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Türk Hijyen ve Deneysel Biyoloji Dergisi

Identification and prevalence of *Coenurus cerebralis* in sheep: Diyarbakır example

Koyunlarda *Coenurus cerebralis*'in belirlemesi ve yaygınlığı: Diyarbakır örneği

Delil Adem ÇİFTÇİ[1] (ID), Banuçicek YÜCESAN[2] (ID)

ABSTRACT

Objective: *Taenia multiceps* is a cestode that lives in the small intestines of domestic and wild carnivores such as dogs, foxes and jackals. The larval stage of *T. multiceps*, *Coenurus cerebralis*, develops in the central nervous system. The parasite is usually seen in rural areas; it is the most contagious agent in dog-sheep contact, including foxes and wild ruminants.. It can be transmitted when the farmers open the skulls of sick animals and feed the cysted tissues to dogs. The presence of the cyst results in typical neurological symptoms and, in most cases, leads to the death of the animal. Coenurosis caused by *C. cerebralis* is a parasitic disease that causes high economic losses in small ruminants. In this study; it was aimed to find *C. cerebralis* by molecular methods in nine of the samples found to have coenurosis.

Methods: This study was conducted on 1200 sheep slaughtered in Diyarbakır province between September 2022 and April 2023. Phylogenetic studies were conducted with nine of the 122 sheep considered positive.

Results: Coenurus cysts were observed in 12 out of 122 animals (9.8%). Cysts of nine out of 12 sheep were genetically analyzed and confirmed as *T. multiceps*

ÖZET

Amaç: *Taenia multiceps*, köpek, tilki ve çakal gibi evcil ve vahşi etoburların ince bağırsaklarında yaşayan bir sestoddur. *T. multiceps*'in larva evresi olan *Coenurus cerebralis*, merkezi sinir sisteminde gelişir. Parazit genellikle kırsal alanlarda görülmekte; tilki ve yabani geviş getiren hayvanlar da dahil olmak üzere köpek-koyun temasında en bulaşıcı etkendir. Çiftçiler hasta hayvanların kafataslarını açıp kistli dokuları köpeklere yedirdiğinde bulaşabilir. Kistin varlığı tipik nörolojik semptomlara neden olur ve çoğu durumda hayvanın ölümüne yol açar. *C. cerebralis*'in neden olduğu coenurosis, küçük ruminantlarda yüksek ekonomik kayıplara neden olan paraziter bir hastalıktır. Bu çalışmada; coenurosis olduğu tespit edilen örneklerin dokuzunda moleküler yöntemlerle *C. cerebralis* tespiti amaçlanmıştır.

Yöntem: Bu çalışma, Eylül 2022 ile Nisan 2023 arasında Türkiye'de Diyarbakır ilinde kesilen 1200 koyun üzerinde yürütülmüştür. Filogenetik çalışmalar, pozitif kabul edilen 122 koyundan dokuzu ile yapılmıştır.

Bulgular: 122 hayvandan 12 (%9,8)'sinde coenurus kisti gözlenmiştir. 12 koyundan dokuzunun kistleri genetik olarak analiz edilmiş ve COX1 (sitokrom c oksidazı alt ünitesi 1 gen dizisi analizi ile *T. multiceps*

[1]Farmer Veterinary Clinic, Private Veterinarian, Diyarbakir, Türkiye
[2]Department of Veterinary Parasitology, Postgraduate Education Institute (Health Sciences), Çankırı University, Çankırı, Türkiye

İletişim / Corresponding Author : Banuçicek YÜCESAN
Çankırı Üniversitesi, Lisansüstü Eğitim Enstitüsü (Sağ. Bil.), Veteriner Parazitoloji AD., Çankırı - Türkiye
E-posta / E-mail : yucesanbanu@yahoo.com

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metacestodes by COX1 (cytochrome c oxidase subunit 1) gene sequence analysis. Accession numbers; OR239756, OR239757, OR239758, OR239759, OR239760, OR239761, OR239762, OR239763, OR239764. Similarities of these data with other strains in NCBI (National Center for Biotechnology Information) were compared.

Conclusion: For a definitive identification of *T. multiceps* in Türkiye, further studies may be required using various molecular protocols on biological characteristics, long sequence genes in the mitochondrial and nuclear genome, other hosts and samples from different geographical regions to improve our understanding of the epidemiological distribution in Türkiye.

Key Words: *Coenurus cerebralis*, sheep, *Taenia multiceps*, Southeast Anatolia, Türkiye

metacestodes olduğu doğrulanmıştır. Erişim numaraları; OR239756, OR239757, OR239758, OR239759, OR239760, OR239761, OR239762, OR239763, OR239764 olarak kaydedilmiştir. Bu verilerin NCBI'daki diğer suşlarla benzerlikleri karşılaştırılmıştır.

Sonuç: Türkiye-Diyarbakır ilindeki bazı koyunlarda coenurus kisti belirlenmiştir. *T. multiceps*'in doğru tanımlanması için, Türkiye'deki epidemiyolojik dağılıma göre biyolojik özellikler, mitokondriyal ve nükleer genomdaki uzun dizili genler, diğer konaklar ve farklı coğrafi bölgelerden alınan örnekler üzerinde moleküler protokoller kullanılarak daha fazla çalışma yapılması gerekebilir.

Anahtar Kelimeler: *Coenurus cerebralis*, koyun, *Taenia multiceps*, Güneydoğu Anadolu, Türkiye

INTRODUCTION

Small ruminants play a significant role in animal production systems worldwide (1). Helminth-borne infections are among the most important global diseases affecting sheep and goats productivity (2). *C. cerebralis*, the larva of *T. multiceps*, has been known for more than 300 years to cause coenurosis, a disease that negatively affects the productivity of sheep and goats (3,4).

T. multiceps is a taeniid cestode that lives in the small intestines of dogs and other carnivores in its adult form. Its larvae, *C. cerebralis*, settle in the major nervous systems of domestic and wild ruminants, including their brains and spinal cord.

C. cerebralis, the larva of *T. multiceps*, causes coenurosis, which is a disease that negatively impacts the productivity of sheep and goats. The life cycle of the parasite is generally in rural areas, and dog-sheep contact, including foxes and wild ruminants, is the most contagious area. It can be transmitted when farmers open the skulls of sick animals and feed the cysted tissues to dogs (4). The presence of the cyst results in typical neurological symptoms and, in most cases, leads to the animal's death. Lambs whose immune systems are not yet fully developed can contract the disease by eating contaminated grass leading to significant economic losses to sheep farms (5-7).

C. cerebralis is a slowly progressing infection and can take a very long time to develop. Symptoms may temporarily resolve on their own. Therefore, clinical symptoms may appear three months after infection (6,8). The clinical picture during this period depends on the number of infected eggs ingested by the host, the immune status of the host, the severity of the inflammatory response and the location of the parasite in the central nervus system (CNS) (9). The acute phase of the disease causes high fever within one to three weeks. Movement disorders, incoordination and abnormal head holding, opisthotonus, circular rotation and teeth grinding may be observed (9,6,5). Single lesions located in sensitive areas in the cerebral hemispheres are more dangerous than multiple lesions. After the acute phase, *C. cerebralis* enters a quiescent phase in which cysts mature and transient symptoms can be observed (5). Diagnosis of *C. cerebralis* can be made by necropsy in the acute

form. In this case, it is difficult to detect localized changes made by the larvae as they migrate along the brain and spinal cord.

The chronic phase is more evident in 9-18 month old sheep (10). In infected animals, udder edema, blindness, agitation, paralysis, abnormal posture, abnormal head holding, nystagmus, lethargy and death may occur (6,8). In sheep, thinning and swelling of the skull indicate progressive infection of the brain (9,11). This thinning is important for the epidemiology of the parasite. Other symptoms are retinal hemorrhage and papillary edema. It has been reported that the most preferred location for *C. cerebralis* is the right frontal lobe of the brain. In symptomatic sheep, *C. cerebralis* has been observed to develop to completely cover the left hemisphere of the brain. Sudden death can occur even in clinically normal animals.

Zoonotic diseases may occur when *T. multiceps* eggs are accidentally ingested by humans along with contaminated food and water (12). The change in demographic and socioeconomic structures due to migration in the last decade increases poverty and poor hygiene conditions. This provides an environment for the development of parasitic, bacterial and vector-borne zoonotic diseases. Today, zoonotic pathogens pose a rapidly increasing threat in Türkiye and European countries (12,13).

Prevalence of coenurosis infections has been reported from countries (14,15). In the Middle East, coenurosis is a significant endemic disease affecting small ruminants, most notably in Türkiye, Egypt, Iraq, and Jordan (16-22).

There has been a significant amount of attention given to the ecological, epidemiological, and taxonomic studies of *T. multiceps*, mainly due to their medical and veterinary importance. While molecular characterization of several species within the Taeniidae family, particularly within the *Echinococcus* genus, has been extensively studied, research on the genetic variability of coenurus has been scarce. Moreover, there are documented studies on the genetic variability of *T. multiceps* in Türkiye.

In the present study, the prevalence of *C. cerebralis* in sheep was investigated and molecular characterizations of *T. multiceps* metacetodes from naturally infected sheep are described. DNA sequence variability was investigated within the COX1 mitochondrial genes.

MATERIAL and METHOD

The study was conducted from September 2022 to April 2023 in Diyarbakır province is located in southeast part of Türkiye. The brains of sheep were examined in slaughterhouses in Diyarbakır province and its surroundings, and biosafety rules were followed during sampling and transportation of positive samples, as infected material was used. The study implicated 1200 sheep slaughtered at a local abattoir in Diyarbakır. Throughout the study period of eight months, the abattoir was visited once every week. At the abattoir, 30-50 sheep were examined for coenurosis before slaughter each week, and the heads of coenurosis suspicious animals were purchased.

During each visit, after the slaughtering process, the skulls of each sheep were dissected, and the brain of each sheep was systematically inspected by visual inspection, palpation, and incisions against *C. cerebralis* cysts.

The cysts obtained from the brain tissues were opened with a sterile scalpel, and 20 mg pieces were cut from the cyst walls and taken into sterile 1.5 mL falcon tubes. Genomic DNA isolation was performed using the 'Thermo Scientific GeneJET Genomic DNA Purification Kit.' Isolation was performed in accordance with the company's recommendations (23).

For molecular analysis, the mitochondrial COX1 gene region was amplified by PCR using primers JB3 (TTTTTTTGGGCATCCTGAGGTTTTAT) and JB4.5 (TAAAGAAAGAACATAATGAAAATG) (23). PCR reaction mixtures were adjusted to a total volume of 50 µL containing 10 x PCR buffer, 2.5 mM MgCl2, 0.2 mM

dNTP, 20 pmol of each primer and 2 U Taq DNA polymerase. PCR amplification was performed under conditions of 35 cycles of initial denaturation at 95°C for 5 min, 94°C for 50 s, 45°C for 45 s and 72°C for 50 s and final extension at 72°C for 7 min.

The product obtained from the polymerase chain reaction was mixed with 10 µL PCR product and 2 µL loading solution in a 1.4% agarose gel, loaded into the wells of the gel and allowed to react in 1X TAE buffer at 100 volts for 40 minutes. A 100 bp marker was used to determine the molecular weight of the bands and analyzed for the presence of bands with an agarose gel UV transilluminator. In each PCR reaction, *T. multiceps* DNA samples previously obtained from Dicle University Faculty of Veterinary Medicine Parasitology Laboratory were used as positive control, while distilled water was used as negative control.

The isolates obtained both directions using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). PCR amplicons were sequenced in Multiple sequence alignments were made with the ClustalWmethod with MEGA7 genetic software program. Phylogenetic trees and pairwise calculations were obtained by using MEGA 7 software (24). The sequence results obtained were analyzed in the GenBank database blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and registered in GenBank.

According to Article 8 ([19]-k- 2) of the "Regulation on the Working Principles and Procedures of Animal Experiments Ethics Committees" published in the Official Gazette dated 15.02.2014 and numbered 28914, it is stated that "Procedures performed with dead animals or their tissues, slaughterhouse materials, waste fetuses" are not subject to the permission of the Local Animal Experiments Ethics Committee (HAYDEK).

RESULTS

122 out of 1200 sheep had clinical signs such as depression, moving in a circle, head deviation, ataxia and blindness of ceornorisis. The skulls of these animals were removed opened and their brains examined. 12 out of 122 (9.8%) animals *C. cerebralis* cyst was observed. *C. cerebralis* cysts were found on the cerebellum, right and left cerebral hemisphere. Figure 1 shows *C. cerebralis* obtained from sheep brains. Of all positively detected animals, two were

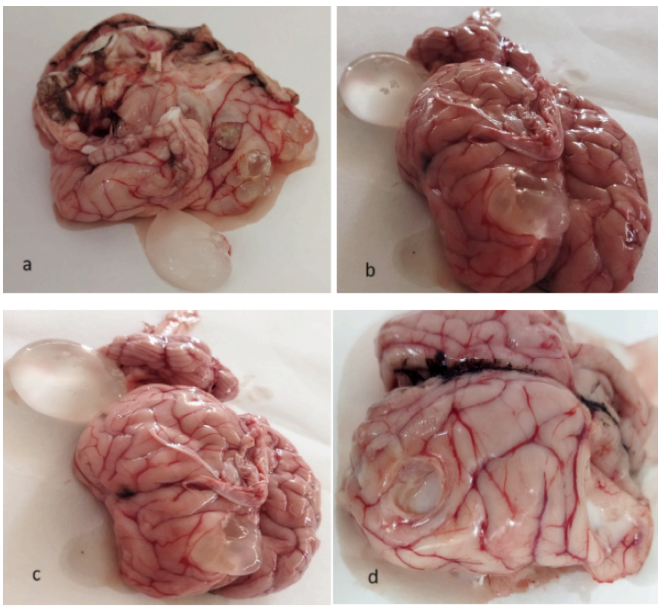

Figure 1. Appearance of *Coenurus cerebralis* cysts of various sizes located in the brain tissue

female (16.7%) and 10 were male (83.3%). Again, all of the positive animals were under 1 year old.

The cysts from nine of infected sheep were genetically analyzed and confirmed to be *T. multiceps* metacestodes by COX1 gene sequence analysis. The sequences were deposited to GenBank with the following accession numbers; OR239756, OR239757, OR239758, OR239759, OR239760, OR239761, OR239762, OR239763, OR239764. The phylogenetic tree of isolates obtained from sheep is given in Fig 2.

Dyb 2 isolate, OR239757, showed 100% homology with our dyb4, dyb7, dyb8, dyb9 isolate, OR239759, OR239762, OR239763, OR239764, and dyb 1 isolate OR239756, 100% identic dyb5 isolate OR239760 in the present study. intraspesifik divergence is 0.0024 (0.000-0.066).

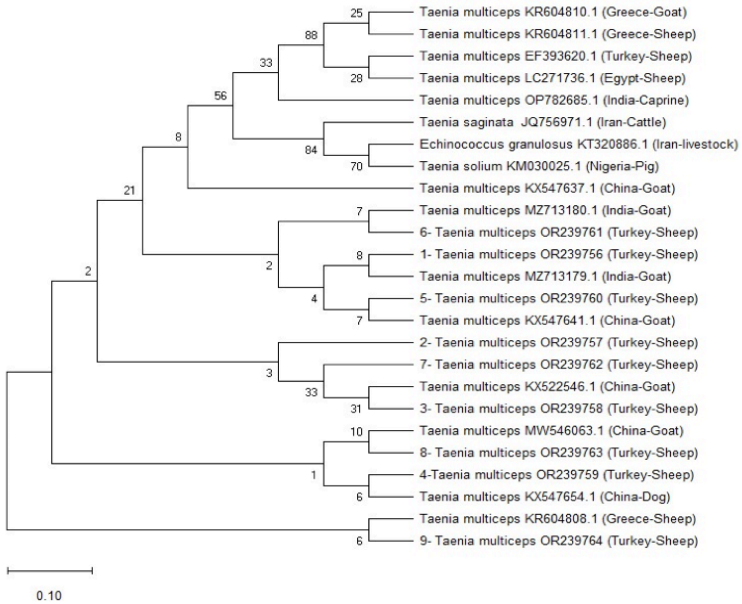

Figure 2. Relationships of *T. multiceps* isolates from sheep of Diyarbakır, Türkiye with some other species of *Taenia* based on COX1 sequence data using the Maximum Likelihood method (MCL). The accession numbers of individual sequences determined in the present study are shown in each tree. A scale bar indicates estimated distance. There was concordance in the topology between trees constructed using the MCL method

DISCUSSION

The disease is more common in lambs and less common in adult sheep, but adaptive immunity has been reported to play an important role (25). In this study, *C. cerebralis* was observed in the brain tissue of 4-6 month old lambs. In previous studies on this parasite, it was reported that the larvae mainly settled in the cerebral hemispheres (18, 26-28). In this study, as in previous studies, large *C. cerebralis* cysts embedded within the cerebellum were observed in the cerebellum of sheep. Clinical findings in affected animals vary depending on the location, size and pressure of the cysts in the brain (28,29).

In studies conducted on sheep in different regions of Türkiye, the prevalence of *C. cerebralis* was reported as 16.3% in Konya (18), 15.5% in Kars (27), 12% in Kırıkkale (30) and 64.7% in Van (31). The present study showed occurrence of *C. cerebralis* cysts in 9.8% of sheep examined in Diyarbakır, Türkiye. This rate is lower than infection rates of previously reported in sheep in Türkiye. In

other countries, prevalence of *C. cerebralis* was reported 4.5% in sheep in Ethiopia (32,33), 2.88% in India (34), in 9.8% and 18.65% in sheep in different areas in Iran (34,35), 14.8% in Mozambique (36) and 42.1% in sheep and goats in Tanzania (37). It is thought that the differences in the rates reported in our country and around the world are related to feeding and care conditions, animal population sizes and especially the presence of uncontrolled dogs.

Based on the BLAST analysis, it was found that the COX1 sequence (dyb isolate) of *T. multiceps* (COX1) larval stage had a high percentage identity of 100-98.82% (with a query coverage of 100-96%) with the COX1 sequences of *T. multiceps* isolates in GenBank that were collected from various countries around the world. The 1st group (OR239757, OR239759, OR239762, OR239763, OR239764) was identical to sequences MZ713180 and MZ713179 obtained from *T. multiceps* from goat in Indian, and %99 identical to sequence KR604808 from sheep in Greece. The second group (OR239756, OR239760) was showed 100% homology with China isolate JX507230 from goat, and 99% homology with Greece isolate (KR604808) from sheep. OR239761 showed 99% and 100% identity with isolates from dog of China KX547654, and KX547641, respectivy. Phylogenetic relationship among *T. multiceps* isolates from sheep Diyarbakır and the other Taenia isolates as inferred by neighbor-joining analysis of the COX1 sequences are presented in Fig 1.

Mitochondrial DNA COXI gene region cestode species has been successfully used in the differentiation of species (38). There is limited data available on DNA sequence information of *T. multiceps*. An initial detection of genetic variation in *T. multiceps* was found in a study on Italian sheep. The genetic variants Tm1, Tm2, and Tm3 were identified through analysis of COX1 and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NAD1). Likewise, analysis of sheep isolates from inner Mongolia and China using COX1 gene sequences revealed three genotypes with variation rates between 0.25% and 0.75%, consistent with findings from Italy (39). Low genetic diversity was observed in Egyptian sheep due to a low number of haplotypes in the COX1 and NAD1 genes of *T. multiceps* (40). Similarly, in our study, *T. multiceps* isolates showed low genetic variation (up to 0.24%) among COX1 gene sequences. In opposite, *T. multiceps* isolates from sheep and goats In Iran exhibited significant genetic heterogeneity. The low genetic diversity detected in our study suggests that new variants and even strains may emerge as a result of new mutations that may occur in the future.

The current study presents findings on the prevalence, localization of cysts, and molecular characterization of *C. cerebralis* (the larval stage of *T. multiceps*) found in the brains of slaughtered sheep in Diyarbakır, Türkiye. We identified genetic differences between isolates of the same species that we submitted to GenBank and other species. To achieve a more precise identification and characterization of *T. multiceps* in Türkiye, further research on biological traits, long sequence genes in the mitochondrial and nuclear genome, from other hosts, and from different geographical areas, using various molecular protocols may be necessary to enhance our understanding of the epidemiological distribution in Türkiye.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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