Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.186

RESEARCH ARTICLE

Molecular Prevalence of *Coenurus cerebralis* in Sheep with Neurological Symptoms in Iğdır Province, Türkiye

Emrah Akkuş and Fatma Ertaş Oğuz

[1]Iğdır University, Institute of Postgraduate Education, Department of Animal Science, Iğdır, Türkiye
[2]Iğdır University, Tuzluca Vocational School, Department of Medical Services and Techniques Iğdır, Türkiye
*Corresponding author: Fatma.ertas@ığdır.edu.tr, vtr.fatma@gmail.com

ARTICLE HISTORY (24-190)

Received:	March 30, 2024
Revised:	May 13, 2024
Accepted:	May 14, 2024
Published online:	May 23, 2024

Key words:
Coenurus cerebralis
Iğdir Province
PCR
Sheep
Phylogenetic analysis

ABSTRACT

Coenurus cerebralis is a metacestode larval stage of *Taenia multiceps* that inhabits the central nervous system of their intermediate host, especially sheep and goats. This study aimed to investigate the molecular prevalence of *C. cerebralis* in sheep in Iğdır province. Out of 1300 sheep brought to the slaughterhouse, 300 sheep were selected based on the clinical signs of coenurosis. After slaughtering, the sheep heads were brought to the laboratory to observe cysts' presence and collect the specimens for PCR analysis. Cysts were present in 246/300 sheep brains, with 112 cysts in the left hemisphere, 73 in the right hemisphere, 12 in the cerebellum, and 9 in the brain stem. However, 40 cysts were observed in both hemispheres. PCR analysis by amplifying COX1 gene revealed that 243 samples were truly positive for *C. cerebralis.* Sequencing and phylogenetic analysis of two sequences (PP713059, PP711257) showed 100% similarity with the sequence of *C. cerebralis* (KX547654.1) and one sequence (PP711299) showed 99.19% similarity with the sequence of *C. cerebralis* (KX547656.1) isolated from China. Our obtained sequences were included in the same clade with six other clade members in the phylogenetic tree. Among different parameters, it was observed that the prevalence was higher in males and animals with less than 1 year of age. Similarly, the prevalence was higher in winter, followed by autumn, spring, and summer. The current study illustrated a high rate of coenurosis infection in the sheep population of Iğdır, Turkey. Preventive measures should be followed to avoid the transmission of this parasite to healthy sheep and other intermediate hosts including humans.

To Cite This Article: Akkuş E and OĞUZ FE, 2024. Molecular prevalence of *Coenurus cerebralis* in sheep with neurological symptoms in iğdir province, Türkiye. Pak Vet J, 44(2): 499-503. http://dx.doi.org/10.29261/pakvetj/2024.186

INTRODUCTION

Endoparasitic attacks have always been a serious threat to the sheep industry causing huge economic losses (Ertaş *et al*., 2022; Öner *et al*., 2022) Among these parasitic diseases, babesiosis, fasciolosis and coenurosis are of significant concern in the sheep industry (Ertaş and Kırmızıgül, 2021; Attia *et al*., 2022).

Coenurosis is a zoonotically significant disease caused by the larvae, i.e., *Coenurosis* (*C.*) *cerebralis*) of *Taenia multiceps.* The parasite lives in the small intestines of carnivores such as dogs, foxes and jackals but mostly targets the central nervous system of its intermediate host, especially sheep and goats (Biçek *et al*., 2019; Abdollahi *et al*., 2023). Sheep and goats get infected when they consume contaminated food and water. The oncospheres reach the central nervous system through the blood and cause cyst formation associated with neurological disorders (Tüfekçi *et al*., 2022; Varcasia *et al*., 2022).

The signs and symptoms of the disease vary according to the location and size of the cysts as well as the pressure they exert on the cerebrum (Abdollahi *et al*., 2023). Coenurosis exists in two forms, acute and chronic. Acute coenurosis usually occurs approximately ten days after consuming the contaminated food and ingesting many *T. multiceps* eggs (Johri *et al*., 2023). In the acute phase, symptoms include fever, fatigue, stagnation, loss of appetite, convulsions and animal death (Soliman *et al*., 2022). Chronic form leads to paralysis, blindness, eye tremors, drowsiness, circling movements, deviation of the head to the side (head tilting), lack of response to stimuli and head shaking (Mohammed, 2020). There is no definite treatment for *T. multiceps* after the symptoms of the disease appear in the intermediate host. Even if surgical treatment is possible, the cost and difficulty of the operation are a big problem. Moreover, by the time the diagnosis is made, it is too late to treat the disease (Bıyıkoğlu and Doğanay, 1998; Yılmaz *et al*., 2014).

Coenurosis in sheep is diagnosed chiefly during post-mortem examination of suspected cases. Therefore, there is no definitive method to clinically differentiate this disease from cysticercosis, echinococcosis or other CNS (central nervous system) infections. In addition, some patients have sterile cysts, making diagnosis difficult with some imaging techniques (Al-Zaidi *et al.*, 2021). Therefore, the availability of accurate and feasible serologic field tests associated with sonography and other clinicopathologic examinations may facilitate diagnosis. The ELISA test is convenient and can evaluate many samples simultaneously. However, molecular tools like PCR are needed to diagnose this disease more specifically and observe the genetic variation (Al-Zaidi *et al.*, 2021).

The data regarding the molecular epidemiology of *C. cerebralis* in the sheep population in Turkey is limited with no molecular study in Iğdır Province. The current study was designed to detect *C. cerebralis* in sheep with nervous signs through PCR in Iğdır Province, Turkey, and to analyze the parasite genetically through sequencing and phylogeny.

MATERIALS AND METHODS

Selection of animals: From 2022-2023, 1300 sheep were brought to the slaughterhouse for slaughtering. These animals were physically examined for the clinical signs of coenurosis. Of 1300 sheep, 300 showed clinical signs of coenurosis and were selected to be included in this study. These animals were registered in the Provincial Directorate of Agriculture and Forestry in the Iğdır Province. The selected animals were older than six months and showed nervous signs including turning movement, unstable walking, lethargy and anorexia.

Collection of specimens: After slaughtering, the heads of selected sheep were collected and brought to the laboratory in a cold chain for necropsy and collection of samples for PCR analysis. Din doing so, the skull specimens were opened with the help of an electric saw and meninges were incised with the scalpel blade. The exposed brains were carefully examined for the gross pathological lesions, size, location and the number of cysts in each compartment of the brain. As the dozens of invaginated scolexes clustered in places were attached to the germinative membrane, cell walls were obtained in Eppendorf tubes to extract DNA as described by Özger (2020).

DNA extraction: 20 mg sample of cyst walls were taken into eppendorf tubes for the extraction of DNA. For this purpose, Invitrogen PureLink™ Genomic DNA Mini Kit (USA, K182002) was used and the instructions were followed provided by the manufacturer. The DNA samples were then stored at -20ºC until use.

Polymerase Chain Reaction (PCR): 446 bp-cytochrome oxidase subunit I (COX1) gene of *C. cerebralis* was amplified through PCR using previously published primer pair i.e., Forward primer: 5′-TTTTTTTTTGGGCATCCTGAGGTTTTAT-3') and Reverse primer: (5′-TAAAG AAAGAACATAATATGAAAATG-3') (Rostami *et al*., 2013). The protocol was carried out in 25 µL of reaction mixture containing 1.75 mM $MgCl_2$, 125 mM of dNTP, 10 pmol of each primer, 1 U Taq polymerase and 2 µL (16-50 ng/mL) DNA from each sample. The reaction conditions were set to initial denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 40 seconds, annealing at 50ºC for 40 seconds, extension at 72ºC for 35 seconds and a final extension step of 10 minutes at 72ºC. A 1.5% agarose gel was prepared and PCR products were run on the gel at 90 volts for 1 hour. Gel Images were taken under a UV transilluminator (EC3 ChemiHR 410 Imaging System, UVP, USA).

Sequence and phylogenetic analyses: PCR amplicons were sent to BM Labosis Ankara for bidirectional sequencing using the same primers. The quality of the forward and reverse AB1 tracing files was checked using the Bioedit software (Hall and Bioedit 1999). COX1 sequences were aligned with the alignment algorithm using MEGA11 software (Tamura *et al*., 2021), and all variable sites in the original trace files were double-checked for confirmation. The phylogenetic relationship between species was performed using Hasegawa-Kishino-Yano (HKY) models determined by J model test (version 2.0) for COX1 alignments. *Taenia granulosus* COX1 sequence (LC068958.1) was used as an outgroup in phylogenetic analysis.

RESULTS

Necropsy findings: Results of this study indicated that 300/1300 animals were showing clinical signs of coenurosis including ataxia, circling movement, head pressing, seizures, anorexia and lethargy. Necropsy findings of these 300 sheep heads indicated the presence of cyst formation in 246 brains. These cysts were oval or spherical with variable sizes. Cysts were covered by a thin fragile membrane and were filled with clear fluid showing a translucent appearance (Fig. 1).

Around 1-5 cysts were observed in different compartments of each brain. It was observed that the highest number of cysts i.e., 112(45.5%) were present in the left hemisphere of the cerebrum followed by the right hemisphere 73(29.6%). However, 40(16.2%) cysts were observed in both brain hemispheres. Similarly, 12(4.87%) and 9(3.6%) cysts were found in the cerebellum and brain stem (Table 1).

PCR results and phylogenetic analysis: PCR analysis of this study indicated that 243/300 (81%) samples were positive for *C. cerebralis* in these animals (Fig. 2). Sequence analysis revealed that our sequences with accession numbers PP713059 and PP711257 were 100% similar to a sequence of *C. cerebralis* isolated in China with accession number KX547654.1. Similarly, our sequence with accession number PP711299 showed 99.19% similarity with a sequence of *C. cerebralis* (KX547656.1) published in a study conducted in China.

Phylogenetic analysis of these sequences showed that our sequences PP713059, PP711257 and PP711299 were included in the same clade with six other clade members, i.e., KX547656.1, KR604809.1, KR604808.1, KX547654.1, LC271736.1 and LC271737.1. The members of this clade were descendants of LC440873.1. *Echinococcus granulosus* COX1 sequence (LC068958.1) was used as an outgroup in this phylogenetic analysis (Fig. 3).

Table 1: Location of the *C. cerebralis* cysts in the brain of positive sheep

Regions of Brain	Frequency of *C. cerebralis*	Percentage (%)
Right hemisphere	73	29.6 (%)
Left hemisphere	112	45.5 (%)
Both hemisphere	40	16.2 (%)
Cerebellum	12	4.87 (%)
Brain stem	9	3.6 (%)
Total	246	

Table 2: Age and gender-wise prevalence of *C. cerebralis* in positive sheep

Age	0-1 years old	1-3 years old	Older than 3 year	Total
Male	80 (32.9%)	51 (20.9%)	23 (9.46%)	154 (63.3%)
Female	45 (18.5%)	32 (13.1)	13 (5.34%)	89 (36.6%)
Total	125 (51.4%)	83 (34.1%)	36 (14.8%)	243

Table 3: Season-wise prevalence of *C. cerebralis* in sheep

Validity	Frequency	Percentage (%)
Autumn	73	30 (%)
Winter	120	49.3 (%)
Spring	34	13.9 (%)
Summer	16	6.5 (%)
Total	243	

Fig. 1: Sampling method and necropsy findings (A: Animal showing clinical signs of Coenurosis, B: Collection of sheep heads, C & D: Autopsy and cyst formation in sheep brain)

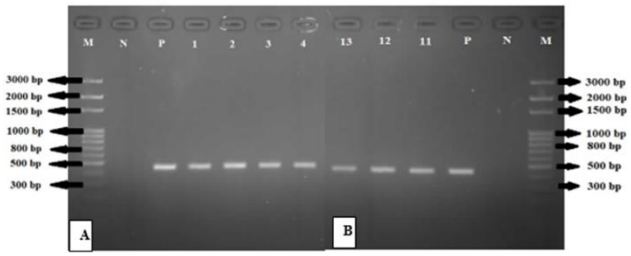

Fig. 2: Gel electrophoresis showing positive bands for the amplified COX1 gene of *C. cerebralis* (Product Length: 446bp; M: Marker, N: Negative control, P: Positive control, 1,2,3,4,11,12&13: Positive bands)

Determinants of disease: Among the associated risk factors, the prevalence of *C. cerebralis* was higher in male animals i.e., 63.3% as compared to female animals. As far as the age of animals was concerned, the prevalence was higher (51.4%) in animals with less than 1 year, followed in decreasing order by the 1-3 years age group (34.1%) and older than 3 years (14.8%) (Table 2). Among different seasons, the prevalence of *C. cerebralis* was higher in winter (48.7%). Moreover, in autumn, spring and summer, the prevalence rate was observed at 30%, 13.8% and 6.5%, respectively (Table 3). Month-wise prevalence showed that *C. cerebralis* was higher in December and January while lowest in May and June (Fig. 4).

DISCUSSION

Sheep farming is an important component of the livestock sector and plays a significant role in any country's economy, providing meat, milk, wool and leather. Sheep farming is also common in the Eastern Anatolia region of Türkiye. However, these animals have always been encountered with various parasitic diseases resulting in huge economic losses. (Acıöz, 2019; Bhowmik *et al*., 2020).

Coenurosis is a severe parasitic disease caused by the larvae (*C. cerebralis*) of *Taenia multiceps* which attacks the central nervous system of the sheep. Coenurosis is prevalent in almost all regions of the world including North Africa, Central Africa, Iran and East Asia including Turkey with a variable prevalence rate (Özger, 2020). The prevalence of *C. cerebralis* has been reported as 3% in Jordan (Abo-Shehada *et al.*, 2002), 3.7% in Egypt (Abbas and Elbeskawy 2016), 4.7% in Iraq (Karim, 1979), 6% in Italy (Varcasia *et al*., 2009) and 5% in Greece (Al-Riyami *et al.*, 2016).

Previously, epidemiological studies regarding the occurrence of *C. cerebralis* have also been carried out in Samsun showing 7.16% (Zeybek, 1977), Diyarbakır with 1.11% (Kalkan, 1978), Kars with 15.5% (Gıcık *et al*., 2007), Konya with 1.5-36% (Uslu and Güçlü, 2007), Kırıkkale with 11.1% (Gökpınar and Yıldız 2012), Istanbul with 24.6% (Uslu and Güçlü, 2007), Erzurum with 0.46% (Avcioglu *et al.*, 2011), Van with 67.7% (Biçek *et al*., 2019), Konya Karacabey with 20% (Uslu and Güçlü, 2007) in Turkey. In the current study, the prevalence of *C. cerebralis* was observed to be 81% which is comparatively high. This is because the animals included in this study were selected based on the clinical signs of coenurosis.

In this study, 300 sheep heads were opened to observe the location of cysts in the brain. It was observed that 45.5% of cysts were located in the left hemisphere of the cerebrum and 29.6% were located in the right hemisphere of the cerebellum. Similar results were reported by Tavassoli *et al*., 2011 in Iran, where 54.63% of the cysts were observed in the left lobe, 40.20% in the right lobe and 5.5% in the cerebellum. Our results also coincided with the previous studies of Gıcık *et al*. (2007) and Gökpınar and Yıldız (2012), who reported a higher number of cyst formation in the cerebrum with 83.3 and 96.7%, respectively. However, we also observed 16.5% of cyst formation in both hemispheres of sheep brains.

The prevalence of *C. cerebralis* is greatly influenced by the season. Biçek *et al*. (2019) reported the highest prevalence of *C. cerebralis* in autumn. Our results coincide with this study as the infection rate was higher in autumn. However, Biçek *et al*. (2019) reported a higher prevalence in November and Tavassoli *et al*. (2011) in February. Our results were controversial to these studies concerning

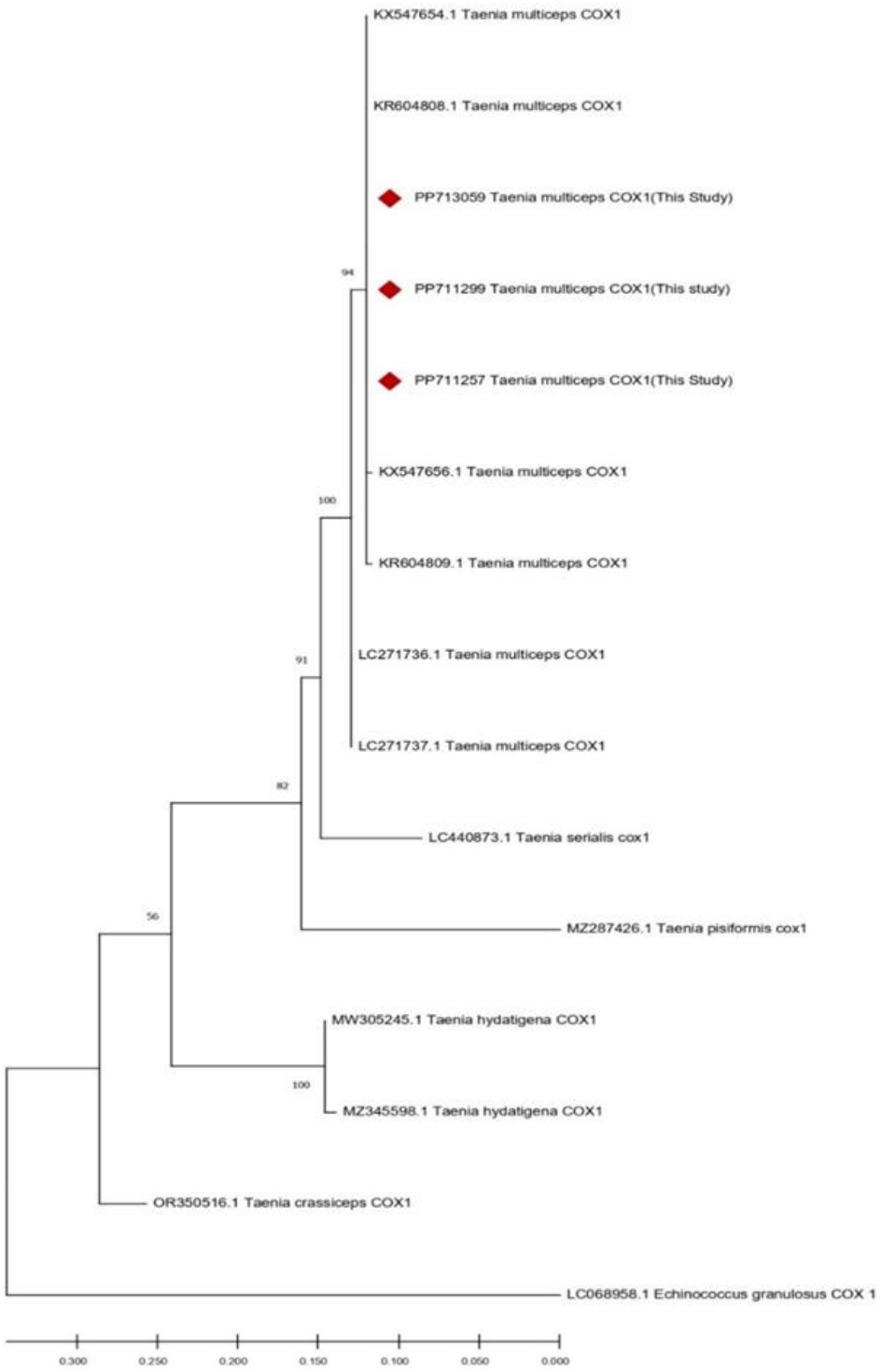

Fig. 3: Phylogenetic tree of obtained sequences of COX1 gene by using Maximum likelihood method with bootstrap value of 1000 replicates

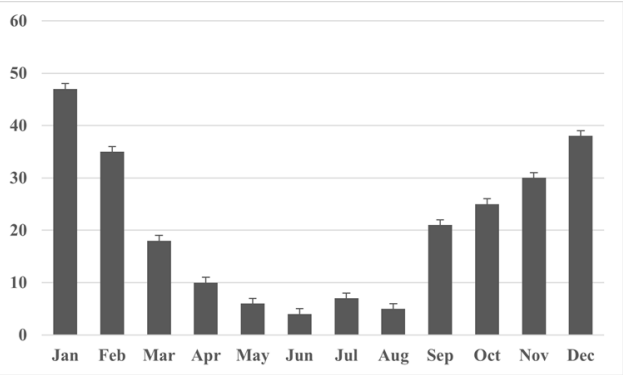

Fig. 4: Month-wise prevalence of *Coenurus cerebralis* in sheep

the month-wise prevalence because in our study the highest prevalence was observed in December and January. These controversial results may be due to the different geographical regions where the studies have been carried out.

As far as the age and gender of the animal were concerned, Biçek *et al*. (2019) and Kızıltepe and Ayvazoğlu (2022) reported previously that the prevalence of *C. cerebralis* was higher in younger animals (<1 year of age) as compared to older animals. They reported the prevalence of *C. cerebralis* in younger animals as 50% and 46%. Our results were in accordance with studies indicating a higher prevalence of *C. cerebralis* (51.4%) in younger animals. However, our results were not in

accordance with the findings of Biçek *et al*. (2019) which observed a higher prevalence in female animals (24%) than in male animals (8%).

Sequence analysis revealed that our sequences with accession numbers PP713059 and PP711257 were 100% similar to a sequence of *C. cerebralis* isolated in China with accession number KX547654.1. Similarly, our sequence with accession number PP711299 showed 99.19% similarity with a sequence of *C. cerebralis* (KX547656.1) published in a study conducted in China. Phylogenetic analysis of these sequences revealed that our sequences (PP713059, PP711257 and PP711299) were included in the same clade with six other clade members, i.e., KX547656.1, KR604809.1, KR604808.1, KX547654.1, LC271736.1 and LC271737.1. The members of this clade were descendants of LC440873.1.

Conclusıons: In this study, 246/300 sheep heads showed cyst formation in the brain indicating the presence of *C. cerebralis.* However, PCR results showed 243 samples were positive for *C. cerebralis*. It indicated that not all the animals showing nervous signs have coenurosis infection, there could be some other kind of problem or infection. This study concluded that coenurosis is highly prevalent in the sheep population in Iğdır province but animals should be diagnosed and treated properly rather than sent to slaughterhouses.

Financial disclosure: This study was financially supported by Iğdır University Scientific Research Projects Coordination Unit as Project no: TMY0923Y16.

Ethical statement: This study was authorized by KAÜ-HADYEK/2023-022 approval number.

Conflict of interest: There is no conflict of interest.

Author's contributions: EA: Conceptualization, Investigation, Writing-Original Draft, Performed parasitological tests. FEO: Planned, designed and supervised the research. performed parasitological tests. checked and finalized the manuscript.

Acknowledgement: This article is ''Molecular Investigation of the Prevalence of *Coenurus cerebralis* in Sheep with Neurological Symptoms in Iğdır Province, Türkiye'' abbreviated from the master's thesis.

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