



Seroprevalence of Toxoplasmosis in Small Ruminants from Cholistan Desert and Agricultural Areas of Rahim Yar Khan and Rajan Pur (Punjab) Pakistan

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ABSTRACT

The present study was conducted to assess the seroprevalence of toxoplasmosis in small ruminants in Cholistan desert, Rahim Yar Khan and Rajan Pur districts of Southern Punjab, Pakistan to compare the rates of infections at species level and also to evaluate the relationship of *T. gondii* infection with age, gender and flock size of animals. The blood samples were collected randomly from 1200 small ruminants and examined for the prevalence of *Toxoplasma gondii* infection by using latex agglutination test. Out of total of 1200 blood samples collected from small ruminants (31.41%) were found positive for anti-*T. gondii* antibodies. The infection were found higher in sheep (37.31%) than in goats (29.13%), whereas the infection were almost the same in male and female small ruminants. A positive correlation was found in the age and flock size of small ruminants and prevalence of toxoplasmosis.

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Authors' Contributions

SA collected the blood samples and analysed sera. ZT supervised the study.

Key words

Toxoplasmosis, small ruminants, flock size, latex agglutination test.

INTRODUCTION

The domestic small ruminants, sheep (*Ovis aries*) and goats (*Capra hircus*) are the small artiodactyls that have immense importance for meat production and in running the fabric and leather (Park *et al.*, 2007) industries thus assisting the exchequer of a country (Lebbie, 2004). However, the menace of parasitic diseases is the main obstacle in the way of promotion of livestock production (Bilal *et al.*, 2009; Ijaz *et al.*, 2009). Parasitic infections pose threats to health and limit the productivity due to the associated mortality due to early death in the embryonic life, abortion and stillbirth (Edwards and Dubey, 2013; Gebremedhin *et al.*, 2013). One of these infections is toxoplasmosis caused by *Toxoplasma gondii* (Innes *et al.*, 2009) which causes premature birth or abortion in small ruminants (Dubey, 2009). In sheep and goats toxoplasmosis not only causes the considerable economic losses, but also has proved to be a source of zoonotic transmission and consequently a hazard for human (Ryan and Ray, 2004).

In case of transmission to human in the form of tachyzoites in milk (Dehkordi *et al.*, 2013), bradyzoites in meat (Montoya and Liesenfeld, 2004; Dubey *et al.*, 2004), and/or oocysts from environmental sources *T. gondii* poses a spectrum of health disorders such as schizophrenia (Flegr, 2013) and encephalitis (Boothroyd and Grigg, 2002). The latent toxoplasmosis in humans are known to be at higher risk of traffic accidents possibly

due to behavioral modifications (Flegr, 2013). Some studies have presented the evidences relating toxoplasmosis with autism in human patients (Flegr, 2013) and cannibalism in some animal species (Prestrud *et al.*, 2008) with probable occurrence in man suggesting to speculate that some patients might develop the habit of carcassophagy as well. In cases of acute infections, toxoplasmosis can cause the even death in all the susceptible species (Jones *et al.*, 2001).

Since *Toxoplasma* is transmitted through zoonosis particularly from sheep and goats (Edwards and Dubey, 2013), there was a dire need to screen the animals whose meat is used by human as food. In addition, the epidemiology of toxoplasmosis in sheep and goats coming from the current study area has not been investigated sufficiently (Ramzan *et al.*, 2009). Therefore, no data about this serious disease is available in our study area except the work of Ramzan *et al.* (2009) and Ahmad and Tasawar (2015). Cholistan desert, district Rahim Yar Khan and Bengla Iccha area of district Rajan Pur, the southernmost region of the Punjab Province was, therefore, focused to ascertain the seroprevalence of *T. gondii* infection among sheep and goats.

MATERIALS AND METHODS

A total of 1200 sera were collected by random sampling technique from small ruminants reared in Cholistan desert, Agricultural region of Rahim Yar Khan and Rajan Pur region along the River Indus. The blood samples (3 to 5 ml) were collected from the jugular vein of each animal in vacuum tubes without addition of anticoagulant. The blood samples were left for about one hour to allow the coagulation. The coagulated samples

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were centrifuged at 3000 RPM for 10-15 minute to separate the sera from blood cells. The commercial kits, "Toxoplasmosis Latex" manufactured by "ANTEC DIAGNOSTIC PRODUCTS-UK for 50 or 100 tests were used for serological detection of anti-*T. gondii* antibodies in each serum sample. The results were statistically analyzed by using Chi-square test for qualitative variables such as infection rates in sex and species of animals while the quantitative variables were analyzed by computing the correlation via Pearson's test through SSPS version 20.

RESULTS AND DISCUSSION

Seroprevalence of *T. gondii*

In the present study, out of total of 1200 blood samples collected from small ruminants, 377 were found positive for anti-*T. gondii* antibodies at a rate of 31.41% (Table I) which is in agreement with the average prevalence reported by Ueno *et al.* (2009) and Oncel and Vural (2006) and much higher than reported by Wanga *et al.* (2011) in China (3.3%) and Ramzan *et al.*, (2009) in Pakistan (19%), Vesco *et al.* (2007) had reported prevalence in Sicily (49.9%).

Table I.- Overall seroprevalence of *Toxoplasma gondii* in small ruminants.

Parameter	Sheep	Goat	Total
Animals examined	335	865	1200
Animals positive	125	252	377
Prevalence (%)	37.31	29.13	31.41

Chi-square, 7.50; P value, 0.0575; OR, 1.4479; CL, 1.1109, 1.8871.

The higher rates of seroprevalence of *T. gondii* infection can be attributed to the environmental factors (Othman and Al-Azuheir, 2014), higher population of felines (Cavalcante *et al.*, 2008) in the sampling localities and poor management techniques (Wanga *et al.*, 2011) adapted by subsistence farmers of Cholistan desert, Rahim Yar Khan and Rajan Pur practiced by flock holders. Another factor responsible for an overall higher incidence of *Toxoplasma* infection might be the use of organic manure in the study area that might be rich in the cat oocysts consequently reaching the GIT of small ruminants (Baumgartner and Belevi, 2001).

On comparison at species level, the infection incidence rates were found insignificantly higher in sheep (37.31%) than in goats (29.13%) (Table I), which is almost in line with Ghazi *et al.* (2006) who have earlier reported insignificantly higher prevalence in ovines

(31.10%) than in caprines (30.0%). Statistically speaking, our findings differed from those of Abu-Dalbouh *et al.* (2012) who have reported significant variance in *T. gondii* prevalence between sheep and goats reared in Jordan. This resemblance in the infection rates can be associated to similar environmental conditions (Ghazi *et al.*, 2006). Higher *Toxoplasma* infection in ovines (44.13%) compared to caprines (42.28%) have been reported by Ali (2013) in selected countryside areas of Mardan.

Ramzan *et al.*, (2009) have reported higher prevalence of *T. gondii* infection in sheep (11.2%) and goats (25.4%) of Rahim Yar Khan. Ahmed *et al.* (2008) have reported 35.6% prevalence in ovine and 43.7% in caprines. Shah *et al.* (2013) earlier reported prevalence of *Toxoplasma* infection higher in caprines (53.8%) compared to ovines (36.0%). The higher rates of *Toxoplasma* infection in sheep might be due to the reason that this species of small ruminants is nurtured, at the most, by grazing while the caprines are fed in comparatively well cared flocks (Nasrullah *et al.*, 2013).

Moreover, the overall occurrence of ovine toxoplasmosis (37.31%) in our findings we in a close agreement with that of Shaapan *et al.* (2008) in Egypt (37%), Asghari *et al.* (2011) in Iran (37.5%) and Shah *et al.* (2013) in Pakistan (36.0%). On the other hand, the ovine toxoplasmosis prevalence was relatively lesser than that of Brazil (57%) (Tembue *et al.*, 2009), but higher than that of Portugal (33.3%) (Lopes *et al.*, 2013^b) and that of Italy (33.3%) (Cenci-Goga *et al.*, 2013).

The overall incidence rate of caprine toxoplasmosis (29.13%) was almost in line with that reported by Balea *et al.* (2012) (32.5%) in Rumania. The infection values was found comparatively higher in goats (3.8%) (Wanga *et al.*, 2011) in China and 19.88% in Multan (Lashari and Tasawar, 2010), 22.7% (Asghari *et al.*, 2011) in Iran and 13.4% in Palestine (Othman and Al-Azuheir, 2014) while comparatively lesser seroprevalence values were obtained than some preceding studies such as 42% in Grenada (Chikweto *et al.*, 2011) and in Egypt 43.7% (Ahmed *et al.*, 2008).

Age and toxoplasmosis

Table II shows the prevalence of *T. gondii* infection in different age groups of small ruminants. The results as a whole indicated a positive correlation ($r=0.2038$) between *T. gondii* infection and increasing age of animals which is in agreement with findings reported in the several earlier studies (Ntafis *et al.*, 2007; Tembue *et al.*, 2009; Wanga *et al.*, 2011) in small ruminants. However, the animals with age >25 months did not follow the increasing trend probably due to lesser number of animals available for sampling.

Table II.- Relationship between age and toxoplasmosis in small ruminants.

	Animals examined	Animals positive	Prevalence (%)
1-6	374	64	17.11
7-12	338	75	22.18
13-18	289	127	43.94
19-24	114	65	57.01
>25	85	46	54.11
Total	1200	377	31.41

The positive correlation between *Toxoplasma* infection and age suggest the increasing contamination of environment (Othman and Al-Azuheir, 2014) with oocysts which reach the digestive tract of animals thus causing the infection with the passage of time. The genital transmission, on the other hand cannot be ruled out (Williams *et al.*, 2005). Several other factors can be taken into consideration in this respect but the wild fauna including cats (Cavalcante *et al.*, 2008), rodents and birds (Dubey, 2009) suspected to be infected horizontally must be playing the vital role in the spread of toxoplasmosis. Almost every year the farmers inhabiting the Barani areas along the course of Indus River migrate along with their animals to the agricultural areas region of Rahim Yar Khan due to the flood. The flood small ruminants had to put up with the unhygienic conditions in terms of food and space that must have facilitated the propagation of toxoplasmosis.

Table III.- Relationship between sex and toxoplasmosis in small ruminants.

Parameter	Male ruminant	Female ruminant	Total
Animals examined	344	856	1200
Animals positive	109	268	377
Prevalence (%)	31.68	31.30	31.41

Chi-square=0.0162, P value=0.8985, OR=1.017, CL=0.997, 1.038

Sex and toxoplasmosis

Table III shows seroprevalence of *T. gondii* at gender level in small ruminants collectively. Our results are consistent with Ntafis *et al.* (2007) for Greece, Caballero-Ortega *et al.* (2008) for Mexico and Cavalcante *et al.* (2008) for Brazil who have reported no association between gender of animals and *Toxoplasma* infection rates. Wanga *et al.* (2011) and Ramzan *et al.* (2009) have however shown that female animals exhibit higher seroprevalence of toxoplasmosis than males.

Moreover, these results ruled out the view that male

animals are more exposed to infection than female animals due to testosterone, which promotes susceptibility to parasites (Morales-Montor *et al.*, 2004). The uniform rates of seroprevalence of *T. gondii* infection in our results can be attributed to the transmission of infection to and from male and female small ruminants (Lopes *et al.*, 2013^a). This fact can be associated with the evidence that the males (bucks and rams) selected for breeding are kept for many years that transmit the *T. gondii* infection via semen to the female animals (Santana *et al.*, 2010). Subsequently the female animals vertically transmit the toxoplasmosis infection in the offspring from generation to generation (Othman and Al-Azuheir, 2014).

The infection could horizontally spread causing zoonosis via slaughter animals (Gebremedhin *et al.*, 2013) to the human population of Cholistan desert, Rahim Yar Khan and Rajan Pur districts where the human toxoplasmosis has not been reported so far. In this regard our suggestion calls for the investigation of incidence of *T. gondii* infection in human in the study area where the awareness about toxoplasmosis was found very poor. The core reason of unawareness about the disease among the flock farmers in the study area must also be associated with the asymptomatic prevalence of toxoplasmosis (Dubey, 2009).

Table IV.- Relationship between toxoplasmosis and flock size of small ruminants.

Group	Flock size	No. of flocks	Infected*	Prevalence %
Group I	1-25	4	16	4.24
Group II	26-50	4	26	6.89
Group III	51-75	5	96	25.46
Group IV	76-100	8	239	63.30
Total		21	377	99.90

Correlation coefficient r,0.9280

*average No. of infected animals

Toxoplasmosis and flock size

Table IV shows significantly positive correlation (r=0.9275) between the incidence of toxoplasmosis infection and flock size of small ruminants which is in agreement with the data for Yu *et al.* (2007) for China, Sechi *et al.* (2013) for Italy and Abu-Dalbouh *et al.* (2012) for Jordan. The analogous relationship between *T. gondii* infection and flock size has been demonstrated by Gebremedhin *et al.* (2013) in Ethiopia. On the other hand, our findings differed from the study report of Cenci-Goga *et al.* (2013) who reported no correlation between the increasing size of herd and the prevalence of toxoplasmosis in small ruminants in Italy.

The existence of strong correlation ($r=0.9275$) between toxoplasmosis infection and flock size in the current study was influenced by the gap between management practices adapted by the farmers (Lashari and Tasawar, 2010) and differential application of vaccination of animals in different flocks (Katzer *et al.*, 2011). The flocks of small ruminants in the study area varied in the availability of veterinary health facilities (Farooq *et al.*, 2012) and also the differential literacy rate fortified with unawareness with the disease was noted which influenced the prevalence rates of toxoplasmosis. The use of water contaminated with oocysts also cannot be overviewed in dispersal of toxoplasmosis in flocks (Sechi *et al.*, 2013).

CONCLUSIONS

The 31.41% seroprevalence of toxoplasmosis in the current study area Cholistan desert, Rahim Yar Khan and Rajan Pur is alarming, which can be attributed to insufficiencies of the basic health facilities in the study area. The age wise differential rates of occurrence of infections in small ruminants warrant the vertical transmission of the disease in the important meat producing animals. The strong correlation between *T. gondii* infection and the flock size of small ruminants speaks of the crude management practices adapted by the farmers of the study area.

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Conflict of interest

Authors of present manuscript declare that there is no conflict of interest.

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