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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 93ewes) 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-Azarbaijan 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 93ewes) 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 -20ºC 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* 5×10^6(Rh –strain from Pasture'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) aliquted in 50 µl volumes and stored at -20°C. We considered each µl of the antigen preparation equivalent to the protein content of 10^4 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 ºC until used.

2.5. Evaluation of positive serum

One micro liter of prepared whole tachyzoite antigens were dotted on the one corner of each 1x 1 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 horseradish-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 1.
Seroprevalence of *T.gondii* in sheep in Tabriz

	No. tested	No. positive (%)
Sex	93	6(6.45)
Male	93	7(7.52)
Female		
Age		
<2yr	101	10(9.9)
>2yr	85	3(3.52)
Overall	186	13(6.98)

4. Discussion

The aim of this study was to study the seroprevalence of *T.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 (northwestern 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 (Keshavarz *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). Hoghooghi-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. Khanmohammadi (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 (Khanmohammadi 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 seroposivity to *Toxoplasma gondii* and the age over two year. This finding was also found in previous studies (Figiliuolo *et al.* 2004; Romanellie *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

Asgari, Q., Sarnevesht, J., Kalantari, M., Adnani-Sadat, S.J., Motazedian, M.H., Sarkari, B. (2011). Molecular survey of *toxoplasma* infection in sheep and goat from Fars province, southern Iran. *Tropical Animal Health Production.* 43, 389–392.

Caballero-Ortega,H.,Palma, J.,Garcia-Márquez, L., Gildo-Cárdenas, A., Correa, D. (2008). Frequency and risk factors for toxoplasmosis in ovines of various regions of the State of Colima,Mexico. *Parasitology.* 135, 1385-1389.

Cavalcante, A., Carneiro, M., Gouveia, A., Pinheiro, R., Vitor, R. (2008). Risk factors for infection by *Toxoplasma gondii* in herds of goats in Ceará, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia.* 60, 36-41.

Dubey, J.P. (2009). Toxoplasmosis in sheep-the last 20 years. *Veterinary Parasitology*. 163, 1–14.

Duncanson, P., Terry, R.S., Smith, J.E., Hide, G. (2001). High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock. *International Journal of Parasitology.* 31, 1699–1703.

Figliuolo, L.P.C., Rodrigues, A.A.R., Viana, R.B., Aguiar, D.M., Kasai, N., Gennari, S.M. (2004). Prevalence of anti-*Toxoplasma gondii* and anti- *Neospora caninum* antibodies in goats from Sao Paulo State. Brazil. *Small Ruminant Researches.* 55, 29–32.

Ghazaei, C. (2006). Serological survey of antibodies to *Toxoplasma gondii*. *African Journal of Health Sciences.* 13, 131–134.

Ghorbani, M., Edrissian, G.H., Assad N. (1993). Serological survey of toxoplasmosis in the northern part of Iran, using indirect fluorescent antibody technique. *Transaction Royal Society of Tropical Medicine and Hygiene* 72, 369–371.

Hamzavi, Y., Mostafaie. A., Nomanpour, B. (2007). Serological prevalence of toxoplasmosis in meat producing animals. *Iranian Journal of Parasitology.* 2, 7–11.

Hashemi-Fesharki, R. (1996). Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats in Iran. *Veterinary Parasitology*. 61, 1–3.

Hoghooghi-Rad, N., Afraa, M. (1993). Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, capital of Khoozestan province, south-west Iran. *Journal of Tropical Medicine Hygiene.* 96, 163–168.

Jittapalapong, S., Sangvaranond, A., Pinyopanuwat, N., Chimnoi, W., Khachaeram, W., Koizumi, S., Maruyama, S. (2005). Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province Thailand. *Veterinary Parasitology* 127, 17–22.

Keshavarz, H., Mohebali, M., Shahnazi, V., Zarei, Z. (2007). Frequency of *Toxoplasma* infection in livestock of Meshkin Shahr, by IFATand its health importance. *Tabriz Medicine Journal* 2:115–118.

Khanmohammadi, M. (2011). Seroprevalence survey of *Toxoplasma gondii* antibodies among sheep in Tabriz district, northwest Iran. *Annual Biology Researches.* 6, 484–488.

Masala, G., Porcu, R., Madau, L., Tanda, A., Ibba, B., Satta, G., Tola, S. (2003). Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. *Veterinary Parasitology.* 117, 15–21.

Navidpour, S.H., Hoghooghi-rad, N. (1998). Seroprevalence of anti-*Toxoplasma gondii* antibodies in buffaloes in Khoozestan province, Iran. *Veterinary Parasitology.* 77, 191–194.

Nematollahi, A., Jafari, R., Shahbazi, P., Khoshkerdar, M ., Zaboli, M., Nouruzi, M. (2013). Survey on ovine toxoplasmosis by IFAT and double-tube nested-PCR in Tabriz, Iran. *Comparative Clinical Pathology* [online]. Available from: http ;// www.springer.com/journal /580.

Raeghi, S., Akaberi, A., Sadeghi, S. (2011). Seroprevalence of *Toxoplasma gondii* in sheep, cattle and horses in Urmia north-west of Iran. *Tropical Animal Health Production.* 43, 389–392.

Raj, G.D., Latha, B., Chandrasekhar, M.S., Thiagarajan, V. (2004). Production, characterization and application of monoclonal antibodies against chicken IgY. *Veterinary Archive.* 74:189-199.

Ramzan, M., Akhtar, M., Muhammad, F., Hussain, I., Hiszczyńska-Sawicka, E., Haq, A., Mahmood, M., Hafeez, M. (2009) Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Tropical Animal Health and Production.* 4, 1225-1229.

Romanelli, P.R, Freire, R.L., Vidotto, O., Marana, E.R., Ogawa, L., De Paula, V.S., Garcia J.L., Navarro, I.T. (2007). Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State. *Brazil Researches Veterinary Sciences.* 82(2), 202-207.

Sharif, M., Gholami, S.H., Ziaei, H., Daryani, A., Laktarashi, B., Ziapour, S.P., Rafiei, A., Vahedi, M. (2007). Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. *The Veterinary Journal.* 174, 422–424.

Teshale, S., Dume tre, A., Darde , M.L., Merga, B., Dorchies, P. (2007). Serological survey of caprine toxoplasmosis in Ethiopia: prevalence and risk factors. *Parasitology.* 14, 155–159.

Van der Puije, W., Bosompem, K., Canacoo, E., Wastling, J., Akanmori, B. (2000). The prevalence of anti- *Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Tropica.* 76, 21-26.