldı ve NC5-NC2 primer çifti ile iki yönlü dizi analizi yapıldı. *T. trichiura* Türkiye izolatı (KC877992) ile diğer bölgelere ait Çin (AM992981, AM992985, AM992998), Çek Cumhuriyeti (JF690940, JF690941, JF690950), Hollanda (JF690948) ve Güney Afrika Cumhuriyeti (GQ301551, GQ301553) izolatlarının tüm ITS gen bölgesi arasındaki uzaklık indeksi %0.1 ile %1.2 arasında değişiklik gösterdi. Bu çalışma ile Türkiye'de *T. trichiura*'nın ITS bölgesinin ilk kez moleküler karakterizasyonu yapılmıştır.

Anahtar sözcükler: *Trichuris trichiura, Macaca mulatta, Papio hamadryas, Moleküler Karakterizasyon, Samsun, Türkiye*

INTRODUCTION

Trichuriasis is caused by the nematode Trichuris trichiura that is a gastrointestinal nematode *in non-human primates and human. Trichuris* egg is frequently identified during routine faecal examination but is rarely clinical significance [1]. The life cycle is direct and infection is by ingestion of embryonated eggs. Humans and primates become infected directly by ingesting the embryonated eggs from contaminated hands, food, soil or water. PCR molecular techniques demonstrated that *T. trichiura* from primates and humans can be identified by their ITS sequences [2,3]. The ITS of the rDNA has been the target locus in many studies on helminths and is considered a good target locus for diagnosing indistinguishable stages of parasites, known to be different species. Generally, the entire ITS-sequence variation among individuals of the same species as well as the intra individual variation has shown to be ≤ 1% for a range of nematodes [4]. Little is known of the molecular characteristics of *T. trichiura* from Turkey. No

İletişim (Correspondence)
+90 362 3121919
cbolukbas@omu.edu.tr

rDNA ITS region study has previously been reported on the genus *Trichuris* in Turkey. In the present study, the entire first and second internal transcribed spacer (ITS-1 and ITS-2) regions of nuclear ribosomal DNA (rDNA) of *T. trichiura* from Turkey were amplified by polymerase chain reaction (PCR) and sequenced.

MATERIAL and METHODS

Parasitological Examination

The studied animals which naturally died were *Macaca mulatta* (rhesus macaque (one ♀) and *Papio hamadryas* (Hamadryas baboon) (one ♀) from Samsun Zoo, Turkey. Post mortem helminthological examinations of two primates were carried out between 2006 and 2010. Parasites were counted and identified morphologically. The collected parasites were preserved in 70% alcohol and were deposited in the collection of Department of Parasitology of the Veterinary Faculty of Ondokuz Mayis University, Samsun, Turkey (voucher OMUPAR.53.12.01).

DNA Extraction, PCR Amplification, and Sequencing

One male nematode from *Papio hamadryas* was randomly selected among the total samples for the molecular identification. Genomic DNA was extracted from individual male nematode using the DNA purification kit (Genomic DNA Purification Kit, Thermo Scientific) according to manufacturer's instructions. PCR targeting the ITS region (ITS-1, 5.8S, ITS-2) were performed. DNA content was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260 nm. PCR was carried out in a final volume of 100 µl, 1.5 µl of DNA template (72.2 ng/µl), 10 µl of 10×Taq Buffer with KCl (Thermo Scientific), 6 µl of 25 mM of $MgCl_2$ (Thermo Scientific), 2 µl of 10 mM dNTPs (Thermo Scientific), 5 µl of forward and reverse primers (0.5 µM each), 0.5 µl of 2.5 U *Taq* DNA Polymerase (Thermo Scientific), and 70 µl of autoclaved distilled water. ITS-1, 5.8S and ITS-2 region were amplified using the forward primer NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and reverse primer NC2 (5′-GGTTAGTTTCTTTTCCTCCGCT-3′) [3]. Negative control consisted of autoclaved distilled water. The PCR was performed in a Thermo PxE 0.2 thermal cycler (Thermo Scientific) and the conditions were as follows: 3 min at 94°C, then 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C followed by a final elongation of 10 min at 72°C. PCR products were electrophoresed in 1.5% agarose gel (Prona) in a TBE buffer (Thermo Scientific), stained with ethidium bromide (Sigma) and visualized by UV illumination. The size of the amplified fragments was estimated by comparisons with the 200 bp DNA Ladder (Thermo Scientific). The ITS region products were sent to sequencing company (Genoks Ankara, Turkey) for purification and sequencing in both directions using NC5-NC2 primers.

Data Analysis and Phylogenetic Tree Construction

The obtained sequences were verified by forward and reverse comparisons, assembled and edited with using Contig Express in Vector NTI Advance 11.5 (Invitrogen). Resulting sequence data were identified via GenBank and aligned with previously characterized sequences of nematodes, using ClustalW in Mega 5.0 multiple sequence alignments [5]. Nucleotide composition was calculated using Mega 5.0 [6]. Genetic distances were calculated using the Kimura two-parameter model with pairwise deletion in Mega 5.0 [6]. Phylogenetic relationships of the parasite lineages were estimated using the neighbor joining (NJ) method in Mega 5.0 [6]. The NJ analysis was performed using a Kimura two-parameter correction model [7] and pairwise deletion option for gaps. Confidence in the NJ trees was determined by analyzing 1.000 bootstrap replicates [8] using the Mega program. The ITS region sequence of *T. trichiura* was deposited in GenBank under accession no. KC877992.

RESULTS

Parasitological Result

All specimens were morphologically determined to be *T. trichiura*. Number of infected animals and parasites are presented in *Table 1*.

Molecular Results

The amplification of the ITS region produced a fragment of approximately 1400 bp from nematode *(Fig. 1)*. The ITS PCR products were subjected to direct sequencing giving products 1248-bp long. Pairwise comparison between the entire ITS region of the *T. trichiura* isolates from Turkey (KC877992) and other *T. trichiura* and *Trichuris* sp. isolates China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553) showed differences ranging from 0.1 to 1.2 % *(Table 2)*. Phylogenetic relationships among *T. trichiura* isolates from *P. hamadryas* of the Turkey and the other *T. trichiura* isolates and *Trichuria* sp. as inferred by neighbor joining analysis of the ITS sequence, and based on the entire ITS fragment including ITS-1, 5.8S, and ITS-2 sequences are presented in *Fig. 2*.

Table 1. *Number of infected animals and parasites*
Tablo 1. *Enfekte hayvan ve parazit sayıları*

Hosts	*Trichuris trichiura*			
	♀	♂	Immature	Total
Macaca mulatta (♀ 4 years old)	7	12	-	19
Papio hamadryas (♀ 6 months old)	783	641	1458	2882

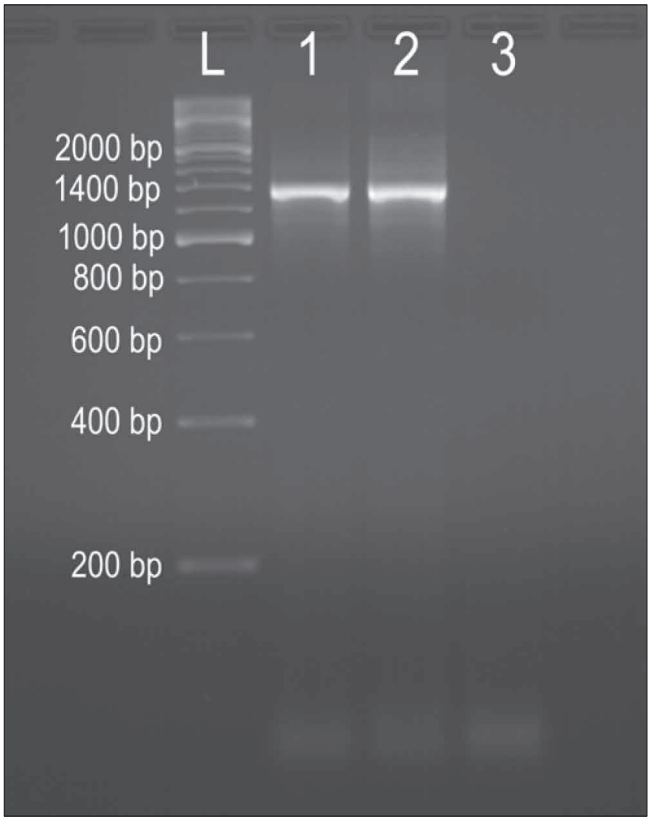

Fig1. PCR amplification of *Trichuris trichiura*. L: Ladder; 1-2: *T. trichiura*; 3: negative control

Şekil 1. *Trichuris trichiura*'nın PZR amplifikasyonu. L: Ladder; 1-2: *T. trichiura*; 3: negatif control

Table 2. *Pairwise comparison of nucleotide sequence differences (in percent) in the ITS among Trichuris isolate (Turkey) and various geographical isolates*
Tablo 2. *Trichuris Türkiye izolatı ile değişik coğrafik bölgelere ait izolatların ITS bölgesindeki nükleotit sekans farklılıklarının birbirlerine olan uzaklık indeksleri*

Accession Number	1	2	3	4	5	6	7	8	9	10
1. AM992981 (China)										
2. AM992985 (China)	0.005									
3. AM992998 (China)	0.003	0.005								
4. JF690940 (Czech Republic)	0.009	0.011	0.011							
5. JF690941 (Czech Republic)	0.003	0.003	0.000	0.011						
6. JF690948 (Netherlands)	0.005	0.005	0.002	0.012	0.002					
7. JF690950 (Czech Republic)	0.003	0.003	0.000	0.011	0.000	0.002				
8. GQ301551 (South Africa)	0.004	0.005	0.002	0.011	0.000	0.002	0.000			
9. GQ301553 (South Africa)	0.006	0.002	0.003	0.011	0.000	0.002	0.000	0.003		
10. KC877992 (Turkey)	0.006	0.002	0.004	0.012	0.002	0.003	0.002	0.004	0.001	

DISCUSSION

Zoological gardens exhibit wild animals for aesthetic, educational and conservation purposes. However, parasitic diseases constitute one of the major problems causing even mortality in these animals while in captivity, the effects of which range from sub-clinical to death [9]. In Turkey, there were a few studies about parasites of non-human primates [10-12]. In this study, we necropsied two non-human primates and identified parasites as *T. trichiura*. According to results of the study, parasites burden of monkeys were seen that too much. We think that the reason of this is the direct life cycle of *T. trichuira*. The other hand some human parasites originated in prehominid ancestors in Africa. Nematode species, such as *Enterobius vermicularis*, hookworms and *T. trichiura* are shared by humans and other close phylogenetic primates [13]. Furthermore, a phylogenetic analysis based on ITS sequence was performed for *T. trichiura* from *Papio hamadryas* in the present study. The phylogenetic trees showed all the sequences of *T. trichiura* and *Trichuris* sp. clustered together and separated from *T. ovis* and *T. discolor*. In the present study, *T. trichiura* from *Papio hamadryas* were characterized for the first time by sequencing of the ITS rDNA. The analyses revealed

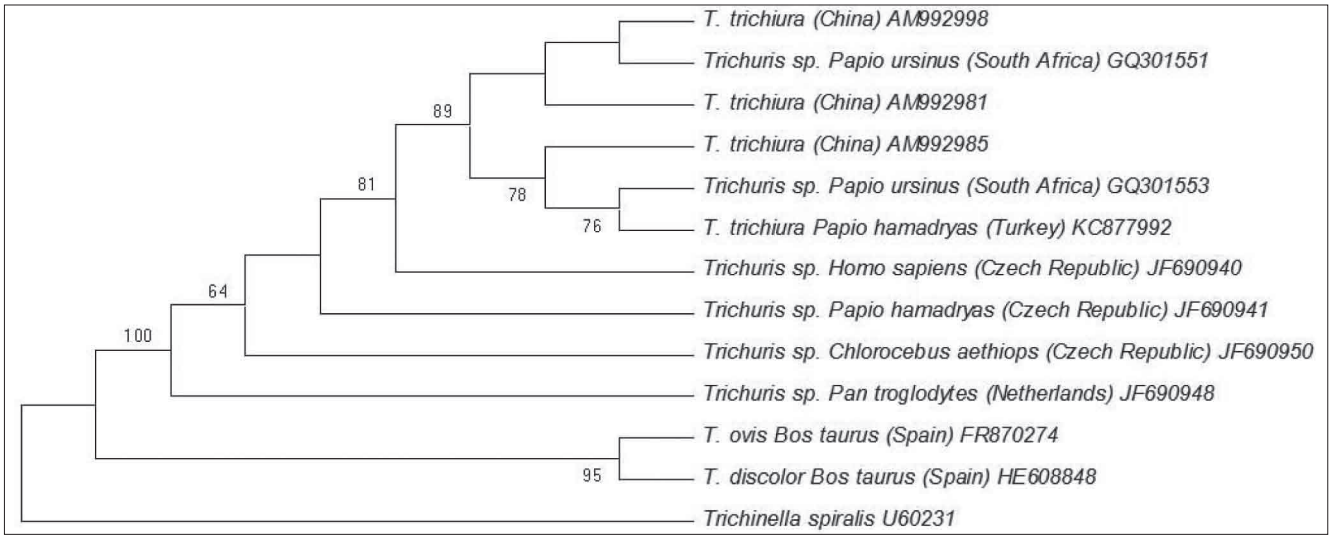

Fig 2. Neighbor joining analysis of the ITS sequence
Şekil 2. ITS dizisinin neighbor joining analizi

that the sequence obtained are highly similar to those of previously published for *T. trichiura* and *Trichuris* sp. from China (AM992981, AM992985, AM992998), Czech Republic (JF690940, JF690941, JF690950), Netherlands (JF690948) and South Africa (GQ301551, GQ301553). The high genetic similarity between the sequence of *T. trichiura* from the Turkey and those published data, from different geographical isolates, represents a wide range of distribution for this nematode and its humans and primates specificity. The presence of *T. trichiura* in monkeys at the zoo is a high risk to zoo keepers and also visitor's welfare because of its zoonotic character. Thus, we suggest that effective parasite control program should be established and stool control should be done regularly for primates.

REFERENCES

1. Purcell JE, Philipp MT: Parasitic Diseases of Non-human Primates. **In**, Wolfe-Coote S (Ed): The Laboratory Primate. 1st ed., Elsevier Academic Press, London, 2005.

2. Nissen S, Al-Jubury A, Hansen TVA, Olsen A, Christensen H, Thamsborg SM, Peter N: Genetic analysis of *Trichuris suis* and *Trichuris trichiura* recovered from human and pigs in a sympatric setting in Uganda. *Vet Parasitol*, 188, 68-77, 2012.

3. Ravasi DF, O'Riain MJ, Davids F, Illing N: Phylogenetic evidence that two distinct *Trichuris* genotypes infect both humans and non-human primates. *PLoS ONE,* 7 (8): e44187, DOI: 10.1371/journal.pone.0044187, 2012.

4. Blouin MS: Molecular prospecting for cryptic species of nematodes: Mitochondrial DNA versus internal transcribed spacer. *Int J Parasitol*, 32, 527-531, 2012.

5. Thompson JD, Higgins DG, Gibson TJ: CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positing-specific gap penalties and weight matrix choice. *Nucleic Acids Res,* 22, 4673-4680, 1994.

6. Tamura K, Peterson D, Peterson N, Steker G, Nei M, Kumar S: MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol,* 28, 2731-2739, 2011.

7. Saitou N, Nei M: The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol,* 4, 406-425, 1987.

8. Felsenstein J: Confidence intervals on phylogenies: An approach using the bootstrap. *Evolution,* 39, 783-791, 1985.

9. Opara MN, Osuji CT, Opara JA: Gastrointestinal parasitism in captive animals at the Zoological Garden, Nekede Owerri, Southeast Nigeria. *Report and Opinion*, 2, 21-28, 2010.

10. Tigin Y, Burgu A, Doganay A, Oge S, Umur S: Ankara hayvanat bahçesindeki bazı memeli ve kanatlı dışkılarının helmint yönünden incelenmesi. *Ankara Univ Vet Fak Derg,* 36, 646-64, 1989.

11. Gurler AT, Beyhan YE, Acici M, Bolukbas CS, Umur S: Helminths of mammals and birds at the Samsun Zoological Garden, Turkey. *J Zoo Wildlife Med,* 41, 218-223, 2010.

12. Oge H, Oge S: Maymunlarda *Enterobius vermicularis* ve *Trichuris trichiura*'nın mebendazol ile sağaltımı. *Türkiye Parazitol Derg,* 19, 562-570, 1995.

13. Araújo A, Reinhard K, Leles D, Sianto L, Iñiguez A, Fugassa M, Arriaza B, Orellana N, Ferreira LF: Paleoepidemiology of intestinal parasites and lice in Pre-Columbian South America. *Rev Chil Antropol,* 43, 303-313, 2011.