Serological and molecular detection of *Toxoplasma gondii* in sheep and goats in Kashan, Central Iran

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Abstract
Toxoplasmosis is one of the most common meat-borne parasitic infections worldwide. Consumption of raw or undercooked meat which contains *Toxoplasma gondii* tissue cysts is an important route of human infection. In this study, we investigate the serological and molecular prevalence of *T. gondii* infection in sheep and goat samples in Kashan, Iran, from 2015 to 2016. Serological (IgG antibody) and molecular detections were performed by the enzyme-linked immunosorbent assay and polymerase chain reaction on the sera and heart samples of sheep (n = 90) and goats (n = 90), respectively. *T. gondii*-IgG antibody was detected in 12.2% of sheep and 4.4% of goat samples. The parasite’s DNA was detected in 17.8 and 8.9% of sheep and goat samples, respectively.

Practical applications
The results of this study emphasize on the role of the sheep and goat as reservoirs of *T. gondii* infection. Hence, consumption of adequately cooked meat should be considered for prevention of *T. gondii* infection in humans.

1 | INTRODUCTION

*Toxoplasma gondii* is one of the most common meat-borne pathogens worldwide (Schlüter et al., 2014). Toxoplasmosis is an important cause of congenital infections, abortion, and stillbirth in humans and animals (Schlüter et al., 2014). Toxoplasmosis is usually asymptomatic in immunocompetent individuals, but the infection is life threatening in immunocompromised patients (e.g., HIV/AIDS, transplant recipients, or cancer patients) (Abdoli, Barati, Dalimi, Pirestani, & Hoseini Shokouh, 2016). The cat is the definitive host of *T. gondii* and a wide variety of warm blooded animals, including human, sheep, and goat are intermediate hosts (Schlüter et al., 2014). The cat feces are the most important source of *T. gondii* infection in humans (Cook et al., 2000; Jones et al., 2009; Kapperud et al., 1996). Sheep and goats are two important intermediate hosts of *T. gondii*, because of the parasite cysts are persisted in the skeletal and heart muscles of these animals (Schlüter et al., 2014). Hence, consumption of raw or undercooked meats containing tissue cysts (Kijlstra & Jongert, 2009; Schlüter et al., 2014). Sheep and goats are two important intermediate hosts of *T. gondii*, because of the parasite cysts are persisted in the skeletal and heart muscles of these animals (Schlüter et al., 2014). Hence, consumption of raw or undercooked meats containing tissue cysts (Kijlstra & Jongert, 2009; Schlüter et al., 2014). Sheep and goats are two important intermediate hosts of *T. gondii*, because of the parasite cysts are persisted in the skeletal and heart muscles of these animals (Schlüter et al., 2014). Hence, consumption of raw or undercooked meats containing tissue cysts (Kijlstra & Jongert, 2009; Schlüter et al., 2014).
and humid temperature ranges than the hot and dry areas (Sharif et al., 2015). The prevalence rate of *T. gondii* infection has been reported in a range of 10–38% in sheep and goats in Iran (summarized in Table 1).

Although some studies conducted on prevalence on toxoplasmosis in human (Rasti et al., 2015, 2016) and cat (Hooshyar, Rostamkhani, Talari, & Arbabi, 2007) in Kashan, there is not any information about prevalence of toxoplasmosis in meat of food producing animals in this area. Because sheep and goats are two major sources of meat production in Kashan, the aim of this study was molecular and serological detection of *T. gondii* in sheep and goats in Kashan during 2015–2016.

### 2 | MATERIALS AND METHODS

#### 2.1 | Study area

Kashan is located in the central region of Iran with nearly 400,000 populations. Kashan is located at 33°59’ longitude north, 51°27’ longitude east with an altitude of 982 m, covers an area of about 9,647 km². Kashan has an arid climate with a hot summer and mild winter (Modarres & de Paulo Rodrigues da Silva, 2007; Tabari & Talaee, 2011a,b). The annual rainfall of Kashan is estimated to be 0.039 mm/year (Tabari & Talaee, 2011a,b).

#### 2.2 | Sample collection

The sample size was determined by the statistical formula (Naing, Winn, & Rusli, 2006) based on the previous article that reports prevalence of toxoplasmosis in sheep and goat in Iran (Moazeni Jula, Moazeni Jula, Nowzari, Kavari, & Hashemzadeh, 2013). Venus blood samples were taken from 90 sheep to 90 goats before slaughter in Kashan abattoir, and then their sera were separated and stored in 220°C until used for serologic evaluation. Whole heart of 90 sheep and 90 goats were purchased from the same abattoir. The study protocol was approved by the Ethical Committee of Kashan University of Medical Sciences, Kashan, Iran.

### Table 1 | A snapshot on prevalence of *T. gondii* in sheep and goat in Iran

<table>
<thead>
<tr>
<th>Location</th>
<th>Animals</th>
<th>Serology</th>
<th>PCR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilan Province, North of Iran</td>
<td>Sheep</td>
<td>Dye Test</td>
<td>36.8%</td>
<td>Havakhah et al. (2014)</td>
</tr>
<tr>
<td>Gilan Province, North of Iran</td>
<td>Goat</td>
<td>Dye Test</td>
<td>12.9%</td>
<td>Havakhah et al. (2014)</td>
</tr>
<tr>
<td>Mazandaran province, North of Iran</td>
<td>Sheep</td>
<td>IFAT, 35%</td>
<td>–</td>
<td>Sharif et al. (2007)</td>
</tr>
<tr>
<td>Mazandaran province, North of Iran</td>
<td>Goat</td>
<td>IFAT, 30%</td>
<td>–</td>
<td>Sharif et al. (2007)</td>
</tr>
<tr>
<td>Urmia, North-West of Iran</td>
<td>Sheep</td>
<td>MAT 21.1%</td>
<td>–</td>
<td>Raeghi, Akaberí, and Sedeghi (2011)</td>
</tr>
<tr>
<td>Kermanshah, West of Iran</td>
<td>Sheep</td>
<td>IFA, 22.5%</td>
<td>–</td>
<td>Hamzavi, Mostafaie, and Nomanpour (2007)</td>
</tr>
<tr>
<td>Kermanshah, West of Iran</td>
<td>Goat</td>
<td>IFA, 23.7%</td>
<td>–</td>
<td>Hamzavi et al. (2007)</td>
</tr>
<tr>
<td>Tabriz, North-West of Iran</td>
<td>Sheep</td>
<td>24.8%</td>
<td>–</td>
<td>Kavari, Nowzari, Moazeni Jula, Moazeni Jula, and Hashemzadeh (2016)</td>
</tr>
<tr>
<td>Tabriz, North-West of Iran</td>
<td>Goat</td>
<td>10.6%</td>
<td>–</td>
<td>Kavari et al. (2016)</td>
</tr>
<tr>
<td>Jahrom, South of Iran</td>
<td>Sheep</td>
<td>35.94%</td>
<td>Heart and diaphragm 34.32%</td>
<td>Armand, Solhjoo, Shabani-Kordshooli, Davami, and Sadeghi (2016)</td>
</tr>
<tr>
<td>Fars province, South of Iran</td>
<td>Sheep</td>
<td>–</td>
<td>Meat, 37.5%</td>
<td>Asgari et al. (2011)</td>
</tr>
<tr>
<td>Fars province, South of Iran</td>
<td>Goat</td>
<td>–</td>
<td>Meat, 22.7%</td>
<td>Asgari et al. (2011)</td>
</tr>
<tr>
<td>Chaharmahal va Bakhtiary, South-West of Iran</td>
<td>Sheep</td>
<td>–</td>
<td>Blood, 33.33%</td>
<td>Khamesipour, Doost, Iranpour Mobarakheh, and Komba (2014)</td>
</tr>
<tr>
<td>Chaharmahal va Bakhtiary, South-West of Iran</td>
<td>Sheep</td>
<td>–</td>
<td>Tongue, brain, femur muscle and liver, 38%</td>
<td>Azizi, Shiran, Boroujeni, and Jafari (2014)</td>
</tr>
<tr>
<td>Kerman, Southeastern Iran</td>
<td>Sheep</td>
<td>IFAT, 3.3%</td>
<td>–</td>
<td>Derakhshian and Mousavi (2014)</td>
</tr>
<tr>
<td>Kerman, Southeastern Iran</td>
<td>Goat</td>
<td>IFAT, 1.7%</td>
<td>–</td>
<td>Derakhshian and Mousavi (2014)</td>
</tr>
<tr>
<td>Markazi, Center of Iran</td>
<td>Sheep</td>
<td>IFAT, 29.1%</td>
<td>–</td>
<td>Bonyadian, Hematzade, and Manuchehri (2007)</td>
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<tr>
<td>Isfahan, Center of Iran</td>
<td>Sheep</td>
<td>–</td>
<td>Blood, 8.47%</td>
<td>Khamesipour et al. (2014)</td>
</tr>
<tr>
<td>Kashan, Center of Iran</td>
<td>Sheep</td>
<td>ELISA, 12.2%</td>
<td>Heart, 17.8%</td>
<td>Current study</td>
</tr>
<tr>
<td>Kashan, Center of Iran</td>
<td>Goat</td>
<td>ELISA, 4.4%</td>
<td>Heart, 8.9%</td>
<td>Current study</td>
</tr>
</tbody>
</table>
2.3 | Serological detection

*T. gondii*-IgG antibody was detected from the sera of each animal by the enzyme-linked immunosorbent assay, with IDvet enzyme-linked immunosorbent assay (ELISA) kit (IDvet, 310, rue Louis Pasteur-Grabels, France) according to the manufacturer’s instruction.

2.4 | Molecular detection

The whole heart of each sheep and goat were individually rinsed with distilled water, and then approximately 200 g from different segments of the hearts were crushed with a pestle and mortor and digested with acid pepsin solutions according to the previously described protocol (J. P. Dubey, 2009). The digested samples were filtered through soft tissues and then washed with phosphate buffered saline three times. DNA was extracted from a commercially available kit (DNK kit, Sinaclon, Tehran, Iran) according to the manufacturer’s instruction, and then the DNAs were stored in −20° until used. Polymerase chain reaction (PCR) was conducted using a pair of *T. gondii*-specific primers TOX4 (5′-CGCTGAGGGAGGAAGACGAAAGTTG-3′) and TOX5 (5′-CGCTGAGCACAGCTGATCGATT-3′) that specifically amplify a repetitive region of 529 bp DNA fragment (GenBank Accession No. AF146527) that repeated 200–300 times in *T. gondii* genome (Homan, Vercammen, De Braekeleer, & Verschuuren, 2000). Amplification was conducted in a final volume of 20 μL reaction mixtures containing 10 pmol of each forward and reverse primers, 10 μL of 2x Taq DNA polymerase master mixes with 2 mM MgCl2 (Cat. no. A170301, Ampliqon, Denmark), 7 μL of distilled water, and 1 μL of template DNA. For each amplification reaction, a positive control (DNA extracted from the RH strain of *T. gondii*) and a negative control (double distilled water) were included. The amplification conditions were performed according to the described program (Abdoli et al., 2016) and the PCR products were electrophoresed in a 1.5% agarose gel that stained with safe stain (Sinaclon, Tehran, Iran) and visualized under UV transillumination.

2.5 | Statistical analysis

The data were analyzed using chi-square and Fisher exact tests by SPSS version 11.5 (SPSS, Inc., Chicago, IL).

3 | RESULTS

*T. gondii*-IgG antibody and DNA were detected in 11 (12.2%) and 16 (17.8%) of the sheep sera and heart samples, that their differences were not statistically significant. Also, both of the IgG antibody and DNA were detected in 4 (4.4%) of the samples (Table 2 and Figure 1). In goat samples, IgG seropositivity and DNA were detected in 4 (4.4%) and 8 (8.9%) of the sera and heart samples and their differences were not statistically significant. Additionally, both of the IgG antibody and DNA were detected in 2 (2.2%) of the goat samples (Table 2 and Figure 1).

4 | DISCUSSION

Sheep and goats are major sources of meat production in Iran. These animals are suitable reservoir hosts of *T. gondii* as well (Schlüter et al., 2014). In the current study, *T. gondii*-IgG antibody and DNA were detected in 12.2 and 17.8% of the sheep samples and 4.4 and 8.9% of the goat samples, respectively. The sensitivity of molecular methods is much higher than serological methods for detection of active *T. gondii* infection (Robert-Gangneux & Dardé, 2012) so that is consisted with our results.

In previous studies in Kashan, *T. gondii*-IgG and IgM seropositivity were detected in 42.7 and 0.63% of pregnant women (Rasti et al., 2015). Also, Hooshyar et al. detected *T. gondii*-IgG antibody in 86% of stray cats in Kashan (Hooshyar et al., 2007). Arbabi and Talari (Arbabi & Talari, 2002) investigated the seroprevalence of toxoplasmosis among pregnant women and men in Kashan. The results showed the seroprevalence rate was 61 and 46.7% in women and men, respectively. Interestingly, the results revealed the infection rate was significantly increased in men who worked in the meat industry or among them who consumed undercooked or raw meat productions (p < .0001) (Arbabi & Talari, 2002). Eating undercooked or raw meat is a major risk factor of toxoplasmosis (Schlüter et al., 2014). In this regard, Jones et al., reported that working with meat and eating undercooked meat are one of the important risk factors for *T. gondii* infection in the United States (Jones et al., 2009). Ghasemi et al. found that eating undercooked or raw meat was a major risk factor of toxoplasmosis in pregnant women with abortion and stillbirth in Tehran, Iran (Ghasemi et al., 2016). Mardani and Tavalla (Mardani & Tavalla, 2015) investigated the seroprevalence rate of *T. gondii* infection among butchers and control group in Khuzestan province, sought of Iran. According to the results, *T. gondii*-IgG

![FIGURE 1](image-url)
antibody was detected in 41.8% of butchers and 28.8% of the control group. Also, IgM seropositivity was detected in 2.2% of butchers and none of the control group (Mardani & Tavalla, 2015).

According to previous studies, the rate of T. gondii infection in sheep and goats has been reported in a range of 10–38% in different part of Iran (Table 1). Toxoplasmosis is an important cause of abortion and stillbirth in sheep and goats as well (J. Dubey et al., 1980; Edwards & Dubey, 2013), while a higher rate of the infection was reported in fetoplacental tissues of aborted sheep due to congenital toxoplasmosis in Iran (Danehchin, Razmi, & Naghibi, 2016). The seroprevalence rate of toxoplasmosis in sheep and goats was reported 33 and 27% in Ghana (Van der Puije, Bosompem, Canacoo, Wastling, & Akanmori, 2000), 23 and 11.6% in Ethiopia (Bekele & Kasali, 1989), 47.8 and 40.5% in Brazil (Rêgo et al., 2016), and 33.6 and 18.5% in Portugal (Lopes et al., 2013), respectively.

It should be noted that serological assays primarily detect chronic T. gondii infection in animals, and these assays alone do not show the prevalence of viable parasites. Hence, PCR-based techniques have been developed to detect T. gondii DNA in meat samples (Robert-Gangneux & Dardé, 2012). Belluco et al. (2016) performed a systematic review and meta-analysis of T. gondii prevalence in meat of food producing animals worldwide. According to their results, pooled prevalence of T. gondii was 14.7, 12.3, and 2.6% in sheep, pigs, and cattle, respectively. Furthermore, the prevalence of toxoplasmosis depends on climate conditions because the parasite’s oocysts remain infective more time in temperate and humid climate, and consequently risk of the infection in the parasite’s hosts (e.g., sheep and goats) increases due to consumption of contaminated forage with infective oocysts (Robert-Gangneux & Dardé, 2012; Yan et al., 2016). It is also postulated that the changing environmental conditions may lead to increase of T. gondii prevalent in some regions of Europe (Meerburg & Kijlstra, 2009). It seems that our results are influenced by climate condition, because Kashan has an arid climate conditions and the prevalence rates of toxoplasmosis in our study are lower in comparison with some regions of Iran with humid environment. While, a higher seroprevalence rates were reported in sheep and goat in north of Iran (Gilan and Mazandaran provinces) which have a humid climate condition (Havakshah et al., 2014; Sharif et al., 2007), but a little seroprevalence rate was reported from sheep (3.3%) and goat (1.7%) in Kerman (southeastern Iran) which have an arid climate situation (Derakhshan & Mousavi, 2014) (see details in Table 1).

5 | CONCLUSION

Our results revealed that sheep and goat are important reservoirs of T. gondii in Kashan, Iran. Inasmuch as the sheep and goat are important sources of meat production in Kashan and also in Iran, consumption of adequately cooked meat should be recommended for prevention of the infection.

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