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Toxoplasmosis in sheep from Kurdistan province, Iran

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Toxoplasmosis is one of the most threatening parasitic zoonoses in Iran. The causative agent, *Toxoplasma gondii*, uses a wide range of warm-blooded intermediate hosts in its life cycle, including sheep. This study was conducted on the seroprevalence of *T. gondii* infection in sheep from different regions of Kurdistan Province from December 2008 to September 2009. The main risk factors associated with the infection were analyzed. Sera was extracted from 368 sheep and examined for anti-*T. gondii* IgG antibodies by indirect ELISA test. According to results, the seropositive rates of sheep were 21.74%. Significant statistical differences were found between different geographical locations. For example central and southern provinces had the highest infection rates with 35.56% being infected, whilst western areas has the lowest infection rates with only 13.25%. Antibodies were detected in 20.87% of males and 22.13% of female sheep but no significant differences between sexes were found. Also, no significant differences between age groups were observed. The results indicated that the seroprevalence of *T. gondii* infection in sheep is relatively high.

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

INTRODUCTION

Toxoplasmosis is a worldwide-established parasitic zoonoses capable of causing clinical manifestations such as abortion, stillbirths, fetal death or birth of weak, non-viable animals (Tenter et al., 2000).

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 nonpasteurized milk containing tachyzoites, and by transplacentally (Jittapalapong et al., 2005; Sukthana, 2006; Clementino et al., 2007).

In sheep and goats, toxoplasma infection is a major cause of abortion and stillbirth. Subclinical infections are also quite common in adult animals of affected flocks and herds. Seropositive reactions reported in different countries vary widely. In a review by Tenter et al. (2000) of surveys carried out in Europe, values range from 4 to 92% in farmed sheep. Seropositivity is logically found correlated with age, increasing from (22%) lambs to (65.6%) ewes (Dumetre et al., 2006).

In animals, *T. gondii* infection results 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, because *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 et al., 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,

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

Parameter	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
Seroprevalence rate (%)	22.60	21.34	21.97

Table 2. Seroprevalence of Toxoplasma gondii antibodies in sheep between ages in Kurdistan province, Iran.

Age groups (month)	No of examined	No of positive	Seroprevalence rate (%)
> 6	53	15	28.3
6 ≥ 18	96	12	12.5
< 18	219	53	24.2

sex and various geographical regions.

MATERIALS AND METHODS

Study area and sampling

Kurdistan covers a territory of $28,203 \text{ km}^2$, all sample areas were between 750 and 3300 m above sea level and rainfall ranges from 250 to 800 mm/year. The climate in the northern/eastern and central region is typically continental with cold winters, and hot and dry summers. The southern/western regions have a slightly milder continental climate.

A total sheep of different ages (> 6 months old, $6 \ge 18$ 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 5ml venous blood samples, by centrifugation at 2000g for 10 minutes and were stored at -20°C prior to testing.

Serological assay

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% \leq 40% should be regarded as negative, between 40 and 50% as doubtful, between 50% \leq and < 200% as positive, and \geq 200% as strong positive.

Statistical analysis

The data were analyzed using Chi-square test and Fisher's exact tests (SPSS 11.5, Standard version, Copyright SPSS Inc., 1982 to 2002). The P value less than 0.05 were considered as significant.

RESULTS

T. gondii antibodies were detected in 80 out of 386 serum samples (21.74%), though the seroprevalence of antibodies in female and male were 22.60% and 21.34% respectively (Table 1).

The association of age with the presence of antibodies against *T. gondii* is shown in Table 2. There was no significant difference in all age groups.

The seroprevalence of *T. gondii* in different regions is displayed in Table 3. There were variations in the rate of *T. gondii*-positive samples in different regions.

DISCUSSION

Prevalence of toxoplasmosis across the world is variable, with prevalence rates from 0 to 100 percent 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), Silva et al. (2006), Sabry et al. (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 Barbera'n, (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

Region	No of serum samples	No of positive	Seropositive rate (%)
Center (Sanandaj, Dehgolan)	55	15	27.12
Northern (Divandareh, Saquez)	117	27	21.17
Southern (Kamyaran)	25	11	44
Western (Baneh, Marivan)	68	9	13.25
Eastern (Bijar, Ghorveh)	102	18	19.93
Total	367	80	21.74

Table 3. Seroprevalence of Toxoplasma gondii antibodies in sheep from various regions of Kurdistan province, Iran.

et al. (2007) in South Africa, Ramazan 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 (Ramazan 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 Bonyadin et al. (2007), Oncel et al. (2006), Gorman et al. (1999), and, While in contrast with the results of Ramazan et al. (2009), Lashari et al. (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' for example, changes in sex-associated hormones, environmental factors, age, nutrition and pregnancy (Martin, 2000; Craig et al., 2001; Kelly 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; 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). 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), Chegini et al. (2002), in center, Sharif et al. (2007), Youssefi et al. (2007), in North, Hamidinejat et al. (2008) in South-West, 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 (Ramazan et al., 2009).

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

Conclusion

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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