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RESEARCH ARTICLE

Seroprevalence of *Toxoplasma gondii* in Four Ovine Breeds of Cholistan Desert of Pakistan

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ABSTRACT

Seroprevalence of *Toxoplasma* (*T.*) *gondii* in four ovine breeds was carried out in Cholistan desert and adjacent regions of Southern Punjab, Pakistan from May 2012 to April 2013 to compare the rates of infection in different ovine breeds so that the infection resistant breeds could be recommended to the farmers of these regions. The study was also aimed to evaluate the infection rates at gender level in all ovine breeds to infer the vertical transmission of *Toxoplasma* infection. Serum samples were collected from 20 spatially reared ovine herds and examined by Latex Agglutination Test. Out of total sampled sera, 37% were found positive for anti-*T. gondii* antibodies. At breeds level, the non significant differences in seroprevalence rates were found as 45.09%, 44.18%, 39.68%, and 28.69% in Kajli, Thalli, Cholistani and Lohi breeds, respectively. At gender level, the findings showed varied trends of seroprevalence in male and female animals. Moreover, the results established the overall high rates of seroprevalence of *T. gondii* in sheep that warranted the threat of public health via zoonotic transmission of toxoplasmosis.

INTRODUCTION

The domestic ovines (*Ovis aries*) are the source of milk and meat particularly in the rural areas (Pugh, 2002). Sheep milk is an outstanding unprocessed material for the milk industry particularly in the manufacture of cheese (Park et al., 2007). These small animals are vulnerable to a wide range of infectious diseases (Bilal et al., 2009) caused by various pathogens including parasites (Ijaz et al., 2009) that pose menace to their health and create limitations in their productivity (Nwosu et al., 2007) due to early embryonic life death, mummification, abortion, stillbirth or the adult mortality (Panadero et al., 2010). One of the potential infectious diseases faced by ovines species is toxoplasmosis, caused by an apicomplexan parasite, *Toxoplasma* (*T.*) *gondii* (Edwards and Dubey, 2013). The infection of toxoplasmosis, whether acute or chronic, can be recognized by detection of anti-*T. gondii* IgG, IgM, IgA and/or IgE antibodies from infected animals (Pignanelli, 2011) depending upon different genetic types (strains) of *T. gondii* (Singh, 2003; Pignanelli, 2011). It is widely accepted that *Toxoplasma* has a number of genetic types but about 95% of them are categorized into three classes of strains simply named as Type I, II and III (Sibley, 2003). These genotypes are considered to be clonal in their structural features (Dubey et al., 2010). Type I causes infection in rodents. Type II has been established as the infectious agent of toxoplasmosis in small ruminants while Type III has yet not been proved as infectious strain. All the three genetic types I, II and III can cause toxoplasmosis in human (Boothroyd and Grigg, 2002). Recently, a newly discovered genotype (Type IV) has also been reported in some game animal species (Khan et al., 2011). The infection of *T. gondii* is more detrimental to humans as compared to other mammalian species. The humans contact this parasite mainly by consuming the infected edible items such as mutton or beef carrying bradyzoites (Qiu et al., 2012), water polluted with oocysts of *T. gondii* (Montoya and Liesenfeld, 2004) or by intake of unpasteurized milk (Higa et al., 2010). On infecting the human body, *T. gondii* causes not only abortion and reproductive issues in women (Dubey, 2009) but also a variety of diseases in both men and women such as schizophrenia (Flegr, 2013), ocular infection and encephalitis (Boothroyd and Grigg, 2002).

No study has been reported on this disease in ovines breeds of Cholistan area of Pakistan. Nevertheless, the sheep reared in nearby area of Rahim Yar Khan were reported by Ramzan et al. (2009) for the infection of *T.*

gondii. As Cholistan desert is the far reached and the most neglected area in connection with the study of toxoplasmosis due to lack of infra structure, it was hypothesized that the parasite might be multiplying vertically (Lopes et al., 2013[a]) due the little attention by the authorities leading to the serious consequences of zoonosis (Stormoen et al., 2012) with the passage of time. Moreover, the disease might be causing harms to the economy of people. It is anticipated that the present study would enable the authorities to save the human from economic losses as well as imminent zoonosis. Furthermore, the study would also provide an insight to the livestock farmers for rearing the disease resistant breeds of sheep in the study area.

MATERIALS AND METHODS

Blood samples (n=335) were collected from four different sheep breeds viz Kajli, Thalli, Cholistani and Lohi for detection of anti-*T. gondii* antibodies. Three to five ml of blood was collected from the jugular vein of each animal in a vacutainer and allowed for about 1 hour to coagulate. Sera were isolated by centrifugation (3000 rpm) for 15 minutes and examined for detection of anti-*T.* antibodies (IgG) by Latex Agglutination Test using commercially available kits, "Toxoplasmosis Latex" manufactured by "ANTEC DIAGNOSTIC PRODUCTS-UK. The results were statistically analyzed by applying Chi-square test for evaluation of infection rates in male and female sheep as well as in the selected breed of sheep via Pearson's test through SSPS version 20.

RESULTS

The overall seroprevalence in all the sampled ovine breeds was 37.31% (n=125/335). The seroprevalence rates of anti-*T. gondii* antibodies were 30% and 66.66% in males and females of Kajli sheep breed, respectively. In Thalli breeds, seroprevalence rates were 46.66% and 42.85% in males and females, respectively. The seroprevalence rates of anti-*T. gondii* antibodies in rams of Cholistani (also called Buchi) breed were 30.95% while infected ewes were 44.04%. On the other hand, these seroprevalence rates were 28.04% and 30.30% in males and females of Lohi breed of sheep (also called Parkanni), respectively.

DISCUSSION

In the present study, four selected ovine breeds namely Kajli, Thalli, Cholistani and Lohi with varied populations in the study area were investigated. The overall seroprevalence rate of anti-*T. gondii* antibodies in sheep breeds was 37.31% (Table 1) that was in agreement with several previous studies such as 37% in

Table 1: Seroprevalence of *Toxoplasma gondii* in four breeds of sheep

Sheep breed	No. of sheep examined	No. of sheep infected	Prevalence (%)
Cholistani	126	50	39.68
Kajli	51	23	45.09
Lohi	115	33	28.69
Thalli	43	19	44.18
Total	335	125	37.31 *

Chi-Square = 2.863, P-Value = 0.413, *P<0.05

Egyptian sheep (Shaapan et al., 2008), 37.5% in Iranian ovines (Asgari et al., 2011) and 36.0% in ovines of Khyber Pakhtoon Khawah (Shah et al., 2013). Contrary to this, current findings also indicated higher seroprevalence rate as compared to some previous studies conducted in different parts of the world (Ramzan et al., 2009; Yang et al., 2013; Lopes et al., 2013[b]; Cenci-Goga et al., 2013) and lower than seroprevalence rate reported in Brazilian population of sheep (57%) (Tembue et al., 2009). The deviations in the seroprevalence rates can be attributed to differences of resistance to parasitic infection in different ovine breeds included in the present study and those examined in other parts of the world. The varied rates of toxoplasmosis might be the outcome of varied serological techniques used in different regions of the world. Furthermore, different geo-climatic conditions might also be speculated as one of the possible reasons behind varied seroprevalence rates of toxoplasmosis in different regions of the world (Chacin-Bonilla and Sanchez-Chavez, 2000; Tenter et al., 2000; Kniel et al., 2002; Hill et al., 2005).

Results of present study revealed varied rates of seroprevalence of toxoplasmosis in different sheep breeds (Table 1). The highest anti-*T. gondii* antibodies prevalence rates were observed in Kajli breed (45.09%) followed by Thalli (44.18%), Cholistani (39.68%) and Lohi (28.69%), respectively. Although the seroprevalence rates ranged from 28.69% to 45.09% in all the four breeds yet differed non-significantly (P-value= 0.523) (Table 1). This trend of non significant differences in seroprevalence rates was coincident with 31.1% and 43.2% evaluated through ELISA and IFAT correspondingly by Carneiro et al. (2009) in Brazil. However, the results showed a disagreement with findings of Lashari and Tasawar (2010) who studied Lohi and Kacchi breeds of sheep and found the significantly (P<0.05) different rates of seroprevalence of toxoplasmosis in the two selected breeds. Further, the seroprevalence rate in Lohi breed was higher than that reported in an earlier study 15% (Lashari and Tasawar, 2010). The lowest rates in Lohi breed of sheep can be associated with the popularity of this ovine breed (Ahmad et al., 2001) earning more attention of the farmers with commercial point of view and being good performer in terms of meat and milk

Table 2: Toxoplasmosis in different ovine breeds at gender level

Sheep breed	Sex	No. of sheep examined	No. of sheep infected	Prevalence (%)	Chi-Square	P-Value	OR	CL
Kajli	Male	30	9	30	6.708	0.010	0.2142	0.667-0.6875
	Female	21	14	66.66				
Thalli	Male	15	7	43	0.057	0.811	1.1666	0.3291-4.1357
	Female	28	12	19				
Cholistani	Male	42	13	30.95	163.9929	0.000	0.6956	0.6580-0.7353
	Female	84	37	44.04				
Lohi	Male	82	23	28.04	0.699	0.403	1.4523	0.6054-3.4837
	Female	33	10	30.30				

OR= Odd's Ratio, CL=Confidence Limits

production earning the highest exchequer for the farmers. The Cholistani sheep are reared most commonly in desert region where the persistent stress caused by scarcity of food and water is faced by animals. Owing to the unrelenting stress conditions, the susceptibility to parasitic infections by these animals might be higher (Khan, 2006). One of the vital factors rendering the animals more vulnerable to infections was the closed housing in the area of study as is evident from a previous study reported by Sajid et al. (2012). Amongst the variety of determinants accounted for the higher prevalence of infections in all the four breeds of sheep, the most considerable one was the interbreeding in different breeds of sheep without screening of the breeding rams for pathogenic organisms. This proliferation of infection might be taking place due to mixed farming of different breeds (Ali et al., 2009) as is in vogue in the current study area.

At gender level, Kajli males 30% (n=9/30) showed significantly lower (P-Value= 0.010; OR= 0.214; CL= 0.0667, 0.6875) seropositive rate for ant-*T. gondii* antibodies as compared to female Kajli sheep (66.66%) (Table 2). Significantly higher prevalence rate in Kajli ewes as compared to rams was in a disagreement with Tasawar et al. (2011) who reported higher prevalence in rams than in ewes. These differences in the findings might be due to differential techniques in both studies. Lower rates of toxoplasmosis in rams as compared with the ewes can be attributed to the differential feeding practiced for both genders. The Kajli rams with beautiful eyes, curved nose and attractive stature are considered as financial darlings. These are perhaps the most costly rams in Pakistan and thus are usually fed at pens with concentrate fodder while the ewes are nourished through pasture grazing that probably renders them more vulnerable to the ingestion of oocysts from environment (Gangneux and Darde, 2012).

Contrary to Kajli breed, males of Thalli breed showed higher seropositivity for anti-*T. gondii* antibodies as compared to females but difference was statistically non-significant (P-value= 0.811; OR= 1.166; CL= 0.3291, 4.1357). This finding was in line with many previous studies on ovine toxoplasmosis (Williams et al., 2005; Ntafis et al., 2007; Carneiro et al., 2009). The analogous non-significant results were also reported in ovine population of China by Wanga et al. (2011). Further, these findings were also in disagreement with some previous studies (Asgari et al., 2011; Abu-Dalbouh et al., 2012). The non significant variation in occurrence of toxoplasmosis at gender level in Thalli breed of ovines was possibly due to the exchange of infection by male and female animals, during natural mating, primarily infected from the contemporary breeds of ovines (Tenter et al., 2000; Lopes et al., 2013[a]) or environment including soil and water polluted with oocysts of *T. gondii* (Al-Jebouri et al., 2013). It is noteworthy that Thalli sheep is reared by poor communities of farmers depending almost totally upon pasture feeding, a well known cause of higher toxoplasmosis in animals due to *T. gondii* oocysts contamination (Kniel et al., 2002; Al-Jebouri et al., 2013).

In Cholistani breed, overall seroprevalence rate was 39.68%, whereas, at gender level, females showed significantly higher (P=0.029) rates as compared to males. These findings were consistent with the results of overall previous study on ovine populations of Rahim Yar Khan (Ramzan et al., 2009) and in another study on mixed breeds of ovines reported by Lashari and Tasawar (2010) from Multan region. The overall higher seroprevalence of anti-*T. gondii* antibodies in Cholistani ovine breed might be the outcome of lack of health facilities available for animals (Farooq et al., 2012), crude management techniques and prototype subsistence farming in the Cholistan region. The animals consume a bulk of stored energy to access the sources of water called Dhands which are only seasonal lakes (Ahmad and Tasawar, 2015) thus being more exposed to parasitic infection due to attenuated physical conditions and consequently the poor immunity level.

Lohi breed showed higher rates of seropositivity for *T. gondii* in females as compared to males but the difference was non-significant (P-Value= 0.403; OR= 1.452; CL= 0.6054, 3.4837) and was in consistence with findings of Carneiro et al. (2009) who reported non-significant relationship between genders of ovines and toxoplasmosis. Similar non-significant values of *T. gondii* infections were reported by Williams et al. (2005) and Ntafis et al. (2007) in ovines.

The higher incidence of toxoplasmosis is facilitated by the persistent stress inflicted by lack of natural foliage, the only fodder source and water as well in the most part of the year. Additionally, the dhands in this region provide an ephemeral stay to the Russian migratory avian species that follow the Indus fly way during their nuptial flight towards Manchar lack, Keenjhar lack and Kalari lack in Pakistan. It is also suspected that the Cholistani mammalian species together with Cholistani ovine breeds must share the infection left by the migratory birds (Cabezon et al., 2011) via felines (Boothroyd, 2009).

Conclusions and recommendations

The seroprevalence of *T. gondii* in the ovine population reared in different localities of Cholistan desert was considerably high that demands further investigations and application of control measures to avoid the proliferation of toxoplasmosis. Lohi and Cholistani (buchi) breeds have been found comparatively more resistant to toxoplasmosis in the study area. Therefore these two breeds are recommended for rearing in Cholistan. Moreover, mixed farming is in vogue in Cholistan, the screening of animals of other species of the study area is recommended to evaluate the prevalence of *T. gondii* infection transmitted via horizontal fashion. At butcheries, the leftover meat of enormous number of *T. gondii* infected animals reaches the intestinal tract of cats which add the oocysts in the environment leading to the infestation of populations of same species through a sort of theoretical 'positive feedback infestation'. Therefore, it is recommended that along with the other measures, the stray cats' study evaluating the infestation status must be conducted in the study area.

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