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Tropical Biomedicine 35(4): 1028–1040 (2018)

Seroprevalence, associated risk factors and hematological impacts of toxoplasmosis in small ruminants of Multan, Punjab-Pakistan

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Received 25 June 2018; received in revised form 30 August 2018; accepted 3 September 2018

Abstract. Toxoplasmosis is a protozoal infection of zoonotic potential with worldwide geographical distribution which affects nearly all warm-blooded animals including mammals and birds. Keeping in view, this study was conducted to determine the seroprevalence of toxoplasmosis along with associated risk factors and its haematological impacts in small ruminants of district Multan, Pakistan. In this study, a total of 250 sera samples collected from sheep (n=125) and goats (n=125) from three tehsils of Multan were examined using commercially available Latex agglutination test kit for the presence of anti-*T. gondii* antibodies. The haematological profiles of Toxoplasma seropositive and seronegative animals were determined by using automated haematology analyser. Overall seroprevalence of toxoplasmosis in small ruminants was 42.80% with a higher prevalence rate (44.80%) in sheep as compared to goats (40.80%). Sex, existence of co-morbid conditions, feeding pattern and presence of pet cats and dogs were identified as significant ($P<0.05$) risk factors associated with the presence of antibodies against toxoplasmosis. The breed was found to be a significant (P=0.026) risk factor for the seroprevalence of toxoplasmosis in goats but not in sheep. Haematological analysis revealed significantly altered leukocytic counts ($P<0.05$) in seropositive sheep and goats as compared to seronegative ones. Our findings showed that small ruminants of the Multan District in Pakistan are toxoplasma seropositive and may pose a serious threat of public health concern in the region.

INTRODUCTION

Toxoplasma (*T.*) *gondii* is an obligatory intracellular apicomplexan protozoan parasite of zoonotic potential with worldwide geographical distribution. It can infect nearly all warm-blooded animals such as mammals and birds (Zhao *et al.*, 2011). Human beings may be infected either by the ingestion of food stuff contaminated with oocysts shed by the cats or by accidental ingestion of raw/undercooked meat containing tissue cysts containing bradyzoites (Akhtar *et al.*, 2014). According to an estimate, approximately one third of the world's population has been infected with toxoplasmosis (Liu *et al.*, 2015). In healthy individuals, primary infection is usually sub-clinical; although cervical lymph-adenopathy and/or ocular disorders had also been reported in some previous studies (Montoya & Liesenfeld, 2004). In pregnant females, it may cause severe damage to the developing foetus whereas in immunocompromised patients, like in HIV infection, reactivation of latent disease can cause life-threatening encephalitis (Li *et al.*, 2016).

The wild and domestic cats serve as a definitive host for *T. gondii* which harbour the sexual parasitic stages and spread the infection by shedding oocysts through their excreta (Kaushik *et al*., 2014). Additionally, various mammalian and avian species which are consumed by the human population may act as intermediate hosts and serve as a potential source for the transmission of toxoplasmosis in human beings (Zhao *et al*., 2011). Previous studies indicated a wide variation in the prevalence of toxoplasmosis in different parts of the world that may depend upon various factors including the host species involved, geographical conditions, husbandry practices, sampling techniques and diagnostic assays etc. (Liu *et al*., 2015).

Sheep and goats contribute a major share of animal protein among the food animals throughout the world. At the same time, these animals have a greater impact on the transmission of toxoplasmosis towards human population. Besides public health concern, toxoplasmosis induces health and production losses through abortion and neonatal mortality (Li *et al*., 2016) leading to reproductive losses and thus, exert a negative impact on the profitability of sheep/goat farming business. The literature revealed high prevalence rates of toxoplasmosis ranging from 48-68% in small ruminants in different parts of the world including Ethiopia (Tegegne *et al*., 2016), Italy (Gazzonis *et al*., 2015), Zimbabwe (Hove *et al*., 2005), Romania (Balea *et al*., 2012), Sudan (Khalil & Elrayah, 2011), Northeast Brazil (Rêgo *et al*., 2016), Bulgaria (Prelezov *et al*., 2008), Bangladesh (Rahman *et al*., 2015), Czech Republic (Bartova & Sedlak, 2012) and United states (Hill & Dubey, 2013), representing a serious threat to sheep/goat populations in addition to an increasing public health risk worldwide.

Under the circumstances, there is a dire need to devise and adopt certain preventive and curative measures to counteract this important threat of veterinary and public health importance. In this scenario, epidemiological surveys will be able to capture the exact picture on infection load in animal and human populations along with its associated risk factors in particular geographical regions so that countermeasures can be implemented to control the occurrence of toxoplasmosis in susceptible populations. Keeping this in view, this study was conducted to determine the seroprevalence, associated risk factors and haematological impacts of toxoplasmosis in small ruminants of Multan District, in Pakistan, one of the highly populated districts with respect to sheep and goat population.

MATERIALS AND METHODS

The study area

The study was conducted in the Multan District (30°1124422N, 71°2823122E), a highly-populated region with respect to livestock population in the Punjab province of Pakistan. It has a total area of 3,721 km^2 and is situated in the southern part of the Punjab with an altitude of 710 feet from sea level. It has a total population of 0.67 million heads of small ruminants containing 0.15 million sheep and 0.52 million goats. It has arid climatic conditions with extremely hot summer and cold winter seasons. The average annual temperature of Multan is 25°C with an annual rain fall of 187 mm [The information was obtained from official website of Multan District (http://www.multan.gov.pk; accessed on February 15, 2015) and Office of the District Livestock Officer, Multan, Livestock and Dairy Development Department, Govt. of the Punjab, Pakistan].

Sampling and collection of descriptive epidemiological data

The sample size was calculated by using the EpiTools epidemiological calculator directly from the website (http://epitools.ausvet.com.au/content.php?page=home; accessed on Feb 18, 2015) with respect to the population of small ruminants in different Tehsils of study area using expected prevalence rate of 20%. A total of 250 sera samples were collected from sheep (n=125) and goat (n=125) populations

from three different tehsils of Multan, Pakistan. Depending upon population dynamics of small ruminants, the tehsil wise distribution of samples was calculated as 110, 80 and 60 from Multan, Shujabad and Jalalpur tehsils, respectively. Among different tehsils, the sampling was done through random sampling technique using the lottery method. All the samples were properly labelled and brought to the Immunoparasitology Laboratory, Faculty of Veterinary Sciences, Bahauddin Zakariya University (BZU), Multan, Pakistan by placing in ice packs and stored at -20°C till further analysis. Along with collection of sera samples, the descriptive epidemiological data regarding sex, age, area, body weight, breed, health status, deworming status, feeding pattern and presence of pet animals kept, if any, was also recorded on a well-designed questionnaire form. The study was approved by FVS Ethical Committee and Advance Studies and Research Board of BZU, Multan, Pakistan.

Serological detection of anti-*Toxoplasma gondii* antibodies using Latex agglutination test (LAT)

The collected sera samples were analysed for anti-*Toxoplasma gondii* antibodies by using Toxoplasmosis Latex Test Kit (ANTEC®, USA; Catalogue No. TOXO/012; Sensitivity 3-7 IU/mL). The assay was performed according to the manufacturer's instructions as follows;

The kit reagents and collected sera samples were thawed to room temperature prior to the start of assay. The sera samples were diluted in physiological saline (PBS) by 1:2 ratio. A quantity of 50 µL of test sera was placed on the test slide through pip-stirrers provided with the kit followed by the addition of 50 µL of latex reagent using a micro dispenser. The test serum and latex reagent were mixed with stirrer followed by incubation for 4-5 minutes to allow the binding and agglutination of the sera with reagents. The presence or absence of agglutination as endpoint was observed in comparison with positive and negative controls.

Haematological analysis of blood samples

The blood samples (n=10) from each of seropositive and seronegative sheep and goats were collected for haematological analysis to demonstrate the impact of toxoplasmosis on blood parameters. The blood samples were collected randomly from seropositive and seronegative animals that were free from any other infection(s). The blood samples were analysed using the automated Haematology analyser (MEK-6450K, Nihon Kohden, Japan) for different blood parameters including Red Blood Cells Count (RBCs; 10^6 /µl), Haemoglobin Concentration (Hb; g/dl), Packed Cell Volume (PCV; %), Mean Corpuscular Volume (MCV; fl), Mean Corpuscular Haemoglobin (MCH; pg), Mean Corpuscular Haemoglobin Concentration (MCHC; g/dl), Total leukocyte Count (TLC; $\times 10^6$/µl), Neutrophils (N; %), Lymphocytes (L; %), Monocytes (M; %) and Eosinophils (E; %).

Statistical analysis

Data thus obtained was analysed using statistical analysis software Minitab Version 16.0. The percentage values were analysed by Odds ratio and Chi-square tests; whereas, haematological parameters were compared by using Student's T-test. The differences were considered significant at $P<0.05$.

RESULTS

Overall prevalence of anti-*T. gondii* antibodies in small ruminants of Multan District

Out of 250 sera samples, 107 were found positive for anti-*T. gondii* antibodies with an overall seroprevalence of 42.80% in the population of small ruminants of the Multan District, Pakistan. In sheep population, 56 samples were found positive out of 125 samples with seroprevalence rate of 44.80%; whereas, in goat population, 51 out of total 125 samples were positive with seroprevalence rate of 40.80% (χ^2=0.408; P=0.523; Sheep vs goat OR=1.178; Table 1).

Table 1. Overall and specie-wise prevalence of anti-*Toxoplasma gondii* antibodies in small ruminants of district Multan, Pakistan

Total No. of samples	Seropositive samples (n)	Seronegative samples (n)	Seroprevalence (%)
250	107	143	42.80
Sheep Population 125	56	69	44.80
Goat Population 125	51	74	40.80

χ^2= 0.408; P = 0.523; Sheep vs goat OR = 1.178.

Prevalence of anti-*T. gondii* antibodies and different risk factors in sheep population (data shown in Table 2)

Data collected from the serological analysis was arranged into four categories of different age groups including; <1 year; > 1 year but <1.5 years; >1.5 years but < 2 years and > 2 years. The results revealed a higher seroprevalence rate of 60% (n=30/50) in the sheep group aging > 1 year but <1.5 years followed by those of >1.5 years but < 2 years (37.28%; n=22/59), <1 year (27.27%, n= 3/11) and >2 years (20%; n=1/5) age groups (χ^2=3.318; P=0.345). Results of sex-wise analysis revealed a higher prevalence of anti-*T. gondii* antibodies in female sheep (64.52%; n=40/62) as compared to male population with a seroprevalence rate of 25.39% (n=16/63) (OR=2.540; χ^2=7.492; P=0.006). The body weight wise analysis of serological data showed that sheep population with a body weight of 51-60 kg showed the highest seroprevalence (57.14%; n=04/07) followed by those of 41-50 kg (48.27%), 31-40 kg (44.23%) and 21-30 kg (16.67%) whereas, none of the serum samples of the age group 10-20 kg was found positive. Further, differences between different groups was statistically non-significant (χ^2 =1.151; P=0.765).

The sera samples from three sheep breeds including Lohi, Kacchi and Kajli were analysed in this study. Results showed that seroprevalence of toxoplasmosis was highest in the Lohi breed (53.33%; n= 32/60) followed by those of the Kajli (41.37%; n= 12/29) and the Kachhi (33.33%; n=12/36) breeds; whereas the difference in the seroprevalence rates between these breeds was non-significant (χ^2=1.482; P=0.477). The data collected from the study area was categorized in two groups including apparently healthy animals and those with some minor co-morbid conditions. But this study was not focused on the correlation of toxoplasmosis with some specific disease conditions. So, samples were not collected from the animals suffering from severe disease conditions with pronounced clinical signs and symptoms. In sheep population, a higher seroprevalence rate (57.98%; n= 40/69) was recorded in sheep with some co-morbid conditions followed by those of the healthy group (28.58%; n=16/56) and the difference was found statistically significant (OR=2.028; χ^2=4.252; P=0.039).

The correlation of seroprevalence of toxoplasmosis with the feeding system was also examined and results showed that animals on open grazing had significantly higher rate of prevalence of anti-*T. gondii* antibodies (50.47%) as compared to those on stall feeding (15%) (OR=3.365; χ^2=3.949; P=0.047). In the sheep population, the data showed that presence of pet cats on sheep farms/flocks had significant impact on the prevalence of anti-*T. gondii* antibodies. The sheep flocks reared in the presence of cats showed highest seroprevalence rate (84%) followed by those reared with both cats and dogs (50%), with dogs only (31.42%) and without any pet dogs and cats (16%) (χ^2=9.874; P=0.02).

Table 2. Prevalence of anti-*Toxoplasma gondii* antibodies in sheep population of district Multan, Pakistan

Parameter	Seropositive samples (n)	Seronegative samples (n)	Seroprevalence (%)
Age			
< 1 year	11	03	27.27
>1 but <1.5 year	50	30	60.00
> 1.5 but < 2 years	59	22	37.28
>2 years	05	01	20.00
	χ^2=3.318, DF=3, P-Value=0.345		
Sex			
Male	63	16	25.39
Female	62	40	64.52
	χ^2=7.492, DF=1, P-Value=0.006, OR= 2.540		
Body weight (Kg)			
10-20	02	00	00
21-30	06	01	16.67
31-40	52	23	44.23
40-50	58	28	28.27
51-60	07	04	57.14
	χ^2=1.151, DF=3, P-Value=0.765		
Breed			
Nachi	60	32	53.33
Teddi	36	12	33.33
Beetal	29	12	41.37
	χ^2=1.482, DF=2, P-Value=0.477		
Location (Tehsil)			
Multan	55	20	38.46
Shujabad	40	18	45.00
Jalalpur	30	18	60.00
	χ^2=1.608, DF=2, P-Value=0.448		
Health status			
Apparently healthy	56	16	28.58
Co-morbid	69	40	57.98
	χ^2=4.252, DF=1, P-Value=0.039, OR=2.028		
Deworming status			
With deworming	56	25	44.65
Without deworming	69	31	44.93
	χ^2=0.000, DF=1, P-Value=0.984, OR=1.006		
Feeding pattern			
Stall feeding	20	03	15.00
Open grazing	105	53	50.47
	χ^2=3.365, DF=1, P-Value=0.047, OR=3.365		
Pet animal			
Cats	25	21	84.00
Dogs	35	11	31.42
Both cats and dogs	40	20	50.00
Without any pet	25	04	16.00

χ^2=9.874, DF=3, P-Value=0.020.
These values are for pet animals analysis only. It should be presented like those mentioned for all above risk factors as a 5th row in Pet Animals Cell.

Prevalence of anti-*T. gondii* antibodies and different risk factors in goat population (data shown in Table 3)

Age-wise analysis of prevalence of anti-*T. gondii* antibodies showed that goats with age group >1 year but <1.5 years showed the highest seroprevalence rate (55.56%) followed by those of >1.5 years but < 2 years (38.99), >2 years (16.67%) and <1 year (11.11%) age groups (χ^2=4.796; P=0.187). In the sheep population, females showed a higher seroprevalence (58.06%) as compared to male goats (23.81%) (OR=2.439; χ^2=6.466; P=0.011). In body weight wise analysis, the group with body weight of 41-50 kg showed the highest seroprevalence (48.98%) followed by those of 31-40 kg (48.94%), 51-60 kg (25.00%) and 21-30 kg (09.53%) (χ^2=6.040; P=0.110).

In case of goats, the sera samples from three goat breeds including Nachi, Teddi and Beetal were analysed for serological detection of anti-*T. gondii* antibodies. Results showed that seroprevalence of toxoplasmosis was highest in the Beetal breed (60.00%) followed by those of Nachi (52.27%) and Teddi (19.61%) breeds and the difference between these breeds was statistically significant (χ^2=7.320; P=0.026). In the goat population, the group of co-morbid animals showed a higher seroprevalence rate (60.00%) as compared to the healthy group (23.07%) (OR=2.333; χ^2=6.212; P= 0.013).

The feeding pattern wise analysis showed significantly higher (53.69%) prevalence rate in sheep fed on open grazing system as compared to those on stall feeding (10%) (OR=5.368; χ^2=8.531; P=0.003). Like sheep population, a significant correlation was detected between the seroprevalence of toxoplasmosis and presence of pet cats and dogs reared with goat flocks (χ^2=11.118; P=0.011)

Haematological impacts of toxoplasmosis in small ruminants

The blood samples from seropositive and seronegative sheep and goats were collected and analysed for haematological parameters including RBC count, TLC, DLC and haemoglobin index. In sheep, results showed a significantly higher neutrophil (P=0.035) and eosinophil (P=0.005) counts in seropositive sheep as compared to negative ones (Table 4).

In goats, a significantly higher (P<0.05) MCHC value was recorded in seropositive animals as compared to negative ones. Further, a highly significant difference (P<0.005) was also detected between the total leukocytic, neutrophil, lymphocyte and monocyte count of both groups (Table 4).

DISCUSSION

Toxoplasmosis is a globally distributed zoonotic disease that infects more than 10 million people annually (Guo *et al.*, 2017). In immuno-compromised individuals it may lead to severe pathological outcomes in the form of encephalitis, pneumonia and myocarditis (Bal *et al.*, 2014). It is one of the leading causes of abortion, still birth and certain other reproductive disorders in different animal species including human beings (Hill & Dubey, 2013). The cats act as definitive host for *T. gondii* and farm animals may contact infection by the ingestion of feed/water contaminated with its oocyst (Rodríguez-Ponce *et al.*, 2017; Hill & Dubey, 2013). Due to its public health importance, *T. gondii* has been intensively studied for screening, occurrence, prevalence and determination of potential associated risk factors in different food animals including small ruminants (Guo *et al.*, 2017; Hanafi *et al.*, 2014) and ranged from 0-100% in different host species in different parts of the world (Tasawar *et al.*, 2012; Ramzan *et al.*, 2009). This wide range in prevalence had been associated with customary and traditional differences, geoclimatic conditions, age of the host and husbandry practices in addition to certain socio-economic factors (Ahmed *et al.*, 2016).

Food animals including small ruminants (sheep and goats) are one of the most affected mammalian species with *T. gondii* (Ahmad and Tasawar, 2015) and are one of the potential sources for the transmission of disease to human beings (Satbige *et al.*,

Table 3. Prevalence of anti-*Toxoplasma gondii* antibodies in goat population of district Multan, Pakistan

Parameter	Seropositive samples (n)	Seronegative samples (n)	Seroprevalence (%)
Age			
< 1 year	09	01	11.11
>1 but <1.5 year	45	25	55.56
> 1.5 but < 2 years	59	23	38.99
>2 years	12	02	16.67
	χ^2=4.796, DF=3, P-Value=0.187		
Sex			
Male	63	15	23.81
Female	62	36	58.06
	χ^2=6.466, DF=1, P-Value=0.011, OR= 2.439		
Body weight (Kg)			
10-20	00	00	00
21-30	21	02	09.53
31-40	47	23	48.94
40-50	49	24	48.98
51-60	08	02	25.00
	χ^2=6.040, DF=3, P-Value=0.110		
Breed			
Nachi	44	23	52.27
Teddi	51	10	19.61
Beetal	30	18	60.00
	χ^2=7.320, DF=2, P-Value=0.026		
Location (Tehsil)			
Multan	55	21	21.82
Shujabad	40	12	30.00
Jalalpur	30	18	60.00
	χ^2=2.641, DF=2, P-Value=0.267		
Health status			
Apparently healthy	70	18	23.07
Co-morbid	55	33	60.00
	χ^2=6.212, DF=1, P-Value=0.013, OR= 2.333		
Deworming status			
With deworming	62	24	38.71
Without deworming	63	27	42.87
	χ^2=0.094, DF=1, P-Value=0.760, OR= 1.107		
Feeding pattern			
Stall feeding	30	03	10.00
Open grazing	95	51	53.69
	χ^2=8.531, DF=1, P-Value=0.003, OR= 5.368		
Pet animal			
Cats	20	16	80.00
Dogs	36	05	13.89
Both cats and dogs	56	27	48.21
Without any pet	13	03	23.07

χ^2=11.118, DF=3, P-Value=0.011.
These values are for pet animals analysis only. It should be presented like those mentioned for all above risk factors as a 5th row in Pet Animals Cell.

Table 4. Comparison of hematological parameters in *T. gondii* seropositive and seronegative sheep and goats

Blood parameter	Sheep			Goats		
	Seropositive (Mean±SE)	Seronegative (Mean±SE)	P-value	Seropositive (Mean±SE)	Seronegative (Mean±SE)	P-value
RBCs (10^6 /µl)	9.72±0.47	9.16±0.71	0.529^{NS}	9.24±0.31	9.88±0.41	0.250^{NS}
Hb. (g/dl)	10.24±0.43	10.14±0.70	0.906^{NS}	9.68±0.31	10.14±0.26	0.290^{NS}
PCV. (%)	30.54±0.98	29.82±2.13	0.077^{NS}	30.84±1.15	30.92±0.74	0.955^{NS}
MCV. (fl)	31.50±0.59	33.22±2.20	0.472^{NS}	31.92±0.47	31.40±0.83	0.602^{NS}
MCH. (pg)	10.56±0.22	11.18±0.66	0.401^{NS}	10.22±0.14	10.30±0.24	0.780^{NS}
MCHC. (g/dl)	33.48±0.50	33.64±0.50	0.827^{NS}	32.20±0.20	32.78±0.12	0.038^{*}
T.L.C. ($\times 10^6$/µl)	7.10±0.79	8.32±0.85	0.324^{NS}	13.92±0.25	6.42±0.27	0.000^{**}
Neutrophils (%)	46.32±5.22	22.52±7.83	0.035^{*}	55.64±3.01	35.84±6.71	0.027^{*}
Lymphocytes (%)	74.32±1.04	74.34±7.15	0.998^{NS}	74.86±1.73	59.16±3.80	0.006^{**}
Monocytes (%)	4.68±0.53	2.68±1.37	0.211^{NS}	5.52±0.86	0.30±0.00	0.000^{**}
Eosinophils (%)	7.46±1.85	0.26±0.09	0.005^{*}	17.68±8.12	0.24±0.07	0.064^{NS}

NS=Non-significant (P>0.05); *=Significant (P<0.05); **=Highly significant (P<0.01).

2016). In the present study, overall seroprevalence of toxoplasmosis in small ruminants of Multan District, Pakistan was 42.80%. Previous studies revealed a higher seroprevalence rate in small ruminants in different parts of the world viz. 57.60% in Southwestern Ethiopia (Tegegne *et al.*, 2016), 50.81% in Northern Italy (Gazzonis *et al.*, 2015), 70.48% in Oromia Regional State, Central Ethopia (Gebremedhin *et al.*, 2013), 67.9% in Zimbabwe (Hove *et al.*, 2005). Contrary to this, lower prevalence rates had also been reported in some countries including Southern regions of Ethopia (26.09%; Gebremedhin and Gizaw, 2014), Northeastern China (16.8%; Xu *et al.*, 2015) and Rahim Yar Khan, Pakistan (19%; Ramzan *et al.*, 2009). Results of species-wise analysis revealed non-significant difference in seroprevalence of *T. gondii* in sheep and goat populations. Previous studies revealed higher seroprevalence rates of toxoplasmosis in sheep like those in Ahvaz, South-West Iran (69.30%; Hamidineja *et al.*, 2008), State of Piauí, Northeast Brazil (48.7%; Rêgo *et al.*, 2016) and Stara Zalgoria, Bulgaria (48.2%; Prelezov *et al.*, 2008). On the other hand, some other studies also revealed lower prevalence rates in sheep in different parts of the world including Tabriz, North-West of Iran (6.98%; Nematollah *et al.*, 2014), Western Cape, South Africa (8%; Hammond-Aryee *et al.*, 2015), Northern Punjab, Pakistan (18.16%; Ahmed *et al.*, 2016) and Colima, Western State of Mexico (29.1%; Caballero-ortega *et al.*, 2008). Similarly, previous studies also revealed higher prevalence rates of toxoplasmosis in goats like those in Rajshahi, Bangladesh (55.1%; Rahman *et al.*, 2015), and Czech Republic (66%; Bartova & Sedlak, 2012); whereas, literatures also revealed lower prevalence rates in goats in different parts of the world including Northern Punjab, Pakistan (14.32%; Ahmed *et al.*, 2016), Northern Palestine (13.4%; Othman & Al-Zuheir, 2013), Southern Tunisia (34.5%; Lahmar *et al.*, 2015), Dir Upper, Pakistan (19%; Perveen & Shah, 2015), Myanmur (11.4%; Bawm *et al.*, 2016), Nile Delta regions of Egypt (17.11%; Mahboub *et al.*, 2013) and Hanan Province of China (12%; Li *et al.*, 2016).

Sex-wise analysis revealed a significantly higher prevalence of toxoplasmosis in female population of both sheep and goats of district Multan. Our findings are consistent with those reported by Tegegne *et al.* (2016) and Gebremedhin *et al.* (2013). It might be speculated that immunosuppression associated with lactation and pregnancy stress due to hormonal differences might be responsible for higher seroprevalence of toxoplasmosis in female

sheep and goats (Tegegne *et al*., 2016). Additionally, age, poor nutrition and physiological status might also lead to poor immunity, providing opportunity to various pathogens to develop infection (Akhtar *et al*., 2014). Conversely, Gebremedhin & Gizaw (2014) reported a non-significant impact of sex on the prevalence of toxoplasmosis in sheep; whereas, some studies had also reported higher seroprevalence in males as compared to females (Tasawar *et al*., 2012). The age-wise analysis of seroprevalence of toxoplasmosis demonstrated a non-significant difference in various age groups. In contrast, Tegegne *et al*. (2016) and Gebremedhin *et al*. (2013) reported higher seroprevalence in adult as compared to young ones. Since less developed or compromised immune functions are considered responsible for high susceptibility and seroprevalence of toxoplasmosis in different age groups of sheep and goat (Gazzonis *et al*., 2015); it could be hypothesized that different age groups of sheep and goat exhibited uniform immune efficiency against toxoplasmosis that might be associated with various factors regarding husbandry practices in the study area (Krejci *et al*., 2013). The correlation of body weight and seroprevalence of toxoplasmosis showed a non-significant difference. No previous data is available in this regard. Anyhow, it could be hypothesized that weight gain has a positive correlation with increasing age (Tizabi *et al*., 2012) so, a similar response was found in weight wise analysis of toxoplasmosis. Results showed a non-significant difference in breed-wise distribution of toxoplasmosis in sheep. Although Dubey & Welcome (1988) reported the higher susceptible to *T. gondii* infection in some sheep breeds as compared to others. It could be hypothesized that sheep breeds of district Multan (Lohi, Kajli and Kachhi) showed a similar immunogenic response to *T. gondii* due to which no significant difference was recorded in this study. In case of goat population, a significant difference was recorded among different breeds of goat. Similarly, Gazzonis *et al*. (2015) and Lopes *et al*. (2013) also reported a significant difference in seroprevalence of toxoplasmosis in different goat breeds in Northern Italy and Northern Portugal, respectively. The difference in the seroprevalence of toxoplasmosis in different goat breeds might be attributed to the differences in genetic resistance to toxoplasmosis in different goat breeds (Gazzonis *et al*., 2015). The difference might also be attributed to the rearing system as Teddi is mostly reared as domestic breed and is kept on stall feeding; whereas, Beetal and Nachi are mostly reared on grazing system in the study area, so are more prone to getting the infection by the ingestion of oocysts from contaminated pastures (Akhtar *et al*., 2014). A non-significant correlation was detected between location and seroprevalence of toxoplasmosis. Anyhow, some previous studies revealed a significant difference in the seroprevalence of toxoplasmosis which might be due to variations in geo-climatic conditions and conventional husbandry practices in different geographical regions (Ahmed *et al*., 2016). The analysis regarding the seroprevalence of toxoplasmosis with co-morbid conditions revealed a significant correlation of toxoplasma seropositivity with other co-morbid conditions in both sheep and goat populations. The stress on the immune system induced by different disease conditions might be speculated as an important factor responsible for higher seroprevalence of toxoplasmosis with various co-morbid conditions (Awais *et al*., 2011).

The correlation of seroprevalence of toxoplasmosis with the feeding system revealed higher seropositivity in sheep and goat populations reared on open grazing system as compared to those on stall feeding. This might be due to the reason, that stray and wild cats and dogs have direct access to the grazing pastures and may contaminate the pastures by shedding oocysts through their excreta (Lindsey *et al*., 1997). The sheep and goats carry infection by ingesting the oocysts while grazing on such contaminated pastures. It is worth mentioning that cats and dogs are the most abundant pets in this area which accompany the sheep and goat flocks when they are sent out for grazing which may act as potential

source to contaminate the grazing pastures (Gebremedhin *et al*., 2013). On the other hand, there are less chances of contamination of feed of sheep and goats reared on stall feeding. The cats act as definitive host for *T. gondii* so a correlation of toxoplasmosis with pet dogs and cats was also determined which revealed that presence of pet cats on sheep and goat farms/flocks had significant correlation with seroprevalence of toxoplasmosis. It might also be correlated with the fact that cats shed the oocysts and contaminate the soil and water with *T. gondii*. Previous studies also showed an important role of cats which are the definitive host of *T. gondii*, in the transmission of disease to the different animals including sheep and goats (Zhao *et al*., 2011).

Results regarding the impact of toxoplasmosis on haematological parameters showed a significant difference in leukocytic profile in seropositive sheep and goats as compared to negative ones. Earlier, Advincula *et al*. (2010) also reported altered erythrocyte, neutrophil and monocyte counts and haemoglobin concentrations in serologically *Toxoplasma* positive cats in the Philippines. The increased neutrophil count might be speculated as an indicator of active infection (Al-Gwaiz & Babay, 2007) in seropositive sheep and goats under study. Anyhow, an altered total leukocytic count (TLC) is an alarming condition which might impair the host immunity making the affected animals more prone to secondary diseases conditions (Rosales *et al*., 2016) making this disease more complicated. So, keeping in view, the adverse impact of toxoplasmosis on various blood parameters, particularly TLC in goats demands much more attention towards the effective control and early treatment of *Toxoplasma* infected animals to safeguard this important segment of meat and wool industry.

In conclusion, the sheep and goat populations of Multan District, Pakistan are positive for anti-*T. gondii* antibodies. The associated risk factors for the seroprevalence of toxoplasmosis in study area include sex, breed (in goats only), pet cats and dogs, farming system and co-morbid conditions. Sheep and goat are one of the most important sources of meat for the human consumption and the presence of the *T. gondii* in these animals may pose a serious public health concern. Under the circumstances, it is suggested to devise some effective local and global *Toxoplasma* control programs and strategies to cope up with this very important disease of public health importance. It will help to step forward to ensure and strengthen the human and animal health care systems in the society.

Acknowledgements. The authors are highly thankful to Livestock & Dairy Development Department, Govt. of Punjab and sheep/goat farmers of Multan District for their cooperation in conducting this survey.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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