



Evaluation of the Effects of *Toxoplasmosis* in Small Ruminants on the Epidemiology of Human *Toxoplasmosis* by Indirect ELISA in Kaleybar Region, North West of Iran

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Abstract

Introduction: *Toxoplasma gondii* is an intracellular protozoan that causes toxoplasmosis in humans and domestic animals. It causes abortion, especially in small ruminants. Sheep and goats have a significant role in the maintenance and survival of the parasite as well as its transmission to humans. Therefore, the aim of this study was to evaluate the seroprevalence of toxoplasmosis in small ruminants in Kaleybar region, East Azerbaijan province, using Indirect ELISA.

Materials and Methods: In this study, blood samples of 270 sheep and goats were collected from October 2019 to December 2019 and anti-*Toxoplasma* IgG antibodies in sera were evaluated using indirect ELISA. Data analysis was performed using chi-square test in SPSS version 24.0. A *P* value of less than 0.05 was considered statistically significant.

Results: Totally, 26 of 270 (9.62%) animals had anti-*Toxoplasma gondii* antibodies in their sera. Analysis showed that sheep were more susceptible to toxoplasmosis than goats and 21 (10.5%) sheep and 5 (7.14%) goats were seropositive for *T. gondii*; however, there was no statistically significant difference (*P*=0.413). The highest frequency (15.87%) of infection was observed in sheep and goats aged 3–4 years, respectively. Moreover, the rate of infection was higher in female (22/205) animals than in males (4/65); however, the relationship between gender and age and the frequency of positive samples was not significant (*P*=0.276 and *P*=0.121).

Conclusions: The results of this study indicated that the seroprevalence of *Toxoplasma gondii* infection in small ruminants of the Kaleybar region (North West of Iran) is relatively low compared to other parts of the country. Further studies should be conducted on the rate of infection in definitive hosts of *T. gondii* in the region as a potential source of human infection.

Keywords: Epidemiology, Toxoplasmosis, ELISA, Kaleybar region, Iran

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Introduction

Toxoplasma gondii, an obligate intracellular protozoan parasite (phylum Apicomplexa, subclass Coccidia), infects many different types of hosts and different host tissues, and it is the causative agent of toxoplasmosis (1). The parasite is only able to complete its sexual life cycle in felids as definitive hosts and oocysts produced are excreted through the feces of the host. Sporulated oocysts contaminate the surrounding environment and pastures (2,3). Toxoplasmosis is a serious health problem in many parts of the world (4).

According to studies, the global rate of human toxoplasmosis is between 1.3% and 98.4% (5), in addition to the fact that more than 30% of Iranians are infected with *Toxoplasma gondii* (6).

Toxoplasmosis is one of the most important parasitic zoonotic diseases, so it has been the most commonly studied coccidian parasite (7).

Sheep and goats acquire the infection after contact with infected cats and ingesting oocytes scattered in the environment (8) or by ticks (9). In the intermediate hosts, even the ingestion of 10 sporulated oocysts can cause infection.

In addition, lambs and goat kids can be exposed to *T. gondii* by consuming the milk of ewes and goats naturally infected with the parasite and the establishment of *T. gondii* infection in the muscles of lambs and even eating small pieces (5–10 g) of their uncooked meat can transmit the infection to humans (10). Human infection occurs in a variety of ways, such as ingesting sporulated oocytes



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released in water and food or by consuming undercooked or uncooked meat containing viable *T. gondii* cysts (10-12), as well as vertical transmission (13). Toxoplasmosis causes serious complications in humans, especially neonates, as well as immunocompromised individuals (14, 15).

Generally, small ruminants are highly susceptible to *T. gondii* infection. The infection is not associated with any specific symptoms (16); however, the main problem for pregnant animals is that the parasite causes early embryonic death, foetal resorption, mummification, stillbirth (10,17) that lead to significant economic and reproductive losses (18,19). In sheep, infection with the parasite in early pregnancy poses a greater risk to the fetus compared to late pregnancy (20). In many countries including Iran, breeding livestock such as sheep and goats is booming and the milk and meat of these animals are widely used as food sources (21). Toxoplasmosis of goats and sheep is affecting the lives of people whose source of income is the production of ruminant meat and milk (22).

Considering the role of sheep and goats in maintaining and transmitting the infection to humans, research on the detection of *T. gondii* infection in livestock for human consumption is very important (2).

Many studies on toxoplasmosis in small ruminants have been performed in different regions of the world.

According to previous studies, the prevalence of toxoplasmosis ranges between 3% and 98% (2) in sheep herds and it ranges from 5.6% to 62% in goats around the world (2). In seroprevalence studies conducted on sheep and goats in different areas of Iran for *T. gondii*, seropositivity was determined to be between 3.3% and 36.8% in sheep (23) and 27% in goats, respectively (19). In general, various methods are used to diagnose *toxoplasmosis*, including direct methods (such as immunohistochemistry, histopathology, and PCR) and indirect methods (such as indirect fluorescent antibody test, Sabin-Feldman dye test, ELISA, and hemagglutination test) (24). In live animals, the ELISA method can be used as a suitable test to detect antibodies against *T. gondii* (25). Considering the economic importance of abortion in sheep and goat herds and the possibility of transmitting the disease to humans via the consumption of livestock products, this study was performed to evaluate the seroprevalence of toxoplasmosis in small ruminants for the first time in Kaleybar region, East Azerbaijan province.

Materials and Methods

Study Area

Kaleybar region is located in the northeast of East Azerbaijan province with a population of about 48837, at 38° 52' North latitude and 47° 25' East longitude from the meridian, and its altitude from sea level is 1144 m. Its area is about 3,702 km² with a mean annual rainfall of 372 mm and its mean temperature varies from 2.2 to 23.9°C. The climate of Kaleybar is cold and temperate. Agriculture

and animal husbandry are flourishing in this region.

Blood Sample Collection

Blood samples of 270 randomly selected animals (200 sheep and 70 goats) were collected from October to December 2019. For the evaluation of seropositivity, 5 mL of blood was drawn from the jugular vein using sterile disposable syringes. After blood sampling, specimens were centrifuged at 3000× for 10 minutes and immediately after the separation of serum, these serums were kept in sterile microtubes at -20°C until use for serological examinations. Serum samples were evaluated in the laboratory by ELISA.

Indirect ELISA

Indirect ELISA method was used according to the protocol provided by Izadyar et al in 2019 (26) with some modifications. In brief, 96-well Strip Microplate (SPL, Korea) was coated with 100 µL of antigen (90 µg/mL) in buffer (NaHCO₃ – Na₂CO₃, pH 9.6) and the overnight incubation was performed at 4°C. After the wells were washed thrice with TBS, the blocking was performed at room temperature for 2 hours using 1% bovine serum albumin in PBS. The wells were then washed with TBS three times as mentioned above and then 100 µL of serum samples from sheep and goats and from positive and negative controls were added to each well after dilution and incubated at 37°C for 1 hour. After incubation, the wells were washed and 100 µL of 1:5000 dilution of HRP (rabbit anti-sheep/goat IgG-horseradish peroxidase) conjugated antibody (Razi BioTech, Iran) was added to each well and incubated for 1 hour at 37°C. Eventually, the wells were washed five times with TBS, and 50 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (Razi BioTech, Iran) was added and incubated in the dark for 20 minutes at room temperature. To stop the reaction, 25 µL of 2 M H₂SO₄ (stop solution) was added to each well after 10 minutes. Optical density (OD) at 450 nm was measured using ELISA reader (Stat Fax, USA).

Statistical Analysis

Statistical analysis of the data was performed using chi-square test in SPSS version 24.0.

Results

Totally, the sera of 270 animals (200 sheep and 70 goats) were examined and 10.5% (21/200) of sheep and 7.14% (5/70) of goats were seropositive for *T. gondii*; however, no statistically significant relationship was found between animal species and serum positivity ($P=0.413$).

Serological results are summarized in Table 1.

Considering the age of animals, the highest and lowest number of positive cases were found in the age groups of 3-4 years (15.87%) and 1-2 years (5.26%), respectively; however, the relationship between age and frequency of positive cases of toxoplasmosis was not significant

Table 1. Prevalence of *T. gondii* in Small Ruminants of Kaleybar Region (Northeast of East Azerbaijan province)

Risk factors	Total	No. of Positive samples	No. of Negative samples	P Value
Species				0.413*
Sheep	200	21 (10.5%)	179 (89.5%)	
Goat	70	5 (7.14%)	65 (92.85%)	
Total	270	26 (9.62%)	244 (90.37%)	
Age				0.121
1-2 years	57	3 (5.26%)	54 (94.73%)	
>2 years	150	13 (8.66%)	137 (91.33%)	
3-4 years	63	10 (15.87%)	53 (84.12%)	
Gender				0.276
Female	205	22 (10.73%)	183 (89.26%)	
Male	65	4 (6.15%)	61 (93.84%)	

*Statistically significant at $P < 0.05$.

($P=0.121$).

As shown in Table 1, the infection rate of the female (22/205) animals was higher than that of males (4/65); however, there was no significant relationship between gender and the frequency of positive samples ($P=0.276$).

Discussion

Toxoplasma gondii is a zoonotic intracellular parasite that infects many warm-blooded animals and humans (16).

It is estimated that anti-*Toxoplasma* antibodies are present in one-third of the people in the world (7,27). The ingestion of the meat of farm animals infected with *T. gondii* tissue cysts as well as drinking the milk of infected goats can cause human infection (28).

Since the consumption of meat has a special role in providing dietary protein in Iran, eating uncooked or undercooked meat is an important risk factor for parasitic infections in humans, so the seropositivity of *T. gondii* is lower in people who consume thoroughly cooked meat than in people who consume it raw (29). Among meat-producing domestic animals, sheep and goats are more susceptible to *T. gondii* infection than other livestock (7,30).

In Iran, sheep and goats are mostly raised by free grazing, so these animals increase the chance of cyst formation in their tissues by ingesting sporulated oocytes scattered in the environment.

Various studies revealed that the prevalence of toxoplasmosis in sheep and goats in different regions of Iran ranges from 33.62% (153/455) to 36.41% (130/375) (26), 24.8% to 10.6% (31), 29.5% to 18.8% (32), 16.7% to 8.08% (33), 13.8% to 13.1% (34), 30% to 15% (35), 3.3% to 1.7% (36), 12.2% to 4.4% (37), and 30% (60/200) to 15% (30/200) (38), respectively. According to reports, anti-*Toxoplasma* antibodies are present in 39.3% of Iranians and 43% of pregnant women also have anti-*T. gondii* antibodies; therefore, 57% of these people were

susceptible to acute toxoplasmosis (6). According to previous studies conducted in different countries of the world, the overall estimated prevalence was reported to be 49.35% (3.0–95.7%) (39) for sheep and 22.9% (5.6–89.0%) (2) for goats.

In this study, the serological prevalence of toxoplasmosis in sheep and goats in Kaleybar region (East Azerbaijan province) was detected to be 10.5% and 7.14%, respectively. In addition to the method used to detect the infection, weather conditions, gender, age, cat density, as well as livestock breeding can affect the prevalence of *T. gondii* (23,40). A previous study performed in Tabriz (located in north-western Iran) showed that the prevalence of *T. gondii* in sheep and goats was 24.8% and 10.6% (31), respectively, which is higher compared to the present study. This may be related to the reasons mentioned above.

In the present study, it was found that the frequency of infection is higher in animals aged 3-4 years (15.87%) than those aged 1-2 and 2-3 years (5.26% and 8.66%). However, no significant relationship was found between age groups and frequency of infection ($P=0.121$).

Numerous studies have reported higher rates of *T. gondii* infection in adult animals than in younger ones (26,41-43), which is similar to the findings of the present study. This can be related to the exposure of adult animals to predisposing factors compared to younger animals (44, 45).

Some studies including those conducted by Bahrieni et al on small ruminants in southeastern Iran (46), de Moura et al on goats in Brazil (47), and Gebremedhin et al on sheep in Ethiopia (48) show that susceptibility to toxoplasma is higher in females than in males. However, in the current study, no statistically significant relationship was found between gender and the seroprevalence of toxoplasmosis (0.276), which is consistent with many other studies (49-53). It seems that gender does not appear to play an important role in toxoplasmosis, and in epidemiological studies, it is considered as a confounding factor (54).

Conclusions

The findings of this study showed that the seroprevalence of toxoplasmosis in small ruminants in the Kaleybar region is relatively low compared to other parts of Iran, which may be due to climatic conditions and the way of keeping and breeding livestock. In addition, in this study, it was found that the rate of infection and susceptibility of sheep is higher compared to goats, which is important given that consumption of less cooked or uncooked mutton increases the risk of human infection with *T. gondii*.

High seropositivity in adult livestock indicates the acquired contamination of the livestock by horizontal transmission as well as exposure to most of these intermediate hosts and oocytes scattered in the

environment and pasture. Numerous factors affect the epidemiology of toxoplasmosis in humans and animals, such as livestock breeding, spread of oocytes by infected cats in the environment, favorable situations for oocyte sporulation (such as climate, etc.), the hygiene status of slaughterhouses, food processing technology, and feeding habits.

Although in many cases, the source of human infection is unknown, preventive measures such as cooking meat thoroughly (67°C), freezing it before consumption (-12°C and less), and improving kitchen hygiene can prevent the transmission of infection by food. In some cases, the implementation of educational programs for farmers and other members of society, especially women, has an effective role in reducing the rate of infection. Further studies are required to investigate the infection of domestic and stray cats (a potential cause of oocyte proliferation) with the parasite and determine the percentage of contamination of meat consumed (an important factor in the infection of people, especially people at risk), which can help assess the overall prevalence of toxoplasmosis in this area.

Conflict of Interests

The authors declare no competing interests.

Ethical Issues

In this research, ethical considerations have been fully observed.

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Authors' Contributions

YF did the writing and editing of the manuscript. BKA designed and did the data collection. MA designed the study and did data collection and statistical analysis.

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