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Toxoplasma gondii infection in sheep, goats and farmers from Bahawalpur (Pakistan)

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ABSTRACT

Toxoplasmosis is a disease that has been reported from all over the world. The objective of the current study was to evaluate the prevalence of Immunoglobulin-G (IgG) antibodies specific to *Toxoplasma gondii (T. gondii)* simultaneously in sheep, goat and humans associated with the sheep and goat farming from Bahawalpur, Pakistan from May, 2016 to April 2017. 640 blood samples were taken from sheep, goats, farmers and non-farmer humans (160 each). Blood sera were examined to detect anti-*Toxoplasma* antibodies (IgG) by employing latex agglutination test (LAT). The findings showed the seroprevalence of *T. gondii* 36.25% (58/160) for sheep, 28.1% (45/160) for goats, 21.2% (34/160) for farmers and 6.8% (11/160) for non-farmers. A significant difference (P-value 0.00; OR: 3.655; CL: 1.77-7.51) was found between seroprevalence of toxoplasmosis in farmers and non-farmers. Furthermore, the infection rates higher in females as compared with male subjects. The results suggested that milk and meat of infected sheep and goats might be responsible for transmission of *T. gondii* from sheep and goats to farmers of study area.

Keywords: Seroprevalence, *Toxoplasma gondii,* Sheep, Goats, Humans, Latex agglutination test

INTRODUCTION

Sheep and goat farming is one of the major activities of the farmers for earning their livelihood and meeting the milk and meat requirements in the South Punjab, Pakistan. Sheep and goat farming is not only vital for the livelihood of the farmers but also for the betterment of exchequer of the country. The farmers, however, face the financial losses due to a spectrum of diseases infecting their livestock animals (Dubey, 2009). Some of the mammalian diseases of parasitic origin are almost equally shared by sheep, goats and humans in the developing countries such as Pakistan. One the important parasitic infections, toxoplasmosis, is inflicted by a protozoan *Toxoplasma gondii* (*T. gondii*). It has been identified as the cause of several human diseases including ocular disease (Dubey, 2009), it was previously considered as the infectious agent of animals since a study on ovine abortion reported by Hartley *et al.* (1954). However, the subsequent studies established *T. gondii* as the agent causing abortion in goats (Tenter *et al.,* 2000), horses & donkeys (Yang *et al.,* 2013) and almost all the warm blooded animals (Urquhart *et al.,* 2003; Dubey, 2009). The later studies proved toxoplasmosis as the second major parasitic disease distributed in almost all the parts of the world that is transmitted to humans through zoonosis via animals (Bisson *et al.,* 2000; Chen and Tan, 2009). In the humans, *Toxoplasma* infection can result in retinitis, myocarditis encephalitis (Dubey, 2009), AIDS (Schwartzman**,** 2001) and the disorders of central nervous system leading to behavioral changes (Flegr, 2013) such as schizophrenia and autism (Flegr, 2013). Some other studies have also reported the association of *T. gondii* infection with human disorders such as Parkinsonism (Miman *et al.,* 2010), Alzheimer's dementia (Kusbeci *et al.,* 2011), tendency towards suicide (Arling *et al.,* 2009), the bipolar disorder

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(Pearce *et al.,* 2012) and the elevation of androgens levels in men while reduction in women (Flegr *et al.,* 2008). To add more, the reproductive issues caused by this parasite are equally grave in animals as well as humans (Aspinall *et al.,* 2002).

Apart from environmental sources, *T. gondii* reaches the human digestive tract through unpasteurized milk contaminated with tachyzoites. This parasite can reach the human body via undercooked meat containing tissue cysts (Tenter *et al.,* 2000; Chen and Tan, 2009). The tissue cysts can also enter the human digestive tract when occurring in offal (Figueiredo *et al.,* 2001). The major reasons of successful pathogenicity of *Toxoplasma* are attributed to its resistance to pepsin and trypsin, the enzymes of human digestive tract (Metheny *et al.,* 1997). A peculiar metabolic adaptations *T. gondii* such as production of oxidative energy and generation of a large amount of its ATP independent of mitochondrion (Blader & Saeij, 2009). In addition, many properties such as the existence and completion of sexual as well as asexual life cycles in felids (Boothroyd, 2009) and successful survival and accomplishment of asexual part of life cycle in secondary host species of homoeothermic animals including humans (Urquhart *et al.,* 2003) and transmission as tissue cyst from meat and meat products, as tachyzoites in milk and sporocysts from environment has enabled *T. gondii* to rank as an agent of one half of the food borne diseases in humans (EFSA, 2007). The survival value of this parasite is also escalated by its ability to infect almost all the tissues such as coronary, cerebral, pulmonary or hepatic tissue of animals including humans (Dubey, 2008).

The study reports on toxoplasmosis from around the world have linked the food animals with the horizontal (Boothroyd, 2009) and vertical zoonosis of *T. gondii* (Urquhart *et al.,* 2003; Chen and Tan, 2009). Several reports on toxoplasmosis in food animals from different regions of Pakistan (Chaudhary *et al.,* 2006; Ramzan *et al.,* 2009; Ahmad & Tasawar, 2015[a]; Ahmad *et al.,* 2015) have suggested the evaluation of zoonotic transmission of *T. gondii* to humans. *T. gondii* sustains well in warm and humid environment (Rorman *et al.,* 2006) such as that exists in the current study area where toxoplasmosis has been established to occur in different ovine and caprine breeds, in the recent past (Ahmad & Tasawar, 2015[b]; Ahmad & Tasawar, 2016[a]). Therefore, we conceived the hypothesis that toxoplasmosis might be proliferating horizontally from sheep and goats to the human population. Furthermore, no study of toxoplasmosis has been reported in the current study area so far, therefore, we carried out the seroepidemiological investigations to test the hypothesis.

MATERIALS AND METHODS

Study area

Bahawalpur districts has the warm climatic conditions in the South Punjab region, a hub between Sindh, Baluchistan provinces of Pakistan and India covering 14,177 mile² lying in the subtropical zone. The current study area shares the boundaries in south west with district Ghotki of Sindh province; in the west the river Indus marks the boundary with Dera Ghazi Khan and Muzaffargarh districts and in the north are districts Bahawalnagar and Khanewal, Pakistan. A large part of study area consists of Cholistan desert.

Blood sampling

From the selected localities in the urban and countryside areas, a total of 640 subjects were selected for sampling including domestic sheep (n=160), goats (n=160), humans (farmers) (n=160) associated with the sheep and/or goat herds and also humans (non-farmers) (n=160) from urban areas.

Exclusion criteria

The animals and human subjects showing the symptoms of any other disease were excluded from sampling to avoid the errors in the results.

Chemical analysis

Five mL of blood samples were collected from jugular veins of sheep and goats; and from the median cubital veins of humans by using vacuum tubes. The blood samples were allowed to coagulate. After coagulation the samples were centrifuged at 3000rpm for 15 to 20 minutes. The sera were separated in eppendorf tubes and stored below -20°C. The chemical analysis was carried out by using Latex agglutination test (LAT) kit manufactured by ANTEC DIAGNOSTIC

PRODUCTS-UK® for detection of *T. gondii* antibodies (IgG).

Statistical analysis

The data were statistically analyzed to find the significance (P-values) of variance; and odds ratios (OR) through Pearson's Chi-Square test employing SPSS version 28.

RESULTS AND DISCUSSION

Toxoplasmosis has been studied in many animal species by employing various methods. The current study was carried out by detection of *Toxoplasma* antibodies using latex agglutination test (LAT), the technique supported by several authors for different species of animals such as Yang *et al.* (2013) in horses and donkeys, Ahmad & Tasawar (2015[b]) in ovines, Zewdu *et al.* (2013) Ahmad & Tasawar (2016a) in goats, Tzanidakis *et al.* (2012) in sheep and goats, Jadoon *et al.* (2009) in dogs and Yu *et al.* (2007) in buffalos. Our findings showed that out of total samples (n=640), 36.2% in sheep, 28.1% in goats, 21.2% in farmers and 6.8% in the non-farmer subjects were positive for *Toxoplasma* antibodies (IgG) at cutoff 1:16 (Table 2 & 3).

The study results on evaluation at gender level demonstrated the nonsignificant differences in infections in sheep (P value: 0.44), goats (P value: 0.34), farmers (P value: 0.18) and non-farmers (P value: 0.76) (Table 1), suggesting the sexual transmission of *T. gondii* to and from male and female. The uniform infection rates can be justified through the evidence that the farmers of the current study area did not know about occurrence, effects or transmission of toxoplasmosis.

Table 1: Overall *Toxoplasma* infection in sheep, goats, farmers and non-farmers

Subject category	Gender	Subjects tested	Subjects infected	Prevalence (%)	Chi-Square	P-Value	OR	CL
Sheep	Male	40	11	27.5	1.767	0.184	0.589	0.269-1.291
	Female	120	47	39.1				
Goats	Male	40	12	30.0	0.093	0.761	1.130	0.515-2.480
	Female	120	33	27.5				
Farmers	Male	80	15	18.7	0.598	0.440	0.741	0.346-1.587
	Female	80	19	23.7				
Nonfarmers	Male	80	7	8.7	0.879	0.349	1.822	0.512-6.486
	Female	80	4	5				

Toxoplasmosis in sheep, goats and farmers

In the current study, the overall *T. gondii* infection values in sheep (Table 2) were found closely coincident with several studies in the recent and past in different countries of the world such as 37.5% in Iran (Asgari *et al.,* 2011), 37.31% in Pakistan (Ahmad & Tasawar, 2015[b]) and 37% in Egypt (Shaapan *et al.,* 2008). Another study on sheep toxoplasmosis by employing IFAT also reported closely related seroprevalence rates 38.62% (Sechi *et al.,* 2013). However, the current toxoplasmosis infection rates in ovines were obtained higher than 33.3% in Italy (Cenci-Goga *et al.,* 2013), 33.6% from Portugal (Lopes *et al.,* 2013) and 18.16% from Pothohar region of Pakistan (Ahmad *et al.,* 2015).

The higher rates of toxoplasmosis can be attributed to the climatic determinants particularly high temperature in the study area (Ahmad & Tasawar, 2015[a]) that promotes the successful survival of *T. gondii* as compared with the regions with low average temperature (Rorman *et al.,* 2006). On the other hand the infection rates in sheep were lower than 66.6% evaluated through Sabin-Feldman Dye Test computed by Oncel & Vural (2006). These disparities in the infection rates

can be linked with different techniques used in different reports (Vesco *et al.,* 2007; Nasrullah *et al.,* 2013). Moreover, the differential level of immunity in various breeds of sheep (Ahmad &Tasawar, 2015[b]) or disparities in farm management practices also cannot be ruled out (Zhao *et al.,* 2011).

The current findings showed the overall 28.12% toxoplasmosis infections in goats (Table 2) in a close coincidence with 27.9% for Thai goats (Jittapalapong *et al.,* 2007), 29.13% for Pakistanis (Ahmad & Tasawar, 2016[b]) and 30.7% for Greek goats (Tzanidakis *et al.,* 2012). Our findings showed the *T. gondii* seroprevalence values in goats higher than 9.0% in China (Xu *et al.,* 2014) and 14.32% in Pothohar, Pakistan (Ahmad *et al.,* 2015) but lower than 42% in Caribbean Islands (Chikweto *et al.,* 2011). These disparities in the *T. gondii* seroprevalence rates might be linked with the differences in study methods (Yu *et al.,* 2007; Vesco *et al.,* 2007) and/or ecological factors prevailing in various study regions (Ahmad & Tasawar, 2015[a]; Ahmad *et al.,* 2015). The results further revealed the significant (p-value= 0.012) differences between sheep, goats and farmers (Table 2) leading to the insight that the infection of *Toxoplasma* is strongly associated with the hygienic condition of the subjects.

The *T. gondii* infection rates 21.2% (Table 3) evaluated in farmers were in close agreement with 20% (Chaudhary *et al.,* 2006) in Pakistan and 23.9% (Kamani *et al.,* 2009) in Nigeria but lower than 32% in Egypt (Ghoneim *et al.,* 2009) and 47% in France (Fromont *et al.,* 2009). The foremost reason of almost analogous results obtained in our study area and Nigeria can be attributed to similar climatic conditions (Tenter *et al.,* 2000; Ahmad & Tasawar, 2015[a]; Ahmad *et al.,* 2015). While the higher rates of infections in France can be due to the pet cats' role in the promoting zoonotic transmission of *Toxoplasma gondii* (Lopes *et al.,* 2008; Neto *et al.,* 2008). On the other hand, non-farmers subjects had less infections 6.8% (Table 2) coincident with 6.7% (Shin *et al.,* 2009) in Korea.

The analysis of current findings showed the significantly varied infections (P-value 0.00; Chi-Square: 13.67) in farmers and non-farmers (Table 3). The higher rates of toxoplasmosis in farmers as compared with the non-farmers might be explained ingestion of infected milk and meat (Ghoneim *et al.,* 2009) of sheep and goats transmitting *T. gondii* to the humans associated with the animal farming profession. The hygienic conditions of farmers and non-farmers might also be the important determinants for differential infection rates in small ruminant farmers and control subjects. Moreover, the non-farmer subjects were sampled from the urban areas who usually use the processed milk while the farmers using the milk and meat of their livestock animals.

Table 2: *Toxoplasma* infection in sheep, goats and farmers

Parameters	Sheep	Goats	Farmers
Subjects examined	160	160	160
Subjects infected	58	45	34
Prevalence (%)	36.2	28.1	21.2
Chi-Square: 8.846 P-Value 0.01			

Table 3: Comparison of *Toxoplasma* infection in farmers and non-farmers

Parameters	Farmers	non-farmer
Subjects examined	160	160
Subjects positive	58	11
Prevalence (%)	36.2	6.8
Chi-Square: 13.67 P-Value 0.00 OR 3.655 C.L. 1.77-7.51		

CONCLUSION

The results showed the higher infections in the small ruminant farmers than in the non-farmer humans inhabiting the study area (Table 3). Our findings led to the conclusion that humans associated with the sheep and goat farming were being infected with *T. gondii*.

RECOMMENDATIONS

The 21.25% occurrence of *Toxoplasma* antibodies in human population associated with the sheep and goats leads to the insight that the horizontal transmission of the disease must be taking place. Therefore, it is recommended that the large ruminants in the study area used for milk and meat must also be screened to ascertain the toll of toxoplasmosis.

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