



## Research Article

# LAT-Based Diagnosis of Toxoplasmosis in Small Ruminants in Punjab, Pakistan

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**Abstract** | Toxoplasmosis is an infectious disease of protozoan origin infecting wide range of domesticated animals and poses significant threat to human population as well. *Toxoplasma gondii* is transmitted to ruminants through ingestion of oocytes-contaminated herbage. The purpose of the current study was to report prevalence and access the risk factors associated with toxoplasmosis. A total of 652 specimens of blood were taken from 9 regions situated within the jurisdiction of Punjab province, Pakistan. The collected samples were labeled in order to have randomization and to pull 384 samples out of 652 harvested samples. Survey Tool Box was used to have representative samples. Latex agglutination test (LAT) was performed on 384 the collected sera. Research data showed that there was high prevalence in sheep than in goats at 29.35% and 13.11%, respectively and there was high prevalence in Mundri breed and lowest in Thalli at 40% and 10.96%, respectively. The district wise data showed that Bhakkar has highest prevalence 31.30% and Khanewal showed lowest prevalence 11.40%. Data showed that Non-lactating animals showed high prevalence 35.29% and lactating animals have low prevalence 10.74%. Pregnant and non-pregnant animals have big differences of 10.68% and 38.66% prevalence, respectively. Prevalence rate was high by using the Bio-Scien kit at 29.5% and was low by using Atlas kit at 13.04%. Parity base prevalence was highest in animals more than 4th parity (22.65%) and lowest in animals 1-2 parity was 11.11%. Females showed more prevalence rate 31.53% as compared to male animals 8.02%. The results indicate that the disease is prevalent in the small ruminant population hosted in Punjab and more studies are needed to molecularly characterize *T. gondii* variants.

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**Keywords** | *Toxoplasma*, Small ruminants, Risk factor, Prevalence, Latex agglutination test



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## Introduction

In Pakistan, there are 78.2 million goats and 31.2 million sheep, making it the third-largest breeding nation in Asia. The livestock industry in Pakistan has grown to be the largest contributor to agriculture. During 2021–2022, it comprised over 60.6% of the country's agricultural output and 11.7% of its gross domestic product (GDP). The country also benefits greatly economically from its goats and sheep. Pakistan's GDP increased in the fiscal year 2019–2020 by almost 1 million, 0.28 million, 0.29 million, 0.75 million, and 0.47 million tons of hair, beef, wool and milk, respectively, additionally 59.5 million pounds of skin from the ruminant (Mumtaz *et al.*, 2022).

In sheep and goats, the serological response to *Toxoplasma gondii* has been investigated. As a defense mechanism against toxoplasmosis, both humoral and cell-mediated immunity grow. Under experimental conditions, IgM and IgG antibodies against *Toxoplasma gondii* can develop 9 to 15 days following oral or subcutaneous infection, peaking at 35 days. After being immunized against *Toxoplasma gondii*, sheep showed seroconversion after seven days, reaching a peak by day thirty-five, by day two-hundred and-seventeen, decreasing further, and staying at a low point until day five-hundred and forty-six, which is comparable to challenge infection with pathogenic *Toxoplasma gondii* as previously described (Ahaduzzaman and Hassan, 2022).

Other farm animals are at lower risk of Toxoplasmosis than goats. Numerous nations view sheep and goats as a significant concern for human infection. Immunocompetent sheep and goats typically show no symptoms of the disease. The damaged tissue and the quantity of released tachyzoites determine the clinical signs in immunocompromised animals. Toxoplasmosis in sheep and goats has no pathognomonic symptoms. Aside from mortality, non-specific symptoms include fever, coughing, dyspnea, icterus, weakness, and neurological problems. Because the parasite can infect the fetus by overcoming the placental barrier, it is accountable for large economic losses (Ahaduzzaman and Hassan, 2022). Immunocompromised human patients can be diagnosed with *Toxoplasma gondii* using polymerase chain reaction, hybridization assays, isolation, and histology research. Amniotic fluid can also be utilized to identify congenital Toxoplasmosis (Ahaduzzaman and Hassan, 2022).

The primary factor causing miscarriages and stillbirths in ruminant animals is infection with *Toxoplasma gondii* which results in huge economic losses for the world's sheep, goat, and cow sectors. Fever, fatigue, diarrhea, chorioretinitis, and lymphadenopathy are just a few of the many non-specific and specific symptoms it can produce. For efficient domestic animal breeding and public health, *Toxoplasma gondii* infection in goats, sheep, and cattle can be avoided (Bentum *et al.*, 2019).

According to estimates, this parasite affects one-third of all persons on the planet. The main risk factor for human Toxoplasmosis is consuming raw or undercooked meat items with tissue cysts. Even though *Toxoplasma gondii* infection in humans typically goes unnoticed, it can have devastating effects on immunocompromised individuals and pregnant women. Abattoir staff, butchers, and hunters who may contract the disease when handling and eviscerating meat are also thought to have Toxoplasmosis as an occupational disease (Almería *et al.*, 2018). The zoonotic protozoan *Toxoplasma gondii* has a heterogeneous life cycle that uses warm-blooded mammals like cats as its definitive host (with asexual and sexual stages in the intestinal epithelium) and humans as its intermediate host (with an asexual extra-intestinal stage mostly in the brain and muscles) (Ramzan *et al.*, 2009). Both humans and other animal species are susceptible to Toxoplasmosis, an infection brought on by the apicomplexan protozoan *Toxoplasma gondii*. There have been reports of the illness in numerous nations and regions (Ramzan *et al.*, 2009).

There is need of study on toxoplasmosis in small ruminants in Punjab province of Pakistan to prevent and control toxoplasmosis by knowing disease prevalence, gathering epidemiological data and various risk factors correlated with toxoplasmosis. Furthermore, economic losses including profitability, reproductivity and productivity losses can be minimized by addressing various control factors to farmers. By knowing its prevalence in infected small ruminant population of livestock is crucial for assessment of risk associated with human health. The data generated from this study will greatly help in maintaining animal health and reducing disease burden.

Keeping in view the global prevalence rates, economic

losses posed by the disease, and scarcity of data about the prevalence of disease at institutional small ruminants raising farms, the current study has been designed with the following objectives: to access the seropositivity for *Toxoplasma gondii* in small ruminant population hosted at Government Livestock Experimental Stations of Punjab and to determine the potential associated risk factors of toxoplasmosis.

## Materials and Methods

### Samples collection

Blood Samples were drawn directly into the vacuum tubes (Improvacuter, Hamberg, Germany) from the jugular vein using a 21-gauge needle attached to the collection cup. Each tube was labeled for identification and transported to the laboratory. Sera then separated on centrifugation, transferred into cryovial (Cryo. STM, Greiner Bio-one, and GmbH, Germany), and stored at -20 °C till serological analysis.

### Procedure

Serum samples were placed out from the refrigerator and thawed at room temperature for 4 minutes. After that 100 µl serum samples were collected from each vial using a micropipette and placed in an ELISA plate. After that serial dilutions were made using distilled water up to 32 (Figure 1). After that, a 30 µl sample was collected and added to 30 µl antigen and mixed gently. Control positive and control negative tests with the same procedure were performed and after 4 minutes observed for agglutination.

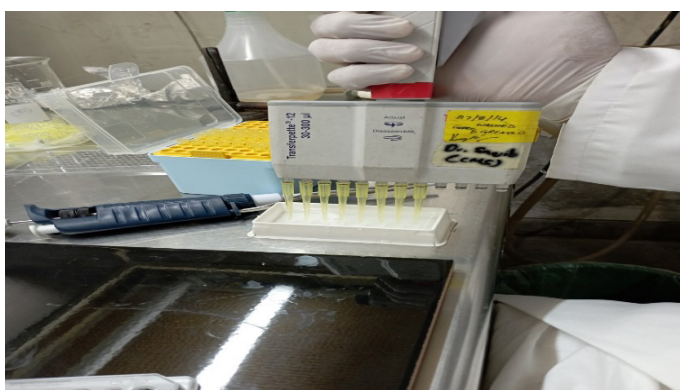


Figure 1: Serial dilution using a pipette.

### Latex agglutination test (LAT)

All sera samples were subjected to Latex agglutination test. Toxo Latex Kit (Atlas Medical GmbH, Germany) and Slide Latex test (BioScien, Egypt) were used. All the procedures were performed as per the guidelines given by the manufacturing companies.

### Statistical analysis

The data generated was subjected to Chi-square test for finding out significant difference among age, sex and location based prevalence. Univariate analysis was used to calculate Odds ratio (OR) for different determinants of toxoplasmosis.

## Results and Discussion

Out of 384 suspected sheep and goats with *T. gondii*, 83 had positive LAT results (Table 1). Sheep (29.35%) had a greater incidence rate than goats (13.11%). In sheep, 59 out of 201 were positive for *T. gondii*, demonstrating a significant ( $p < 0.05$ ) variation in occurrence.

In this research investigation, the role of sex in the prevalence of toxoplasmosis was studied and found that females (31.53%) had a higher prevalence rate than males (8.02%). Out of 222 females, 70 showed positive tests for toxoplasmosis, while in males, 13 samples were found positive out of a total of 162, with the predominance change being statistically significant ( $p < 0.05$ ) (Figure 2).

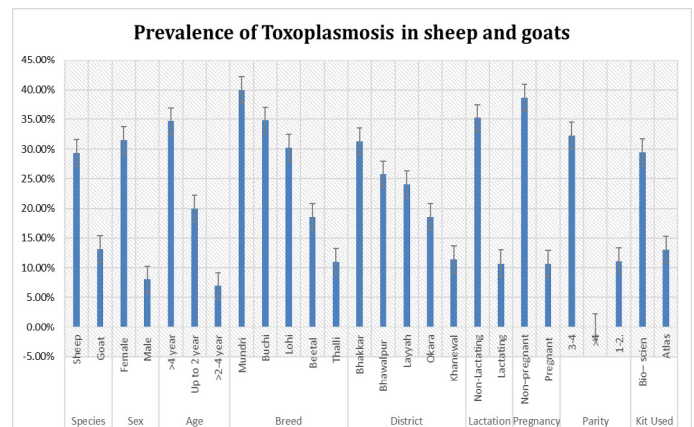


Figure 2: Seroprevalence of *Toxoplasma gondii* in Sheep and Goats in Pakistan.

Similarly, age factor was also considered and the data related to age is shown in Table 1, with three age groups: up to 2 years, 2 to 4 years, and more than 4 years. Prevalence was higher in older animals with more than 4 years of age (34.72%), followed by animals up to 2 years (20%), and lastly, animals between 2-4 years (6.95%). The alteration in dominance was statistically substantial ( $p < 0.05$ ).

Breed-wise prevalence was also investigated and found the highest prevalence (40%) in Mundri breed, while Thalli breed had the lowest prevalence (10.96%).



**Table 1:** Prevalence of *Toxoplasmosis* in sheep and goats with associated risk factors.

S.No	Category	Variable	Positive tested	% Prevalence	95 % C. I	Odds Ratio	Chi-square	P- value
1	Species	Sheep	59/201	29.35	23.49– 35.99	2.24(1.34-3.74)	9.71	0.0018
		Goat	24/183	13.11	8.97– 18.77	-		
2	Sex	Female	70/222	31.53	25.78– 37.91	3.93(2.11-7.33)	20.49	0
		Male	13/162	8.02	4.75 -13.24	-		
3	Age	>4 year	50/144	34.72	27.43 –42.8	4.99(2.28-10.92)	22.95	0
		Up to 2 year	25/125	20	13.93–27.86	2.87(1.25-6.61)		
		>2-4 year	8/115	6.95	3.57–13.13	-		
4	Breed	Mundri	10/25/	40	23.4-59.26	1.2 (0.52 -3.19)	15.69	0.0035
		Buchi	20/58	34.80	23.56– 47.33	3.14(1.55 - 6.40)		
		Lohi	23/76	30.26	21.09– 41.33	2.76(1.40 - 5.46)		
		Beetal	13/70	18.57	11.19– 29.22	1.6(0.78 - 3.66)		
		Thalli	17/155	10.96	6.96– 16.86	-		
5	District	Bhakkar	36/115	31.30	23.55– 40.26	2.75(1.39 - 5.43)	9.32	0.0537
		Bahawalpur	9/35	25.71	14.16–42.06	2.25(0.90 - 5.67)		
		Layyah	12/50	24	13.52-37.41	2.1(0.90 - 4.91)		
		Okara	13/70	18.57	11.19–29.22	1.63(0.72 - 3.70)		
		Khanewal	13/114	11.40	6.48 –18.53	-		
6	Lactation	Non-lactating	60/170	35.29	28.5 -42.73	3.28 (1.95- 5.52)	21.44	0
		Lactating	23/214	10.74	7.27 –15.61	-		
7	Pregnancy	Non-Pregnant	58/150	38.66	31.25– 46.65	33.6 (2.17 - 6.03)	26.64	0
		Pregnant	25/234	10.68	7.34 –15.29	-		
8	Parity	3-4	29/128	32.23	16.27– 30.65	0.7(0.41- 1.21)	11.03	0.004
		>4	15/135	22.65	6.85– 17.52	0.34(0.18 - 0.66)		
		1-2	39/121	11.11	24.56–40.99	-		
9	Kit Used	Bio- scien	59/200	29.50	23.61-36.16	2.26 (1.35 - 3.78)	9.98	0.0016
		Atlas	24/184	13.04	8.92 –18.67	-		

Buchi and Lohi breed showed 34.8% and 30.26% prevalence respectively, and with the prevalence difference being statistically significant ( $p < 0.05$ ) (Figure 2).

On district-wise comparison, the results showed that the prevalence was highest in District Bhakar at 31.30% and lowest in Khanewal at 11.40%. Bahawalpur and Layyah showed 25.71% and 24%, respectively.

The study also looked at the occurrence of toxoplasmosis in lactating and non-lactating animals. The highest prevalence (35.29%) was found in non-lactating animals, while the lowest prevalence (10.74%) was found in lactating animals. A significantly different trend in recurrence was observed ( $p < 0.05$ ).

The predominance of toxoplasmosis in pregnant

and non-pregnant animals was also investigated in this study. Non-pregnant animals had a higher prevalence rate of 38.66% while pregnant animals had a prevalence rate of 10.68%. A substantial ( $p < 0.05$ ) variation in occurrence was observed.

The study also looked at the prevalence of *Toxoplasma gondii* in different parities. Parity 3-4 had the highest prevalence (33.23%), followed by animals with >4 parities (22.65%), and lastly, animals with 1-2 parities (11.11%). The difference in prevalence was statistically significant ( $p < 0.05$ ). Finally, the study compared the prevalence of toxoplasmosis in different samples tested by Bio-scien and Atlas *gondii* kits. The prevalence by using Bio-scien was higher than Atlas, with 29.5% and 13.04%, respectively, and the difference in prevalence was statistically significant ( $p < 0.05$ ).

Worldwide, toxoplasmosis distribution varies, with transmission rates ranging from 0% to 100% in various nations (Olivier *et al.*, 2007; Selim *et al.*, 2023; Elaadli *et al.*, 2023), based on the population's habits, customs, and way of life, as well as the climate, the lifespan of the animals, and the methods used to raise animals. In addition, the overall rate could be linked to felines that eliminate eggs, which spread to humans and other animals upon sporulation (Dubey, 2004).

It is worth mentioning that in our study the investigated region is Pakistan's drought-prone area, where pasture supply is typically limited year-round, and animals frequently experience nutritional deficiencies that make them more susceptible to illnesses. However, in the present investigation overall prevalence is 21.61% which showed prevalence increased with time.

In current study, the 13.11% severity of infection of *T. gondii* in caprine is less than what other researchers have discovered in goats from around the globe; rates of infection of 28.9 to 92% in Brazil (Bisson *et al.*, 2000; Ragozo *et al.*, 2008), 42% in Germany, 59.8% in Bulgaria (Prelezov *et al.*, 2008), 80.61% in the Van region of Turkey (Karaca *et al.*, 2007), 63% in Canary Island, Spain, and 27.9% in Satun Province, Thailand. However, the estimated incidence rate in the present research is higher than that derived from multiple authors' observations in goats from different parts of the earth; prevalence rates of 3.2% in India (Sharma *et al.*, 2003), 11.6% in central Ethiopia (Bekele and Kasali, 1989), and 12.5% in Italy (Masala *et al.*, 2003) are also lesser than the average incidence rate found in this work and have previously published.

The seroprevalence of 29.35% for toxoplasmosis in sheep was documented in this research study is less than that which has been documented in several Turkish locales (33.2% to 55.6%); Ghana (33.2%), India (30%) and Canada (57.6%) (Hasemi-Fesharki, 1996; Hamzavi *et al.*, 2007; Sharif *et al.*, 2007; Oncel *et al.*, 2005). The variations in seropositivity observed across the various nations suggest that the animals bred in these regions were subjected to varying degrees of biological contamination containing *T. gondii* oocytes. It may also be connected to variations in the methods employed in every research to track the *T. gondii* antibodies. According to reports, female animals are more vulnerable to protozoan parasites than their male counterparts (Alexander and Stinson,

1988; Puije *et al.*, 2000). Regarding seroprevalence in different sexes, current data recorded a higher prevalence in females as compared to the male sex i.e. 31.53% and 8.02%, respectively.

These discrepancies may result from variances in the quantity of animals examined, the finding techniques employed, the times and locations of sampling, or both. To enhance the accuracy of this investigation, we also use local and worldwide evaluations of our findings about *Toxoplasma gondii* distribution in a similar background. The greatest infection rate among the examined animals in our investigation was 38.74% positive for sheep, less than the 57.5% seropositive utilizing LAT in Sudan and according to current research overall prevalence of sheep and goats is 31.30%, 25.71%, 24%, 18.57 % 11.40% in district Bhakkar, Bahawalpur, Layyah, Okara, and Khanewal, respectively. Current study results indicate a lower prevalence of *Toxoplasma gondii* seropositivity in this study compared to previous studies.

Research demonstrates that utilizing serological testing, the overall seroprevalence of *Toxoplasma gondii* infection is 16% and 27% in sheep and goat stillbirths and aborted fetuses. The incidence of *Toxoplasma gondii* infection in bovine fetuses that were aborted has not been investigated using serological assays. Although *Toxoplasma gondii* is one of the major factors contributing to reproductive failure in sheep and goats, this research shows that the prevalence in non-pregnant sheep and goats was 38.66% and 10.68% in pregnant animals.

6.8% (6 of 88) of the animals were younger than a year old, according to the research, while 16.3% (15 of 92) of the animals were older than that. The correlation between age and prevalence of infection was not statistically noteworthy (Bahreh *et al.*, 2021). According to age in current research revealed that the prevalence is quite high more than year old and then low in animals up to 2 years old and then 2- 4 years old sheep and goats 34.72%, 20%, and 6.95%, respectively. The p-value is 0.0000 it's showed a significant value.

In the current study parity base data showed that animals of 3-4 parity showed the highest prevalence 32.23%, whereas, animals of parity more than 4 showed higher prevalence (22.65%) and animals of 1-2 parity showed the lowest prevalence 11.11%. In the present study, 2 different LAT kits are used. One is

Bio-Scien and the other one is Atlas. The comparison between these two kits showed the highest prevalence in samples by Bio-Scien than by Atlas at 29.5% and 13.04%, respectively, and previously published data not found related to kits. P-value was less than 0.05 and that's showed results are significant. In current research non-lactating animals showed 35.29% which showed high prevalence than lactating animals 10.74% and no previous data was found related to lactation. The P-value is less than 0.05 and that showed significant results. In the current study, non-pregnant animals showed high prevalence of 38.66% as compared to pregnant animals 10.68% and no previous data found related to pregnancy prevalence in small ruminants p-value is less than 0.05 and that showed significant results.

## Conclusions and Recommendations

Toxoplasmosis, an infectious disease caused by the protozoan *T. gondii*, poses a significant threat to both domesticated animals and the human population. Toxoplasmosis is considered one of the leading causes of abortion in sheep and goats. However, before this study, there was a lack of province-wide surveys on the prevalence of toxoplasmosis in sheep and goats. Therefore, this study aimed to fill this research gap by reporting the prevalence and identifying the risk factors associated with the disease. The study concluded that various risk factors are associated and are found to be significant with the prevalence of toxoplasmosis. Moreover, the kit Bio-scienc has more sensitivity as compared to the Atlas kit. The results of this study provided useful insights into the true picture of the prevalence of toxoplasmosis in small ruminant population in Punjab.

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## Novelty Statement

The research and experimental work on the subject title is original and new in the field of veterinary sciences in Punjab, Pakistan.

## Author's Contribution

**Muhammad Tahir:** Investigation, writing-original draft preparation.

**Muhammad Saqib:** Supervision, methodology.

**Shahbaz ul Haq:** Conceptualization.

**Shahrood Ahmed Siddiqui:** Project administration.

**Khurram Ashfaq:** Validation.

**Urfa bin Tahir:** Software.

**Mughees Aizaz Alvi:** Data curation.

**Shujaat Hussain:** Resources.

**Talha Javaid:** Visualization.

**Raheela Taj and Muneeb Islam:** Writing-review and editing.

**Imad Khan, Asad Ullah and Shakirullah Khan:** Formal analysis.

### Data availability

All data related to this study is presented in the manuscript.

### Conflict of interest

The authors have declared no conflict of interest with respect to research, authorship, and/or publication with the work submitted.

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