

Role of toxoplasmosis in abortion of ewes in western Iran: a serological study

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Abstract. The aim of the current investigation was to determine the seroprevalence of *Toxoplasma gondii* infection in ewes and that role in abortion from Hamedan province, western Iran. 360 blood samples from ewes with recent abortion history and 148 samples from sheep with no abortion history (total=508) from different rural regions of Hamedan province were evaluated for the presence of IgG-antibodies against *T. gondii* using enzyme linked immunosorbent assay. Sixteen (3.1%) samples were seropositive (3.08%, CI95<3.11). The results indicated that there was a significant correlation between infection rates and the presence of abortion history in ewes ($p=0.04$, $OR=6.39$), but the difference between the fetal age groups was not statistically significant ($p=0.688$). The seropositive samples were reported only in ewes between 2 and 4 years ($p=0.004$). The current investigation is the first report of *T. gondii* seroprevalence in ewes from Hamedan province, western Iran. Although the rate of infection is low, the results indicate that *T. gondii* infection may partly be responsible for abortion and economic losses in sheep husbandry in this region. Further comprehensive studies for *T. gondii* in sheep and other hosts in this region are necessary.

Keywords: *Toxoplasma gondii*; Ewe; Sheep; Abortion; Iran

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Introduction

Toxoplasmosis is caused by the infection with *Toxoplasma gondii*, an obligate intracellular protozoan parasite (Dubey, 2008). The parasite was first recognized in tissues of a hamster-like rodent from Tunis in 1908 (Nicolle and Manceaux, 1908). *Toxoplasma gondii* has a

global distribution and is mainly transmitted by food contaminated with oocysts dispersed by felid definitive hosts, via uncooked meat containing tissue cysts, via non-pasteurized milk containing tachyzoites or transplacentally (Khezri et al., 2012). Most sheep acquire *T. gondii* infection after birth and less than 4% of

persistently infected sheep transmit the parasite vertically to the next gestation (Dubey, 2009).

In sheep, the infection with *T. gondii* may cause early embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. Severity of infection is associated with the stage of pregnancy at which the ewe becomes infected, the earlier in gestation, the more severe the consequences. Abortion is the most important economic loss due to toxoplasmosis in sheep and goat worldwide (Dubey, 2009). The diagnosis of *T. gondii* infection is usually based on histopathological, bioassay and serological examination, such as modified agglutination test (MAT), indirect fluorescent antibody (IFA) and enzyme linked immune-sorbent assay (ELISA) (Habibi et al., 2012).

Serological surveys of *T. gondii* infection were performed in different regions of Iran. This parasite was also isolated from ovine fetuses and brain using nested-PCR and bioassay methods from Iran (Zia-Ali et al., 2007; Habibi et al., 2012). Past surveys indicate that a wide range of animals such as sheep have been exposed to *T. gondii* in Iran. Sheep are important to economy of many countries because they are a source of food for humans. However, there is no published information of *T. gondii* infection in the sheep from Hamedan province.

The principal aim of this study was to evaluate the seroprevalence of *T. gondii* infection in ewes and subsequently to estimate the role of this parasite in the abortion of sheep in Hamedan province, Western Iran.

Materials and methods

Study area

Hamedan province is located in west part of Iran (34.77°N; 48.58°E) with mountainous and mild climate. This province is economically impressed by an agricultural and animal husbandry, including sheep and cattle breeding. According to veterinary office report, the sheep population in this area is approximately 1,480,000.

Sample collection

A cross-sectional study was performed during one year (April 2011 to April 2012). Five hundred eight blood samples were collected in native ewes (360 samples in ewes with recent abortion history and 148 samples from ewes without abortion history) in different rural regions of Hamedan province. Information about the ewes' age and fetal age were obtained from owners and by physical examination. The exact age of gestation was calculated with the use of the formula by Noakes et al. (2009): $[X=2.1(Y+17)$; X = developmental of fetal age in days, Y = length of fetal crown to anus in centimetres]. The ewes and fetuses were categorized into three age groups (<2 years, 2-4 years and >4 years in ewes and ≤2.5 months, >2.5 months and 24 hour after birth in fetuses).

Serology and statistical analysis

All sera were removed after centrifugation at 800×g for 15 minutes and stored at -20°C until laboratory testing. Anti-*Toxoplasma* IgG-antibodies were detected using a commercially available *T. gondii* ELISA kit (CHEKIT-TOXOTEST®; IDEXX Laboratories; Switzerland) in the Parasitology laboratory of the Veterinary Faculty, Bu-Ali Sina University of Hamedan. The kit was used according to the manufacturer's instructions.

Statistical analysis was performed by using the software package SPSS version 16.0 for windows. The differences among variables were evaluated by *Chi-square* test. *p*-value of less than 0.05 was considered statistically significant.

Results

Sixteen out of 508 (3.1%) of the serum samples were seropositive to *T. gondii* (3.08% CI95<3.11) (table 1). The results indicated that there was significant correlation between infection rates and the presence of abortion history in ewes ($\chi^2=4.19$, $p=0.04$, $df=1$, $OR=6.39$), but the difference between the fetal age groups was not statistically significant ($\chi^2=0.746$, $p=0.688$, $df=2$). The seropositive samples were reported only in age group of 2-4 years ($p=0.004$).

Table 1. Seroprevalence of *T. gondii* in ewes from Hamedan province, western Iran

	Ewe age groups			AB*	Fetal age groups			Total
	<2yr	2-4yr*	>4yr		a	b	c	
NS	134	281	93	360	90	242	28	508
(%)	(26.4)	(55.3)	(18.3)	(78.2)	(25)	(67.2)	(7.8)	(100)
NP	0	16	0	15	4	11	0	16
(%)	(0)	(5.7)	(0)	(4.2)	(4.4)	(4.5)	(0)	(3.1)

NS = number of sample, NP = number of positive, AB = abortion history, a = equal or less than 2.5 months, b = greater than 2.5 months, c = 24 hour after birth, * = significant difference was seen between this seropositive.

Discussion

This study is the first report of *T. gondii* seroprevalence in ewes in Hamedan province, Western Iran. Antibodies to *T. gondii* have been found in sheep worldwide (Dubey, 2009). The lowest and highest seroprevalence of *T. gondii* were reported in Pakistan (3% using Latex Agglutination Test) and Kars (95.7% using ELISA), respectively (Dubey, 2009).

There were some reports on seroepidemiology of *T. gondii* in sheep from different region of Iran. In previous investigations, the seroprevalence rate were reported between 21.7% and 22.5% in West, 20.9%-35% in North, 13.8%-72.6% in South-West, 21.1%-59% in North-East, 26.5% in South, 24.7% in South-East and, 29.1% in Central of Iran (Hoghooghi-Rad and Afraa, 1993; Hashemi-Fesharki, 1996; Bonyadian et al., 2007; Sharif et al., 2007; Bahrieni et al., 2008; Hamidinejat et al., 2008; Asgari et al., 2009; Raeghi et al., 2011).

The results of this research showed that 3.1% of ewes in Hamedan province were seropositive, using ELISA. Similar rates of infection were reported in Pakistan and India (Zaki, 1995; Sharma et al., 2008). A high prevalence of toxoplasmosis in hot and humid environments compared to cold and dry ones was attributed to the longer viability of *T. gondii* oocysts under humid conditions (Hamidinejat et al., 2008). Different serological tests and cut-off values, study design, climatic variations and frequency of felines on the farms are the main causes of the varied results (Hamidinejat et al., 2008; Dubey, 2009).

Our study showed that the prevalence of *T. gondii* antibodies in ewes with abortion history was significantly higher than in those without

abortion history ($p=0.04$, $OR=6.39$) (table 1). *Toxoplasma gondii* DNA was detected in up to 23% of aborted fetuses. The parasite has been recognized as one of the main causes of infective ovine abortion in New Zealand, Australia, UK, Norway, USA and the Iran (Dubey, 2009; Habibi et al., 2012). Detection of *T. gondii* antibodies in fetal fluids or serum is useful in the diagnosis of ovine abortion (Dubey, 2009).

The results of the current study, showed that the highest seroprevalence (5.7%) was in the age group of 2-4 years and the lowest (0%) in <2 years and >4 years. The difference was statistically significant ($p=0.004$). This finding is opposite to a study in Kurdistan, west of Iran ($p>0.05$) (Khezri et al., 2012). Dubey (2009) reported that age is an important factor in sheep toxoplasmosis. In this research, the prevalence of *T. gondii* was higher in younger animals than other age groups. This could be explained on the basis that the animals included in this age group were less resistant to *T. gondii*. Seroprevalence was shown to increase with age, reaching 95% in 6-year-old ewes in some flocks, suggesting that most animals acquire infection after birth (Dubey and Kirkbride, 1989). In general, most sheep acquired infection before 4 years of age, but one-third of old ewes were still seronegative in highly endemic flocks (Dubey and Kirkbride, 1989).

In the present study, the difference between the fetal age groups was not statistically significant ($p=0.688$) (table 1). In early pregnancy, the fetus is unable to mount any immune response, and so cannot inhibit parasite multiplication. The fetus rapidly dies and is resorbed. In a flock, this is clinically visible as large numbers of barren ewes. In mid-gestation (70-120 days) infection can again be lethal. This causes a mummified fetus which is often twinned with a lamb that is

stillborn or weak. Abortion due to infection at 70-120 days gestation tends to occur in very late pregnancy (Noakes et al., 2009).

Toxoplasmosis in sheep is a potential risk of transmission to humans through consumption of meat contaminated with tissue cysts of *T. gondii* in Iran (Shahmoradi et al., 1993). Most of the sheep breeding farms are traditional in Iran and animals have a direct contact with cats. The presence of cats and abortion history is a risk factors associated with *T. gondii* infection in sheep. Oocyst-contaminated pastures, fodder, and drinking water are regarded as potential sources of postnatal infection in sheep (Dubey, 2008).

The results of this survey can provide baseline information for the future studies. According to our results, study on *T. gondii* infection in final hosts such as cats in this region is recommended. In conclusion, toxoplasmosis exists among sheep in Hamden province. Although the rate of infection is low but the result indicates that *T. gondii* infection may partly be responsible for abortion and economic losses in Sheep husbandry in this region. Therefore, further additional researches (molecular and bioassay examination) and designing control strategies for improving management in sheep flocks and humans are necessary.

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