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# Seroprevalence of *Toxoplasma Gondii* Infection Among Pregnant Women and Small Ruminant Populations in Sistan Region, Iran

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## Abstract

**BACKGROUND:** Toxoplasmosis is one of the most prevalent parasitic diseases in human societies and animal populations, particularly pregnant women and domestic animals. This life-threatening infection may cause severe consequences in the fetus.

**OBJECTIVES:** This study was aimed to estimate the prevalence of anti-parasite antibodies in pregnant women and sheep and goat populations of Zabol city, Sistan and Baluchistan Province, Southeast Iran.

**METHODS:** Ninety serum samples of pregnant women and 184 serum samples of sheep and goats were collected and anti-*Toxoplasma* IgG and IgM antibodies were examined using a commercial enzyme-linked immunosorbent assay kit. Subsequently, the correlation between the seroprevalence of infection and socio-demographic data was statistically calculated.

**RESULTS:** Among pregnant women, 13/90 (14%) samples were IgG positive and seroprevalence was significantly correlated to history of abortion ($P<0.05$). Among examined sheep and goats, 34 sheep (24.6%) and 3 goats (6.5%) out of 184 (138 sheep and 46 goats) serum samples were positive for parasite-specific IgG. Also, the seroprevalence of infection was significantly associated with animal species ($P<0.05$), history of abortion ($P<0.05$) and parity ($P<0.05$).

**CONCLUSIONS:** According to the findings of this study, despite the relatively low prevalence of infection in pregnant women in Sistan, given the high prevalence of infection in the small ruminant population of the region, more careful monitoring and control of transmission of infection from small ruminants along with other common vectors of the disease are essential. However, more precise investigations are needed to reveal the epidemiological aspects of the parasite in Sistan.

**KEYWORDS:** Abortion; Human; Sheep; Goats; Toxoplasmosis; Zoonosis

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## Introduction

Toxoplasmosis, due to an intracellular obligatory protozoan parasite, *Toxoplasma gondii (T. gondii)*, is one of the most important parasitic infections which is widespread throughout the world and overwhelms nearly all warm-blooded animals as intermediate hosts and it is prevalent globally even in the arctic (Dubey, 2016). The Center for Disease Control and Prevention (CDC) has assigned toxoplasmosis as one of the most neglected parasitic infections (NPIs) with high prevalence and chronic nature (Foroutan-Rad *et al*., 2016). On average, 25-30% of the world's population has acquired the infection at least once; the highest seroprevalence of the disease among human belongs to Latin America, Central and Eastern Europe, and Southeast Asia (75-85%), while the prevalence rate in the Middle East ranges 30-50% (Daryani *et al*., 2014). The infection dominance also differs in different geographical regions and among various populations of a country. According to some meta-analysis studies, the mean prevalence of toxoplasmosis in the general population of Iran is approximately 40% (Daryani *et al*., 2014), whereas it is higher among Iranian immunocompromised patients with 50% (Ahmadpour *et al.*, 2014). Furthermore, the prevalence rate of infection in cat, sheep, goat and cattle populations in Iran reaches 33.6%, 31%, 27% and 18%, respectively (Rahimi *et al*., 2015; Sarvi *et al*., 2015; Sharif *et al.*, 2015). Among domestic animals, sheep and goats are easily infected with *T. gondii* (Sharif *et al*., 2015). Owing to their milk and meat products, sheep and goats are crucial for livestock industry, particularly in developing countries (Cenci-Goga *et al*., 2011; Dubey, 2009). Toxoplasmosis was first described in sheep and goats by Hartley and Feldman (Garcia *et al*., 2012). Oocyst-infected water and food supplies serve as the major sources of infection for sheep and goats. According to seroprevalence studies, antibodies against *Toxoplasma* are commonly detectable among sheep and goat populations worldwide (Sharif *et al*., 2015).

Toxoplasmosis is frequently prevailed in lowlands with warm and humid weather; these climatic conditions are necessary for oocyst sporulation (Dabritz and Conrad, 2010). The primary routes of human infection include: 1) ingesting raw or undercooked meat containing bradyzoites, 2) consuming oocyst-contaminated vegetables and water, 3) vertical transmission, 4) tissue grafts, and 5) blood transfusion (Khademvatan *et al*., 2017). The infection has two clinical phases: the parasite tachyzoites (rapid, invading form) are predominant in acute phase, while bradyzoites (slow, non-invading form) are developed in tissue cysts mostly in muscle and brain during chronic phase (Dubey, 1998). Pregnant women are one of the susceptible individuals to toxoplasmosis, as the infection may render serious sequel in their offspring (Jones *et al*., 2001). The time of toxoplasmosis establishment in gestation course is central to the severity of consequences in the fetus. As the end of pregnancy approaches, more parasites are capable of trans-placental transmission, although harsh outcomes often emerge in first trimester of conception (Kravetz and Federman, 2005). Congenital infection in the fetus may lead to mental retardation, deafness, microcephaly, hydrocephaly as well as retinochoroiditis. Also, toxoplasmosis yields some serious consequences in sheep and goats such as mummification, abortion, stillbirth, fetal and neonatal death, inflicting considerable financial burden annually (Foroutan-Rad *et al*.,

2016; Sharif *et al*., 2015).

Based on seroepidemiologic evidence, one-third of the world population are seropositive, though asymptomatic in terms of toxoplasmosis (Torgerson and Mastroiacovo, 2013). Regarding the fact that Iranians are the major consumers of the milk and meat of sheep and goats, they may be at risk of toxoplasmosis infection. With respect to unknown aspects of toxoplasmosis epidemiology in Sistan region, we decided to investigate the seroprevalence of this infection among pregnant women and the population of sheep and goat herds in Zabol city, Sistan & Baluchestan Province, Southeast Iran.

## Materials and methods

**Study area**

This cross-sectional study was done in Zabol region, Sistan and Balushestan Province, from December 2016 to February 2017. Bordering Afghanistan, this city possesses two major water reservoirs, i.e. Chahnimeh and Hamoon lakes. However, the drying up of the lake Hamoon on the one hand, in addition to the hot and dry climate in the last few decades on the other hand, have led to harsher environments in this territory (https://en.wikipedia.org/wiki/Zabol).

**Sample collection**

In order to assess the role of some possible risk factors, socio-demographic information of each pregnant woman, enclosing age, month of gestation, history of abortion, contact with dog, cat and other domestic animals, consuming raw foods, level of education and place of residence were obtained, according to a pre-designed questionnaire. Subsequently, 90 blood samples were collected. Then, the serum of each sample was separated by means of centrifugation at 3000 rpm for 6 min and stored at -20 °C for further use. Additionally, blood samples were collected from 184 sheep and goats by venipuncture. Briefly, after shaving and disinfecting the skin next to the jugular vein, a volume of 10 ml blood was taken in specific tubes without anticoagulant agents. All taken blood samples were transferred to the Parasitology laboratory, College of Veterinary Medicine, Zabol University, observing the cold chain. Similar to human samples, serum was separated and kept at -20 °C.

**ELISA assay**

A single test was carried out on human blood samples to evaluate the anti-*Toxoplasma* IgM and IgG antibodies using a commercial ELISA kit (Pishtaz Teb Zaman, Tehran, Iran).

The serological assay was performed based on the manufacturer's protocols. Once completed, the results were read at 540 nm by an ELISA reader device (Hiperion microplate reader, England). The experiment was accomplished as single test. Also, three standard solutions with various concentrations were used in the experiment for kit validity.

On animal blood samples, a single test was used to evaluate the anti-*Toxoplasma* IgG antibodies in animal serum samples using a commercial ELISA test (ID screen® Toxoplasmosis Indirect Multi-species ELISA kit, IDVET, Montpellier, France). The P30 (SAG1) antigen of *Toxoplasma gondii* is used as coated antigen in this assay. The serological assay was performed based on the manufacturer's protocols. Briefly, a volume of 100 µl of 1:10 diluted sera and control samples were embedded in P30-coated microtiter plates. Then, the plate was maintained in ambient temperature (21 ± 5 °C) for 45 min. After a phase of three times washing, 100 µl of prepared conjugate 1X was appended to each well and the plate was incubated at room temperature for 30 min. Subsequently, each well was washed three times with 300 µl of the wash solution. Next,

100 µl of the substrate solution was added to the wells. After incubation of the plate in dark room for 15 min, 100 µl of stop solution was incorporated into each well in order to stop the reaction. Finally, the optical density (OD) of each well was read by an ELISA reader device at 450 nm. The interpretation of obtained results was accomplished using the following formula:

$$S/P\ \% = \frac{OD\ (sample) - OD\ (negative\ control)}{OD\ (positive\ control) - OD\ (negative\ control)} \times 100$$

Positive samples were those with an S/P percentage equal to or greater than 50%.

**Statistical analysis**

Obtained data were analyzed by Chi-Squared and Fisher's exact tests. Also, 95% confidence interval for serum prevalence was calculated by binomial distribution. The level of statistically significant results was considered as $P<0.05$.

## Results

### Pregnant women

The seroprevalence of IgM and IgG anti-*Toxoplasma* antibodies in blood serum of pregnant women referred to the reference laboratory of Zabol was 0% (0/90) and 14% (13/90), respectively (Table 1). Moreover, correlation between dependent and independent variables is shown in Table 2. Toxoplasmosis seroprevalence in women with a previous history of abortion was significantly higher than women without abortion ($P<0.05$). There was no statistically significant relationship between *T. gondii* infection and other risk factors.

**Table 1**. Anti-*Toxoplasma* IgG and IgM prevalence in pregnant women of Sistan region

| Anti-Toxoplasma antibody | No. of examined samples | No. of infected animals | Prevalence of serum infection (95% Confidence interval) |
|---|---|---|---|
| IgM | 90 | 0 | 0 % (0% - 4%) |
| IgG | | 13 | 14% (8% - 23%) |

### Sheep and goat

In this study, 37/184 cases (20.1%, CI: 14.6% to 26.6%) were previously exposed to the organism and possessed specific anti-*Toxoplasma* antibodies. Additionally, Table 2 represents the correlation between independent variables and the presence of anti-*T. gondii* antibodies in serum of examined sheep and goats. Accordingly, the prevalence of serum infection was significantly associated with animal species ($P<0.05$), history of abortion ($P<0.05$) and frequency of parturition ($P<0.05$). There was no statistically significant correlation between other risk factors and serum prevalence of toxoplasmosis (Table 3).

**Table 2**. The seroprevalence status of toxoplasmosis in Sistan pregnant women, based on risk factors

| Variable | Level | No. of examined women | No. of infected women | Prevalence of serum infection (%) | P value |
|---|---|---|---|---|---|
| Age | <30 | 52 | 10 | 19 | 0.131 |
| | >30 | 38 | 3 | 8 | |
| Gestation Period | First trimester | 39 | 6 | 15 | 0.824 |
| | Second trimester | 51 | 7 | 14 | |

| Variable | Level | No. of examined women | No. of infected women | Prevalence of serum infection (%) | P value |
|---|---|---|---|---|---|
| History of abortion | No | 71 | 7 | 10 | 0.027* |
| | Yes | 19 | 6 | 32 | |
| Contact with domestic animals | No | 80 | 10 | 13 | 0.155 |
| | Yes | 10 | 3 | 30 | |
| Raw vegetable consumption | No | 48 | 8 | 17 | 0.521 |
| | Yes | 42 | 5 | 12 | |
| Level of education | Illiterate | 5 | 2 | 40 | 0.215 |
| | Primary school | 14 | 2 | 14 | |
| | Secondary school | 28 | 4 | 14 | |
| | Academic | 43 | 5 | 12 | |

*significantly different between groups

**Table 3**. The seroprevalence status of toxoplasmosis in small ruminants of Sistan, based on risk factors

| Risk factors | description | No. of examined animals | No. of infected animals | Prevalence of serum infection (%) | P value |
|---|---|---|---|---|---|
| Animal species | sheep | 138 | 34 | 24.6 | 0.008* |
| | goat | 46 | 3 | 6.5 | |
| Age | 1-3 years | 118 | 21 | 17.8 | 0.295 |
| | 4-6 years | 66 | 16 | 24.2 | |
| Month of gestation | first two months | 74 | 19 | 25.7 | 0.122 |
| | last three months | 110 | 18 | 16.4 | |
| History of abortion | no | 159 | 28 | 17.6 | 0.033* |
| | yes | 25 | 9 | 36 | |
| Parity | 0th | 30 | 1 | 3.3 | 0.035* |
| | 1st | 53 | 11 | 20.8 | |
| | 2nd | 48 | 11 | 22.9 | |
| | 3rd | 32 | 8 | 25 | |
| | 4th | 13 | 4 | 30.8 | |
| | 5th | 8 | 2 | 25 | |

*significantly different between groups

## Discussion

Toxoplasmosis is a zoonotic protozoal disease with probable congenital manifestations in the fetus of pregnant women and small ruminants such as still-birth, sudden abortion and brain calcifications (Foroutan-Rad *et al*., 2016; Sharif *et al*., 2015); hence, it is recommended to clarify the seroepidemiology status of this infection for better preventive measures. This serological research was conducted to elucidate the seroprevalence of anti-*Toxoplasma gondii* antibodies in serum samples of pregnant women and small ruminant populations of Sistan region, Southeast Iran. Based on the results of human samples, the prevalence rate of anti-parasite IgM and IgG antibodies was 0% and 14%, respectively. Positive IgG and negative IgM titers often represent a previous (more than a year ago) exposure to the parasite (Robert-Gangneux and Dardé, 2012). During the last decade, several studies have investigated the *T. gondii* seroprevalence in pregnant women globally. Toxoplasmosis infection is more dominant in the Americas and Africa continents, where seroprevalence in pregnant women ranges from 6.1-77.5% and 25.3-75.2%, respectively, whereas serological rates in Asia and Europe are confined to 0.8-60.4% and 9.1-63.2%, respectively (Pappas *et al*, 2009). In Iran the overall prevalence rates have been as follows: Mazandaran 75% (Panah *et al*., 2013), East Azerbaijan 71.61% (Rajaii *et al*., 2015), Kurdistan 55.22% (Parvizpour *et al*., 2010), Khuzestan 45.45% (Saki *et al.*, 2017), Golestan 41.8% (Sharbatkhori *et al*., 2014), Ghazvin 31% (Tabatabaie *et al*., 2015), Alborz 30% (Akhlaghi *et al*., 2014), Khuzestan 29.35% (Yad *et al*., 2014), Hamedan 29.01% (Hamidi *et al*., 2015) and Sistan and Baluchistan 10.3% (Mousavi *et al.*, 2014). Accordingly, a lower seroprevalence was achieved in our study, in comparison to other parts of the country and similar to a serological investigation in Nikshahr (10.3%) (Mousavi *et al*., 2014). Climate is a substantial parameter for the development of parasite oocysts. In contrast with the southern and northern parts of Iran, where there is sufficient humidity for parasite sporulation and higher prevalence rates have been documented in pregnant women, there exists low prevalence in Sistan and Baluchistan province due to hot and dry climate, particularly in Sistan district (Daryani *et al*., 2014; Foroutan-Rad *et al*., 2016). Pertinent to our findings, only one out of six evaluated risk factors, i.e. history of abortion, was significantly correlated ($P<0.05$) to toxoplasmosis seroprevalence in pregnant women, which is consistent with the results of Sarkar et al (Sarkar *et al*., 2012). Besides, there was no statistically significant association between *Toxoplasma* seropositivity and other risk factors such as age, conception course, contact with domestic animals, level of education and consumption of raw vegetables.

Based on the results of the present study, it seems that there is a direct relationship between the history of abortion and seropositivity against *T.gondii* infection in pregnant women of Sistan region. Alteration in immune system responses and presence of some hormones such as 17β-estradiol and low levels of progesterone and estrogen are the likely reasons of abortion in acute toxoplasmic infection, particularly in first trimester of pregnancy (Galván-Ramírez *et al*., 2014).

Regarding Toxoplasmosis seroprevalence among small ruminants, 34 sheep (24.6%) and 3 goats (6.5%) out of 184 (138 sheep and 46 goats) serum samples were posi-

tive for parasite-specific IgG. Up to now, there were multiple studies that reported the prevalence of anti-*Toxoplasma* antibodies in sheep and goats. During a cross-sectional study in Spain, parasite-specific antibodies were found in 49% of sheep and 25% of goats (Garcia-Bocanegra *et al*., 2013). In a research conducted in Greece, 48.6% and 30.7% of sheep and goats, respectively, were positive for toxoplasmosis (Tzanidakis *et al*., 2012). In Kerman province it was determined that 1.7% of goats and 3.3% of sheep were positive for *Toxoplasma*-specific antibodies, using fluorescent antibody test (Derakhshan and Mousavi, 2014). Akhoundi and Youssefi, (2017) demonstrated that 28.2% of sheep in Golestan province have antibodies against *T.gondii* using IFA method. In a study performed in the center of Iran, Kashan, two separate tests were used to evaluate sheep and goats, resulting in 12.2% and 17.8% of sheep and 4.4% and 8.9% of goats testing positive by ELISA and PCR, respectively (Rasti *et al*., 2018). These studies represent similar results with our study ($P$<0.05). Some authors also reported contrary results; for instance, agglutination latex test was used to examine 200 serum samples, showing 25.4% and 11.2% of goat and sheep infection, respectively (Ramzan *et al*., 2009). Higher infection seroprevalence in sheep and goats were also reported (Wang *et al*., 2011). Regarding open husbandry system in the area, small ruminant exposure to contaminated pastures is inevitable. Meanwhile, seroprevalence rate was higher among sheep, which may be due to higher susceptibility of these animals to toxoplasmosis (Sharif *et al*., 2015). On the other hand, lower goat infection may be due to their eating habits, as they feed on upper plant parts, hence they are less exposed to lower, and probably more-contaminated plant parts (Lu, 1988).

Based on our findings, 36% of seroprevalence was in animals with a previous history of abortion, which was statistically significant ($P$<0.05). In Italy, following 31 cases of toxoplasmic abortion in a farm, anti-parasite IgG prevalence ranged from 31.5% in first sampling to 62.6% in fourth sampling (Zedda *et al*., 2010). In a study on 48000 sheep in Brazil, the prevalence of anti-*Toxoplasma* antibodies in animals with abortion history was reported to be 67.8% (Cosendey-KezenLeite *et al*., 2014). In Pakistan, the seroprevalence rate of infection in sheep populations with abortion history was 51.66% (Ramzan *et al*., 2009). Also, in separate studies the seroprevalence rate among sheep herds with previous abortion was 17.35% and 97.4% in Iraq, and 32.3% in Jordan (Abu-Dalbou *et al*., 2011; Issa and Omer, 2011; Khadi *et al*., 2009). According to studies, the seroprevalence rate is lower in current research, contrary to neighboring countries. This may be dependent on the climate condition in Sistan region, which probably leads to decreased sporulation rate of *Toxoplasma* oocysts in the environment. Then, lesser animal infection would occur. Furthermore, the mechanism of toxoplasmic abortion in small ruminants is not completely understood as yet, but it was substantiated that the blood levels of progesterone in sheep declines following Toxoplasma infection. This hormone is vital for maintaining pregnancy and its reduction exposes the risk of abortion (Galván-Ramírez *et al.*, 2014).

Moreover, in our study we found that frequency of parturition is relatively directed to the rate of seroprevalence infection of toxoplasmosis in examined animals ($P$<0.05). This relationship could be justified by the fact that seroprevalence increases with age (Dubey, 2016), although the correlation be-

tween age and seroprevalence was not statistically significant in our work. Nevertheless, seroprevalence rates are close in second to fourth labor and decreases in the following. So, further studies are recommended. In our work, seroprevalence increases with age, but it is not statistically significant ($P$<0.05), while some studies in Pakistan and Brazil show that this correlation is significant (Cosendey-KezenLeite *et al*., 2014; Hanif amd Tasawar, 2016). Lack of significant rates in our research may result from low sampling pool and inappropriate categorizing of age groups.

Despite the improved diagnostic techniques for toxoplasmosis, serological methods are still convenient and accessible experiments for initial screening of animal and human populations. The results of current research among pregnant women and small ruminant populations of Zabol city represented a relative prevalence of Toxoplasma infection in the area. Regarding traditional animal breeding as well as non-sanitary animal slaughter in some parts, the risk of infection is considerable in Sistan region. It is recommended to exclude cats from animal farms, inform people, particularly pregnant women, about parasite transmission and disease symptoms, perform regular serological tests and molecular confirmations as well as boil goat milk and consume cooked sheep meat. Also, determination of Toxoplasma genotypes in different animal and human populations of this territory is necessary.

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## Conflict of interest

The authors declared that there is no conflict of interest.

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# شیوع سرمی انگل توکسوپلاسما گوندی در خانم های باردار و جمعیت نشخوارکنندگان کوچک منطقه سیستان

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## چکیده

**زمینه مطالعه:** توکسوپلاسموز یکی از شایع ترین بیماری های انگلی در جوامع بشری و جمعیت حیوانات به ویژه زنان باردار و حیوانات اهلی است. این عفونت خطرناک برای زندگی ممکن است عواقب شدیدی را در جنین ایجاد کند.

**هدف:** این مطالعه با هدف تعیین شیوع آنتی بادی های ضد انگل در زنان باردار و جمعیت گوسفند و بز شهر زابل ، استان سیستان و بلوچستان ، جنوب شرقی ایران انجام شد.

**روش کار:** ۹۰ نمونه سرم از زنان باردار و ۱۸۴ نمونه سرم نشخوارکنندگان کوچک جمع آوری و آنتی بادی های ضد توکسوپلاسما IgG و IgM با استفاده از کیت الیزا مورد بررسی قرار گرفت. پس از آن ، همبستگی بین شیوع عفونت و داده های اجتماعی و جمعیت شناختی از نظر آماری محاسبه شد.

**نتایـــج:** در بین زنان باردار ، ۱۳/۹۰ درصد (۱۴ درصد) نمونه IgG مثبت بودند و میزان شیوع آن با سابقه سقط ارتباط معنی داری داشت ($P<۰/۰۵$). در بین حیوانات مورد بررسی ،۳۴ رأس گوسفند (۲۴/۶٪) و ۳ رأس بز (۶/۵٪) از بین ۱۸۴ رأس (۱۳۸ گوسفند و ۴۶ بز) حیوان به لحاظ IgG ضد توکسوپلاسما مثبت بودند. همچنین شیوع آلودگی سرمی با گونه های حیوانی ($P<۰/۰۵$)، سابقه سقط ($P<۰/۰۵$) و فراوانی زایمان ($P<۰/۰۵$) ارتباط معنی دار داشت.

**نتیجه گیری نهایی:** براساس یافته های مطالعه حاضر، علیرغم پایین بودن نسبی شیوع عفونت در زنان باردار در منطقه سیستان، با توجه به اینکه شیوع عفونت در جمعیت نشخوارکننده کوچک منطقه قابل ملاحظه است، مراقبت و نظارت دقیق تر در کنترل انتقال آلودگی از گوسفند و بز در کنار سایر ناقلین معمول بیماری ضروری می باشد. هرچند تحقیقات دقیق تری برای آشکار ساختن جنبه های اپیدمیولوژیک انگل در منطقه سیستان مورد نیاز است.

**واژه‌های کلیدی:**

انسان، توکسوپلاسموز، زئونوز، سقط، گوسفند و بز.

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