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Toxoplasmosis in Sheep from Kurdistan Province, Iran

[1]M. Khezri, [1]B. Mohammadian, [2]K. Esmailnia and [3]O. Khezri
[1]Agricultural and Natural Resources Research Center, Kurdistan, Iran
[2]Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran
[3]Shahid Beheshti University of Medical Students, Tehran, Iran

Corresponding Author: M. Khezri, Jame-Jam Intersection, Pasdaran Blvd, Sanandaj, Postal Box 714, Kurdistan, Postal Code 6616936311, Iran Tel: 989181710608 Fax: 988716623351

ABSTRACT

Toxoplasmosis is one of the most common parasitic zoonosis in Iran. The causative agent, *Toxoplasma gondii*, uses a wide range of warm-blooded intermediate hosts in its life cycle, including sheep. Sheep is an important domestic animal in the Kurdistan province of Iran due to its minimal rearing and maintenance costs and production of meat, milk and wool. This study was conducted on the seroprevalence of *T. gondii* infection in sheep from different regions of Kurdistan province between December 2008 and September 2009 and analyzed the main risk factors associated with the infection. Sera from 368 sheep were examined for anti *T. gondii* IgG antibodies by indirect enzyme-linked immunosorbent assay ELISA test. According to the results, the seropositive rates of sheep were 21.74%. Though, the southern with 44% and western with 13.25% had the highest and lowest infection rates, respectively; the differences between geographical locations were statistically significant ($p<0.05$). Although, the specific antibodies were detected in 20.87% of males and 22.13% of female's sheep but there were no significant differences between two genders. On the other hand, no significant differences were observed between age groups. These results indicate that the seroprevalence of *T. gondii* infection in sheep is relatively high.

Key words: Iran, ELISA test, sheep, toxoplasmosis, seroprevalence

INTRODUCTION

Toxoplasmosis is worldwide-established parasitic zoonosis capable of causing clinical manifestations such as abortion, stillbirths, fetal death or birth of weak, non-viable animals (Tenter *et al*., 2000; El-On and Peiser, 2003). The parasite has a worldwide distribution and it is mainly transmitted by food contaminated with Oocysts dispersed by definitive hosts, cats and other felines, uncooked meat containing tissue cysts or non-pasteurized milk containing tachyzoites stages and transplacentally (Sacks *et al*., 1982; Dubey, 1996; Jittapalapong *et al*., 2005; Sukthana, 2006; Clementino *et al*., 2007). Toxoplasmosis is a parasitic disease that causes serious reproductive problems and economic losses to the sheep industry all over the world (Buxton *et al*., 2007).

Sheep are important to the economy of many countries because they are a source of food for humans sheep are commonly infected with the protozoan parasite, *Toxoplasma gondii*. Infection with the parasite may cause early embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death. Severity of infection is associated with the stage of

pregnancy at which the ewe becomes infected, the earlier in gestation, the more severe the consequences. Infected sheep meat is a source of *T. gondii* infection for humans and carnivorous animals. Most sheep acquire *T. gondii* infection after birth and less than 4% of persistently infected sheep transmit the parasite vertically to the next generation (Dubey, 2009).

In a review by Tenter *et al.* (2000) of surveys carried out in Europe, values range from 4 to 92% in farmed sheep. Antibodies to *T. gondii* were found in (22.0%) lambs and in (65.6%) ewes slaughtered in Haute-Vienne district (Dumetre *et al.*, 2006).

In animals, *T. gondii* infection not only in significant reproductive and hence economic losses but also has implications for public health since consumption of infected meat or milk can facilitate zoonotic transmission. *T. gondii* can be transmitted directly by animal-human contact or through contact with contaminated feces, soil or herbage, it can also be transmitted through contaminated food or water (Jittapalapong *et al.*, 2005).

In Iran, studies have shown the presence and importance of *T. gondii*, especially in sheep and goats (Ghazaei, 2005; Hamidinejat *et al.*, 2008; Hoghooghi-Rad and Afraa, 1993; Sharif *et al.*, 2007; Zia-Ali *et al.*, 2007).

Since, there is little information on the prevalence of infection in Kurdistan province (west Iran) the objectives of the present study were, therefore, to investigate the prevalence of *T. gondii* and its relationships between age, sex and various geographical regions.

MATERIALS AND METHODS

Kurdistan covers a territory of 28,203 km^2, all sample areas were between 750 and 3300 m above sea level and rainfall ranges from 250 to 800 mm $year^{-1}$. Kurdistan has three types of weather. Southern and central parts of the temperate climate and average rainfall of 450 mm. Eastern and northern part shave cold and relatively dry climate, with rainfall less than 300 mm. Western part is cold and wet climate and average rainfall is 700-800 mm. A total sheep of different ages (>6 months old, 6 = 18 months old and <18 months old) and sex from various locations in the Kurdistan province were selected for study. Blood samples were obtained from 386 sheep (115 male and 253 female) between December 2008 and September 2009 from the various geographical regions of Kurdistan province, Iran. Sera were extracted from 5 mL venous blood samples, by centrifugation at 2000 g for 10 min and were stored at -20°C prior to testing. Sheep IgG antibodies against *T. gondii* were tested using an enzyme-linked immunosorbent assay (ELISA). The indirect ELISA (ID. VET. Innovative diagnostics, France) was performed by commercial kit. Optical densities (OD) were read at 450 nm. The results were expressed as the percentage of the mean absorbance values of sample (S) to the mean absorbance value of the positive (P) control sample provided with the diagnostic kit. The resultant S-P ratio was expressed as a percentage (S/P %). According to the manufacturers recommendation, sera with S/P% = 40% should be regarded as negative, between 40 and 50% as doubtful, between 50% = and <200% as positive and = 200% as strong positive.

Statistical analysis: The Chi-squared test was used for statistical analysis using SPSS, version 11.5, $p<0.05$ was considered significant.

RESULTS

Using the cELISA, from 386 tested samples, IgG prevalence of toxoplasmosis among sheep was 21.74% (80 sheep) in Kurdistan province, Western Iran (Table 1). The results of age, sex and different regions with positivity to *T. gondii* are summarized in Table 2, 3 and 4, respectively. The

Table 1: Seroprevalence of *Toxoplasma gondii* antibodies in sheep between sexes in Kurdistan province, Iran

Parameters	Male sheep	Female sheep	Total
No. of sheep examined	115	253	368
No. of sheep Doubtful	7	17	24
No. of sheep infected	26	54	80
Infection (%)	22.60	21.34	21.74

Table 2: Seroprevalence of *Toxoplasma gondii* antibodies in sheep between ages in Kurdistan province, Western Iran

Age groups (months)	No.	Positive	Infection (%)
>6	53	15	28.3
6≥18	96	12	12.5
<18	219	53	24.2

Table 3: The rate of sheep *Toxoplasma* infection in different districts of Kurdistan, Western Iran

Districts	No.	Positive	Infection (%)
Center	55	15	27.27
Northern	117	27	23.08
Southern	25	11	44
Western	68	9	13.24
Eastern	102	18	17.65
Total	368	80	21.74

Table 4: The rate of toxoplasmosis infection of sheep in different cities of Kurdistan province

City	No.	Seropositive	Infection (%)
Sanandaj	27	5	18.52
Dehgolan	28	10	35.71
Divandareh	74	21	28.38
Saquez	43	6	13.95
Kamyaran	25	11	44.00
Baneh	30	4	13.33
Marivan	38	5	13.16
Bijar	74	11	14.86
Ghorveh	29	7	24.14
Total	368	80	21.74

highest frequency of infection was seen in Southern parts (44%, $\alpha = 0.05$) and lowest Western localities (13.24%, $\alpha = 0.0.5$) (Table 3). The highest prevalence (44%) was observed in Kamyaran. However, the lowest prevalence (13.16%) was found in Marivan (Table 4).

There was no difference among seropositivity for *T. gondii* in serum samples in all age groups ($p>0.05$). The seroprevalence of antibodies in female and male were 22.60 and 21.34%, respectively ($p>0.05$). There were variations in the rate of *T. gondii*-positive samples in different regions ($p<0.05$).

DISCUSSION

Prevalence of toxoplasmosis across the world is variable, with prevalence rates from 0 to 100% in different countries (Olivier *et al*., 2007; Tenter *et al*., 2000), depending upon their customs, traditions, life styles of the inhabitants, weather conditions, age of the animals and husbandry practice (Smith, 1999).

This study showed, the prevalence of sheep toxoplasmosis from Kurdistan province, for the first time in Iran. The seroprevalence of *T. gondii* in sheep was 21.74%. The rate of toxoplasmosis in our study is close to some studies (Zia-Ali *et al*., 2007; Sabry and Reda, 2008), respectively, who reported 20.9, 22 and 25.6% of *T. gondii* infection in sheep in Iran, Brazil and Egypt. However, higher incidence rates 40.4, 41.7, 50, 51.5, 52.2, 67.7, 72.6 and 84.5% were recorded by Mainar-Jaime and Barberan (2007) in Spain, Shaapan *et al*. (2008) in Egypt, Mason *et al*. (2010) in UK, Romanelli *et al*. (2007) in Brazil, Sanad and Al-Ghabban (2007) in Saudi Arabia, Hove *et al*. (2005) in Zimbabwe, Hamidinejat *et al*. (2008) in Iran and Klun *et al*. (2006) in Serbia, respectively but the lower values of 3.8, 4.3 and 11.2% detected by Sharma *et al*. (2008) in India, Samra *et al*. (2007) in South Africa, Ramzan *et al*. (2009) in Pakistan, respectively.

These differences in seropositivity between the different countries indicate that animals bred in these areas were exposed to different environmental contamination with *T. gondii* oocysts.

Further, it can be related to differences in techniques used in each study to monitor the *T. gondii* antibody (Ramzan *et al*., 2009).

The results of this study showed that, there was no significant difference in sex for antibodies to *T. gondii* (Table 1). This finding is similar to Bonyadian *et al*. (2007), Oncel and Vural (2006) and Gorman *et al*. (1999), While in contrast with the results of Ramzan *et al*. (2009), Lashari and Tasawar (2010) and Clementino *et al*. (2007).

However, Alexander and Stinson (1988) reported that female animals were more susceptible to be infected with *T. gondii*. The literature generally indicates that females have more immunity than males which may be due to the presence of estrogen in females which normally increases the immunity, while androgen in males decreases the immunity Romanelli *et al*. (2007). However, there are various other factors which may break down the immunity in females' e.g., changes in sex-associated hormones, environmental factors, age, nutrition and pregnancy (Martin, 2000; Messingham *et al*., 2001).

This study shows no positive association between the presences of anti-*T. gondii* antibodies and sheep age. It is widely accepted that animals acquired Toxoplasma infection with the acquisition of age through ingestion of infective Oocysts from the environment (Ramzan *et al*., 2009; Figliuolo *et al*., 2004; Van der Puije *et al*., 2000) as apparently opportunity for exposure to *T. gondii* is routinely available. As animal ages, its cumulative likely hood for exposure increases. Due to some reasons, the age of animals is considered an important factor in determining prevalence rate of toxoplasmosis in animals (Dumetre *et al*., 2006; Van der Puije *et al*., 2000). Older sheep have a higher prevalence of toxoplasmosis than younger sheep. According to the results of the prevalence of *T. gondii* in present study was higher in younger animals than adult ones. This could be explained on the basis that the animals included in this age group were less resistant to *T. gondii* (Yung, 2000; Pawelec *et al*., 2002).

Seroprevalence for various regions of the province is shown in Table 3. Significant differences were indicated for seroprevalence of sheep in different areas ($p<0.05$). Our study showed that sheep from southern Kurdistan were at an increased risk of infection to all other regions, possibly associated with its hot and humid environment Higher prevalence rate of toxoplasmosis in warm, moist areas compared to those which are cold and dry is attributed to the longer viability of *T. gondii* oocysts in moist or humid environments southern Kurdistan is a warm and moist area which helps *T. gondii* oocysts to maintain their viability (Van der Puije *et al*., 2000).

The 21.74% seropositivity rate detected in 368 sheep in Kurdistan (West Iran) is lower than those reported by Bonyadian *et al*. (2007) and Roberts *et al*. (2001), in center, Sharif *et al*. (2007)

and Youssefi *et al.* (2007), in North, Hamidinejat *et al.* (2008) in South-West and Hamzavi *et al.* (2007) in west, Asgari *et al.* (2009) in south of Iran, respectively, 29.1, 25.5, 35, 31.2, 58, 22.5 and 26.4%. These differences from different countries indicated that animals bred in these areas were exposed to different environmental contamination with *T. gondii* Oocysts. Furthermore, it may be due to differences of techniques used condition to monitor *T. gondii* antibody (Ramzan *et al.*, 2009).

For this reason, the lower *T. gondii* prevalence could be attributed to the low relative humidity, cold and dry weather (Hashemi-Fesharaki, 1996).

In conclusion, the results of this study confirm the presence of Toxoplasma antibodies in sheep in Kurdistan. As some of the infected animals play a distinct role as a source of human infection, adequate management might be useful and essential to control the toxoplasmosis in the sheep herds of Kurdistan, Iran.

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