



RESEARCH ARTICLE

Sero-Surveillance and Risk Factors Analysis of Caprine Toxoplasmosis in Faisalabad Punjab, Pakistan

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ABSTRACT

Toxoplasma gondii, a protozoan parasite can infect all warm-blooded animals such as mammals and birds, worldwide. Infection in domestic goats is caused by consumption of oocytes contaminated food and water. Infection due to *T. gondii* causes reproductive losses in goats and may play a key role in transmission of *T. gondii* infection to humans due to consumption of milk and meat of infected animals. The present study was aimed at evaluating the sero-prevalence of toxoplasmosis and its associated risk factors in goats of peri urban areas of Pakistan. A total of 240 sera samples were collected and tested by Latex Agglutination Test. The results of the study were subjected to Chi square test, relative risk and odds ratio to assess the association of risk factors. According to the results age, breed and purpose of breeding the goats had no association with the prevalence of disease. However, gender, pregnancy, management practices and pen flooring exhibited the association with toxoplasmosis ($P < 0.05$). The other strongly associated risk factors were domestic cats ($P = 0.001$, $OR = 13.06$), use of stagnant water ($P = 0.001$, $OR = 10.68$), Number of cats ($P = 0.001$, $OR = 5.81$) and access of cats to food storage ($P = 0.001$, $OR = 8.96$). This study concludes that prevalence of *Toxoplasma gondii* infection is still high in goats of Pakistan. The highest seroprevalence of *T. gondii* was recorded in autumn (29.5%) and the lowest in spring (10.3%). This infection is transmitted to humans through contaminated milk and meat posing a serious Public health problem. Awareness about risk factors in farmers can be helpful to reduce this serious infection.

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INTRODUCTION

Toxoplasma gondii infection is a zoonosis causing severe fetal damage and immune deficiency among individuals. Cats (family, felidae) include the definitive host and also reservoir of afflicted oocysts (Dubey and Jones, 2008). Vertical transmission in humans has always existed related to miscarriage, stillbirth, and different morbidity among goats. *T. gondii* is as an opportunistic agent for the patients with HIV that can damage heart, lungs, bone marrow, vision and cause potentially fatal encephalitis in these patients (Rafique *et al.*, 2017). The protozoon *T. gondii* is an obligatory intracellular parasite that can cause disorders like abortion, birth of deformed fetuses, mummification of fetus, weakness of new young

ones, embryonic reabsorption and other reproductive disorders in small ruminants (Lopes *et al.*, 2013).

Indirect laboratory techniques are primarily used to diagnose antibodies against *Toxoplasma* through serological techniques like latex test, direct agglutination, indirect immunofluorescence antibody test (IFAT) and (ELISA) enzyme-linked Immunosorbent assay (Morales *et al.*, 2011).

The key source of *T. gondii* infection for strict herbivores such as goat is the oocysts shed by cats in their faeces. In goats, it leads to reproduction and economic losses, which plays a significant role in public health due to usage of meat and milk leading to zoonotic transmission (Dubey *et al.*, 2011). In past years, prevalence of *Toxoplasma* among goats i.e. 12.3% was observed in

Sardinia, Italy (Masala *et al.*, 2003), 30% in Iran (Sharif *et al.*, 2007) and 74.8%, in Ethiopia (Teshale *et al.*, 2007).

The goat is very important in many countries and is the main source of Islamic meat and milk (Neto *et al.*, 2008). Milk and meat obtained from goats meet the nutritional needs of the developing world's population, especially in areas where the population is increasing rapidly (Boyce *et al.*, 2006). *Toxoplasma* cysts are predominantly found in goats among ruminant and are rarely seen in farmed cattle and buffaloes. Transmission of *T. gondii* from goats and sheep to human beings is possibly through the consumption of unprocessed milk and under cooked meat (Dubey *et al.*, 2011).

Pakistan lacks modern agricultural system like many other countries, which increases the prevalence of *T. gondii*. After being transported in the slaughterhouse, meat is easily contaminated with *T. gondii*. Open markets are common in Pakistan and are a cause of pathogen contamination because stray cats, mice, etc. are commonly found in the markets. There are comparatively fewer reports on seroprevalence of *T. gondii* in distinct parts of Punjab, Pakistan (Ramzan *et al.*, 2009).

Keeping in view the importance of *Toxoplasma gondii* and above-mentioned facts, the present study was aimed to investigate the seroprevalence of *T. gondii* in domestic goats of peri-urban areas of Punjab and to assess risk factors associated with this infection (Bachan *et al.*, 2018).

MATERIALS AND METHODS

The study was conducted in Peri urban areas of Punjab, Pakistan. Punjab province is the second largest and most populated province of Pakistan with an estimated population of 110012442 individuals. The total area of Punjab is 205,344 km² having a total of 36 districts. Majority of goat population is residing in Punjab, Pakistan (Muner *et al.*, 2018).

Blood samples (2–3 ml) from goats were collected aseptically from the jugular vein by using sterile syringes and shifted to test tubes without anticoagulant for the separation of serum from goats (n=240). The study was sanctioned by the animal ethics committee of Faculty of Life Sciences, Government College University Faisalabad. Permission was obtained from animal owners before collecting blood samples. Samples were collected by a professional veterinarian, according to animal husbandry research and education guidelines. The samples were brought to Department of Zoology, Government College University, Faisalabad. Serum was separated by centrifugation at 3000 rpm for 10 minutes and was stored at -20°C until the serological analysis was performed. The sampling was done during September, 2016 to February, 2017 from Peri-urban areas of Punjab using non probability convenience sampling technique. The samples were collected from different breeds of goats including Beetle (n=90), Nachi (n=95) and Teddy (n=55), respectively. The goats having age less than 3 months were excluded from the sampling frame. A questionnaire was prepared having information regarding general characteristics of animals and risk factors. The questionnaire was administered to the owners of animals at the time of blood collection after explaining the objectives and purpose of the study as reported by Rego *et al.* (2016).

The collected sera were subjected to detection of anti-*T. gondii* antibodies using the Latex Agglutination Test. The test was determined as a screening serologic test for toxoplasmosis in animals (Rafique *et al.*, 2017). The test procedure was carried out according to the method described by manufacturer. The commercial “Toxoplasmosis Latex Kit” (Antec Diagnostic Product TM UK) was used having +ve and –ve controls. Briefly, fifty microliters of 1:8 diluted sera was mixed with the (LAT) buffer. A positive result was expressed by agglutination.

The observed data was analyzed using the statistical programming language and environment R version 3.4.2. The cross tabulation and percentages of positive cases for different categories of different risk factors were used to get an insight into the observed data. We tested the association of different characteristics of the goats, and some possible risk factors for the prevalence of toxoplasmosis in goats, using chi-squared test-statistics. Furthermore, relative risks and odds ratios were also computed with their confidence intervals to study the odds of suffering from the disease due to different risk factors.

RESULTS

A total of 240 serum samples from goats were collected from peri-urban areas of Punjab and tested by Latex Agglutination Test Kit. Out of the 240 samples, 43 samples were found positive making the overall prevalence in domestic goats 17.9% as shown in (Table 1). The cross tabulation of the observed data regarding general characteristics of goats exhibited that the percentage of female goats with toxoplasmosis (21.47%) was almost three times higher than those of male goats (7.94%). As the age increases, the prevalence percentage of *T. gondii* also increases. The results also showed that all the breeds of goats were equally infected with toxoplasmosis. The results on the basis of age and breed were found statistically non-significant (P>0.05). The seroprevalence of toxoplasmosis in pregnant goats (27.62%) was found double than those in non-pregnant goats (12.50%) which was statistically significant (P=0.03). The results regarding risk factors of toxoplasmosis exhibited that the use of management practices notably decreased the percentage of *T. gondii* infection (P=0.04). The seroprevalence of goats kept on a dirty floor was highest (48.61%) than those living on a slat (4.31%) or cemented floor (3.16%) which was highly significant (P<0.001). The presence of cats at farms was also strongly associated with the prevalence of toxoplasmosis as the prevalence was 54.29% in farms having cats and 8.33% without cats [P=0.001: OR=13.06 and RR= 6.51]. Similarly, the goats which were allowed to drink stagnant water showed 46.5% prevalence while the goats that drink fresh flowing water showed 7.43% prevalence [P=0.001: OR=10.68 and RR=6.21]. The role of cat's access to feed storage [P=0.001: OR=8.96 & RR=5.46] and number of cats [P=0.001: OR=5.81 & RR=3.90] was also found highly significant with the occurrence of toxoplasmosis in goats. On the basis of breeding purpose, the goats were divided into three groups (Milk, Meat and dual purpose). The results of present study showed highest prevalence of toxoplasmosis in meat purpose goats (23.73%) while the lowest was detected in milk purpose goats (12.94%) and this difference was found statistically non-significant (P>0.05).

Table 1: Relative Risk (RR) and Odds Ratios (OR) with their Confidence Intervals (CI) for different attributes/characteristics of the goats to study the prevalence of toxoplasmosis in goats in peri-urban areas of Faisalabad city, Pakistan

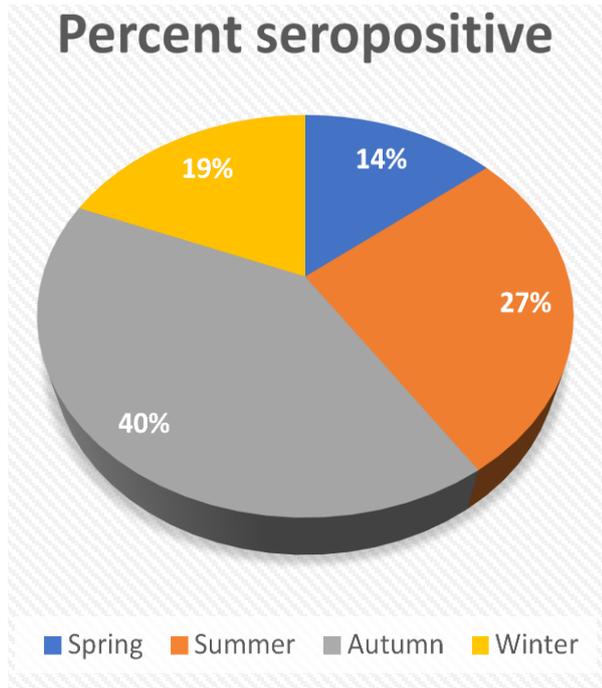
Variable	Categories	+ive	-ive	+ive %age	Chi-squared	p-value	RR	CI	OR	CI
Gender	Female	38	139	21.47	4.90 ⁺	0.03 [*]	0.37	(0.15,0.90)	0.32	(0.12,0.84)
	Male	5	58	7.94						
Age	<1	5	49	9.26	4.03	0.13	2.10	(0.86,5.15)	2.37	(0.87,6.47)
	1-2	29	120	19.46						
	>3	9	28	24.32						
Breed	Beetle	16	74	17.78	0.004	0.99	1.01	(0.54,1.87)	1.01	(0.47,2.14)
	Nachii	17	78	17.89						
	Teddy	10	45	18.18						
Pregnancy	No	9	63	12.50	4.93 ⁺	0.03 [*]	2.21	(1.11,4.38)	2.67	(1.18,6.06)
	Yes	29	76	27.62						

⁺ Chi-squared values computed using Yates' continuity correction: ^{*} Significant at 5% level of significance: The overall seroprevalence was 17.9%.

Table 2: Relative Risk (RR) and Odds Ratios (OR) with their Confidence Intervals (CI) for the potential risk factors associated with the prevalence of toxoplasmosis in goats in peri-urban areas of Faisalabad city, Pakistan

Variable		+ive	-ive	+ive %age	Chi-squared	p-value	RR	CI	OR	CI
Purpose of breeding	Meat & Milk	18	77	18.59	2.84	0.24	1.25	(0.67,2.32)	1.33	(0.60,2.93)
	Meat	14	45	23.73						
	Milk	11	74	12.94						
Management practices	No	29	82	26.13	8.45 ⁺	0.004 [*]	0.42	(0.23,0.75)	0.34	(0.17,0.69)
	Yes	14	115	10.85						
Pen Flooring	Dirt	35	37	48.61	83.74	<0.001 [*]	0.09	(0.04,0.22)	0.05	(0.02,0.13)
	Slat	5	111	4.31						
	Cement	3	92	3.16						
Domestic Cats	No	15	165	8.33	60.99 ⁺	<0.001 [*]	6.51	(3.83,11.07)	13.06	(6.44,26.50)
	Yes	38	32	54.29						
Stagnant Water used	No	13	162	7.43	45.73 ⁺	<0.001 [*]	6.21	(3.46,11.15)	10.68	(5.06,22.53)
	Yes	30	35	46.5						
Cats' access to food storage	No	14	160	8.05	39.51	<0.001 [*]	5.46	(3.08,9.67)	8.96	(4.31,18.61)
	Yes	29	37	43.94						
No. of Cats	<2	18	159	10.17	25.55	<0.001 [*]	3.90	(2.29,6.65)	5.81	(2.88,11.72)
	>2	25	38	39.68						

⁺ Chi-squared values computed using Yates' continuity correction: ^{*} Significant at 5% level of significance.

**Fig. 1:** Percentage of Seasonal seropositivity of *Toxoplasma gondii* among goat

The hypotheses of independence of different attributes with the presence of absence of toxoplasmosis were tested using chi-squared test statistic. The associated results given in Tables 1 and 2 showed that age, breed of the goat, and the purpose of breeding had no association with the disease. For all other risk factors, the hypothesis of independence was rejected with p-value <0.05, and an association with the prevalence of disease was concluded.

The relative risks (RR) were computed with corresponding confidence intervals (CI) to compare the risk between two exposures. Similarly, the odds ratios (OR) with their confidence intervals were also computed to know the strength of different categories of risk factors associated with the presence of the disease. The results of CIs for RR and OR also supported the results obtained from testing the hypothesis of independence of risk factors and presence/absence of the disease. The proportion of being +ive for males was 0.37 times the proportion of females being positive. In terms of OR, the odds for a male being +ive are 0.32 times the odds of +ive results for a female, or alternatively, we can conclude that the odds for a female being +ive are 3.12 times the odds of +ive results for a male. The risk of toxoplasmosis increases with increasing age. The odds for the disease in the age group "1-2" are 2.10 times than in goats aged less than one year. The risk further increases in goats with age greater than three years. The goats older than three years have odds 3.15 times higher than those for a goat less than one year old. All three types of breed i.e., Beetle, Nachii and Teddy, have equal chances of suffering from the disease. Pregnancy was observed to be a significant risk factor, and exhibited a significant increase in the risk of suffering from the disease as compared to non-pregnant animal. The odds for the disease in a pregnant animal were 2.67 times than the odds for a non-pregnant animal.

Although raising the goats for "meat" or "milk" purpose have different proportion of suffering than those goats who were raised for both "meat and milk" purpose, but the CI for OR for "meat" (0.60,2.93) and milk (0.28,1.44) covering a value one suggested the same outcome in these groups as compared to the "meat and

milk" group. The results showed that management practices can reduce the risk of the disease to 34% than the situation where no management practices were used. In pen flooring, "dirt" has the highest chances of causing the disease. "slat" has only 5% odds, and "cement" has only 3% odds of disease as compared to "dirt". The risk of the disease increased not only with the presence of cats with the goats but their number also played an important role in increasing the risk. The presence of cats increased the odds of disease to 13.06 as compared to the situation where no cats were present. Furthermore, the places where more than two cats were being raised had odds=5.81 for the disease than the situation where only two or fewer cats were being raised. The use of stagnant water is also a major risk and odds in such situation were 10.68 times those for the situation where the stagnant water was not used. The finding indicates that *T. gondii* infection is more likely in females than males. *T. gondii* seroprevalence in goats was higher in autumn (29.5%), followed by summer (19.04%), but lower in winter (13.7%) and spring (10.4%) as shown in Figure 1.

DISCUSSION

In this study, the prevalence of toxoplasmosis among the goats of Punjab, Pakistan was found 17.9% (43/240). The results of current study were found lower when compared with the findings of (Ramzan *et al.*, 200 and Ahmad *et al.*, 2015) from Pakistan, (Iovu *et al.*, 2012) from Romania and (Tzanidakis *et al.*, 2012) from Greece. The findings were identical to results of (Sah *et al.* 2018), who reported 16% prevalence in goats of Bangladesh. It was observed that toxoplasmosis was more pronounced in females than male goats, which is in accordance with the findings of Dubey *et al.*, (2011). Similar types of results were also recorded by (Nunes *et al.*, 2013) indicated that male goats are at lower risk of toxoplasmosis. The reason behind this result might be due to life span of animals. The male animals are often sold to slaughters at less than 1.5 years of age, hence less exposure time towards protozoan parasites in contrast to females which are reserved for the production of milk and breeding purposes for longer durations (Rego *et al.*, 2016).

The odds of toxoplasmosis seropositivity in pregnant goats were almost 2.67 time than that of non-pregnant goats. The female physiology and hormonal differences may be the key factor in consideration of disease. Stress related to the pregnancy and lactation makes the goats more vulnerable towards toxoplasmosis and other diseases (Lopes *et al.*, 2013).

The results revealed that age, breed and purpose of breeding had no association with disease. Resistance against disease might be due to the genetic variation among different breeds. The risk of toxoplasmosis increases with increasing age. A continuous exposure to the pathogen is responsible for the progression of *T. gondii* with age (Sharif *et al.*, 2006; Cosendey-Kezenleite *et al.*, 2014). The seroprevalence of antibodies to *T. gondii* in domestic goats of Satun Province in Thailand were estimated using commercial latex agglutination test kits. Seropositivity was high in older goats than young (under-1 year) goats (Teshale *et al.*, 2007). Older goats were significantly has higher anti *T. gondii* antibodies than kids. Results of the

present study are in agreement with results reported by (Clementino *et al.*, 2007; Guimaraes *et al.*, 2013). This might be explained on the basis that low immunity of older animals is responsible for the low resistance against toxoplasmosis.

Concerning about the number of cats that existed around the animal, multivariate analysis showed chances of *T. gondii* infection increases by 13.06 times the OR with increased number of infected cats around animals. Contamination with faeces of cats is the potential source for the high risk of transmission of toxoplasmosis oocysts to goats. Various studies have shown that, cats are the vital risk factors for the toxoplasmosis infection. (Moraes *et al.*, 2011; Andrade *et al.*, 2013).

In the present study, other variables that could significantly increase feline oocyst exposure (e.g., access of cats to the food storage, water sources) revealed a close correlation with seropositivity of *T. gondii*. Another risk factor *i.e* faeces management practices in rearing goats was also correlated with toxoplasmosis infection. The infection was found to be higher where the faeces were poorly managed ($P < 0.05$), which may be attributed to poor hygienic condition. Other studies revealed that management system of rearing goats is associated as a responsible for toxoplasmosis (Kamani *et al.*, 2010; Rego *et al.*, 2016). The result showed that management practices can reduce the toxoplasmosis to 34% than where no management practices used. Undercooked meat, non-pasteurized milk, and water and food contaminated with oocysts of *T. gondii* released by feline are the source of contamination in human beings (Silva *et al.*, 2015; Dubey *et al.*, 2011; Elmore *et al.*, 2010).

Conclusions: These results suggest that *T. gondii* infection in goats is prevalent all year round. This is likely to be associated with the moist and warm climate in temperate climate of Pakistan, which is favorable for survival of the oocysts (Van Wormer, 2013). The identified risk factors are sex, age, access of cats to the food storage and water sources. These are the preventive measures to reduce toxoplasmosis among goats the high prevalence of *Toxoplasma* infections results among goats may have cysts in muscle tissue, which is important for the parasite transmission in humans that consume poorly cooked meat. The risk of toxoplasmosis can be lessened by management practices through education. Other aspects are not studied or assessed in detail in this study, including, mixing of animal species, the density of animals, visiting of wandering cats and contact with food and water, the dietary and immune system of the goats and the incidence of other related diseases may be vital in construe these results. Further research is needed to determine these factors, Further research should also be conducted to explore the impact of disease among humans' segment that is in contact with goats.

Authors contribution: AR, SN, and AA conceived, designed and executed the study. FMZ analyzed the data. ZN, AA and SM reviewed the manuscript critically. All authors have interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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