# Sero-Prevalence of Toxoplasmosis in Herbivorous Livestock in Northern Iran

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**ABSTRACT**

*Toxoplasmosis is a zoonosis that distributed in worldwide caused by the coccidian parasite Toxoplasma gondii. In present Study, Sero-Prevalence of T. gondii in Cattle, sheep and goats were investigated in three adjacent geographical areas within Mazandaran province in northern Iran. Serum samples were collected randomly from 384 Cattle, 384 sheep and 384 goats in three counties; Chalous with a humid subtropical climate, Babol with sub-humid climate and Savadkouh with cold semi-arid steppe climate. The samples were tested by using Sabin- Feldman Dye Test for Toxoplasma antibodies. The prevalence was in Cattle 10.94%, in sheep 27.08% and 20.31% in goats. The overall prevalence in sheep was significantly higher than the goats and Cattle (P<0.001). In addition, the prevalence in sheep was greatly increased with a humid subtropical climate (P<0.001). As a result, the high prevalence of T. gondii have seen in this area shows that herbivorous livestock have an important role in transmission of infection to humans by eating uncooked meat. In addition, the economic losses caused by T. gondii infection in these animals is striking.*

**Keywords: Sero-Prevalence, Toxoplasmosis, Livestock, Herbivorous, Iran.**

## 1. INTRODUCTION

Toxoplasmosis is a zoonosis that distributed in worldwide caused by the coccidian parasite *Toxoplasma gondii* [1-9]. All warm-blooded animals, including small ruminants, humans, marine mammals and birds can infect by this intracellular parasite [4-8, 10-13]. Human infection may occur through the ingestion of food, water or soil contaminated by oocysts excreted in feline feces specially through the ingestion of uncooked or undercooked meat containing tissue cysts or by mother-to-child transmission during pregnancy [2-7, 10, 13, 14]. The most infections in herbivorous animals occurs following the consumption of infective oocysts in the pasture, feed and drinking water [2, 10]. Abortion at any stage of pregnancy, fetal resorption, fetal mummification and stillbirth or birth of weak live offspring are causes by Toxoplasmosis in Cattle, sheep and goats [8, 11]. It can cause congenital disease in all warm-blooded animals, Among humans [6]. Congenital toxoplasmosis may lead to abortion, neonatal death, ocular signs such as chorioretinitis or neurological symptoms include hydrocephalus [2]. The parasite in the tissue, among animals is most common in herbivorous Livestock, also in poultry and rabbits [7]. *T. gondii* infection in cattle naturally does not appear to cause clinical disease or abortion [2, 7]. Tachyzoite, rapidly dividing invasive, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst are the main three infective stages of *T. gondii*. The domestic cats, are the only definitive hosts in which oocyst develop [1, 3, 4, 6, 9-11]. Oocysts of *T. gondii* shows a characteristic resistance against a wide range of disinfectants due to structural stability principally in warm and humid regions of the world, nonetheless having poor survival in arid and cold climatic conditions [8]. The main transmission routes in humans may vary between different geographical areas and ethnic groups; nevertheless, consumption of undercooked meat is a major risk factor and may be 50% or more of the cases of toxoplasmosis[2]. Near to one third of people have been exposed to *T. gondii* [5]. As well as in small ruminants may cause the monitory losses in terms of the reproductive disorders and reduced productivity with lower quality of wool and leather [8]. This disease also causes serious economic losses to Livestock industry worldwide [11]. Infection of *T. gondii* is deleterious in terms of both economy of a country and health of its people and approximately thirty three percent of animals and the human population of the world has been estimated to be infected with *T. gondii* at an average ranging between 1 and 99% rates of infections [8]. Serological studies done in various parts of the world show that in some countries more than a third of the people have antibodies against *T. gondii* [11]. Seroprevalence of *T. gondii* in livestock around the world vary widely, ranging from 3% to 96% in sheep, 4% to 77% in goats, 0.4% to 96% in pigs and 2% to 83% in cattle, with

seropositivity increasing with age [2]. Diagnosis of Toxoplasma infection is usually by serological tests. Sabin-Feldman Dye test (DT) is generally used as the standard test for the diagnosis of *T. gondii* infection and is one of the most widely used tests for screening Toxoplasma infection in animals [14]. The rate of Prevalence of Toxoplasma investigated in different species of animals in most countries with differing results from country to country, region to region, herd to herd and Seasons [8]. Iran is a large country with various weather across the country. Several studies have been conducted throughout the country, however, few studies have investigated the prevalence in livestock in northern Iran and no report is available for Mazandaran province which has the most humid climate. In present Study, Sero-Prevalence of *T. gondii* in Cattle, sheep and goats were investigated in three adjacent geographical areas within Mazandaran province in northern Iran, with great differences in average annual precipitation (AAP); from 400 to 1400 mm. The results could have implications for decreasing the incidence of Toxoplasmosis in animals and human populations.

## 2. MATERIAL and METHODS

### 1.1 Study Area

Mazandaran province is located in the south of the Caspian Sea in northern Iran. Three adjacent counties of Mazandaran province were included in the study; Chalous a coastal town on the coast of the Caspian Sea with a humid subtropical climate; Babol, in the center of Mazandaran province with a sub-humid climate and Savadkouh, a mountainous city in the southern province of Mazandaran with a cold semi-arid steppe climate. The AAP is about 1400 mm in Chalous, 700 mm in Babol and 400 mm in Savadkouh.

### 2.2. Serum Samples

Blood samples were collected randomly according to the Morgan table from 384 cattle, 384 sheep and 384 goats from the three counties among one year in 2018. The samples were collected directly from the cervical vein of each animal by using sterile syringes and tubes and transported to the laboratory. Then Serum samples were extracted from 5 ml blood samples by centrifugation at 2000 g for 10 min, and stored at -20°C before testing.

### 2.3. Sabin-Feldman Dye Test

SFDT is based on methylene blue staining of intact *T. gondii* tachyzoites, whereas parasites killed by complement-mediated lysis do not take up the dye and remain colorless. Live tachyzoites required for the test were prepared from peritoneal fluid of laboratory Swiss mice. The mice were infected with tachyzoites and three days later tachyzoites were collected by repeated flushing of the peritoneal cavity with phosphate-buffered saline (PBS; pH 7.4). Serum samples were heated at 60°C for 20 min to inactivate preexisting complement, and diluted 1:2 with PBS in a flat-bottomed 96-well plate. Then, live tachyzoites were added, and the plate was incubated for 60 min at 37°C. The Methylene blue solution was added to the wells, and the plate was kept at room temperature for 10 min. The SFDT result was regarded positively if more than 50% of tachyzoites remained unstained under the light microscope at 40 × magnifications.

### 2.4. Statistical analysis

Chi-squared test ($\chi 2$) was used to compare the prevalence among the three counties, and the prevalence in Cattle, sheep and goats in each county. The differences were considered statistically significant when probability P-value was $<0.05$. The 95% confidence intervals (95% CI) of seroprevalence rates were calculated. Statistical analysis was performed using IBM® SPSS software version 17.

## 3. RESULTS

In present Study, performed SFDT on serum samples of 384 Cattle, 384 sheep and 384 goats collected from three different regions of Mazandaran province in northern Iran with various weather and annual precipitation. The overall prevalence in Cattle, sheep and goats was 10.94%, 27.08% and 20.31%, respectively, and showed statistically significant difference ($P<0.001$) (Table 1). The prevalence in sheep was significantly ($P<0.001$) higher than the goats (40.62% vs. 27.34%) in Chalous, with AAP of 1400 mm. It is interesting, the prevalence in sheep was greatly different in between the three counties, and increased with the AAP ($P<0.001$); the prevalence in sheep was 40.62% in Chalous (AAP of 1400 mm), 29.68% in Babol (AAP of 700 mm) and 10.93% in Savadkouh (AAP of 400 mm). Alike, the prevalence in goats from Chalous was significantly ($P<0.05$) higher than the goats from Babol and Savadkouh. The prevalence in goats was 27.34% in Chalous (AAP of 1400 mm), 21.09% in Babol (AAP of 700 mm) and 12.5% in Savadkouh (AAP of 400 mm). The prevalence in Cattle was 16.4% in Chalous (AAP of 1400 mm), 10.15% in Babol (AAP of 700 mm) and 6.25% in Savadkouh (AAP of 400 mm) (Table 2). The highest prevalence of infection in sheep and cattle in the lowest pollution was observed. The prevalence in the sheep was considerably more of the cows and goats. The ratio of prevalence of T. gondii in sheep, a total of 46.43% of the infected animals, the ratio of the prevalence

of infection in cows, of the whole of the animals infected with Toxoplasma, 18.75% and the prevalence of infection in goats, of the whole of the animals Infected with 34.82%, respectively.

***Table 1**: Seroprevalence of T. gondii infection in sheep, cattle and goats in three adjacent counties of Mazandaran province, northern Iran*

| County | Climate/AAP | Species | No. of animals tested | No. of Positive animals | Prevalence % (95 % CI) |
|---|---|---|---|---|---|
| Chalous | Humid subtropical | Sheep | 128 | 52 | 40.62% |
| | | Cattle | 128 | 21 | 16.4% |
| | | Goat | 128 | 35 | 27.34% |
| Babol | Sub-humid | Sheep | 128 | 38 | 29.68% |
| | | Cattle | 128 | 13 | 10.15% |
| | | Goat | 128 | 27 | 21.09 |
| Savad kouh | Cold semi-arid | Sheep | 128 | 14 | 10.93% |
| | | Cattle | 128 | 8 | 6.25% |
| | | Goat | 128 | 16 | 12.5% |
| Total | - | Sheep | 384 | 104 | 27.08% |
| | | Cattle | 384 | 42 | 10.94% |
| | | Goat | 384 | 78 | 20.31% |

***Table 2:** Toxoplasma gondii distribution of positive cases by region and type of livestock in the Mazandaran province, northern Iran*

| Species / County | Cattle No. of Positive animals (%) | Sheep No. of Positive animals (%) | Goat No. of Positive animals (%) |
|---|---|---|---|
| Chalous | 21 (16.4) | 52 (40.62) | 35 (27.34) |
| Babol | 13 (10.15) | 38 (29.68) | 27 (21.09) |
| Savadkouh | 8 (6.25) | 14 (10.93) | 16 (12.5) |
| Total Positive animals | 42 (10.94) | 104 (27.08) | 78 (20.31) |
| Total animals tested | 384 | 384 | 384 |

## 4. DISCUSSION

The overall prevalence in sheep was significantly higher than the goats and Cattle (P<0.001). Moreover, the prevalence in sheep was greatly increased with AAP (P<0.001); the prevalence in sheep was 27.08%, in Cattle 10.94% and 20.31% in goats.

Many studies on toxoplasmosis have been investigated in the different animal populations in Iran, where a wide distribution of the parasite has been observed. In addition, the prevalence of *T. gondii* in animals in many parts of Iran is almost a lot [15]. In the west of Iran in Kermanshah Province, *Hamzavi et al. (2007)* Reported Totally about 18.2% of study animals showed anti-toxoplasmic antibodies include 23.7% of goats, 22.5% of sheep and 4.8 % of cattle [16]. *Havakhah et al. (2014)* in the similar Study on Seroprevalence by *T. gondii* infection reported in sheep the overall prevalence in sheep and goats was 36.8 and 12.9%, respectively [14]. *Asgari et al. (2013)* reported Anti-Toxoplasma antibodies were detected 34.9%, include: 55% in cattle, 29.5% in sheep, 18.8% in goats, 40% in horses, 51.5% in dogs and 11.1% in turkey at southern Iran [15]. *Khamesipour et al. (2014)* in a study design the current research aimed to determine the occurrence of the parasite in cattle, camels and sheep in Isfahan and Chaharmahal va Bakhtiary provinces of Iran, reported *T. gondii* infections were detected in 0.00%, 6.60% and 17.9% of the cattle, camels and sheep respectively. In that Survey Sheep were more frequently affected in Chaharmahal va Bakhtiary 33.33% compared to Isfahan 8.47% [17]. *Rahdar et al. (2012)* in a study in slaughtered animals and meat products in Ahvaz, in south of Iran,

reported Total positivity rate for *T. gondii* 4.7% include 14% in lambs and 4% in beef were found as positive [18]. *Sanati et al. (2012)* in one study in Kerman, southeastern province of Iran, reported in cattle 71.3% seropositive [19]. In another study by *Gharekhani (2013)* in Hamedan province, Western Iran; 2.3% of the samples were seropositive [20]. A total of 22 studies since 1983 to 2012 reporting the seroprevalence of toxoplasmosis in cattle from different regions of Iran from over the 30-year period was estimated 18.1%. The Seropositive rate of cattle toxoplasmosis in various regions of Iran was between 1.4% and 71.3% in Kerman and Tehran Province, respectively [21]. The difference in the prevalence is depended to many factors, including relative distribution of stray cats, performance Livestock farms, ambient humidity and farming operation. Among livestock, sheep are highest prevalence of congenital Toxoplasma infection, abortion caused it and neonatal loss [14]. The results from across the world indicated various measures. *Ahmad and Tasawar (2015)* in a study in Punjab, Pakistan, indicated the total infection rates collectively found in small ruminants were 31.41% and the incidence rates of Toxoplasma infection in small ruminants were 38.1%, 32.1% and 14.5% in animals reared in Agricultural Region, Riverside region and Sand Dunes region of Cholistan correspondingly [8]. In a study located in Eastern Mexico, a frequency ranges of 77-84% of *anti-T. gondii* antibodies were found in sheep, using an indirect ELISA and the highest prevalence of toxoplasmosis in environments with warm and humid climates was attributed to the high viability of the T. gondii oocysts. In Northern Mexico, a *T. gondii* seropositivity of 15.1% was found in animals tested. In Southern Mexico, a seroprevalence of 23.1% was found in sheep [4]. *Kalita and Sarmah (2015)* indicated Prevalence of *T. gondii* infection among cattle of Assam, in India, 26.66% seroprevalence against 16.66% recorded by LAT in the sera of open grazing cattle [9]. *Kadle (2014)* in a sero-prevalence survey of Toxoplasmosis in domestic animals was conducted in the Benadir region of Somalia, 15.9% of study animals showed sero-positive such as 6.3% of camels, 7.1% of cattle, 34.5% of sheep and 26.7% of goats [6]. In Sudan reported 32% in cattle and 57.5% in sheep [22]. In a survey carried out to show the prevalence of antibodies of *T. gondii* in sheep by (LAT) in different geographical areas in Libya was 71% [23]. *Negash et al. (2004)* reported in Ethiopia Seroprevalence of Toxoplasmosis 52.6% in sheep [24]. In Pakistan in goat were reacted positively 52% [25]. In Ethiopia 24% in goats were reported [24]. In another study by *Hamilton et al. (2015)* in west Indies, *T. gondii* DNA was detected in heart tissue about 21% of pigs, 16% of sheep and 23% of goats tested [2]. *Yildiz et al. (2015)* in a study in different locations of Ankara and Kirikkale provinces of Turkey, the prevalences of the tissue cysts were detected in 21.2% of the meat samples with Percoll gradient centrifugation as 20.8% of the meat samples obtained from Ankara and 22.4% from Kirikkale [3]. In a serology study by *Holec-Gasior et al. (2013)* in Northern Poland, the prevalence of *T. gondii* among cattle was 3.1% [7]. *Kader and Al-Khayat (2013)* from Erbil city in Iraq, were examined for antibodies to *T. gondii* by using latex test, 25.4% in sheep and 28.4% in goats were seropositive [11]. *Tasawar et al. (2013)* at Punjab, Pakistan reported prevalence of *T. gondii* in Cattle, with an overall prevalence of 43.5% include 47% in female and 39.5% in males [26]. *Ahmad and Qayyum (2014)* in a study in northern parts of Punjab, Pakistan, reported the overall prevalence of infection was 19.75% in cattle and 15.16% in buffaloes [27]. *Wiengcharoen et al. (2012)* in Thailand among beef cattle slaughtered for food in western, the antibodies to *T. gondii* was found in 25.7% [28]. *Song et al. (2011)* indicated the prevalence of Toxoplasma gondii using a latex agglutination test (LAT) in native Korean cattle 3.8% [29]. *Fajardo et al. (2013)* reported an overall seroprevalence of 2.68% of toxoplasmosis in cattle in Brazil [30].

The results of this survey, were consistent with results published from elsewhere in Iran by *Hamzavi et al. (2007)*, *Asgari et al. (2013)* and *Sarvi et al. (2015)*. Also the results of this study were close to the results of similar studies by *Havakhah et al. (2014)* with almost identical weather in Gilan province in northern Iran. But the results of this study indicated that the prevalence in Mazandaran was lower than the Gilan province. Moreover, the results of the present study were significantly less than Study by *Sanati et al. (2012)* in Kerman, southeastern province of Iran. Also the results of the present study were considerably less than the studies by *Ahmad and Tasawar (2015)*, *Tasawar et al. (2013) and Ahmad* and *Qayyum (2014)* that were conducted in Pakistan. Although the results of this study indicated that the seroprevalence of *T. gondii* in the present study was higher than from the results of other researchers in Brazil by *Fajardo et al. (2013)*, Korea by *Song et al. (2011)*, Poland by *Holec-Gasior et al. (2013)* and by *Wiengcharoen et al. (2012)* Thailand. But the results of this study with the results of the investigation in Iraq by *Kader and Al-Khayat (2013)*, Turkey by *Yildiz et al. (2015)*, India by *Kalita and Sarmah (2015)* and *Hamilton et al. (2015)* and Somalia by *Kadle (2014)* were consistent. The Studies that have been done in most African countries indicate that the prevalence of *T. gondii* was more than present study. In the studies have been done in Ethiopia by *Negash et al. (2004)*, Libya by *Al-mabruk et al. (2013)*, Sudan by *Khalil and Elrayah (2011)* and Mexico by *Hernandez-Cortazar et al. (2015)*, prevalence of Toxoplasma infection was considerably higher than my Study.

Lower seropositive in cattle compared to sheep and goats might be attributed to differences in the susceptibility to *T. gondii* infection and to differences in management practices [16]. Nevertheless, it seems that higher ambient humidity favors spore production and survival of oocysts, as a result of that increasing the risk of infection, in the counties with higher AAP. Truly, the outbreak in humans in the provinces of Mazandaran and Gilan, with humid subtropical climates, is about 70%, which is much higher than elsewhere in the country [14].

Generally, in the present study were indicating a high prevalence of the infection with *T. gondii* in studied animals in Mazandaran province. The difference in the prevalence of *T. gondii* in herbivorous livestock in Iran and other countries may be due to the sensitivity of the host, and the methods used to determine the seroprevalence. Moreover, many other factors Like the management and hygienic standards in breeding, the density of cats and environment conditions for effective animal parasite oocysts. As a result, the high prevalence of *T. gondii* have seen in this area shows that herbivorous livestock have an important role in transmission of infection to humans by eating uncooked meat. In addition, the economic losses caused by *T. gondii* infection in these animals is striking.

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