Molecular Survey of Toxoplasma gondii Infection in Aborted Fetuses of Sheep in the Iğdır Province of Türkiye

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ABSTRACT

Toxoplasma gondii, an obligatory intracellular protozoan parasite, can infect a wide range of warm-blooded animals, including livestock species. T. gondii is a zoonotic protozoan parasite that affects both humans and other warm-blooded animals. The aim of this study was to detect T. gondii by using PCR in the brain tissues of 60 aborted sheep fetuses from the Iğdır Province in Turkey. For this purpose, 60 brain tissue samples of sheep were collected within the lambing seasons of 2023 in Iğdır, Türkiye. The DNA extraction was performed using the PureLink™ Genomic DNA Mini Kit from brain samples. The PCR was performed with the appropriate primers from the obtained DNA samples. T. gondii was found in the brain (16.6%) samples of aborted sheep fetuses. According to the present study, T. gondii infection can be one of the causes of fetus abortion in sheep in Iğdır province, Türkiye. This result emphasizes the need for vigilance and preventive measures in managing this potential public and animal health concern.

INTRODUCTION

Toxoplasma gondii, an obligatory intracellular protozoan parasite, can infect a wide range of warm-blooded animals, including livestock species (Innes, 2010). The cats are the primary hosts for T. gondii. Sheep may become infected after consuming feed or pasture contaminated with sporulated oocysts (Elmore et al. 2010). T. gondii affects reproductive system organs resulting in reproductive failure such as abortion, stillbirths, and low viability of offspring in sheep. Therefore, it may cause important economic losses in the sheep industry (Anastasia et al. 2013; Gutiérrez-Expósito et al. 2021). T. gondii can also affect public health negatively. In pregnant women, the main way of transmission of T. gondii is through the consumption of raw or undercooked meat during pregnancy (Kapperud et al. 1996; Bilgili and Haneden, 2019). The prevalence of T. gondii in pregnant women ranges between %13 and %55 (Bilgili and Haneden, 2019). Therefore, it is an important risk factor for pregnant women worldwide. In addition, the serological prevalence of T. gondii is high among farm animal species such as pigs, sheep, and goats (Tenter et al. 2000). Türkiye is one of the important countries in Europe for sheep breeding with 46.1 million head of sheep (TÜİK, 2022). Therefore, sheep are an important source of meat, milk, and wool in Türkiye (Köseman and Şeker, 2015; Behrem and Gül, 2022). There are different studies showing the high seroprevalence of T. gondii in Türkiye (Tutuncu et al., 2003; Oncel and Vural, 2006; Acici et al. 2008; Çakmak and Karatepe; 2017). The high prevalence of T. gondii is an important problem for the sheep industry in Türkiye because it causes reproductive diseases. T. gondii-induced abortions are still reported in different countries (Edwards and Dubey, 2013; Chessa et al., 2014; Nayeri et al. 2021). However, there is limited information on T. gondii-induced abortion in sheep. The objective of the investigation was to detect T. gondii by using PCR in the brain tissues of 60 aborted sheep fetuses from the Iğdır Province in Türkiye.

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Materials and Methods

**Ethical Statement**

This study was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval no: 2023/03-11).

**Study Samples**

Brain specimens were collected from 60 sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in İğdır, Türkiye. The samples were exclusively sourced from Morkaraman breed sheep. Out of 60 aborted ovine fetuses, a total of 60 brain tissue samples were procured. To extract brain samples, each fetus was handled individually, with the calvarium opened and meninges dissected using a fresh disposable scalpel and forceps. Approximately 1 cm³ of brain tissue from the right cerebral hemisphere was excised and subsequently frozen at -20 °C for DNA extraction.

**DNA Extraction**

The DNA extraction from the aborted fetus brain was carried out using the PureLink™ Genomic DNA Mini Kit (Invitrogen™, USA, K182002), and subsequently stored at -20°C.

**PCR Amplification**

The amplification of the 529-bp repetitive element region of *Toxoplasma gondii* was conducted using the TgTox4F (5'-CGCTGAGGGAGGAGCAAGTAAGTGG-3') and TgTox4R (5'-CGCTGAGAGACAGTGCATCTGGAT-3') primers (Sah et al. 2019). In a 20 µl master mix, the following components were used: 8 pmol of both forward and reverse primers, 4 µl of 5x FIREPol® Master Mix (containing 7.5 mM MgCl₂, Solis BioDyne, Estonia), 1.6 µl of DNA, and 12.8 µl of Nuclease Free Water. The PCR protocol involved an initial denaturation step at 95°C for 5 minutes. This was followed by 35 cycles, each consisting of denaturation at 95°C for 60 seconds, annealing at 60°C for 60 seconds, elongation at 72°C for 1 minute and final elongation at 72°C for 10 minutes. Subsequently, a 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were then electrophoresed on the agarose gel, and images were captured using a gel imaging device (Syngene Bio imaging System).

**Results**

In this study, a total of 60 brain tissue samples were chosen from aborted fetuses for the isolation of the *Toxoplasma gondii* parasite through conventional PCR (Figure 1). Positivity for the presence of *T. gondii* was confirmed in 10 out of the 60 samples, accounting for 16.6% of the total.

**Discussion**

*Toxoplasma gondii* infection is a zoonotic protozoan parasite that affects both humans and other warm-blooded animals. Various molecular techniques, including serological methods, cell culture, laboratory animal vaccination, and PCR, are employed to detect toxoplasmosis (Fuente et al. 1996; Greg et al. 1996; Tavassoli et al. 2009). The increased sensitivity of PCR now enables to delve into alterations at the individual cell level, surpassing the typical requirements for parasite-related research. PCR has profoundly influenced advancements in fields such as parasite systematics and epidemiology, as well as in the domains of immunology and interactions between hosts and parasites (Ndao, 2009). PCR stands out for its high sensitivity and specificity, allowing for the detection of a specific segment of *T. gondii* DNA, making it the preferred choice over other techniques (Fuente et al. 1996; Greg et al. 1996; Tavassoli et al. 2009).

Indeed, numerous studies worldwide have employed aborted fetal tissues for diagnosing Toxoplasmosis infection. Molecular investigations have been carried out across different regions to assess the prevalence of *Toxoplasma gondii* in aborted fetuses. Prevalence of Toxoplasma gondii infection in aborted fetuses have been reported in various studies around the world. These include 10% in Germany (Steuber et al. 1995), 13% in Italy (Chessa et al. 2014), 14.3% in Brazil (de Moraes et al. 2011), 13.5% in Iran (Rassouli et al. 2011), 64% in Iran (Shahbazi et al. 2019), 11.8% in Romania (Paștiu et al. 2023), and 5.4% in Spain (Moreno et al. 2012). In our study, a positivity rate of 16.6% was observed.

Molecular studies have been carried out to investigate the prevalence of *Toxoplasma gondii* in aborted fetuses in Türkiye (Özkaraca et al. 2016; Irehan et al. 2022; Oruç Kılıç et al. 2023; Akpinar et al. 2023). Özkaraca et al. (2016) detected positivity in 1 out of the sheep abortion samples brought to Elazığ Veterinary Control Institute using the Duplex PCR method. Irehan et al. (2022) identified positivity in 7.27% of 55 aborted fetus samples from 13 different provinces in the Eastern and Southeastern Anatolia Regions of Türkiye using Real-time PCR. Oruç Kılıç et al. (2023) reported a 35.7% positivity rate in 42 aborted sheep fetuses in the Van region in 2023 using the conventional PCR method. Akpinar et al. (2023) found that 7.7% of 78 sheep fetuses from 9 provinces (Samsun, Sinop, Amasya, Giresun, Ordu, Rize, Tokat, Trabzon, and Sivas) between 2018 and 2020 were positive by the PCR method. In the present study, which focused on brain samples from aborted ovine fetuses, the prevalence of *T. gondii* infection was 16.6% (10 out of 60) based on the conventional PCR method.

![Figure 1. Agarose gel image of Toxoplasma gondii. M - marker, P - positive control, N - negative control; lanes 3, 8, 17, and 29 are positive samples for Toxoplasma gondii (529 bp).](image-url)
The clinical manifestation of toxoplasmosis in pregnant ewes can be influenced by the age and immune status of the fetuses. In the first trimester, when the fetal immune system is relatively immature, the likelihood of fetal demise due to infection is greater compared to later stages of pregnancy. Infections during mid-gestation typically lead to the birth of stillborn or frail lambs. In contrast, infections in the later stages of gestation may result in the birth of a live lamb that appears healthy but is infected (Salehi et al., 2020).

**Conclusion**

Sheep infection with *Toxoplasma gondii* carries significant implications for public health, underscoring the importance of ascertaining its prevalence for implementing requisite precautions. In the current study, which focused on brain samples from select aborted ovine fetuses, the prevalence of *T. gondii* infection was determined to be 16.6% (10 out of 60) using the conventional PCR method. This finding emphasizes the need for vigilance and preventive measures in managing this potential public health concern.

The findings of our study will play a crucial role in enhancing awareness among veterinarians, researchers, and farmers regarding the epidemiology and prevalence of *T. gondii* infection in the Iğdır region. However, further investigations are imperative to delve deeper into understanding the various genotypes of *T. gondii* and their potential connection to abortion and other reproductive complications within the sheep population. This will contribute significantly to a more comprehensive understanding of the infection's impact on the local livestock.

**References**


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