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Original Article

# Seroprevalence of *Toxoplasma gondii* in Cattle and Sheep in Isfahan, Iran

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## Abstract

**Background and aims:** *Toxoplasma gondii* is an obligate intracellular parasite which has the potential to infect all warm-blooded animals. Sheep and cattle play a main role in the economy and their infection to *T. gondii*, besides economic losses, can cause the infection of humans through the consumption of raw meat and other products. In this study, we aimed to survey the prevalence of *T. gondii* in the livestock in Isfahan, Iran.

**Methods:** A total of 384 samples including 144 sheep and 130 bovine serum samples from Isfahan and 50 sheep and 60 bovine serum samples from Kashan slaughterhouses were tested for the presence of *T. gondii* antibody using the indirect immunofluorescence method.

**Results:** In general, the antibody was found in 27.31% of sheep and 22.63% of cattle at a dilution of ≥1:200. In detail, 26.15% of bovine and 26.17% of sheep serums from Isfahan and 15% of bovine and 22% of sheep serums from Kashan were seropositive.

**Conclusion:** The study revealed that sheep and cattle can be a significant intermediate host for the spread of the disease in this region (Isfahan province) and the health and livestock authorities in the province should be given priority in improving animal welfare and nutrition.

**Keywords:** *Toxoplasma gondii*, Sheep, Cattle, Indirect immunofluorescence

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## Introduction

*Txoplasma gondii* is an obligate intracellular parasite, from apicamplex phylum, sporozoa class, eucoccidia order, and Sarcocystidae family. Sarcocystidae family members have a complex life cycle in alternating sexual and asexual replication between two different hosts. One of the morphological characteristics is an apical complex that can be seen by electron microscope and includes subpellicular microtubules, conoid, rhoptries, micronemes, polar ring, and in some stage, micropore.[1]

This protozoa has the potential to infect all warm-blooded animals, and it is estimated that one third of the world's adult population is likely to be infected with this parasite. According to the International Bureau for the WHO reports in 2005, the incidence of infection with this parasite is increasing.[2] In some regions of Brazil, 20% to 80% of women at childbearing age are susceptible to infection and in the United States of America, 10%-50% of adults are exposed to this parasite.[3,4]

The most common manifestation of the disease in majority of immunocompetent hosts is neck lymphadenopathy. Multiple enlarged lymph nodes can be noticed and usually there is no adhesion between the nodes or the tissue around. Lymphadenopathy may also be seen on the lower extremities of the posterior cranial, the subclavicular, the inguinal, or the mediastinal regions. In 20%-30% of cases, pervasive lymphadenopathy is reported. Moreover, 20% to 40% of patients with lymphadenopathy develop more symptoms such as anorexia, weight loss, drowsiness, shortness of breath, eye symptoms, vomiting, diarrhea, jaundice, muscle inflammation, boredom, fatigue, fever usually less than 40°C, abdominal pain, maculopapular rashes, meningococcal fever, perturbing sensation, and abortion. Furthermore, rare complications in immunocompetent hosts include pneumonia, myocarditis, encephalopathy, pericarditis, and polymyositis. The symptoms associated with acute infection usually resolve within a few weeks, although lymphadenopathy may persist for several months.[5] The chronic or acute infection in immunocompromised hosts can lead to many injuries, including encephalitis and even death, and the most common opportunistic infection of the central nervous system in AIDS is caused by this parasite.[6]

When host immune responses successfully control acute

infection, some tachyzoites become drowsy parasites called bradyzoites, and create cysts in some parts of the body, such as the muscles and the brain, leading to chronic toxoplasmosis. These cysts contain hundreds of bradyzoites that increase very slowly for many years in the body. If raw cysts are eaten by a carnivorous host, bradyzoites are released in the intestine and once again cause an acute infection.[7] While bradyzoites are eaten by cat family members, both sexual and non-sexual replication cycles take place and as a result, oocysts are formed in the cat's intestine and then excreted with feces. The 12 × 10 µm sized oocysts are sporulated, which can then infect all warm-blooded animals, including humans, birds, and grass mammals like cattle and sheep.[1]

Congenital toxoplasmosis occurs when the parasite is transmitted from the previously infected mother to the fetus through placenta, causing a severe and irreversible disease of central nervous system, epilepsy, development of motor disorders, calcification of the brain, blindness, corretinitis, strabismus, and sometimes death of the fetus.[1] The livestock are also infected by eating infectious oocysts in water or fodder.[8] Toxoplasma infections in animals are similar to human diseases, and in many of them, in particular, in sheep, goats and pigs, cause abortion, imature childbirth, stillbirth and mummies, thereby damaging the livestock industry.[9] With regard to these, the consumption of infected products such as meat in raw or semi-cooked form, uncooked eggs, or unpasteurized milk may cause toxoplasmosis in humans.[10]

Cows and sheep are among the most important livestock whose products including milk and meat have high level of consumption (http://faostat.fao.org, FAO statistical year book 2013).

*Toxoplasma gondi* is a wide spread protozoan which affects both animals and humans. So far, there have been several reports on the prevalence of toxoplasmosis in different world regions. For example, its prevalence in the north of Italy at the farm level, 63.6% (7/11) of farms housing sows and 6.7% (1/15) housing fattening pigs scored positive, with individual prevalences of 8.6% (13/151) in sows and 0.5% (1/219) in fattening pigs.[11] In the north of Portugal, 7.5% of 161 cattle, 33.6% of 119 sheep, 18.5% of 184 goats, and 9.8% of 254 pigs were seropositive,[12] while in Egypt the prevalence was 38.7% in sheep, 28.7% in goats, 23.6% in cattle, and 22.6% in donkeys.[13] In Babol, Guilan province, north of Iran, the prevalence has been reported 31.2% in sheep.[14] Furthermore, the prevalence of the parasite has been reported 31% in Ardebil in the northwest and 35% in Mazandaran province in the north of Iran.[15]

Sheep has a high economic significance and plays an important role in the nutrition of the general public, so it can be considered as a source of infection for human if it is infected.

For better control of this disease, which may cause a lot of damage to humans, especially the fetus of pregnant mothers as well as the livestock industry, it is essential to have epidemiological information about it in order that preventive actions could be taken based on this information. Therefore, the aim of this study was to determine the seroepidemiology of anti-*T. gondii* antibodies in cattle and sheep in official slaughterhouses of Isfahan province in 2017.

## Materials and Methods

In this descriptive study, 384 blood samples were collected from Isfahan and Kashan slaughterhouses. Respective numbers of samples from Isfahan and Kashan were 144 and 50 for sheep and 130 and 60 for cattle. In this regard, 5 mL blood sample was directly collected from the cranial (jugular)vein using sterilized syringes and tubes for animals. The samples were then transferred to the lab and placed in a 37°C incubator for 30 minutes for serum separation. Subsequently, the serum was transferred to another sterilized tube and then centrifuged for 10 minutes to obtain a clear, free RBC. Each sample was then transferred individually to sterile microtubes (1.5 mL), and after numbering, was kept at -20°C until use.

Serum test was done in the Parasitology Department of Isfahan University of Medical Sciences using the indirect immunofluorescence (IFA) method according to the Waller and Anil method (1971).[16]

### *Toxoplasma gondii* Smears

A *T. gondii* suspension was prepared from *T. gondii* infected Balb/c mice and then organisms were passaged in peritoneum of mice and harvested after three days and washed twice with PBS. To avoid the presence of mouse antibody as much as possible, young (8-10 week old)mice were used.

IFA specific slide wells were covered with suspension not more than 5 mm with 300-400 toxoplasma per high power field, and no formalin fixation was made since their antigens might have been damaged by formalin. Then smeares were air dried and stored at -20°C till being used.

### Titration of Samples Sera

Serial dilution were made in saline, using the same pipette for adding diluent and reagent. Three or four drops of each serum dilution were applied to a separate toxoplasma smear. The slides were put in Pyrex Petri dishes along with moist cotton wool to prevent drying. This procedure was followed by incubation at 37°C for 30 minutes. Afterward, they were momentarily rinsed under a gentle stream of tap water and then washed for 15 minutes in saline-filled jars on a shaker. The saline was removed from around the smeares as far as possible.

Anti-sheep IgG (whole molecule)-FITC and Anti-Bovine IgG (whole molecule)–FITC conjugates were purchased from SIGMA-ALDRICH, Germany. One

large drop of diluted mentioned fluorescent conjugate was added and the smeares were incubated at 37°C for 30 minutes. After rinsing the smeares gently under the tap water and immediately washing by agitation in fresh saline for 30 minutes, they were completely, but gently, rinsed under tap water to remove the saline. Mounting and cover glasses were not necessary and after air drying the slides were ready for microscopy.

To confirm the procedure and determine the serum titres, the positive and negative sera of the control were used.

## Results

In this study, a total of 190 cattle and 194 sheep serum samples were collected from the Isfahan and Kashan slaughterhouses.

In the tested sheep sera, 53 (27.32%)had positive antibody titer (1: ≤ 200)and 141 (72.68%)had negative grade (1: ≥200), and in the bovine sera, 43 (22.63%)were positive for the presence of antibodies (1: ≤200)and 147 (77.37%)had a negative grade (1: ≥200). The results for each city are shown separately in Table 1.

Table 1. Seroepidemiology of *Toxoplasma gondii* in Livestock of Isfahan and Kashan Slaughterhouses in 2017, Determined by IFA Method

| Sample Collection Site | Prevalence of *T. gondii* | |
|---|---|---|
| | Cattle | Sheep |
| Isfahan | 26.15% (34) | 26.17 (42) |
| Kashan | 15% (9) | 22% (11) |
| Total | 22.63% (43) | 27.32% (53) |

## Discussion

The current study aimed to investigate the presence of *T. gondii* in the livestock blood samples collected from the slaughterhouses of two regions, namely Isfahan and Kashan. In this respect, the results indicated that the prevalence of this parasite in serum samples collected from sheep in Isfahan and Kashan slaughterhouses was estimated to be 27.32%. This number agrees with the average prevalence in Iran and other world regions.[17] The prevalence in cows was also 22.63%. Although this number is more than the average prevalence in many regions, it is less than the prevalence in sheep in most other areas of the country.

Cows and sheep breeding makes an important contribution to animal husbandry in providing meat and dairy products, and monitoring their health is also of utmost importance for reproduction purposes. In developed countries of Asia where population growth has been seen since last decade, yearly meat consumption has increased by 3% and dairy consumption has raised by 5%. In 2012, 416 thousand tonnes of meat (except fowl) was produced in Iran, from which 126 thousand tonnes was produced from sheep.[17] Accordingly, livestock health assessment and attention to animal health is needed.

Finding an anti-*T. gondii* antibody in livestock affirms that their infection can occur easily.[18]

Worldwide prevalence of *T. gondii* is reported from 3% in Pakistan to 95.5% in Turkey. The average prevalence is 31% across the world.[17]

Iran is a country in the Middle East with climatological diversity, and the different prevalence rates of this parasite have been reported in various areas. For example, the prevalence has been reported 28.2% in Golestan province and 35% in Mazandaran province in the north of Iran.[19]

Other researches have also reported a prevalence of 3.3% in Kerman province in the southeast,[20] 31% in the sheep in Ardebil in the northwest,15 and 16.07% in the aborted fetuses of the sheep in Khorasan Razavi in the northeast of Iran.[21] In Tehran, the prevalence of toxoplasma infection in the cat as the main host has been reported 63%.[22] It seems that the increase of infected cats may occur due to vertical transmission from the mother to the fetus. Statistically speaking, the prevalence of this parasite in warm and wet areas is more than that in warm and dry areas.[22]

Based on the results of studies conducted on the prevalence of infected livestock in Iran that have been published between 1977 to 2012, the average prevalence rates in sheep and goat are 31% and 27%, respectiely.17 There are also some reports on the infection in cows in Iran. According to these investigations, infection in cows was 0% in Mazanadaran in the north, 14.8% in Ahvaz in the southwest, 1.6% in Urmia, and 15.91% in Tabriz in the northwest of Iran.[17,23-28]

The prevalence of toxoplasmosis in the blood samples taken from the sheep in Isfahan and Chaharmahal and Bakhtiari provinces was determined to be 17.9 using the PCR method. Regarding the fact that the parasite is found in the acute phase of the disease in the blood, the chronic stages of the disease may have been ignored. In this regard, the discrepancy between the results of the present study and those of previous studies could be justified.[26]

According to serological assessments, IFA with 95% sensitivity and 96% specificity is the most sensitive and specific method in diagnosing *T. gondii* in comparison with ELISA (enzyme linked immunosorbent), DAT (direct agglutination test), IHA (indirect hemagglutination assay), and LAT (latex agglutination test).[17]

## Conclusion

In conclusion, the results of this study indicated an almost large exposure of sheep and cows to *T. gondii* in Isfahan. Consequently, there is a higher risk for human infection by the consumption of semi-cooked meat of livestock and non-pasteurized dairy products. For the health of livestock, which ensures more people's health, as well as less economic losses, it is recommended that the health and livestock authorities in the province be given priority in improving animal welfare and nutrition.

## Ethical Approval

This research project was approved by the Ethics Committee of Isfahan University of Medical Sciences with code of ethicsIR.MUI.REC.1395.1.168.

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## Conflict of Interest Disclosures

None.

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