Prevalence and Molecular Analysis of the Metacestode Stage (*Cysticercus tenuicollis*) of *Taenia hydatigena* in Sheep

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DOI: 10.57244/dfbd.1925946
Geliş tarihi/Received: 08/04/2026 Kabul tarihi/Accepted:27/04/2026

Abstract

This study aimed to determine the prevalence and genetic structure of *Cysticercus tenuicollis* (the larval stage of *Taenia hydatigena*) infection in sheep slaughtered in Van province. A total of 100 sheep slaughtered at the Van Metropolitan Municipality Slaughterhouse between June and December 2025 were examined. Prevalence was evaluated by gender and age group, and phylogenetic analysis was conducted on the mt-CO1 gene region. The findings revealed an overall prevalence of 25.0%. No significant difference was observed between genders (males: 28.1%, females: 23.5%; p = 0.62). However, a statistically significant difference was found between age groups (≤3 years: 13.3%; >3 years: 42.5%; p = 0.001). Phylogenetic analysis showed that the isolates from this study (GenBank accession numbers PZ006119.1 and PZ006118.1) were 100% identical to cattle isolates from China. These results indicate that *T. hydatigena* has a largely conserved genetic structure worldwide. Compared with the previously reported prevalence of 72.89% in Van in 2001, the current rate of 25% may suggest a decline in infection pressure; however, this difference should be interpreted with caution due to potential variations in study design, sampling strategy, and animal population characteristics. Nevertheless, the high infection rate, particularly in older animals, suggests that the parasite continues to exert pressure on animal health and the local economy. This study emphasizes the importance of parasite management in dogs and proper disposal of slaughterhouse waste to control infection.

Keywords: *Cysticercus tenuicollis*, prevalence, phylogenetic analysis, *mt-CO1 gene*, *Taenia hydatigena*

Koyunlarda *Taenia hydatigena*'nın Metasestod Evresi'nin (*Cysticercus tenuicollis*) Prevalansı ve Moleküler Analizi

Özet

The skin is a structure that protects the organism from external factors with its multi-layered structure and hair covering. Structures such as hair, feathers, scales, nails, and claws, which are skin extensions protruding from the body surface, contribute to the organism's structure, while skin extensions such as sebaceous glands, sweat glands, and mammary glands, which are distributed throughout the body, confer special physiological functions such as environmental adaptation. Intermediate filaments such as vimentin, desmin, and laminin play important roles in the formation and development of the organism. These IFs have also been reported in many studies as potential markers in tumor formation. Based on the roles of IFs in cell formation and development and their potential use as markers in tumors, in our study, we investigated the expression of vimentin and desmin, which are intermediate filaments, and laminin, which is a connective tissue component, in the skin tissue of adult rats. Our findings showed that these components reacted with varying degrees of intensity in the medulla layer of the hair, some connective tissue cells, the muscle layer of the skin, the retractor muscles of the hair, the medulla of the hair, some blood vessels, and the outer epithelial cells of the hair. As in other mammals, the expression of vimentin, desmin, and laminin in certain cell groups in rat skin indicates that these factors contribute to the cellular skeleton and tissue integrity in the skin.

Anahtar Kelimeler: *Cysticercus tenuicollis*, prevalans, filogenetik analiz, *mt-CO1 geni*, *Taenia hydatigena*

Introduction

Taenia hydatigena is a parasite that lives as an adult in the small intestines of carnivores. Its larval stage, *Cysticercus tenuicollis*, forms cysts in ruminants and pigs (Yıldırım et al., 2015). These larvae typically appear as fluid-filled cysts in the abdominal, pleural, and pelvic cavities, but can also occur in less common sites such as the lungs, kidneys, and brain (Çelik et al., 2021; Hama et al., 2018). When larvae invade host tissues, they can damage the liver and lungs, sometimes causing severe illness, such as *hepatitis cysticercosa*, or even death in young animals (Bozkurt et al., 2015; Mohammed & Kadir, 2020). As a result, this infection causes major economic losses in livestock, especially because livers and carcasses are often discarded at slaughterhouses (Filip-Hutsch et al., 2019).

Studying the parasite's genetics is essential for understanding how it spreads and adapts over time. The mitochondrial cytochrome c oxidase 1 (mt-CO1) gene is a reliable marker for identifying genetic relationships and phylogenetic patterns among isolates (Çelik et al., 2021). Research shows that *T. hydatigena*'s genetic diversity varies by region (Hama-Soor et al., 2021; Mohammed & Kadir, 2020). These findings help guide the development of more effective control strategies and reduce economic losses (Hama-Soor et al., 2021; Khan et al., 2021). For a more complete picture of the parasite's genetics and to detect differences within the species, it is best to use full mitochondrial genome sequencing and to study other gene regions such as nad5, nad6, and atp6 (Çelik et al., 2021; Jia et al., 2010).

Many studies have examined the prevalence of *C. tenuicollis* in sheep worldwide, showing that infection rates vary widely across locations, animal management practices, and stray dog densities. For example, a study in Egypt found infection rates of 19% in goats (67 cases) and 16% in sheep (80 cases) among 500 sheep, 350 goats, and 103 camels. The same study also reported the first case of this larva in a camel liver (1%) in Egypt (Omar et al., 2016). In Bangladesh, 12.9% of goats were infected (Dey et al., 2022). A larger study in Kazakhstan found lower rates: 0.6% in cattle and 1.1% in sheep (Kiyan et al., 2025).

Research in Van province indicates that *C. tenuicollis* infection is a major issue. Earlier studies reported prevalence rates of 72.89% in sheep, 58.85% in goats, and 10.86% in cattle (Değer et al., 2001). Another study in Van found an infection rate of 27.9% (Oğuz & Değer, 2013). A later, more detailed study confirmed that *C. tenuicollis* is among the most common metacestodes in Van, causing significant economic losses (Değer & Biçek, 2005).

Collectively, these studies demonstrate that *C. tenuicollis* infection is widespread. Overall, they show that *C. tenuicollis* infection is common in Van province and poses a serious risk to small ruminant farming. However, more recent data are needed on the current prevalence of the infection, the parasite's genetic makeup, and its transmission among hosts. This study aims to determine whether the prevalence of *C. tenuicollis* in sheep in Van has changed in recent years and to compare the genetic characteristics of local isolates with those reported from other regions.

Materials and Methods

Study Area

The study material consisted of 100 sheep slaughtered at the Van Metropolitan Municipality Slaughterhouse from June to December 2025.

Postmortem Examination and Prevalence Determination

All sheep underwent routine postmortem inspection at the slaughterhouse. The abdominal cavity and visceral organs, particularly the omentum, mesentery, liver surface, and peritoneum, were macroscopically examined for *C. tenuicollis* cysts. Prevalence was calculated as the number of infected animals divided by the total number of examined sheep.

Collection of Cysts

Cysts detected during postmortem examination were carefully collected, and their anatomical locations were recorded. Representative samples were then subjected to molecular analysis.

Animal Data

The animals' ages and sexes were recorded, and the sheep were grouped for statistical analysis.

DNA Extraction

Genomic DNA was isolated from scolices collected from cysts using a commercial kit (DNA Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until PCR analysis. For PCR, primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′) were used to amplify a 446-bp region of the mt-CO1 gene (Bowles et al., 1992). The mt-CO1 gene region was amplified in a 30 µl PCR mixture containing 200 µM of each dNTP, 10 pmol of each primer, 1× PCR buffer, 1.25 U/µl DreamTaq DNA Polymerase (Thermo Scientific), and 3 µl of template DNA. The reaction conditions were as follows: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes. PCR products were visualized on an agarose gel using SafeView dye. Positive amplicons were sequenced in both directions using the same primers (Macrogen, Ankara, Türkiye). The mt-CO1 gene sequence data were analyzed using BioEdit (Hall et al., 2011).

Sequence Analysis

Purified PCR products were sent to Macrogen in Ankara, Türkiye, for Sanger sequencing. The resulting sequences were analyzed in BioEdit (Hall et al., 2011) using

the Clustal/W method (Higgins et al., 1996). Sequence similarity was assessed with NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis of *T. hydatigena* was conducted using the mt-CO1 locus, incorporating additional isolates from GenBank. The phylogenetic tree was constructed in MEGA 12 (Kumar et al., 2024). ModelTest in MEGA 12 was used to identify the best-fit model, and the Tamura 3+I model (Tamura, 1992) was applied for Maximum Likelihood analyses. Bootstrap analysis with 1000 replicates was used to evaluate the reliability of the results.

Statistical Analysis

The data from the study were analyzed using SPSS V16.0 (IBM, Chicago, IL, USA). The relationship between grouped variables was assessed using the chi-square test. Differences were considered statistically significant when $P < 0.05$.

Results

Of the 100 sheep examined, 32 were male and 68 were female. *C. tenuicollis* cysts were detected in 25 animals, yielding an overall prevalence of 25.0%. Infection rates were 28.1% (9/32) in males and 23.5% (16/68) in females, with no significant difference between groups ($p > 0.05$). In contrast, infection was significantly more common in sheep older than 3 years (42.5%, 17/40) than in those aged 3 years or younger (13.3%, 8/60) ($p = 0.001$) (Table 1).

Table 1. Prevalence of *C. tenuicollis* according to gender and age group in sheep.

Variable	Category	n(examined)	n(positive)	Prevalence (%)	Pvalue
Gender	Male	32	9	28.1	0.62
	Female	68	16	23.5	
Age group	≤3 years	60	8	13.3	**0.001***
	>3 years	40	17	42.5	
Total		**100**	**25**	**25.0**	

*Significant at $p < 0.05$.

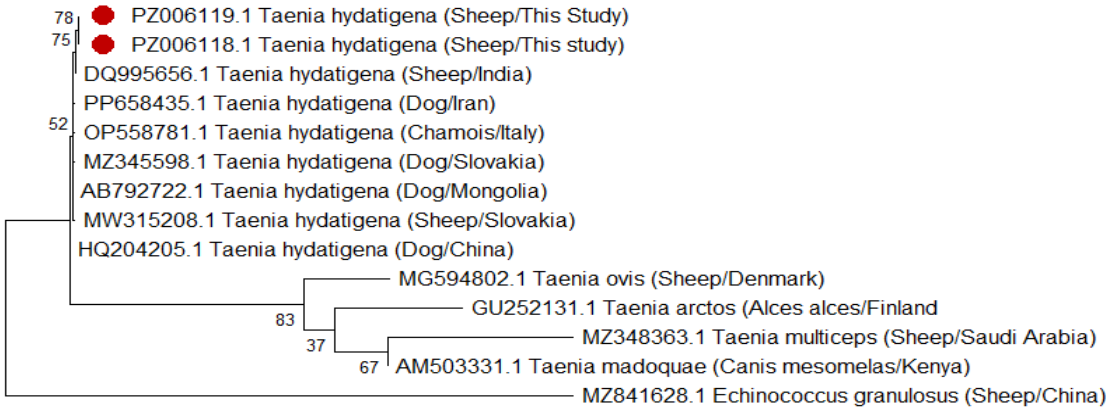

Figure 1. Phylogenetic tree showing relationships among *T. hydatigena* isolates (PZ006119.1, PZ006118.1) based on the mt-CO1 gene region, inferred using the Maximum Likelihood method. *Echinococcus granulosus* (MZ841628.1) served as the outgroup.

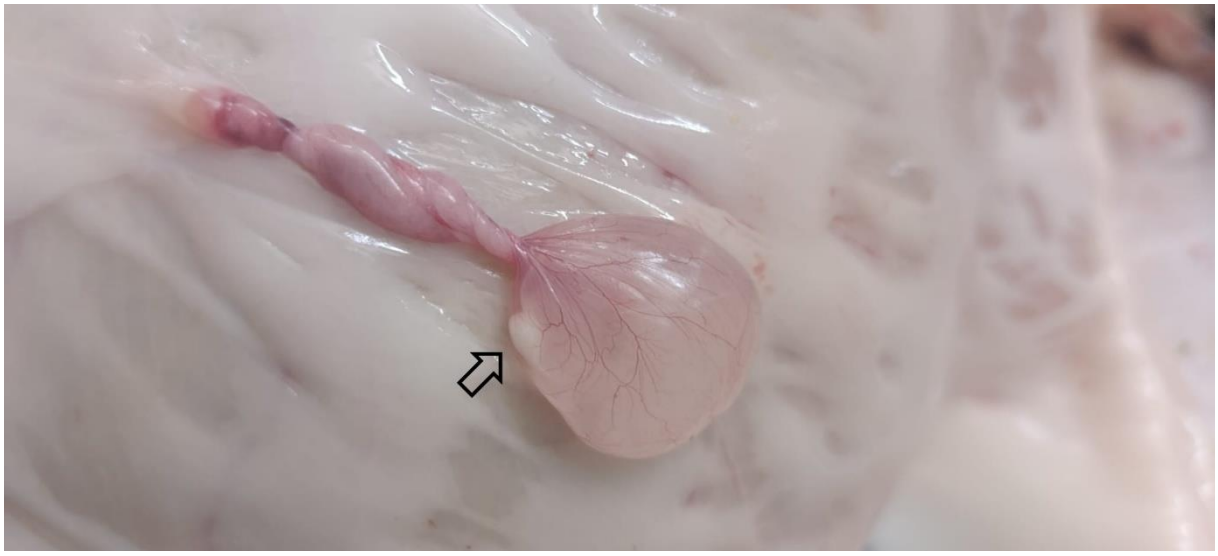

Figure 2. *C. tenuicollis* cyst located in the omentum. Arrow: scolex within the cyst fluid.

Phylogenetic Analysis

Homology and phylogenetic analyses showed that the *T. hydatigena* isolates from this study (GenBank accession numbers PZ006119.1 and PZ006118.1) were 100% identical to cattle isolates from China (OP861564.1, OP861557.1, and OP861563.1). In addition, the study isolates showed high similarity to other *T. hydatigena* sequences in GenBank (DQ995656.1, PP658435.1, OP558781.1, MZ345598.1, AB792722.1, MW315208.1, and HQ204205.1). *Echinococcus granulosus* (MZ841628.1) served as the outgroup in the phylogenetic tree.

Discussion

It is especially common in small ruminants and pigs (Peña-Espinoza et al., 2013; Yıldırım et al., 2015). This study found that 25% of sheep in Van province were infected with *C. tenuicollis*. This result is similar to other studies in Türkiye, where prevalence rates range from 12.3% to 65.67% (Çelik et al., 2021). Worldwide, rates vary widely, with some studies reporting up to 79% in Ethiopia (Sissay et al., 2008), 42.2% in Tanzania (Miran et al., 2016), and 15.17% in India (Nimbalkar et al., 2011). Compared with the earlier rate of 72.89% in Van (Değer et al., 2001; Oğuz & Değer, 2013), the current 25% may indicate a decline in infection pressure in the region. However, this difference should be interpreted with caution, as variations in study period, sample size, sampling strategy, slaughterhouse conditions, and animal population characteristics may have influenced the reported prevalence rates. Therefore, improvements in control practices and slaughterhouse hygiene may be considered among several possible explanations for the lower prevalence observed in the present study, rather than a definitive cause.

By age, infection was much more common in sheep older than 3 years (42.5%) than in those 3 years or younger (13.3%), and this difference was statistically significant ($p = 0.001$). This is likely because older sheep are exposed to parasite eggs for longer periods while grazing (Dyab et al., 2017; Laranjo-González et al., 2016). Some researchers also suggest that older animals may have weaker immune systems, which could increase infection rates (Dyab et al., 2017). For gender, there was no significant difference (28.1% in males and 23.5% in females), indicating similar risk between the sexes. These results align with findings from Tanzania and Iraq, where no significant gender differences were observed (Hama et al., 2018; Miran et al., 2016).

In a study by Oğuz and Değer (2013) on sheep in Van, the infection rate of *C. tenuicollis* was reported as 27.9%. In a molecular study by Ütük and Pişkin (2012) on cysts collected from goats, 99% similarity to the reference sequences (DQ995656.1, HQ204206.1) was reported. In our study, however, 100% similarity was observed with sequences obtained from studies conducted in China and Egypt (OP861564.1, AB513874.1).

The mitochondrial cox1 gene used in this study was a reliable marker for understanding the parasite's genetic structure (Çelik et al., 2021). The isolates were 100% identical to strains from China and Egypt, indicating that *T. hydatigena* has a largely conserved genetic structure worldwide (Hama et al., 2018; Mohammed & Kadir, 2020). Thus, although the parasite is found in many regions and hosts, its main genetic profile remains the same, though new mutations can occasionally arise in certain locations (Mohammed & Kadir, 2020). *C. tenuicollis* is a major economic problem for the livestock industry. Severe infections can cause serious liver damage and *hepatitis cysticercosa*, sometimes leading to death in young animals (Çelik et al., 2021; Yıldırım et al., 2015). Discarding livers and carcasses at slaughterhouses also causes significant financial losses. For example, annual losses in Italy are about €333,657, and in Egypt, liver condemnations cost thousands in local currency (Çelik et al., 2021; Omar, 2021; Ras et al., 2024).

The 25% prevalence found in Van still puts pressure on animal health and the local economy. To control this infection, it is important to manage the parasite in dogs and ensure that slaughterhouse waste is properly disposed of so that dogs cannot access it.

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