**Original Article**

Serological Survey of Antibodies to *Toxoplasma Gondii* in Sheep in North-West of Iran

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**ABSTRACT**

**Objective:** Sera from 186 sheep (93 rams and 93 ewes) from Tabriz, North-West of Iran were evaluated for antibodies to *Toxoplasma gondii* using a commercial ELISA kit.

**Methods:** The animals were divided into two age groups (< 2 years, and > 2 years).

**Results:** Antibodies to *T. gondii* were found in 13 of 186 sheep (6.98%). Infection rate in ewes was higher than rams but this difference was not significant. The seroprevalence of *Toxoplasma* in sheep >2 years old was significantly higher than in other age groups.

**Conclusions:** Results of this study indicate a low prevalence of *T. gondii* in sheep tested in Tabriz by ELISA.

**1. INTRODUCTION**

*Toxoplasma gondii* is known to be highly associated with ovine abortion and neonatal loss worldwide. Clinical ovine toxoplasmosis occurs following primary infection in pregnant sheep primarily as a result of the ingestion of sporulated oocysts. Affected animals subsequently abort or produce stillborn and/or weakly lambs, often including small, mummified fetuses. Also sheep are important to the economy of many countries because they are a source of food for humans. Sheep are commonly infected with the protozoan parasite, *T. gondii*. Whilst the sexual life cycle of the parasite is confined to cats (the definitive host), the asexual cycle occurs in many warm blooded animals, although clinical disease is exhibited only in certain species. The diagnosis of *T. gondii* abortion is based on the detection of specific antibodies in the adult population by indirect fluorescent antibody test (IFAT) or latex agglutination test (LAT) confirmed by characteristic histological lesions in the placenta and brain of aborted fetuses (Dubey, 2009; Masala et al. 2003). However, inconclusive diagnoses for ovine abortion specimens are frequently reported by diagnostic veterinary laboratories despite detailed investigation and testing (Duncanson et al. 2001). This coccidian protozoan is widely prevalent in Iran (Ghorbani et al. 1993; Hoghooghi-rad & Afraa, 1993; Hashemi-fesharki, 1996; Navidpour & Hoghooghi-rad, 1998). Most epidemiological investigations on toxoplasmosis in Iran have been based on the results of serological examination using Sabin–Feldman dye test or LAT both of which also shown the presence of antibodies against *T.gondii*. The objective of this study was to carry out a serum epidemiological study of ovine toxoplasmosis in Tabriz, North-West of Iran.

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2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Tabriz (northwest Iran). Tabriz is located in the East-Azarbajian province (36°43′–39°25′N and 45°3′–48°19′E). The region is mountainous, with an altitude of 1351.4 m. The climate is temperate with relatively hot and dry summers and cold winters.

2.2. Animals

Blood samples from were obtained from 186 sheep (93 rams and 93 ewes) in Tabriz between December 2012 and April 2013. Sera were extracted from 5 mL venous blood samples, by centrifugation at 2000g for 10 min, and was stored at -20°C prior to testing for antibodies to T. gondii using a commercial ELISA test kit (Euro Immune, Germany). The age of animals was recorded. The animals were divided into two age groups (< 2 years, and > 2 years).

2.3. Antigen Preparation

Tachyzoites of Toxoplasma 5×10⁶ (Rh – strain from Pasteur’s Institute) were lysed by a lysis buffer (0.15 M NaCl, 0.05 M Tris-HCl pH=7.2, 0.1% sodium dodecyl sulphate, 1% Triton X-100, 0.1% Sodium deoxycholate, 10G4 M PMSF) and after a short spinning (8000 g at room temperature for 2 minutes) the supernatant (500 μl) aliquoted in 50 μl volumes and stored at -20°C. We considered each μl of the antigen preparation equivalent to the protein content of 10⁴ tachyzoites.

2.4. Positive control preparation

Two adult ewes (Tabriz University, Tabriz, Iran) were immunized subcutaneously with 1 ml prepared tachyzoite antigen, 3 times with 2- weeks' interval. The first immunization was performed using emulsion of 1 ml prepared tachyzoite antigen and 0.5 ml complete Freund’s adjuvant and the second and third immunization was performed with emulsion of 1 ml prepared tachyzoite antigen and 0.5ml incomplete Freund's adjuvant (Raj et al. 2004). Two weeks after the third immunization, serum was prepared from the immunized ewes and stored at -20°C until used.

2.5. Evaluation of positive serum

One micro liter of prepared whole tachyzoite antigens were dotted on the one corner of each 1x1 cm square nitrocellulose membrane. As negative control, purified IgY was used and dotted on other site of the square diagonal to the first dots. Positive serum from the immunized sheep with Toxoplasma tachyzoites antigen was used in different dilutions (1/10, 1/100, 1/1000, 1/10000) for screening. Briefly, the dot blotted nitrocellulose membranes were incubated in 3% skim milk in TBS buffer (20mM Tris base and 0.15 M NaCl in H2O) containing 0.05% Tween 20 for 1h at RT to block the free binding sites on the membranes. Subsequently, the membrane was incubated in the prepared positive serum in different dilutions (1/10, 1/100, 1/1000, 1/10000 in TBS containing 0.05% Tween 20) for 1h at room temperature (RT). The membrane was then washed three times with TBS containing 0.05% Tween 20 for 5 min at RT. After that hors eradish-conjugated rabbit antisheep IgG (Dako, Denmark) (1:2000) were added to the washed membrane and incubated for 1h at RT. After incubation, the membrane was washed three times as described above. The positive reaction was developed using DAB (Sigma, USA) as substrate under visual observation within 5 min.

2.6. ELISA for Detection of Antibodies to T. gondii

All the sera were tested for serodiagnosis of T.gondii by using a commercial ELISA (Anti-Toxoplasma gondii ELISA- IgG kit (human), Euro Immune, Germany). In per kit one positive control serum with one repetition and 2 negative control serums with 2 repetitions are used. To estimate the antibody titer, the positive sera were applied in the same ELISA with different dilutions: 1:100, 1:200 and 1:400. The optical density (OD) of the samples was measured at 450 nm using an ELISA reader.

2.7. Statistical analysis

A chi-squared test was used to analyze the association between infection to Toxoplasma with age and gender of sheep. For the statistical analysis SAS (Version 9.1) software was also used. For interpretation of results Mean±SE is used.

3. Results

From 186 sheep sampled, 13(6.98%) were seropositive to T.gondii. Infection rate in ewes was higher than rams but this difference was not significant. The seroprevalence of Toxoplasma in sheep >2 years old was higher than in other age group, and this difference was significantly (Table 1).

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. tested</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>93</td>
<td>7(7.52)</td>
</tr>
<tr>
<td>Female</td>
<td>93</td>
<td>6.45</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2yr</td>
<td>101</td>
<td>10(9.9)</td>
</tr>
<tr>
<td>&gt;2yr</td>
<td>85</td>
<td>3(3.52)</td>
</tr>
<tr>
<td>Overall</td>
<td>186</td>
<td>13(6.98)</td>
</tr>
</tbody>
</table>

Table 1. Seroprevalence of T. gondii in sheep in Tabriz
4. Discussion

The aim of this study was to study the seroprevalence of Toxoplasma gondii in Tabriz, northwest Iran. T. gondii is considered to be an important abortifacient in sheep and goats worldwide, but little is known about protozoan-induced abortion in Iranian livestock. In the present study, the prevalence of antibodies against T. gondii in sheep was 6.98%. Most epidemiological investigations on toxoplasmosis in Iran have been based on the results of serological examination using Sabin–Feldman dye test or LAT both of which also show the presence of antibodies against T. gondii. Ghazaei (2006) reported 30% prevalence of toxoplasmosis in the Ardabil province (northern Iran). Hashemi-Fesharki (1996) reported prevalence levels of 24.5% and 19.5% in sheep and goats in various parts of Iran using indirect hemagglutination and LATs. In a survey by Hamzavi et al. (2007) in Kermanshah (western Iran), 22.55% of the sheep from a single abattoir were positive by IFAT. Using IFAT, Sharif et al. (2007) reported 35% prevalence of toxoplasmosis in Mazandaran. In another survey in Meshkin Shahr, 59% of sheep were found positive for toxoplasmosis by IFAT (Keshavaz et al. 2007). In northern and northwestern Iran, two surveys showed seroprevalence rates of 32.5% and 17.7% in sheep and goats and 21% in sheep, respectively (Ghorbani et al. 1993; Raeghi et al. 2011). Hoghooeghi-Rad and Afraa (1993) showed a seropositivity of 13.8% and 13.1% in sheep and goats, respectively, in Ahwaz. Asgari et al. (2011) in their study in the Fars province (southern Iran) reported that 20.24% of bovine and 33.3% of sheep tested positive by the IFAT and PCR, respectively. Khammohammadi (2011) showed that 56.8% of the sheep in Tabriz were positive for T. gondii by using the Sabin–Feldman Dye Test. These differences in the prevalence of toxoplasmosis in various regions of Iran may be due to different diagnostic methods, management, and/or procedural standards in breeding livestock, different climates, and the density of cats. Among these factors, the use of a different diagnostic method is probably the most differentiating. The difference in two studies in a year (2011) and in similar zones (Khammohammadi and the present study) confirms this hypothesis.

It has been shown that female sheep and goats are more susceptible than males to Toxoplasma infections (Ramzan et al. 2009). Although there are other reports did not show significant correlation between Toxoplasma infection and the gender of the animals (Caballero-Ortega et al. 2008; Cavalcante et al. 2008). In this study, although the female sheep presented more prevalence of toxoplasmosis than the male sheep, this difference was not significant. Some reports indicate that female animals are more infected by T. gondii than males (van der Puije et al. 2000; Jittapalapong et al. 2005; Teshale et al. 2007). On the other hand, Bisson et al. (2000) and Cavalcante et al. (2008) observed that sex was not a significant factor in determining exposure to T. gondii infection in sheep and goats.

It has been reported that age can be associated with the seroprevalence of toxoplasmosis, as older sheep and goats had a higher prevalence of toxoplasmosis infection compared to younger sheep (Cavalcante et al. 2008; Ramzan et al. 2009). In this study a significant positive association was found between seropositivity to Toxoplasma gondii and the age over two years. This finding was also found in previous studies (Figiliuolo et al. 2004; Romanelli et al. 2007), showing that horizontal transmission by ingestion of sporulated oocysts in the environment seems to be a main transmission route.

In total the results of this study are adverse with our previously recorded high rate of toxoplasmosis in same sheep sera which were done with IFAT and PCR (34.6% and 56.6% respectively) support the impression that IFAT is highly specific and PCR is a sensitive method to detect T. gondii in different biological fluids including blood, aqueous humor, and amniotic fluids (Nematollahi et al. 2013).

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REFERENCES


