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Evaluation of seroprevalence and associated risk factors of toxoplasmosis in sheep and goats in District Jhang-Pakistan

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ABSTRACT: Toxoplasmosis is a zoonotic infection caused by a pathogenic protozoan, *Toxoplasma gondii*, responsible for huge economic and health losses in developing countries. The current study was conducted to assess the seroprevalence of toxoplasmosis and associated risk factors in sheep and goats in District Jhang, Punjab, Pakistan. Blood samples (n=400) were collected from both genders of goats (n=219) and sheep (n=181) from four Tehsils of District Jhang along with a comprehensive questionnaire to evaluate the risk factors associated with the disease endemicity and spread. For assessing the seroprevalence, the samples were examined using Latex agglutination test. Additional data regarding hygienic conditions, water source, gender, breed, age of animal was also collected on a predesigned questionnaire. The overall seroprevalence of *Toxoplasma gondii* was found 34.25% (137/400) in District Jhang. Higher seroprevalence was recorded in goats {36.52% (80/219)} as compared to sheep {31.49% (57/181)}, however, it was non-significant ($p>0.05$). Gender-wise seroprevalence was found 32.59% (44/135) and 35.09% (93/265) in male and female animals, respectively ($p>0.05$). Further, the association of toxoplasmosis between different age groups was significantly higher in older animals having age >24 months 42.75% (62/145) than younger animals with age <12 months 26.60% (29/109) and 11-24 months 31.50% (46/146) ($p<0.05$). The seroprevalence was also higher 40.81% (80/196) in animals drinking water from outdoor water source than in animals drinking from indoor water source 27.94% (57/204) ($p<0.05$). Moreover, seroprevalence was significantly higher 43.11% (97/225) in animals kept in vicinity of cats than in absence of cats 22.85% (40/175) ($p<0.05$). However, reproductive status, breeds, flock size had non-significant impact on the prevalence of *T. gondii*. Thus, it is concluded that the presence of cats near animals, larger flock size, older age of animals, and poor hygienic conditions are main risk factors of toxoplasmosis in sheep and goats and these could be a potential threat of infection for livestock industry and public health.

Keywords: *Toxoplasma gondii*; Goats; Sheep; Latex agglutination test; Cats; Pakistan.

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INTRODUCTION

Toxoplasmosis is a common zoonotic disease caused by a parasite *Toxoplasma gondii* with high prevalence in human population. This parasite is distributed worldwide and is considered as one of the most successful parasite. *T. gondii* infects a large number of species, including domestic animals. Toxoplasmosis is more prominent in small ruminants like goats and sheep and causes huge economic losses due to abortion and neonatal deaths in infected animals. Humans can also be infected with this disease using contaminated and undercooked meat and milk of infected animals (Dubey and Jones, 2008).

Due to zoonotic potential of toxoplasmosis globally, several serological studies show that antibodies against *T. gondii* are found in more than one third of human population. However, prevalence of *T. gondii* in human population varies greatly in different countries due to variations in cultures and customs; and even shows variations among different ethnic groups within one country (Sroka, 2001). Toxoplasmosis has been found 20-30% in USA, 25% in Japan, 60% in Netherlands, 60% in Italy, 35% in Finland and 50%-60% in Poland (Abu-Dalbouh et al., 2012).

T. gondii is an obligate intracellular pathogenic protozoan that belongs to family Sarcocystidae (Sibley et al., 2009). *T. gondii* has a number of strains and are mainly divided into three classes i.e. Type I, Type II and Type III (Boothroyd and Grigg, 2002). Type I is recognized as an infectious agent that causes infection in rodents. Type II is a causative agent of toxoplasmosis in small ruminants like goats, sheep etc. However, it has been found that Type III is not an infectious strain of toxoplasmosis (Boothroyd, 2009).

T. gondii completes its sexual stage in cats because these are definitive hosts (Petersen and Schmidt, 2003). Cats spread oocysts through their feces. Humans, warm blooded animals and large number of other animals act as intermediate hosts of *T. gondii*. Cats or other members of felids shed oocysts after completion of sexual stage where sporozoites are harbored (Bisson et al., 2000). When oocysts contaminated food is ingested, sporozoites found in oocysts enter into the gastrointestinal tract of the secondary host (sheep, goat, buffalo, cattle, mice, birds, and humans) and completes its asexual period of life cycle.

In livestock, the main route of transmission of the parasite is through ingestion of sporulated oocysts. These oocysts are produced in wild and domestic cats

during the sexual stage of their life cycle. These animals are major reservoir of the parasite. However, it has been noted that this infection does not exist or rarely found if cats are absent. So cats have a critical role in spreading this infection (Jones and Dubey, 2012). Sheep and goats are commonly infected with *T. gondii*. In sheep and goats, *T. gondii* infection causes significant losses due to abortion and because of zoonotic transmission through consumption of infected milk and meat (Afonso et al., 2013). *T. gondii* can cause early embryonic death, fetal death, resorption, stillbirth, abortion, mummification and neonatal death in sheep depending on the stage of gestation (Jones et al., 20001).

T. gondii is a zoonotic parasite which may transmit to humans through consuming raw or undercooked meat containing infectious tissue cysts, taking food or water infected with sporulated oocysts, using goat or sheep milk contaminated with *T. gondii* tachyzoites (Ahmad et al., 2015), and parasite may also be transmitted to humans through organ transplantation, blood transfusion and transplacentally transmitted from mother to fetus (Ramzan et al., 2009). In humans, infection does not show any symptoms, however, individuals suffering from AIDS and immunocompromised may show some complications. In humans, toxoplasmosis may be congenital or acquired (Tenter et al., 2000). *T. gondii* causes both acute and chronic infections. The major symptoms of the toxoplasmosis are flu-like mild illness having characteristics such as like fatigue, fever and headache. Sometimes the disease exists without proper signs and symptoms. Immunocompromised people like HIV infected patients and pregnant women may suffer serious illness characterized by diarrhea, weight loss, liver diseases, pneumonia, and central nervous system infection. In case of severe infection, toxoplasmosis can even cause death.

Numerous studies have been conducted on the prevalence of *T. gondii* in different animal species in various parts of the world. Prevalence of *T. gondii* has been reported in goats and sheep in different parts of Punjab, Pakistan. Prevalence of toxoplasmosis was 19.9% in sheep of Southern Punjab (Dubey, 2004) and 14.3% and 18.2% in goats and sheep of Pothohar region, respectively (Ganter, 2008). In Rahim Yar Khan, Pakistan seroprevalence was reported 25.4% and 11.2% in goats and sheep, respectively (Spisak et al., 2010). In District Jhang, rearing of goats and sheep is carried out on large scale in rural areas

where livelihood of a huge community of the farmers depend upon raising of these animals. However, there is no comprehensive data available on seroprevalence of *T. gondii* infection in goats and sheep in District Jhang. Keeping in view the consequences of the disease and its impact, the current study was designed to determine the seroprevalence of *T. gondii* in goats and sheep and associated risk factors e.g., gender, age, breed, water source, presence of cats, flock size, hygienic conditions with the infection.

MATERIALS AND METHODS

Study design

The present study was carried out in four Tehsils (Jhang, Shorkot, Athara Hazari, and Ahmadpur Sial) of District Jhang, Punjab, Pakistan. The study was conducted in accordance with the Ethical Principles in Animal Experimentation, and before starting the research project, necessary ethical approval was obtained from the ethical review committee, Government College University, Faisalabad, Pakistan.

Collection of blood samples

Blood samples were collected from jugular vein of sheep and goats. Blood samples (n=400) were collected from goats (n=219) and sheep (n=181) present in the study area during the period from February, 2018 to July, 2018. To make the scope of study wider, blood samples were collected from male and females of three breeds of goats (teddy, beetal and juttile) and three breeds of sheep (desi, thalli and kajli) present in the area of study. One hundred samples were collected from each of the Tehsil to carry out the study.

Serum separation

After collection of blood, samples were promptly transported to the Microbiology Laboratory, College of Veterinary and Animal Sciences, Jhang for separation of serum. Serum samples were separated through centrifugation at 3000 rpm for 15 minutes. All serum samples were properly labelled and stored at -20°C until further analysis.

Questionnaire surveys

A questionnaire was designed to obtain the information from the farmers about the sampled animals regarding their age, sex, breed, herd size, abortion history, biosecurity, management practices, source of drinking water, and presence of cats in the premises. Based on collected information, animals were grouped into three classes based on their age (<12

months, 13 to 24 months and >24 months), hygienic conditions (low, moderate and high). Low referred to farms that were cleaned after ≤ 2 days, moderate means that farms were cleaned > 2 days and high means that farms were cleaned daily. Sources of water were categorized into an indoor water source (inside farmhouse) and an outdoor water source (ponds). Based on flock size, animals were divided into three classes (flock having < 10 animals, flocks with 11 - 30 animals and flocks >30 animals). Reproductive status of animals (pregnant, non-pregnant and lactation) was also recorded. The data were recorded in a pre-designed questionnaire.

Latex agglutination test

Antibodies specific for *T. gondii* were measured using latex agglutination test (LAT) using a commercially available toxoplasmosis latex kit manufactured by Antech Diagnostic, UK according to the manufacturer's instructions. Latex reagent contains a suspension of polystyrene particles coated with antigens of *T. gondii*. Agglutination appears when the parasite is present in the serum in positive control while negative control does not show such agglutination. Antigen-antibody reactions occur when serum containing antibodies against *T. gondii* were tested and reactions can be easily visualized due to agglutination. The serum and all reagents were brought to room temperature. A drop of diluted serum (40 μ L) was placed into each well of test slide followed by the addition of a drop of latex reagent and mixed well. The presence and absence of agglutination were observed within four minutes. The positive sera indicated clear agglutination while in negative sera, no agglutination was observed.

Statistical analysis

The data was analyzed using SPSS version 23.0 IBM Inc. USA. Odds ratio was calculated using SPSS by comparing healthy and diseased animals to determine *T. gondii* infections. To measure the association between various variables Pearson's Chi-Square test was used. The difference was considered statistically significant at $p \leq 0.05$ and non-significant at $p > 0.05$.

RESULTS

The present study is the first epidemiological study carried out to evaluate the seroprevalence of the *T. gondii* infection in goats and sheep from District Jhang, Punjab, Pakistan using latex agglutination test (LAT). Among 400 serum samples collected

from goats and sheep, 137 samples were found positive. Hence, the overall seroprevalence of *T. gondii* in goats and sheep in district Jhang was 34.25% (137/400) (Figure 1). The seroprevalence of *T. gondii* in goats (36.52%) was higher than seroprevalence in sheep (31.28%). Out of 219 serum samples collected from goats, 80 were found positive, so seroprevalence of *T. gondii* was 36.52% (80/219). While 57 out of 181 blood samples collected from sheep were positive so, the seroprevalence was 31.49% (57/181).

Tehsil-wise seroprevalence of *T. gondii* in goats was 44.64%, 35.38%, 22.72%, and 40.74% in Tehsil Jhang, Tehsil Athara Hazari, Tehsil Shorkot and Tehsil Ahmadpur Sial, respectively. So, the highest seroprevalence (44.64%) was found in goats of Tehsil Jhang and lowest (22.72%) in goats of Tehsil Shorkot. In sheep, the seroprevalence was 38.63%, 25.71%, 30.35% and 30.43% in Tehsil Jhang, Tehsil Athara Hazari, Tehsil Shorkot and Tehsil Ahmadpur Sial, respectively. So, the highest prevalence in sheep was found in Tehsil Jhang (38.63%) and the lowest (25.17%) in Tehsil Athara Hazari.

There has been a close association reported between toxoplasmosis in goats and sheep and various risk factors such as gender, age, presence of cats, flock size, source of water, breed etc. The current data showed that seroprevalence of *T. gondii* in female goats was higher 37.57% (59/157) than male goats 33.87% (21/62), however, this difference was statistically non-significant. However, seroprevalence was little bit higher in male sheep 33.33% (21/63) than female sheep 30.50% (36/118).

It was also found that seroprevalence was higher in older age groups (>24 months) than younger animals

having age <12 months and between 13-24 months. The prevalence of *T. gondii* in goats having age <12, 13-24 and >24 months was 30% (15/50), 39.71% (33/83) and 41.02% (32/78), respectively. Hence, in goats highest seroprevalence was found in animals having age >24 months. In sheep, seroprevalence was 29.72% (11/37), 30% (18/60) and 37.83% (28/74) in above groups, respectively.

Cats are definitive hosts of *T. gondii*. Current study demonstrated that seroprevalence was significantly higher in those animals living in the farms where cats were present than those farms where no cats were present. Prevalence of *T. gondii* in sheep and goats living in vicinity of cats was found 47.61% (60/126) and 38.53% (42/109), respectively. However, seroprevalence in absence of cats was 21.50% (20/93) and 20.83% (15/72) in goats and sheep, respectively.

The current data showed a positive correlation between flock size of goats and sheep and seroprevalence of *T. gondii*. Highest seroprevalence (43.66%) was found in those goats that live in flock size of >30 as compared to animals living in flock comprising of less than 10 (40%) and between 11 to 30 animals (29.59%).

The seroprevalence was also higher 40.81% (80/196) in animals drinking water from outdoor water source than in animals drinking water from indoor water source 27.94% (57/204) ($p < 0.05$). While, based on different breeds of goats, seroprevalence of *T. gondii* in Teddy breed was 50% (23/46), Juttile 46.42% (13/28) and Beetal 30.34% (44/145). Whereas the seroprevalence based on different breeds of sheep was Thalli 44.11% (15/34), Kajli 41.93% (13/31), and Desi 25% (29/116) (Table 1).

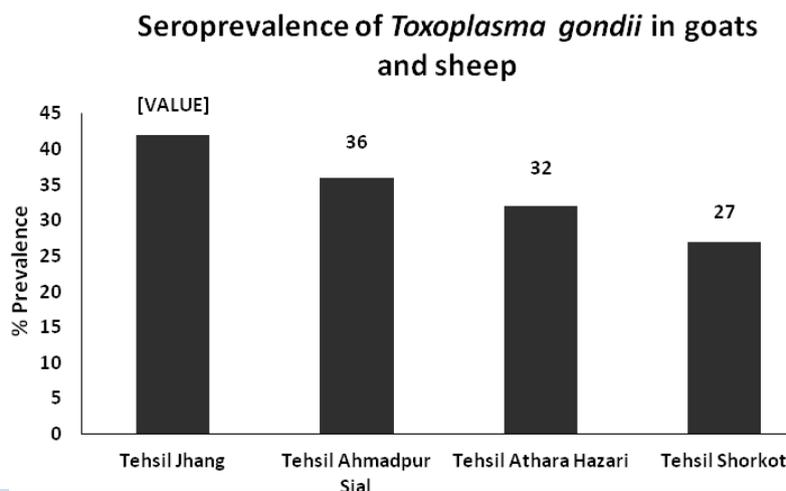


Figure 1. Seroprevalence of *T. gondii* in different Tehsils of District Jhang

Table 1. Demographic and risk factor association of *Toxoplamagondii* in goats and sheep

Risk Factor	Tested	Positive	Negative	Prevalence	Chi-Square	P-Value	Odds Ratio	95% Confidence interval	
								Lower	Upper
1- Animal Type									
Goat	219	80	139	36.52%					
Sheep	181	57	124	31.49%	2.398	0.122	1.392	0.915	2.118
2-Place									
Tehsil Jhang	100	42	58	42%					
Tehsil AtharaHazari	100	32	68	32%					
Tehsil Shorkot	100	27	73	27%					
Tehsil AhmadpurSial	100	36	64	36%	5.362	0.147	--	--	--
3-Breed									
Teddy	46	23	23	50%					
Beetal	145	44	101	30.34%					
Juttile	28	13	15	46.42%					
Desi	116	29	87	25%					
Thalli	34	15	19	44.11%					
Kajli	31	13	18	41.93%	9.700	0.084	--	--	--
4-Gender									
Male	135	44	91	32.59%					
Female	265	93	172	35.09%	0.249	0.618	0.894	0.576	1.388
5-Age (months)									
<12	105	26	79	24.76%					
13-24	143	51	92	35.66%					
>24	152	60	92	39.47%	7.978	0.019	--	--	--
6-Flock Size									
<10	97	27	70	27.83%					
11--30	135	40	85	29.62%					
>30	168	58	110	34.52%	0.010	0.995	--	--	--
7-Cats									
Yes	235	102	133	43.40%					
No	165	35	130	21.21%	17.932	0.000	2.558	1.646	3.974
8-Reproductive status (Female animals)									
Pregnant	91	34	57	37.36%					
Non-Pregnant	116	36	80	31.03%					
Lactation	68	25	43	36.76%	3.161	0.206	--	--	--
9-Water Source									
Outdoor	230	86	144	37.39%					
Indoor	170	51	119	30.00%	7.358	0.007	1.779	1.171	2.701
10- Hygienic Conditions									
Low	124	56	68	45.16%					
Moderate	191	61	130	31.93%					
High	85	20	65	23.52%	11.347	0.003	--	--	--

DISCUSSION

There exist great variations in prevalence of *T. gondii* across the world and rates of infection ranges from 0 to 100 percent in various countries (Villa-gra-Blanco et al., 2019). This difference in prevalence depends upon traditions, customs, weather conditions, husbandry practices, presence or absence of cats and difference in age (Atail et al., 2017). The current study has given an insight on toxoplasmosis and revealed the widespread occurrence of *T. gondii* among the domestic goats and sheep raised in District Jhang, Punjab. In the present study overall seroprevalence including both species was found 34.25%. Current prevalence rate i.e. 34.25% that is higher than those reported by (Andrade et al., 2013) in South Africa and (Romanelli et al., 2007) in North America as 4.3% and 11.2%, respectively. However, present prevalence rate is lower than reported earlier (42.8%) by (Ahmed et al., 2016) in Pakistan, 49.43% reported by (Sechi et al., 2013) in eastern Slovakia, 52% reported by (Gebremedhin et al., 2013) in Multan, Pakistan and 52% reported by Buxton et al., 2007 in El-Gadrfif state. These variations in the seroprevalence rates with the above reports could be due to differences in their customs, traditions, lifestyles of the inhabitants, weather conditions, age of the animals and husbandry practices (Gazzonis et al., 2015).

It has been reported in several studies describing various risk factors have significant association with toxoplasmosis in goats and sheep (Samra et al., 2007; Wang et al., 2011; Ahmad and Tasawar, 2016; Turcekova et al., 2013; Ramzan et al., 2009). These risk factors include source of water, age, flock size, presence of cats, hygienic conditions etc. Cats are definitive host of *T. gondii* and can pass oocysts of the parasite in their feces, hence one of major source of environmental contamination (Rashid et al., 2016; Onyiche and Ademola, 2015). Moreover, the prevalence rate may also be linked with the presence of cats that excrete oocysts, which after sporulation become infectious to man and animals (Zhao et al., 2015). In present study, the association of these risk factors was also checked.

It was found that seroprevalence was higher in older age groups (>24 months) than younger animals having age <12 months and between 13-24 months. The prevalence of *T. gondii* in animals having age <12, 13-24 and >24 months was 24.76% (26/105), 35.66% (51/143) and 39.43% (60/152) respectively. So, the present study revealed the fact that prevalence

increases with an increase in the age of animals. Present results are supported by some previous reports describing that seroprevalence of *T. gondii* increases with an increase of age of animals (Roberts et al., 2001; Raeghi et al., 2011; Lopes et al., 2010; Fu et al., 2009). These results suggest that usually, animals acquire infection post-nataly. The increase in infection rates with the increase in age might be due to fact that old animals have a high opportunity of contact with various predisposing risk factors or ingestion of infective oocysts from the environment (Gottstein, 1995). Furthermore, older animals have low immunity as compared to younger ones and hence, less resistant to infection (Hove et al., 2005).

Cats are definitive hosts of *T. gondii*. So their presence or absence among sampled animals might affect the prevalence of parasite. Current study confirmed the presence of cats as an important factor in the epidemiology of the toxoplasmosis. It is found that cats are positively associated with the infection caused by *T. gondii*. Prevalence was significantly higher in those animals living in the farms where cats were present than those farms where no cats were present. Prevalence of *T. gondii* in sheep and goats living in vicinity of cats was found 43.40% (102/235), while seroprevalence in absence of cats was found 21.21% (35/165). The results are due to the reason that cats shed millions of oocysts in the environment which could be ingested by animals along with food and water thus causing infection. Other studies have also confirmed that a significant association is found between the prevalence of toxoplasmosis and the presence of cats in the vicinity (Ahmad and Qayyum, 2014) reported the results from a study conducted in Poland that the presence of wandering cats is an important risk factor that transmits the infection to sheep and goat. Current results are in agreement with the findings from previous studies reporting an increase in seroprevalence in goats and sheep due to presence of cats (Cenci-Goga et al., 2013; Deyo et al., 2009; Fotiric-Aksic, 2013).

In current study it was found that seroprevalence was high in those animals that live in poor and moderate hygienic conditions as compared to those living in a well hygienic conditions. Seroprevalence in animals was highest 45.16% (56/124) among those who live in poor hygienic conditions while it was 31.93% (61/191) and 23.52% (20/85) in animals living in moderate and hygienic conditions, respectively. These findings are in agreement with those reported by others (Montoya and Liesenfeld, 2004; Camossi

et al., 2011). The risk of contamination of food and water with oocysts is greatly reduced due to proper cleaning at farms, thus decreasing the risk of Toxoplasmosis. While poor cleaning conditions at farms increases the risk of contamination of water and food by oocysts and eventually increases the risk of toxoplasmosis.

The results of current study showed a positive correlation between flock size of goats and sheep and seroprevalence of *T. gondii*. The highest prevalence i.e. 34.52% (56/168) was found in those goats that live in flock size of more than 30 as compared to animals living in a flock comprising of less than 10 i.e. 27.83% (27/97) and between 11 to 30 animals 29.62% (40/135). These results are in agreement with the results reported by (Sadek et al., 2015). They found that there exists a positive correlation ($r=0.9275$) between flock size and prevalence of toxoplasmosis. They suggested that chance of toxoplasmosis increases with large flock size as compared to small flock size. The reason is that in large flock size animals have a greater chance to come in contact with each other and infectious material such as cat's feces because floor space

of the pen per animal is less (Robert-Gangneux et al., 2017). Besides, animals in large flock size might have received less care from managers as compared to smaller flock size where nutrition and care could be better (Vismarra et al., 2017). Similar correlation was also found by others (Tavassoli et al., 2013).

CONCLUSIONS

Present study witnessed the high prevalence of Toxoplasmosis in study area and it is further concluded that presence of cats near animals, large flock size, older age of animals, and poor hygienic conditions are the main risk factors of Toxoplasmosis in sheep and goats in District Jhang which are potential threat of infection for human population of study area. Moreover, further studies are needed for countrywide screening of the food animals that might create awareness in animal farmers and help policymakers to formulate suitable approaches to control this disease.

CONFLICT OF INTEREST

There is no potential conflict of interest among the authors listed in this manuscript.

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