Serological determination of *Toxoplasma gondii* in sheep (*Ovis aries*) in Gilan province, North of Iran

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**ABSTRACT**

*Toxoplasma gondii* is one of the most common meat born protozoan parasites that cause congenital infection, abortion, and stillbirth in humans and animals. Sheep are one of the important sources of meat production in Gilan Province. Therefore, consumption of raw and half-cooked meat is one of the major risk factors for *T. gondii* infection. Toxoplasmosis in patients with intact immune systems is usually asymptomatic, but can be life-threatening in patients with a weak immune system; for example, patients with HIV/AIDS or cancer, and transplant recipients. Gilan is divided into three regions of plains with moderate climatic conditions, hillsides with semi-humid climates, and heights with cold mountainous weather. Climate situations are involved in the prevalence of toxoplasmosis. The present study aimed to investigate the seroprevalence of *T. gondii* infection in sheep in Gilan Province, North of Iran. In a current cross-sectional study, 400 sheep sera samples were tested for *T. gondii*-IgG antibody by the enzyme-linked immunosorbent assay technique (ELISA). The samples were divided into different groups according to the geographical location and animal ages. *T. gondii*-IgG antibody was detected in 166 sheep (41.5%). The highest frequency (72.7%, n=56) was observed in the age group of >4 years, which the difference was statistically significant (P = 0.0001) in comparison with other groups. Also, the seroprevalence of *T. gondii* was significantly higher in plains (53.9%) than those of hillsides and heights (P = 0.0001). Consequently, The seroprevalence of *T. gondii* infection in Gilan Province is high, indicating a significant relationship with geographical location and age of animals.
INTRODUCTION

Toxoplasma gondii is one of the most common zoonotic diseases that can be transmitted through meat throughout the world (Schluter et al., 2014). Toxoplasmosis is a major contributor to abortion, congenital infection, and stillbirth in humans and animals (Schluter et al., 2014). Toxoplasmosis in patients with intact immune systems is usually asymptomatic, but can be life-threatening in patients with a weak immune system; for example, patients with HIV/AIDS or cancer, and transplant recipients (Abdoli et al., 2016). The cat is the final host and a wide range of warm-blooded animals, including humans and ruminants, are the intermediate hosts of T. gondii (Schluter et al., 2014). The T. gondii sexual cycle occurs only within the feline intestine and the oocysts produced are excreted through the host’s feces. Sporulated oocysts can resist environmental conditions for 12-18 months (Schluter et al., 2014). Eating foodstuffs, water, vegetables, or even soils contaminated with oocysts is the major source of transmission of the parasite to humans and animals (Schluter et al., 2014). Formation of tissue cysts are also the most important stage of T. gondii infection. Humans and felines are infected through the consumption of raw or half-cooked meat containing tissue cysts (Schluter et al., 2014). Sheep are one of the most important intermediate hosts for T. gondii since the parasite cysts lie dormant within the cardiac and skeletal muscles. Therefore, consumption of raw and half-cooked meat is one of the major risk factors for T. gondii infection (Schluter et al., 2014). Iran is located in Western Asia and has four main climates, including temperate and humid climate on the coast of the Caspian sea is located in the north of Iran, hot and dry climate in the central plateau of Iran, cold and mountainous climate in the west and northwestern of Iran and warm and humid climate on the southern coast of the Persian gulf. These climatic changes play a major role in changing the prevalence of T. gondii infection in ruminants in different regions of the country. In past serological studies since 2007 to 2019, due to climate changes in different provinces of the country, Gilan and
Mazandaran provinces with temperate and humid climate on the coast of the Caspian sea (Havakhah et al., 2014, Sharif et al., 2007), Tabriz, Urmia, Kurdistan, Kermanshah, Markazi and Qazvin provinces with cold and Mountainous climate (Jula et al., 2013, Raeghi et al., 2011, Khezri et al., 2012, Hamzavi et al., 2007, Bonyadian et al., 2007, Izadyar et al., 2019), Kerman, Jahrom and Kashan provinces with hot and dry climate in the central plateau of Iran (Derakhshan and Mousavi, 2014, Armand et al., 2016, Rasti et al., 2018) and Khuzestan with warm and humid climate. According to published reports, the seroprevalence of *T. gondii* in sheep ranges between 3.3%-36.8% in Iran. It is noteworthy that the prevalence of *T. gondii* is associated with climatic conditions, so that in areas with moderate and humid climates, the prevalence is higher than in hot and dry areas (Sharif et al., 2015). However, in the study from Iran by Havakhah et al., the prevalence of toxoplasmosis in sheep was reported only in three cities of Gilan Province (Havakhah et al., 2014), and there is no information about the prevalence of toxoplasmosis across the province. Since sheep are one of the major sources of meat production in Gilan Province, the current study used serological investigation of *T. gondii* infection in sheep in Gilan Province from 2018 to 2019 to identify prevalence rates of the parasite.

**MATERIAL AND METHODS**

**Study area**

Gilan Province, one of the Northern provinces of Iran with a population of about 2,500,000, is located at 37° 27' North latitude and 49° 58' East longitude from the meridian, and its altitude from sea level is varying in different regions (Figure 1). Its area is about 14,711 km² and the average annual rainfall is 1275 mm. Gilan is divided into three regions of plains with moderate climatic conditions, hillsides with semi-humid climates, and heights with cold mountainous weather.

**Figure 1.** Geographic map of Gilan Province, Iran

**Sample collection**
In the current study, a total of 400 sheep blood samples obtained from the jugular vein were collected according to the geographical distribution of sheep population in Gilan Province. Samples from pregnant female sheep from herds with the history of abortion were prioritized. Blood samples were centrifuged at 3000 rpm for 10 min and the isolated sera stored at -20°C until analysis. According to the climate variation in Gilan Province, the samples were divided into three groups of plains, hillsides, and heights based on the geographical location. The sheep were also divided into three age groups of <2, 3-4, and >4 years. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

**Serological detection**
An indirect enzyme-linked immunosorbent assay method (ELISA) was used to detect IgG antibodies against *Toxoplasma gondii* in sera (multi-species ID Screen® Toxoplasmosis Indirect, IDVet, Montpellier, France), according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm with an ELISA automated plate reader (BioTek, USA). The S/P percentage was calculated according to the following formula:

\[
\text{S/P\%} = \frac{\text{OD sample} - \text{OD Negative Control}}{\text{OD Positive control} - \text{OD Negative Control}} \times 100
\]

Samples presenting a S/P\% ≤ 40% were considered negative, samples with S/P\% between 40% and 50% were considered doubtful and the S/P\% ≥ 50% were considered positive for *T. gondii*.

**Statistical analysis**
The data were analyzed statistically using chi-square test by SPSS version 24 (SPSS, Inc., Chicago, IL).

**RESULTS**
In the current study, a total of 400 female sheep with the mean age of 3.4 ± 1.84 years (range: 1-13) were evaluated. *T. gondii*-IgG antibody was detected in 166 cases (41.5%, n=166). Age was
significantly associated with IgG seropositivity, with the highest frequency percentage (72.7%, n=56) observed in the age group of >4 years (P = 0.0001).

Geographical location and IgG seropositivity also reported a significant relationship (P = 0.0001) with the highest frequency percentage of positive samples (53.9%, n=103) found in sheep from the plain samples (Table 1).

With increasing age from 3 to 4 years and more, frequency of IgG seropositivity was decreased from plain to height. (Table 2).

**DISCUSSION**

Toxoplasmosis is a common disease among animals and humans around the world and is one of the major causes of abortion and stillbirth in sheep (Edwards and Dubey, 2013). Humans, after birth, are infected via taking tissue cysts found in half-cooked meet, consumption of water and food contaminated with oocysts, or accidentally ingestion of oocysts spread in the environment. Contaminated lamb is one of the major sources of *T. gondii* infection in humans and carnivorous animals (Dubey, 2009). These animals are also the intermediate hosts of *T. gondii* (Schluter et al., 2014).

Iran has a wide variety of climates; in the Northern regions of Iran, with moderate and humid climates, the oocysts excreted through felines feces can remain for months or even years in the environment (Subedi et al., 2018). Therefore, the highest prevalence of *T. gondii* infection in humans and animals in Iran is reported from this region (Izadyar et al., 2019, Sharif et al., 2015); this indicates the high importance of this issue in Northern Iran. In previous studies conducted on pregnant women in Northern Iran, the prevalence of *T. gondii* infection was reported at 41.8%–75.02% (Foroutan-Rad et al., 2016). Also, in a serological investigation on rural people in Amol City, Northern Iran, the prevalence of this infection was 75.7%. This high prevalence indicates Northern Iran is an endemic area for toxoplasmosis. There was also a significant relationship in the current study between the lamb
meat consumers (P = 0.015), as well as raw or half-cooked meat consumers (P <0.001) and the presence of *T. gondii*-IgG in their serum (Rostami et al.,2016).

According to previous studies conducted in different parts of the world, the serological prevalence of *T. gondii* in sheep ranges from Portugal 33.6% (Lopes et al.,2013), Mexico 15.1%-84% (Hernández-Cortazar et al.,2015), South Africa 8% (Hammond-Aryee et al.,2015), Brazil 47.8% (Rêgo et al.,2016), Pakistan 26.2% (Ahmed et al.,2016), USA 22.0% (Guo et al.,2016), China 20.71% (Yang et al.,2017), and Northern Iraq 42.1% (Al Hamada et al.,2019). The prevalence of *T. gondii* infection in sheep in different regions of Iran ranges 3.3% to 38.3% according to published reports (Rasti et al.,2018). These differences can be attributed to different climates, age, and livestock breeding conditions, as well as various methods for detecting the parasite (Izadyar et al.,2019). In the present study, the serological prevalence of *T. gondii* in sheep in Gilan Province was detected at 41.5%. In another study in Mazandaran Province, Northern Iran with moderate and humid climate, the seroprevalence of *T. gondii* was 35% (Sharif et al.,2007) which was lower than of the present study. In the study by Havakhah et al. (2014) in Gilan Province, the prevalence of *T. gondii* infection in sheep was 36.8%, which was lower than that of the present study; however, they used Sabin–Feldman serologic dye test only in three counties (Havakhah et al.,2014). This could account for the differences reported as the present study, owing to the population distribution of sheep in Gilan Province, was the first research that investigated most regions of the province by the serological method of ELISA, which has a higher sensitivity than the dye test (Balsari et al.,1980).

In addition to moderate and humid climates, the extensive grazing system of sheep breeding, the presence of wild felines and open crop storage facilities that felines have access to, are other causes of high prevalence of *T. gondii* in Gilan Province, which are similar to the results of the study by Subedi et al., on Nepal sheep (Subedi et al.,2018).

Gilan Province was divided into three regions of plains, hillsides, and heights according to the geographical location. In the current study, the highest and lowest frequency percentages of *T. gondii* infection were observed in plain samples (53.9%) followed by height samples (10%), and a significant
relationship was observed between the geographical location and the frequency of *T. gondii* infection (P = 0.0001). With increasing altitude from the sea level, the amount of humidity and temperature decreases and a dry and cold winter rules over the heights of the province, which reduces the survival rate of oocysts in such climatic conditions; while in the plains, due to the lower altitude from sea level and proximity to the sea, the conditions are suitable for the survival of oocysts during winter. Oocysts can survive in moderate and humid climates for up to 18 months (Gazzonis et al., 2015, Katzer et al., 2011). In the study by Khezri et al. on Kurdistan Province sheep, the prevalence of *T. gondii* infection in the Southern regions due to hot and humid weather was higher than the Western regions, due to cool and dry climate rule over these regions (Khezri et al., 2012). In another study by Sharif et al. on the sheep of Mazandaran Province, the high prevalence of *T. gondii* infection in the Western region was due to the difference in moisture content that increased the number of oocysts compared to Eastern and Central regions (Sharif et al., 2007); the results were consistent with those of the current study.

In the present study, there was a significant correlation between mean age and seropositive (4.2 ± 33.17) and seronegative (2.1 ± 7.2) samples (P = 0.0001). Also, the highest frequency percentage of infection was observed in the age group of >4 years (72.7%) and the lowest frequency percentage in the age group of <2 years (16%); the relationship between age groups and seropositivity was statistically significant (P = 0.0001).

In previous studies, including Gazzonis et al. on Northern Italian sheep (Gazzonis et al., 2015), Katzer et al. on Scottish sheep (Katzer et al., 2011), Hecker et al. on the sheep in Argentina (Hecker et al., 2018), and Izadyar et al. on Qazvin sheep (Izadyar et al., 2019) an increased prevalence of *T. gondii* infection in older animals compared to younger ones was consistently reported as found in the current study (Izadyar et al., 2019, Sharif et al., 2015). These results suggest that contamination with *T. gondii* in sheep mostly occur after birth and in additionally, that horizontal contamination is the main route of transmission of infection in herds (Dubey, 2009, Hecker et al., 2018).

**CONCLUSIONS**
The present study found a high prevalence of *T. gondii* infection in Gilan Province, Iran; this appears to be related to the region’s moderate climate and high humidity. Due to climate variation in Gilan Province, the prevalence of contamination in the plains of the province was higher than other regions. It was also shown that as the sheep age increased, the prevalence of *T. gondii* infection also increased, which confirms horizontal transmission of contamination. A high prevalence of *T. gondii* infection is reported in sheep therefore in order to improve the management of sheep in this region, a comprehensive educational, prevention, and continuous treatment program is recommended.

**Ethics**

We hereby declare all ethical standards have been respected in preparation of the submitted article.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Acknowledgment**

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Table 1. Seroprevalence and associated risk factors for *T. gondii* in sheep of Gilan Province, North of Iran.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Seropositive N(%)</th>
<th>Seronegative N(%)</th>
<th>Total N(%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>21(16)</td>
<td>110(84)</td>
<td>131(100)</td>
<td>0.0001</td>
</tr>
<tr>
<td>3-4</td>
<td>89(46.4)</td>
<td>103(53.6)</td>
<td>192(100)</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>56(72.7)</td>
<td>21(27.3)</td>
<td>77(100)</td>
<td></td>
</tr>
<tr>
<td><strong>Geographical location</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Plain</td>
<td>103(53.9)</td>
<td>88(46.1)</td>
<td>191(100)</td>
<td></td>
</tr>
<tr>
<td>Domain</td>
<td>56(40.3)</td>
<td>83(59.7)</td>
<td>139(100)</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>7(10)</td>
<td>63(90)</td>
<td>70(100)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>166(41.5)</td>
<td>234(58.5)</td>
<td>400(100)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparing effect of age trend and location changes on serum results

<table>
<thead>
<tr>
<th>Age group</th>
<th>Serum result</th>
<th>Location</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 year</td>
<td>Pos N(%)</td>
<td>Plain</td>
<td>4(11.1%)</td>
<td>15 (30.0%)</td>
</tr>
<tr>
<td></td>
<td>Neg N(%)</td>
<td>Domain</td>
<td>32 (88.9%)</td>
<td>35 (70.0%)</td>
</tr>
<tr>
<td>3-4 year</td>
<td>Pos N(%)</td>
<td>Height</td>
<td>49 (56.3%)</td>
<td>35 (42.7%)</td>
</tr>
<tr>
<td></td>
<td>Neg N(%)</td>
<td></td>
<td>38 (43.7%)</td>
<td>47 (57.3%)</td>
</tr>
<tr>
<td>&gt;4 year</td>
<td>Pos N(%)</td>
<td></td>
<td>50 (73.5%)</td>
<td>6 (85.7%)</td>
</tr>
<tr>
<td></td>
<td>Neg N(%)</td>
<td></td>
<td>18 (26.5%)</td>
<td>1 (14.3%)</td>
</tr>
</tbody>
</table>

Pos= positive. Neg= negative. *statistically significant P<0.05