



Seroprevalence of Toxoplasmosis in Sheep in Southern Punjab, Pakistan

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ABSTRACT

The present study was conducted to investigate the prevalence of *Toxoplasma gondii* in sheep in Southern Punjab, Pakistan. Blood samples were collected from 518 sheep of nine localities of three districts (Dera Ghazi Khan, Multan and Khanewal). The samples were tested by using latex agglutination test (LAT) and commercial ELISA method. The overall prevalence of *T. gondii* infection in sheep was 19.88%. In male sheep, the prevalence was higher (30.15%) than in female sheep (18.46%), the difference was significant ($P < 0.05$). The relationship between age and sheep toxoplasmosis showed that the prevalence was the highest (38.88%) in age group of 16-28 months and lowest (8.51%) in age group of 68-80 months. Significant differences in prevalence were observed between age groups ($P < 0.05$). The relationship between body weight and sheep toxoplasmosis revealed that the prevalence of *T. gondii* was highest (20.85%) in weight group of 26-36 Kg and lowest (10.71%) in weight group of >47 Kg, the difference was non significant. The infection of *T. gondii* was significantly higher ($P < 0.05$) in Kacchi breed compared to Lohi breed of sheep (22.72% versus 15.7%). The results of the present investigation suggest that the *T. gondii* parasite is widely spread and may be the cause of sheep abortion in Southern Punjab, Pakistan.

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INTRODUCTION

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). *Toxoplasma gondii* has been recognized as a significant cause of lambing loss (Innes *et al.*, 2009) and food hazard (Kijlstra and Jongert, 2009). The animal offspring may die within weeks after birth. Prevalence of *T. gondii* infection can vary from 0 to 100% in sheep flocks in different countries (Tenter *et al.*, 2000; El-Moukdad, 2002). The estimated worldwide seroprevalence of toxoplasmosis in livestock has been reported as 30% in sheep, 15% in goats and 9% in cattle (Dubey, 2004). The definitive hosts of the parasite are the domestic cats and other felids, as the sexual cycle of the parasite occurs only in these species (Frenkel *et al.*, 1970). The disease is transmitted by the ingestion of oocysts shed by infected cats in contaminated food and water, or bradyzoites (cysts) in the tissues of an infected animal.

Use of different serological tests for the detection of *T. gondii* in sheep has been demonstrated in several countries. Using indirect fluorescent antibody test (IFAT), the prevalence of *T. gondii* was 55% in Swedish pregnant ewes (Uggla *et al.*, 1983) and 33.3% in Australian lambs

(Munday *et al.*, 1987). Using enzyme linked immunosorbent assay (ELISA), the prevalence of infection was 62.5% in the USA (Malik *et al.*, 1990) and 57% in sheep of Northwest Spain (Pandero *et al.*, 2010), while by modified agglutination test (MAT), the prevalence of infection was 64% in 4–6-year-old and 80% in ewes over 6 years of age in the USA (Dubey and Jones, 2008), while 13.9 and 28.5% in sheep kept under an intensive and extensive management system, respectively, in Uruguay (Savio and Nieto, 1995). Using SFT, the prevalence of infection was 33.2% in 0-12-month-old sheep and 47% in sheep older than 1 year in Turkey (Aktas *et al.*, 2000).

Information regarding the seroprevalence of *T. gondii* in sheep is quite scanty in Southern Punjab, Pakistan. The objectives of the present study was, therefore, to investigate the prevalence of *T. gondii* and its relationships between age, body weight, sex and breeds of sheep in Southern Punjab, Pakistan.

MATERIALS AND METHODS

Experimental animals

The present study was undertaken to determine the seroprevalence of *Toxoplasma gondii* in sheep. A total of

518 sheep of both sexes from nine localities of three districts (Dera Ghazi Khan, Multan and Khanewal) were included in the study. These animals had age of 3-80 months with body weight of 15 to >47 Kg and belonged to two breeds i.e. Lohi (n=210) and Kacchi (n=308).

Blood collection

The blood samples (3-5 ml) were collected from the jugular vein of each animal in vacuum tubes without anti-coagulant. The blood samples were transported to the laboratory. After clotting, the samples were centrifuged at 3000 rpm for 10-15 min, the serum was decanted and stored at -20°C until analysis.

Serological analysis

Two serological tests were used for the detection of antibodies to *T. gondii*. These included latex agglutination test (LAT) used for Lohi sheep and the enzyme-linked immuno-sorbent assay (ELISA) for Kacchi.

Latex agglutination test

The commercial "Toxoplasmosis Latex Kit" (Antec Diagnostic Product™ UK) was used for this purpose. Serum samples were diluted in physiological saline (0.9% NaCl). The serum and latex reagent were mixed. A positive result was expressed by agglutination. Sera were serially 2- fold diluted i.e. 1:16, 1:32 and 1:64.

Enzyme linked immuno-sorbent assay

The test procedure was carried out according to the method described by Lind *et al.* (1997). The "EIA Test Kit Toxoplasma IgM" (BioCheck, Inc, USA) was used according to the manufacturer's instructions. In brief, after incubation of antigen-coated microplates with the test sera diluted 1:20, *T. gondii*-specific antibodies were detected by binding the antigen/antibody complex with a peroxidase labeled anti-ruminant IgM monoclonal antibody conjugate for 90 min. Both the positive and negative controls provided in the kit were sheep sera. The optical density (OD) of the reaction was read on an ELX 800 (BioTek, Instruments Inc, USA) on a wavelength of 450 nm. The results were calculated according to the control serum readings; i.e. the percent ratio between the ODs for the sample and the positive control corrected for the OD of the negative control, and interpreted as recommended by the manufacturer, as follows: <0.90% negative, 0.91-0.99% ambiguous, 1.00 and >1.00% positive.

Statistical analysis

The results are expressed in percentages. The prevalence for *T. gondii* was statistically analyzed by the Chi-square test (χ^2) considering the variables sex, age, body weight and breeds. The differences were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The overall prevalence

Out of 518 sheep examined, 103 were found infected with *T. gondii*, showing an overall average prevalence as 19.88%. Bonyadian *et al.* (2007) reported nearly similar results (18%) in sheep of Iran, while lower values of 3.6, 3.8, 4.3, 11.2 and 12.1% were recorded by Maronpot and

Botros (1972) in Egypt, Sharma *et al.* (2008) in India, Samra *et al.* (2007) in South Africa, Ramzan *et al.* (2008) in Pakistan and Dubey and Foreyt (2000) in the North America, respectively. However, higher incidence rates (51.5, 52.2, 67.7, 84.5 and 50%) were recorded by Romanelli *et al.* (2007) in Brazil, Sanad and Al-Ghabban (2007) in Saudi Arabia, Hove *et al.* (2005) in Zimbabwe, Klun *et al.* (2006) in Serbia and Mason *et al.* (2010) in UK, respectively. The differences in the overall prevalence observed among different studies might have been due to differences in the diagnostic techniques used in the different regions, frequency of felines on the farms and the climatic variations from one region to another (Dubey, 2004; Innes *et al.*, 2009).

Relationship between sex and toxoplasmosis

In the present study, out of 63 male hosts, 19 were infected with *T. gondii* with the prevalence of 30.15%. In females, the prevalence was 18.46%. Thus, higher ($P < 0.05$) prevalence was observed in rams than in ewes (Table 1). The significantly higher prevalence in males than in females has also been reported earlier (Silva *et al.*, 2003). However, Alexander and Stinson (1988) reported that female animals were more susceptible than males to infection with protozoan parasites.

The literature generally indicates that females have more immunity than males (Alexander and Stinson, 1988), 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). But 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; Craig *et al.*, 2001; Kelly *et al.*, 2001).

Relationship between age and toxoplasmosis

The relationship between age and sheep toxoplasmosis showed that the prevalence was highest (38.88%) in age group of 16-28 months and lowest (8.51%) in age group of 68-80 months (Table 2), the difference was statistically significant ($P < 0.05$). The prevalence decreased as age of sheep increased from 28 months. Puiji *et al.* (2000) and O Donoghue *et al.* (1987) reported that age is an important factor in the prevalence of *Toxoplasma gondii* in sheep. According to the results of the present study, the prevalence of *T. gondii* was higher in younger animals than adults. This could be explained on the basis that the animals included in this age group were less resistant to *T. gondii*. These results are supported by Yung (2000) and Pawelec *et al.* (2002). Assoku (1979) and Vesco *et al.* (2007) reported that the system of management and health practices have a significant effect on the incidence of blood borne parasites.

Relationship between body weight and toxoplasmosis

The relationship between body weight and sheep toxoplasmosis revealed that the prevalence of *T. gondii* was highest (20.85%) in body weight group of 26-36 Kg and lowest (10.71%) in body weight group of >47 Kg (Table 3). However, difference in prevalence of toxoplasmosis among different body weight groups was

Table 1: Relationships between sex and sheep toxoplasmosis

Parameters	Male sheep	Female sheep	Total
No. of sheep examined	63	455	518
No. of sheep infected	19	84	103
Prevalence (%)	30.15 ^a	18.46 ^b	19.88

a, b = The values differed significantly from each other (P<0.05).

Table 2: Relationships between age and sheep toxoplasmosis

Parameters	Age groups of sheep (Months)						Total
	3-15	16-28	29-41	42-54	55-67	68-80	
Animals examined	88	54	137	117	75	47	518
Animals positive	12	21	33	21	12	4	103
Prevalence (%)	13.6 ^a	38.88 ^b	24.08 ^c	17.94 ^a	16.0 ^a	8.51 ^a	19.88

Values having different superscripts were significantly different (P<0.05).

Table 3: Relationships between body weight and sheep toxoplasmosis

Parameters	Body weight groups of sheep (Kg)				Total
	15-25	26-36	37-47	>47	
Animals examined	n=156	n=211	n=123	n=28	518
Animals positive	32	44	24	3	103
Prevalence (%)	20.5	20.85	19.51	10.71	19.88

There was no significant difference between body weight groups (P>0.05).

Table 4: Prevalence of toxoplasmosis in different breeds of sheep

Sheep breed	No. of sheep examined	No. of sheep infected	Prevalence (%)
Lohi	210	33	15.71 ^a
Kacchi	308	70	22.72 ^b
Total	518	103	19.88

a, b = Difference in prevalence between two breeds was significant (P<0.05).

non significant. In general, as the body weight of the animal increased the infection rate decreased. This might be due to the acquired immunity in old age groups.

Relationship between breeds and toxoplasmosis

The two breeds of sheep, the Kacchi and Lohi, were sampled in this study. The prevalence was found to be 22.72 and 15.71% in the two breeds, respectively (Table 4). It shows that *T. gondii* was more prevalent (P<0.05) in Kacchi breed as compared to Lohi breed. Van Der Puije *et al.* (2000) also found significant breed differences in susceptibility to *T. gondii* infection. They explained that the breed differences could be due to differences in resistance to parasitic infection, because some breeds are more resistant than others.

In conclusion, the results of the present study confirm the presence of anti-*T. gondii* specific antibodies in sheep of Southern Punjab, Pakistan. The impact of toxoplasmosis on the animal industry and losses due to clinical toxoplasmosis in livestock in Pakistan and the potential risk of its transmission to humans through consumption of meat contaminated with tissue cysts of *T. gondii* need further investigations.

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