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Molecular Survey of the *Toxoplasma gondii* and *Neospora caninum* in brain tissue of aborted fetuses of Morkaraman sheep in Muş, Türkiye

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ABSTRACT

Toxoplasma gondii and *Neospora caninum* are obligate intracellular protozoan parasites that can affect different warm-blooded species worldwide. In this study, it was aimed to detect *T. gondii* and *N. caninum* using PCR method in brain tissues of aborted sheep fetuses. Brain specimens were collected from 50 Morkaraman sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in Muş. Approximately 1 cm³ of brain tissue from the right cerebral hemisphere was excised and subsequently frozen at -20°C for DNA extraction. DNA extraction and PCR amplification were then performed. As a result of this study, 11 (22%) of 50 brain tissues were positive. All brain samples examined in this study were negative for *Neospora caninum.* Based on the results of this study, it is possible to say that *T. gondii* is an important abortion agent in sheep in this region. Although *N. caninum* was not detected in this study, larger scale studies are recommended. Moreover, this study provides important information to breeders and veterinarians in the evaluation and management of abortion in the field.

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Introduction

Toxoplasma gondii and *Neospora caninum* are obligate intracellular protozoan parasites that are distributed worldwide, have structural, genetic and immunological similarities, belong to the phylum Apicomplexa and can affect different warm-blooded species (Dubey, 2003; Basso et al., 2022).

Toxoplasma gondii causes toxoplasmosis, one of the most common parasitic diseases in humans and animals worldwide (Tenter et al., 2000; Wang et al., 2011). The definitive hosts are cats and felidae family, and the intermediate hosts are mammals, including humans, and birds (Dubey, 1994; Mor and Arslan, 2007). Infected cats can shed millions of oocysts. This causes widespread environmental contamination and is an important source of infection for herbivores (Innes et al., 2009; Can, 2010). The infective forms of the parasite are tachyzoites, bradyzoites and oocysts of the felidae family, especially in the brain and muscle tissue. (Muz et al., 2013). The disease causes significant economic losses especially in sheep due to pneumonia, enteritis, neurological disorders, encephalitis, premature birth, stillbirth, neonatal losses and abortions (Van der Puije et al., 2000; Bártová et al., 2009; Dubey, 2009; Anğ et al., 2011).

Neospora caninum is an obligate intracellular parasite first isolated in Norway in 1984 in puppies with congenital encephalomyelitis (Dubey et al., 2007; Uzêda et al., 2007). The definitive host of *N.caninum* is domestic and wild canids and the intermediate hosts are herbivores.

Herbivores become infected by ingesting infected oocysts scattered in the feces of the definitive hosts (Sharma et al., 2015; Gharekhani et al., 2016). This disease causes abortions and newborn deaths in cattle, sheep and goats (Dubey, 2003; Figliuolo et al., 2004; Uzêda et al., 2007). In many hosts, transmission is transplacental. Significant economic losses can occur due to abortions and neonatal mortality (Sharma et al., 2015). In experimental studies, congenital infection has been reported in sheep and goats (Gharekhani et al., 2016).

In this study, it was aimed to detect *T. gondii* and *N. caninum* using PCR method in brain tissues of 50 aborted sheep foetuses in the Muş, Türkiye.

Material and Methods

The Study Area and Samples Collection

Brain specimens were collected from 50 sheep fetuses that had undergone abortion at various stages of pregnancy, within the lambing seasons of 2023 in Muş, Türkiye. The samples were exclusively sourced from Morkaraman breed sheep. Out of 50 aborted ovine fetuses, a total of 50 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

DNA extraction was performed in all aborted fetus brain using the PureLink™ Genomic DNA Mini Kit (Invitrogen™, USA, K182002), according to the manufacturer's protocol. The obtained DNAs were 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). Protocol for reactions was performed according to Orunç Kılınç et al. (2023).

For amplification of the Nc5 gene region of *N. caninum*, nested PCR was performed using external (5'-CTGCTGACGTGTCGTTGTTG-3′) forward and (5' CATCTACCAGGCCGCTCTTC-3′) reverse primers inner (5'-GCGTCAGGGTGAGGACAGTG-3′) forward and (5'-CTCTCCGTTCGCCAGCAGTG-3′) reverse primers (Fish et al., 2007). Protocol for both reactions was performed according to Orunç Kılınç et al. (2023).

The reaction was performed in an automatic thermal cycler (Eppendorf Mastercycler® pro) device. Subsequently, 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were run on an agarose gel afterward, and images were obtained on the gel imaging device (Syngene bioimaging system).

Ethical Approval

This study was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval no:2023/03-10).

Results

In this study, a total of 50 brain tissue samples were chosen from aborted fetuses for the isolation of the *Toxoplasma gondii* parasite through conventional PCR. Positivity for the presence of *T. gondii* was confirmed in 11 out of the 50 samples, accounting for 22% of the total. (Figure 1). All brain samples examined in this study were negative for *Neospora caninum.*

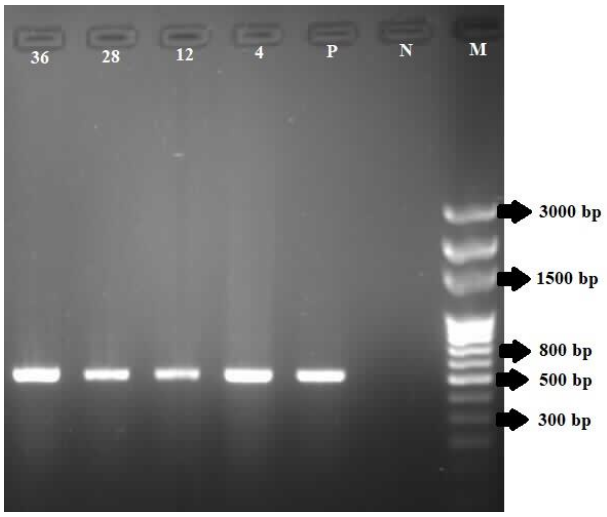

Figure 1. Amplification of *T.gondii* using conventional-PCR. Lanes M: Marker, N: Negative control, P: positive control; 36, 28, 12 and 4 represent *T.gondii*. (529 bp).

Discussion

Toxoplasmosis plays an important role in sheep abortions and causes heavy losses to the sheep industry worldwide (Dubey, 2009). Especially infectious abortions have important effects on public health not only with their economic dimension but also with their zoonotic importance (Har and Başbuğan, 2019). Although *N. caninum* has been reported to cause congenital infections, abortions and deaths in newborn lambs in sheep, it is not considered among the main causes of abortion in sheep (Innes et al., 2001; Koyama et al., 2001; Hässig et al., 2003).

Toxoplasma gondii infections have been recorded in sheep populations worldwide with highly variable seroprevalences (Basso et al., 2022). A prevalence of 51.76% was reported in Egypt (Ibrahim et al., 2017), 38.22% in Brazil (Ueno et al., 2009), 3.76% in India (Sharma et al., 2008) and 1.6% in Iran (Raeghi et al., 2011). In studies conducted in Turkey; 54.65% prevalence was reported in Afyonkarahisar (Çiçek et al., 2004), 66.66% in Yalova (Oncel et al., 2005), 10% in Nevşehir (Çakmak and Karatepe, 2017), 48.4% in Mersin (Öztürk et al., 2002), 95.7% in Kars (Mor and Arslan, 2007) and 13% in Konya (Aköz et al., 2009). It was reported that *T. gondii* DNA was detected in 3 of 20 aborted fetal brain tissues (Hässig et al., 2003), 4 of 74 sheep fetal brain tissues (Moreno et al., 2012), 48 of 75 sheep fetal brains (Shahbazi et al., 2019), 9 of 111 sheep fetal brains (Partoandazanpoor et al., 2020), and 9 of 53 sheep fetal brains (Hurtado et al., 2001). In this study, the brains of 50 aborted sheep fetuses were examined by conventional PCR method and *Toxoplasma*

gondii DNA was detected in 11 (22%) tissues. This result was lower than the findings of some researchers (Öztürk et al., 2002; Çiçek et al., 2004; Oncel et al., 2005; Ueno et al., 2009; Ibrahim et al., 2017) and higher than the results of some studies (Aköz et al., 2009; Çakmak and Karatepe, 2017).

In studies to determine the prevalence of *N.caninum* in sheep in the world; 10.1% in Spain (Panadero et al., 2010), 16.8% in Greece (Anastasia et al., 2013), 8.81% in Brazil (Ueno et al., 2009), 27.7% in Pakistan (Nasir et al., 2012) and 1.53% in Iran (Ezatpour et al., 2015) prevalence was reported. In Türkiye, studies investigating the prevalence of *N. caninum* in sheep are quite limited. Positive rates of 12.4% in Adana (Ekşi et al., 2018), 0% in Van (Har and Başbuğan, 2019), 0% in Elazığ (Özkaraca et al., 2016) and 2.1% in Kars (Gökçe et al., 2015).

In studies conducted on aborted fetal brain tissue, it was reported that *N. caninum* DNA was detected in 4 of 20 aborted fetal brain tissues (Hässig et al., 2003), 5 of 74 sheep fetal brain tissues (Moreno et al., 2012), 3 of 18 fetuses (Howe et al., 2008), 18.9% of 74 fetuses (Hughes et al., 2006). All tissue samples examined in this study were negative for *Neospora caninum*. This result is similar to the findings of researchers (Özkaraca et al., 2016; Har and Başbuğan, 2019). The reasons for the differences between the studies include geographical location, climate, sheep breed, end-host prevalence and the tests used.

Conclusion

Based on the results of this study, it is possible to say that *T. gondii* is an important abortion agent in sheep in this region. It was also concluded that PCR method is an important tool in the diagnosis of protozoal abortion agents. Although *N. caninum* was not detected in this study, larger scale studies are recommended. Furthermore, the results of this study are poised to have a significant impact on increasing awareness among veterinarians, researchers, and farmers regarding the epidemiology and prevalence of *T. gondii* and *N. caninum* in the Muş region. Nonetheless, additional investigations would be advantageous in elucidating the diverse genotypes of *T. gondii* and their potential correlation with abortion and other reproductive challenges in the sheep population. This will make a substantial contribution towards a more thorough comprehension of the influence of these factors on pertinent animals.

Conflicts of interest

Authors state no conflict of interest.

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