Turkish Journal of Agriculture - Food Science and Technology, 11(12): 2390-2393, 2023
DOI: https://doi.org/10.24925/turjaf.v11i12.2390-2393.6322

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

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

Davut Koca[1,a,*], Özlem Orunç Kılınç[2,b], Adnan Ayan[3,c], Fatma Ertaş Oğuz[4,d], Ali Osman Turgut[5,e], Özge Oktay Ayan[6,f]

[1]*Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Van, Türkiye*
[2]*Van Yuzuncu Yil University, Özalp Vocational School, Department of Medical Laboratory Technician, Van, Türkiye*
[3]*Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Genetics, Van, Türkiye*
[4]*Iğdır University Tuzluca Vocational School, Department of Medical Services and Techniques, Iğdır, Türkiye*
[5]*Siirt University, Faculty of Veterinary Medicine, Department of Animal Science, Siirt, Türkiye*
[6]*Van Yuzuncu Yil University, Faculty of Medicine, Department of Parasitology, Van, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 06.10.2023
Accepted : 21.11.2023

Keywords:
Abortion
PCR
Sheep
Toxoplasma gondii
Türkiye

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 Türkiye. 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 of sheep in Iğdır province, Türkiye. This result emphasizes the need for vigilance and preventive measures in managing this potential public and animal health concerns.

[a] *davutkoca@yyu.edu.tr* *https://orcid.org/0000-0002-7962-6959*
[b] *ozlemkilinc@yyu.edu.tr* *https://orcid.org/0000-0001-6233-7109*
[c] *adnanayan@yyu.edu.tr* *https://orcid.org/0000-0002-6564-3416*
[d] *vtr.fatma@gmail.com* *https://orcid.org/0000-0001-5289-071X*
[e] *aosman.turgut@siirt.edu.tr* *https://orcid.org/0000-0001-6863-0939*
[f] *ozgeokty09@gmail.com* *https://orcid.org/0000-0003-2577-3774*

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 Hanedan, 2019). The prevalence of *T. gondii* in pregnant women ranges between %13 and %55 (Bilgili and Hanedan, 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 (TUIK, 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.

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 Iğdır, 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 cm^3 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'-CGCTGCAGGGAGGAAGACGAAAGTTG-3′) and TgTox4R (5'-CGCTGCAGACACAGTGCATCTGGATT-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 minutes 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 (Paştiu 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; Orunç Kılınç 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. Orunç Kılınç 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. Akpınar 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.

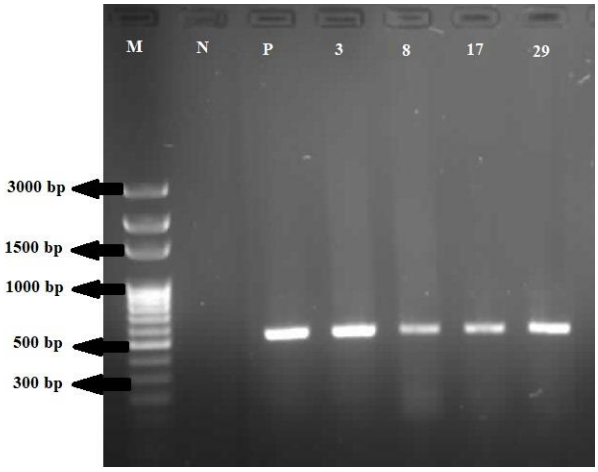

Figure 1. Agarose gel image of Toxoplasma gondii.
M - marker, P - positive control, N - negative control; lanes 3, 8, 17, and 29 are positive samples for Toxoplasma gondii (529 bp).

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

Acici M, Babur C, Kilic S, Hokelek M, Kurt M. 2008. Prevalence of antibodies to *Toxoplasma gondii* infection in humans and domestic animals in Samsun province, Turkey. Tropical Animal Health and Production, 40: 311-315.

Akpinar R, Kiziltepe Ş, Kaya S, Aydin C, Türlek ŞÖ. 2023. Koyun ve Keçi Atık Fetüslerinde *Toxoplasma gondii*'nin Prevelansının PZR ile Taranması. Firat Universitesi Saglik Bilimleri Veteriner Dergisi, 37(1): 43-48.

Bilgili A, Hanedan B. 2019. Importance of toxoplasmosis for human and animal health, present condition, problems and solution proposals in Turkey and the World. World Journal of Advanced Research and Reviews, 4(2): 61-74.

Anastasia D, Elias P, Nikolaos P, Charilaos K, Nektarios G. 2013. *Toxoplasma gondii* and *Neospora caninum* seroprevalence in dairy sheep and goats mixed stock farming. Veterinary Parasitology, 198: 387–390.

Behrem S, Gül S. 2022. Effects of age and body region on wool characteristics of Merino sheep crossbreds in Turkey. Turkish Journal of Veterinary & Animal Sciences, 46(2): 235-247.

Çakmak DÖ, Karatepe B. 2017. Seroprevalence of *Toxoplasma gondii* in sheep from Nevşehir province in Turkey. Türkiye Parazitoloji Dergisi, 41(3): 148.

Chessa G, Chisu V, Porcu R, Masala G. 2014. Molecular characterization of *Toxoplasma gondii* Type II in sheep abortion in Sardinia, Italy. Parasite, 21: 6.

de Moraes ÉBX, da Costa MM, Dantas AFM, da Silva JCR, Mota RA. 2011. *Toxoplasma gondii* diagnosis in ovine-aborted fetuses and stillborns in the state of Pernambuco, Brazil. Veterinary Parasitology,183(1-2): 152–155.

Edwards JF, Dubey JP. 2013. *Toxoplasma gondii* abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype T. gondii from an aborted lamb from a chronically infected ewe. Veterinary Parasitology, 192(1-3): 129-136.

Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, Dubey J. 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends in Parasitology. 26: 190–196.

Fuente E, Rodriguez M, Domingo C. 1996. Urine sample used for congenital toxoplasmosis diagnosis by PCR. Journal of Clinical Microbiology, 34(10): 2368–2371.

Greg S J, Vitali GS, David JD. 1996. Comparison of cell culture, mouse inoculation and PCR for detection of *Toxoplasma gondii*: Effects of storage conditions on sensitivity. Journal of Clinical Microbiology, 34: 1572–1575.

Gutiérrez-Expósito D, Tejerina F, Gutiérrez J, Fernández-Escobar M, Ortega-Mora LM, Mantecón AR, Dagleish MP, Pérez V, Benavides J. 2021. Direct economic losses of *Toxoplasma gondii* abortion outbreaks in two Spanish sheep flocks. Veterinary Parasitology: Regional Studies and Reports, 26: 100623.

Innes E. 2010. A brief history and overview of *Toxoplasma gondii*. Zoonoses Public Health 57: 1–7.

Irehan B, Sonmez A, Atalay MM, Ekinci AI, Celik F, Durmus N, Turkan Ciftci A, Simsek S. 2022. Investigation of *Toxoplasma gondii*, *Neospora caninum* and *Tritrichomonas foetus* in abortions of cattle, sheep and goats in Turkey: Analysis by real-time PCR, conventional PCR and histopathological methods. Comparative Immunology, Microbiology and Infectious Diseases, 89: 101867.

Kapperud G, Jenum PA, Stray-Pedersen B. 1996. Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. American Journal of Epidemiology, 144: 405–412.

Orunç Kılınç Ö, Ayan A, Yumuşak N, Kömüroğlu AU, Aslan B, Çelik ÖY, Göz Y. 2023. Investigation of *Toxoplasma gondii* and *Neospora caninum* in different tissues of aborted foetuses of sheep in Van Province, Türkiye: Analysis by nested PCR, histopathological and immunohistochemical methods. Acta Veterinaria Brno, 92(2): 123-131.

Köseman A, Şeker İ. 2015. Current status of cattle, sheep and goat breeding in Turkey. Van Veterinary Journal, 26(2): 111-117.

Moreno B, Collantes-Fernández E, Villa A, Navarro A, Regidor-Cerrillo J, Ortega-Mora L. 2012. Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Veterinary Parasitology. 187(1-2): 312–318.

Nayeri T, Sarvi S, Moosazadeh M, Daryani A. 2021. Global prevalence of *Toxoplasma gondii* infection in the aborted fetuses and ruminants that had an abortion: A systematic review and meta-analysis. Veterinary parasitology, 290: 109370.

Ndao, M. 2009. Diagnosis of parasitic diseases: old and new approaches. Interdisciplinary perspectives on infectious diseases, 2009.

Oncel T, Vural G. 2006. Occurrence of *Toxoplasma gondii* antibodies in sheep in Istanbul, Turkey. Veterinarski Arhiv, 76(6): 547-553.

Özkaraca M, İrehan B, Parmaksiz A, İtik Ekinci A, Çomakli S. 2016. Koyun ve Keçi Abortlarında *Neospora caninum* ve *Toxoplasma gondii*'nin Dubleks PCR, İmmunohistokimyasal ve İmmunfloresans Yöntemlerle Teşhisi. Ataturk University Journal of Veterinary Sciences, 11(2): 200-206.

Paştiu AI, Mircean V, Mercier A. 2023. *Toxoplasma gondii* infection in sheep from Romania. Parasites Vectors, 16: 24.

Rassouli M, Razmi GR, Bassami MR, Movassaghi AR, Azizzadeh M. 2011. Study on ovine abortion associated with Toxoplasma gondii in affected herds of Khorasan Razavi Province, Iran based on PCR detection of fetal brains and maternal serology. Parasitology. 138(6): 691-697.

Sah RP, Dey AR, Rahman AA, Alam MZ, Talukder MH 2019: Molecular detection of *Toxoplasma gondii* from aborted fetuses of sheep, goats and cattle in Bangladesh. Veterinary Parasitology: Regional Studies and Reports, 18: 100347

Salehi M, Nezami H, Niazkar HR. 2020. Investigation of *Toxoplasma gondii* infection in aborted fetuses of sheep using PCR: A study in North Khorasan Province, Iran. Veterinary Medicine International, 2020: 1-5.

Shahbazi G, Rad NH, Madani R, Matin S, Mortazavi P, Jangjou AH. 2019. *Toxoplasma gondii* in aborted fetuses of sheep in Ardebil Area, North-West of Iran. Iranian Journal of Parasitology, 14(3): 430.

Steuber S, Niu A, Bauer C, Reetz J, Roth A, Janitschke K. 1995. The detection of *Toxoplasma gondii* in abortion tissues of sheep using the polymerase chain reaction. Deutsche Tierarztliche Wochenschrift, 102(2): 91–93.

Tavassoli M, Esmaeilnejad B, Tabatabaei M. 2009. A survey on infection of animals with *Toxoplasma gondii* using PCR and genetic differences using RFLP in Urmia, Iran. Pazhoohesh va Sazandegi, 58: 61–66.

Tenter AM, Heckeroth AR, Weiss LM. 2000. *Toxoplasma gondii*: from animals to humans. International Journal for Parasitology, 30: 1217-1258.

TUIK (2022). Hayvansal Üretim İstatistikleri. Link: https://data.tuik.gov.tr/, accession date: 16.10.2023.

Tutuncu M, Ayaz E, Yaman M, Akkan HA. 2003. The seroprevalance of *Toxoplasma gondii* in sheep, goats and cattle detected by indirect haemaglutinnation (IHA) test in the region of Van, Turkey. Indian Veterinary Journal, 80: 401-403.