



Original Article

The seroprevalence of *Toxoplasma gondii* infection among horses in Northwest Iran

Yaser Jafari-Khataylou^{1*}, Abbas Imani-Baran¹, Hamid Akbari²

1- Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

2- Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

*Corresponding author: y.jafari@tabrizu.ac.ir

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Abstract

Toxoplasmosis is an important health concern for people and farm animals, leading to many studies on its effects on these populations. Despite this, there is a lack of research on the prevalence of *Toxoplasma gondii* infection among horses in Iran. This study aims to determine the seroprevalence of toxoplasmosis using the Modified Agglutination Test (MAT) in northwest Iran horses. In this study, blood samples were taken from 385 horses in Ardabil, East Azerbaijan, and West Azerbaijan provinces. Data regarding the age, gender, and location of each horse was documented to assess their impact on the prevalence of toxoplasmosis. The data was analyzed using the chi-square test, with a significance level of $p < 0.05$. Of the 385 samples, 34 (8.8%) were positive for *Toxoplasma gondii*. Among the 271 male and 114 female horses, 24 (8.9%) and 10 (8.8%) were seropositive, respectively. Of the 116 horses under and 269 over five years old, 15 (12.9%) and 19 (7.1%) were seropositive, respectively. The prevalence rates in Ardabil, East Azerbaijan, and West Azerbaijan were 11.7% (18 out of 154), 5.1% (7 out of 136), and 9.5% (9 out of 95), respectively. According to the statistical analysis, there were no significant differences in the prevalence of *Toxoplasma gondii* infection based on age, gender, or sampling location. This study is the first extensive report on the toxoplasmosis seroprevalence among horses in northwest Iran. The results provide important information that can help control and prevent the spread of toxoplasmosis.

Keywords: Horse, Modified Agglutination Test (MAT), Seroprevalence, *Toxoplasma gondii*

Introduction

Toxoplasma gondii is one of the most major and common zoonotic protozoa in the world. Domestic and wild cats are the definitive hosts, and almost all warm-blooded animals are intermediate hosts of this parasite (Marzok et al., 2023; Dubey et al., 2020). The primary modes of transmission for definitive and intermediate hosts are through the consumption of water or food contaminated with

oocysts, or meat that contains cysts. Congenital transmission is also possible (Ouslimani et al., 2019; Zhang, 2018). Toxoplasmosis in horses is usually subclinical and asymptomatic, unlike in other hosts. However, some horses may occasionally experience encephalomyelitis, fever, retinal degeneration, ataxia, or abortion/stillbirth if pregnant (Miao et al., 2013). In addition, *Toxoplasma* cysts have been found in the muscles

and eyes of horses as well as in the brain tissue and fetal membranes of aborted foals (Shappan and Ghazy, 2007). Horses are at high risk for *Toxoplasma* infection by ingesting oocysts while grazing on pastures. This has led to high levels of seroprevalence being reported in some regions worldwide (Tassi, 2007). A systematic review found that the global pooled seroprevalence of *T. gondii* infection in horses was 11.29% (Li et al., 2020). Cases of human toxoplasmosis have been reported in Europe after consuming horse meat (Pomares et al., 2011). The consumption of infected horse meat by carnivores can also lead to the parasite survival and spread in the environment (Tassi, 2007). As one of the most important intermediate *Toxoplasma gondii* hosts, horses can play a crucial role in the epidemiology and this protozoan life cycle. Therefore, in areas with a close relationship between horse and human populations, it is necessary to investigate the toxoplasmosis prevalence in horses (Zhang et al., 2018). *Toxoplasma gondii* triggers both cell-mediated and humoral immune responses. Cellular immunity, involving macrophages, T lymphocytes, natural killer cells (NKC), and cytokines, is a crucial component in defending against *Toxoplasma* invasion. In addition to their role in the immune response, antibodies are important in diagnosing *Toxoplasma gondii* infection (Filisetti and Candolfie, 2004). Different types of antibodies, like IgM, IgA, IgE, and IgG₂, can be found in human patients. Identifying these antibodies is an effective method for distinguishing between newly infected individuals and those with chronic infections (Dupont et al., 2012). Toxoplasmosis is detected using histopathological, molecular, and serological approaches, including MAT. This serological method exhibits good sensitivity and specificity in identifying *Toxoplasma* antibodies, making it a widely utilized technique for identifying both naturally and experimentally infected animals (Wu et al., 2011). Numerous investigations have been carried out concerning toxoplasmosis in both human and domestic ruminants in Iran (Kalantari et al., 2023; Deljavan et al., 2022; Chaechi Nosrati et al., 2020;

Gharekhani and Yakhchali, 2020; Mousavi-Hasanzadeh et al., 2020; Izadyar et al., 2019; Tavassoli et al., 2013). However, studies on equine toxoplasmosis are limited and have been conducted in some areas such as Qazvin, Hamedan, North Khorasan, Khuzestan, and Urmia (Razmi et al., 2016; Gharekhani et al., 2015; Tavalla et al., 2015; Raeghi et al., 2011; Hajjalilo et al., 2010). The infection caused by *Toxoplasma gondii* is of significant importance due to its impact on human public health and economic losses within the livestock population. Therefore, this study investigates the prevalence of *Toxoplasma gondii* infection among horses in northwest Iran, as they can potentially act as intermediate hosts, contributing to the completion of the parasite's life cycle.

Materials and methods

Blood sampling

This cross-sectional study was conducted between April and August 2015 in the northwest provinces of Ardabil, East Azerbaijan, and West Azerbaijan in Iran. Given the lack of prior studies on the prevalence of equine toxoplasmosis in these regions, a conservative estimate of 50% expected prevalence, a 5% standard error, and a confidence interval of 95% were used to determine the sample size using the formula for cross-sectional studies (Thrusfield & Brown, 2017). 385 horses from three provinces in northwest Iran were chosen for sampling, and ten cc blood samples were drawn from their jugular veins. During sampling, information regarding age (grouped as over or under five years), gender (male or female), and sampling location (Ardabil, East Azerbaijan, or West Azerbaijan provinces) was recorded to examine the impact of these variables on the prevalence of *Toxoplasma gondii*. Blood samples were gathered in plain tubes without anticoagulants (Mediplus, free additive tube, Sunphoria Co., Ltd, China) and transported to the laboratory on ice. After centrifugation at 5000 rpm for 5 min, the sera were extracted and stored at -20°C for subsequent testing.

Modified Agglutination Test (MAT)

The RH strain antigens of *Toxoplasma gondii* were obtained from the Razi Vaccine and Serum Institute, Shiraz branch, to perform the MAT test. The *Toxoplasma gondii* antibodies presence was assessed using the MAT test, following the method described by Tavalla et al. (2015). Sera were diluted in double dilutions, ranging from 1:20 to 1:320, using a phosphate buffer saline solution (PBS=7.2) with 0.2 M mercaptoethanol. 50 microliters of each dilution were added to the wells of a 96-well U-shaped ELISA plate. Then, 50 microliters of formaldehyde-preserved tachyzoites were added to each serum dilution. The wells were thoroughly mixed and covered by pipetting several times before incubating at 37°C for 24 hours. After this period, if a layer of agglutinated parasites formed in the wells at dilutions ranging from 1:20 to 1:320, the test was considered positive. Positive and negative controls were included in the experiment.

Statistical analysis

The data were initially subjected to a descriptive analysis. Subsequently, the chi-square test of independence was employed to examine the relationship between the prevalence of equine

toxoplasmosis and the independent variables of age (grouped as over or under 5 years), gender (male or female), and sampling location (Ardabil, East Azerbaijan, or West Azerbaijan provinces). All analyses were conducted using SPSS software (Version 22.0, SPSS Inc., Chicago, IL, USA), with *p*-values less than 0.05 considered significant.

Results

The findings of this study are presented in Table 1. Of the 385 samples analyzed, 34 (8.8%) tested positive for *Toxoplasma gondii* infection. Among the 271 male horses, 24 (8.9%) were seropositive, while among the 114 female horses, 10 (8.8%) were seropositive. Regarding age, 15 (12.9%) of the 116 horses under five years old were seropositive, and 19 (7.1%) of the 269 horses over five years old were seropositive. The prevalence rates for toxoplasmosis in different regions were as follows: 11.7% (18 out of 154) in Ardabil, 5.1% (7 out of 136) in East Azerbaijan, and 9.5% (9 out of 95) in West Azerbaijan. Statistical analysis revealed insignificant differences in the toxoplasmosis prevalence based on age, gender, or sampling location.

Table 1- The prevalence of toxoplasmosis among horses and the association between *Toxoplasma gondii* infection and age, gender, and sampling location

Variables		Seropositive (%)	Seronegative (%)	Total (%)	<i>p</i> -value	Chi-square
Age	<5 y	15 (12.9)	101 (87.1)	116 (100)	<i>p</i> >0.05	3.47
	>5 y	19 (8.3)	250 (91.7)	269 (100)		
	Total	34 (8.8)	351 (91.2)	385 (100)		
Gender	Female	10 (8.8)	104 (91.2)	114 (100)	<i>p</i> >0.05	0.001
	Male	24 (8.9)	247 (91.1)	271 (100)		
	Total	34 (8.8)	351 (91.2)	385 (100)		
Sampling location	Ardabil	18 (11.7)	136 (88.3)	154 (100)	<i>p</i> >0.05	3.90
	East Azerbaijan	7 (5.1)	129 (94.9)	136 (100)		
	West Azerbaijan	9 (9.5)	86 (90.5)	95 (100)		
	Total	34 (8.8)	351 (91.2)	385 (100)		

Discussion

Toxoplasmosis is an important zoonotic disease prevalent in most regions of Iran. According to the clinical findings of our study, we found that the

Toxoplasma gondii infection prevalence among horses in northwest Iran was 8.8%. A meta-analysis study examining the global prevalence of toxoplasmosis in horses revealed a pooled

prevalence rate of 11.29%, similar to the rate found in our study (Li et al., 2020). In Iran and other parts of the world, there are fewer studies on the prevalence of toxoplasmosis in horses compared to other domestic animals and humans. A survey of 52 horses in Qazvin found a 71.2% infection rate of *Toxoplasma gondii* using the MAT (Hajjalilo et al., 2010). In Hamadan province, the infection rate of *Toxoplasma gondii* in 120 horses and 100 donkeys was 13.3% and 47%, respectively, using the same method (Gharekhani et al., 2015). Another study in the North Khorasan province on 100 Turkmen horses reported a 14% *Toxoplasma gondii* infection prevalence using the Indirect Fluorescent Antibody Test (IFAT) (Razmi et al., 2016). In Khuzestan province, a study on 235 Arabian horses found a 48.5% prevalence of toxoplasmosis using the MAT method (Tavalla et al., 2015). In Urmia, the infection rate of *Toxoplasma gondii* in 26 horses was 11.5% using the same method (Raeghi et al., 2011). The infection rate of *Toxoplasma gondii* in horses in different parts of Iran varies from 11.5% to 71.2%, indicating that the rate found in our study is lower than those reported in other studies. The *Toxoplasma gondii* infection prevalence in horses and donkeys was studied in several countries using different methods. In Turkey, using the Sabin Feldman Dye Test (SFDT) method, the prevalence was 42.8% in 159 horses and 92.2% in 51 donkeys (Sertel and Kirbas, 2022). In Pakistan, the latex agglutination method (LAT) showed a 23.5% infection rate in 183 horses (Qamar and Alsayeqh, 2023). In Egypt, the ELISA method showed a 16.2% prevalence in 420 horses (Marzok et al., 2023). A cross-sectional study of 1399 equids (horses, donkeys, and mules) in Italy, Spain, Great Britain, and Ireland using the MAT method showed an overall prevalence of 18.9%. By country, the prevalence was 27.1% in Italy, 16.6% in Spain, 12% in the United Kingdom, and 7% in Ireland. The infection rate by species was 12.8% in horses, 43.7% in donkeys, and 28.9% in mules (Cano-Terriza et al., 2023). Most studies on the prevalence of toxoplasmosis in equids have found that donkeys have a higher infection rate than other

equines. The lower prevalence of toxoplasmosis found in this study could be partially attributed to the lack of investigation into *Toxoplasma gondii* prevalence among donkeys and mules. However, several other factors may also account for variations in prevalence across different studies. These factors include sample size, sampling time, horse-rearing practices, age, gender, animal sensitivity, environmental contamination, local cat population, the accuracy of serological testing methods, climatic conditions, as well as the health and management practices employed on the farm (Zhang et al., 2018; Tassi, 2007).

The results of this study showed that there is insignificant difference between gender and the toxoplasmosis prevalence in horses, which is consistent with the results of other studies (Sertel and Kirbas, 2022; Saqib et al., 2015). However, it has been shown in another study that the prevalence of *Toxoplasma gondii* infection in mares is significantly higher than in stallions (Marzok et al., 2023). This study found a higher prevalence of toxoplasmosis in Ardabil province compared to East and West Azerbaijan provinces, but the difference was not statistically significant. The relatively humid and moderate climate of Ardabil province may create favorable conditions for the life cycle of *Toxoplasma gondii* (Zhang et al., 2018). *Toxoplasma gondii* is an opportunistic protozoan, and its cysts can persist throughout the life of a host. Therefore, older hosts typically have a higher prevalence of toxoplasmosis than younger hosts (Meng et al., 2015; Zhang et al., 2015). However, this study found a higher prevalence in horses under five years old, although the difference was insignificant. This is consistent with other studies (Bai et al., 2017; Zhang et al., 2015) and may be due to the incomplete immune system in young horses. Additionally, young horses are more active and may have more opportunities to be exposed to *Toxoplasma gondii* oocysts, which could contribute to the higher prevalence of toxoplasmosis in this age group (Zhang et al., 2018).

Conclusion

Using the MAT method, this study found that the prevalence of *Toxoplasma gondii* infection among horses in northwest Iran was 8.8%. These findings provide valuable insights to policymakers on the *Toxoplasma gondii* epidemiology in the region, allowing them to assess the public health risks of this zoonotic disease and implement suitable control and preventive measures. Moreover, further research is suggested to investigate the prevalence of toxoplasmosis in other equines, such as mules and donkeys, and club horses.

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Conflict of Interests

There is no conflict of interest.

Ethical approval

Our research was conducted in accordance with the guidelines and standards of the Animal Research Ethics Committee of the University of Tabriz, which approved our research protocol (No. 154/20160413).

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Corrected Proof
