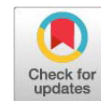


## Research Article



# Influence of Farming Systems on the Incidence of *Toxoplasma gondii* Infection in Cattle and Goats

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**Abstract** | *Toxoplasma gondii* is one of the most widespread protozoan parasites affecting warm-blooded animals. This study aimed to evaluate the effect of farming systems on the seroprevalence of *T. gondii* infection in cattle and goats in Charsadda District. A total of 640 blood samples were randomly collected from cattle (Achai and crossbred) and goats (Desi and crossbred) reared under traditional and commercial farming systems across three tehsils (Charsadda, Shabqadar, and Tangi). The latex agglutination test (LAT) was employed to detect *T. gondii* antibodies. The overall seroprevalence of *T. gondii* in cattle was 16.3% (traditional) and 8.8% (commercial), while in goats it was 24.4% (traditional) and 11.9% (commercial). Across the tehsils, the prevalence in cattle was 18.7%, 8.0%, and 9.4%, and in goats 21.1%, 19.8%, and 12.2%, respectively. Crossbred goats (23.7%) and crossbred cattle (18.8%) showed higher seropositivity than Desi goats (12.5%) and Achai cattle (6.2%). Goats exhibited a higher infection rate than cattle overall ( $\chi^2$  test,  $P < 0.05$ ). Female animals showed greater seropositivity than males. Animals consuming canal water had significantly higher prevalence than those using pump water, while stall-fed animals showed lower prevalence than grazing animals. The findings confirm the circulation of *T. gondii* in cattle and goats in Charsadda District and highlight the influence of farming practices and management factors on its occurrence.

**Keywords** | *Toxoplasma gondii*, Cattle, Goats, Farming systems, Charsadda

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## INTRODUCTION

Agriculture is a cornerstone of Pakistan's economy, and livestock farming plays a pivotal role in rural livelihoods. According to the Pakistan Economic Survey 2023–24, the livestock sector contributes approximately 60.84% to the agricultural value-added and 14.63% to the national GDP. The gross value addition of livestock rose

to PKR 5,804 billion in 2023–24 from PKR 5,587 billion in 2022–23, reflecting a growth rate of 3.9% (Pakistan Economy Survey, 2024). Over 8 million rural families are engaged in livestock production, and the sector also contributes about 1.6% to total national exports. In Khyber Pakhtunkhwa (KP), the livestock population increased from 35.50 million in 2022–23 to 36.10 million in 2023–24, highlighting the province's critical role in national

*Toxoplasma gondii* is an obligate intracellular protozoan parasite and a globally significant zoonotic pathogen that infects a wide range of warm-blooded animals, including cattle, goats, sheep, and humans (Shoukat *et al.*, 2022). Infection in livestock can cause reproductive losses such as abortions, neonatal mortality, and weak offspring, while also posing a public health risk through the consumption of undercooked meat or unpasteurized milk (Qamar *et al.*, 2023). Globally, seroprevalence varies widely, with rates of 20–40% in small ruminants and up to 50% in specific occupationally exposed human populations (Ahaduzzaman and Reza, 2024).

The prevalence and incidence of *T. gondii* are strongly influenced by farming systems. Traditional systems, characterized by mixed herds, free-ranging animals, and minimal biosecurity, generally exhibit higher infection rates than commercial systems with controlled environments (Odeniran *et al.*, 2020). The presence of cats, unhygienic housing, and pasture contamination further increase infection risk (Ayub *et al.*, 2024). The parasite is transmitted primarily through ingestion of oocysts from contaminated feed, water, or the environment, and transplacental transmission may also occur, causing congenital toxoplasmosis (Singh, 2016).

*Toxoplasma gondii* is a ubiquitous protozoan parasite with a complex life cycle involving both definitive hosts (cats and other felids) and intermediate hosts (livestock, birds, and humans). Livestock become infected primarily through ingestion of sporulated oocysts present in contaminated water, feed, or soil. The parasite can also be transmitted vertically from infected dams to their offspring, causing congenital infections that may lead to abortion or weak neonates (De Noya *et al.*, 2020). Goats and cattle are particularly susceptible, and infection often remains subclinical, making detection and control challenging. In addition to its economic impact on livestock production, *T. gondii* poses a significant zoonotic risk, as humans may acquire infection through consumption of undercooked meat, unpasteurized milk, or contaminated vegetables and water (Dubey *et al.*, 2020). Recent global studies report that *T. gondii* infection affects nearly one-third of the world's population, highlighting the importance of integrated surveillance and control strategies in both animal and human populations (Mushtaq *et al.*, 2025). Given the economic importance of livestock and the zoonotic potential of *T. gondii*, understanding its prevalence, risk factors, and transmission dynamics is crucial. Effective control measures, including improved biosecurity, proper feeding management, and public awareness, are essential to reduce both livestock production losses and public health

The present study aimed to evaluate the impact of different farming systems on the seroprevalence of *Toxoplasma gondii* in cattle and goats. By comparing traditional and commercial farming practices, this study seeks to identify key risk factors associated with infection and provide baseline data that can inform effective control strategies to reduce both economic losses in livestock and potential zoonotic transmission to humans.

## MATERIALS AND METHODS

### STUDY AREA AND HERD SELECTION

The study was conducted in Charsadda District (34.145°N, 71.731°E), which spans 996 km<sup>2</sup> and has a population of approximately one million. Administratively, the district is divided into three tehsils: Charsadda, Tangi, and Shabqadar. For this study, ten union councils were randomly selected across the three tehsils. A total of 60 households were included, with 20 households from each tehsil. Herds were stratified by farming system, comprising traditional herds (5–10 cattle or 10–15 goats) and commercial herds (≥15 cattle or ≥25 goats).

### DATA COLLECTION AND SAMPLING

A structured questionnaire was used to collect household- and herd-level information, including demographics, herd size and composition, management practices, feeding, water sources, veterinary care, disease history, waste management, and the presence of cats. Blood samples (3 mL) were collected aseptically from the jugular vein of cattle and goats. In total, 640 serum samples (320 cattle and 320 goats) were collected: 128 from Charsadda, 96 from Tangi, and 96 from Shabqadar. Samples were grouped into two age categories (goats: ≤2 years and >2 years; cattle: ≤3 years and >3 years). Serum was separated by centrifugation (3,000 rpm for 10 minutes) and stored at –20 °C until testing (Table 1). Samples were immediately transported on ice to the Veterinary Research Laboratory, Peshawar, ensuring the cold chain was maintained throughout transportation to prevent degradation.

### SEROLOGICAL TESTING AND BREEDS EXAMINED

Antibodies against *Toxoplasma gondii* were detected using the Latex Agglutination Test (LAT) with a commercial diagnostic kit (Weiner Lab, Argentina), following the manufacturer's protocol. This assay has been widely validated for seroprevalence screening of *T. gondii*. The LAT kit used in this study had a sensitivity of 96% and specificity of 98%, ensuring high diagnostic reliability. Two cattle breeds (Achai and crossbred) and two goat breeds (Dasi and crossbred) were examined, with sampling distributed across tehsils and farming systems (Table 2).

**Table 1:** Distribution of serum samples collected from cattle and goats in Charsadda District.

| Species | Breed        | Tehsil (Charsadda) | Tehsil (Tangi) | Tehsil (Shabqadar) | Total | Farming system (Traditional/ Commercial) |
|---------|--------------|--------------------|----------------|--------------------|-------|------------------------------------------|
| Cattle  | Achai        | 32                 | 24             | 24                 | 80    | Both                                     |
| Cattle  | Crossbred    | 32                 | 24             | 24                 | 80    | Both                                     |
| Goats   | Dasi (Local) | 32                 | 24             | 24                 | 80    | Both                                     |
| Goats   | Crossbred    | 32                 | 24             | 24                 | 80    | Both                                     |
| Total   |              | 128                | 96             | 96                 | 640   |                                          |

**Table 2:** Risk factors and variables assessed for *T. gondii* incidence in cattle and goats in Charsadda District, Pakistan.

| Variable category  | Factors Considered                                                                                                                                                                            |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Animal-related     | Species (cattle, goats); Breed (Achai, Crossbred cattle; Desi, Crossbred goats); Age group ( $\leq 2$ years, $> 2$ years in goats; $\leq 3$ years, $> 3$ years in cattle); Sex (male, female) |
| Management-related | Farming system (traditional, commercial); Herd size; Feeding system (stall-fed, grazing, mixed)                                                                                               |
| Environmental      | Water source (river/canal, tube well, pond); Shelter and hygiene status; Waste removal frequency                                                                                              |
| Health-related     | Veterinary services availability; Disease outbreak history; Mortality; Abortion records                                                                                                       |
| Socioeconomic      | Farmer education level; Main occupation; Household size; Income status                                                                                                                        |

STATISTICAL DESIGN

Sample size was estimated assuming 50% expected prevalence, 95% confidence level, and 5% desired precision, following. Data were entered in Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., USA). Descriptive statistics were calculated, and associations of *T. gondii* seroprevalence with risk factors (species, breed, sex, age, farming system, management practices, and tehsil) were assessed using ANOVA, General Linear Models (GLM), and Pearson’s correlation coefficient. Statistical significance was set at  $p < 0.05$ . All data were double-checked for entry errors to ensure data integrity and reproducibility.

**Table 3:** Impact of farming system on the incidence of *T. gondii* infection in cattle and goats, Charsadda District.

| Specie | Farming system             | Negative     | Positive    | P value |
|--------|----------------------------|--------------|-------------|---------|
| Cattle | Traditional farming system | 83.70% (134) | 16.30% (26) | 0.034   |
| Cattle | Commercial farming system  | 91.20% (146) | 8.80% (14)  |         |
| Goat   | Traditional farming system | 75.60% (121) | 24.40% (39) |         |
| Goat   | Commercial farming system  | 88.10% (141) | 11.90% (19) |         |

RESULTS

A total of 640 serum samples (320 cattle and 320 goats) were screened for *Toxoplasma gondii* antibodies; each species comprised 160 samples from traditional and 160 from commercial farming systems. In cattle, the prevalence was 16.3% under traditional systems and 8.8% under commercial systems ( $\chi^2$ ,  $p = 0.034$ ). In goats, seropositivity

was significantly higher under traditional (24.4%) than commercial (11.9%) systems ( $p = 0.003$ ) (Table 3).

IMPACT OF TEHSIL

The prevalence of *T. gondii* in cattle from Charsadda, Shabqadar, and Tangi tehsils was 18.7%, 8.0%, and 9.4%, respectively. In goats, the corresponding rates were 21.1%, 19.8%, and 12.5%. A significant difference was observed among cattle ( $p = 0.020$ ), whereas the difference was non-significant in goats ( $p = 0.225$ ) (Table 4).

**Table 4:** Impact of tehsil on the prevalence of *Toxoplasma gondii* in cattle and goats in Charsadda District.

| Tehsil    | Specie | Negative     | Positive    | P-value |
|-----------|--------|--------------|-------------|---------|
| Charsadda | Cattle | 81.30% (104) | 18.70% (24) | 0.020   |
| Shabqader | Cattle | 92.00% (88)  | 8.00% (8)   |         |
| Tangi     | Cattle | 90.60% (87)  | 9.40% (9)   |         |
| Charsadda | Goat   | 78.90% (101) | 21.10% (27) | 0.225   |
| Shabqader | Goat   | 80.20% (77)  | 19.80% (19) |         |
| Tangi     | Goat   | 87.50% (84)  | 12.50% (12) |         |

IMPACT OF BREED

Among cattle, Achai and crossbred breeds showed seroprevalence rates of 6.2% and 18.8%, respectively. In goats, Dasi (local) and crossbred breeds exhibited 12.5% and 23.7% prevalence. The association between breed and infection was statistically significant ( $p < 0.05$ ) in both species (Table 5).

SPECIES INFLUENCE

Overall, the prevalence of *T. gondii* was 12.5% in cattle and 18.1% in goats, with the difference being statistically significant ( $p = 0.048$ ), indicating higher infection rates in goats (Table 6).

**Table 5:** Impact of breed on the seroprevalence of *Toxoplasma gondii* in cattle and goats.

| Specie | Breed        | Negative    | Positive   | P-value |
|--------|--------------|-------------|------------|---------|
| Cattle | Achai        | 93.8% (150) | 6.2% (10)  | 0.001   |
| Cattle | Crossbred    | 81.3% (130) | 18.8% (30) |         |
| Goat   | Desi (Local) | 87.5% (140) | 12.5% (20) | 0.009   |
| Goat   | Crossbred    | 76.3% (122) | 23.7% (38) |         |

**Table 6:** Influence of species on the overall prevalence of *Toxoplasma gondii* infection.

| Specie | Negative     | Positive    | P-value |
|--------|--------------|-------------|---------|
| Cattle | 87.50% (280) | 12.50% (40) | 0.048   |
| Goat   | 81.90% (262) | 18.10% (58) |         |

#### IMPACT OF SEX

Among cattle, seroprevalence was 5.6% in males and 19.4% in females. In goats, infection rates were 11.2% in males and 25.0% in females. The association between sex and infection status was highly significant ( $p < 0.05$ ) for both species (Table 7).

**Table 7:** Impact of sex on the seropositivity of *Toxoplasma gondii* in cattle and goats.

| Specie | Sex    | Negative     | Positive    | P-value |
|--------|--------|--------------|-------------|---------|
| Cattle | Male   | 94.40% (151) | 5.60% (9)   | 0.000   |
| Cattle | Female | 80.60% (129) | 19.40% (31) |         |
| Goat   | Male   | 88.80% (142) | 11.20% (18) | 0.001   |
| Goat   | Female | 75.00% (120) | 25.00% (40) |         |

#### IMPACT OF WATER SOURCE

Animals consuming canal water showed higher prevalence (cattle: 23.1%; goats: 24.4%) compared to those using pump water (cattle: 4.0%; goats: 11.5%). These differences were highly significant ( $p < 0.05$ ) (Table 8).

**Table 8:** Effect of water source on the prevalence of *Toxoplasma gondii* in cattle and goats.

| Specie | Water source | Negative     | Positive    | P-value |
|--------|--------------|--------------|-------------|---------|
| Cattle | Pump water   | 96.00% (170) | 4.00% (7)   | 0.000   |
| Cattle | Canal water  | 76.90% (110) | 23.10% (33) |         |
| Goat   | Pump water   | 88.50% (138) | 11.50% (18) | 0.003   |
| Goat   | Canal water  | 75.60% (124) | 24.40% (40) |         |

#### IMPACT OF FEEDING SOURCE

Grazing cattle (21.9%) and goats (22.9%) exhibited significantly higher infection rates compared to stall-fed cattle (2.0%) and goats (12.1%). The association between feeding system and seropositivity was highly significant ( $p < 0.05$ ) (Table 9).

**Table 9:** Impact of feeding source on the seroprevalence of *Toxoplasma gondii* in cattle and goats.

| Specie | Feeding source | Negative     | Positive    | P-value |
|--------|----------------|--------------|-------------|---------|
| Cattle | Stall feeding  | 98.00% (148) | 2.00% (3)   | 0.000   |
| Cattle | Grazing        | 78.10% (132) | 21.90% (37) |         |
| Goat   | Stall feeding  | 87.90% (124) | 12.10% (17) | 0.012   |
| Goat   | Grazing        | 77.10% (138) | 22.90% (41) |         |

## DISCUSSION

The present study demonstrated a significantly higher prevalence of *T. gondii* infection in animals reared under traditional systems compared to commercial systems, with goats showing the highest positivity (24.4%). This finding is consistent with previous reports, where extensive or backyard systems were associated with greater exposure to *T. gondii* due to poor biosecurity and higher likelihood of contact with cats, contaminated pastures, and untreated water (Dubey, 2021; Stelzer *et al.*, 2019). Animals in intensive or commercial farms are usually better protected, which reduces the risk of oocyst ingestion (Kamani *et al.*, 2010; Liu *et al.*, 2022). Prevalence varied across the three tehsils, with Charsadda showing the highest incidence in both species. Such geographical variation may reflect differences in farm density, cat population, climate, and water source contamination (Rahman *et al.*, 2020; Gebremedhin *et al.*, 2014). Similar spatial clustering has been reported in Ethiopia, Brazil, and China, where localized management practices and environmental contamination strongly influenced infection rates (Zhou *et al.*, 2017). Crossbred cattle (18.8%) and goats (23.7%) exhibited significantly higher prevalence than indigenous breeds (Achai cattle, 6.2%; Desi goats, 12.5%). Crossbreeds may be more susceptible due to stress of intensive rearing and possible genetic factors (Ragozo *et al.*, 2009; Wang *et al.*, 2016). Local breeds, adapted to environmental conditions, may possess relative resistance against parasitic infections (Babaie *et al.*, 2017). Our findings agree with Khan *et al.* (2017), who reported higher infection in crossbred small ruminants in South Asia. Goats (18.1%) were more frequently infected than cattle (12.5%), consistent with earlier studies (Dubey, 2021; Gondim *et al.*, 2017). Goats are more susceptible because of their browsing behavior, which increases exposure to contaminated foliage and water (Figueiredo *et al.*, 2018). Cattle, although less susceptible to tissue cyst formation, may still act as mechanical carriers and pose zoonotic risk (Stelzer *et al.*, 2019). Females in both species showed significantly higher prevalence than males. Hormonal changes during pregnancy and lactation may compromise immunity, increasing susceptibility (Wang *et al.*, 2016; Liu *et al.*, 2015). Several studies have documented a higher seroprevalence in female goats compared to males (Ramzan *et al.*, 2009; Dubey, 2021). This highlights the

potential reproductive consequences of *T. gondii*, including abortions, stillbirths, and neonatal losses (Liu *et al.*, 2015). Animals using canal water showed markedly higher prevalence (cattle 23.1%, goats 24.4%) than those using pump water. Water is an established transmission route of *T. gondii* oocysts, particularly where untreated surface water is consumed (Rousseau *et al.*, 2018). Outbreaks linked to contaminated water have been reported in humans and animals (Bahia-Oliveira *et al.*, 2017). This underscores the importance of safe water provision for livestock. Grazing animals had higher prevalence compared to stall-fed animals. This agrees with the findings of Tenter *et al.* (2000) and Guo *et al.* (2015), who reported that access to contaminated pastures significantly increases the risk of infection. Grazing behavior exposes animals to soil, grass, and water potentially contaminated with oocysts shed by cats, the definitive host. The overall higher prevalence in goats, females, and animals under traditional management highlights the critical role of husbandry practices and environmental contamination in the epidemiology of toxoplasmosis. The results confirm that improved farm biosecurity, safe water provision, and limiting cat access to animal environments are essential to reduce the zoonotic risk of *T. gondii* in livestock and humans.

## CONCLUSION

The study revealed a higher incidence of *Toxoplasma gondii* in goats than in cattle, particularly under traditional farming systems. Crossbred and female animals showed greater susceptibility, while canal water use and grazing practices significantly increased infection risk. Overall, goats were more affected, confirming their role as important reservoirs. These findings highlight the need for improved biosecurity, safe water provision, and One Health-based control strategies to protect livestock and human health.

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## NOVELTY STATEMENT

This study is the first to report the comparative influence of traditional and commercial farming systems on the seroprevalence of *Toxoplasma gondii* infection in cattle and goats in Charsadda District, Pakistan. The findings reveal that management practices, water sources, and animal breed significantly affect *T. gondii* transmission dynamics under local field conditions.

MF and AR: Conducted the study and collected data. SA and FAL: Assisted in analysis. AK: Supervised the work, interpreted results, and finalized the manuscript. MU, SL, and MU: Contributed to review and editing. All authors approved the final manuscript.

## GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI or AI-assisted technologies were used in the design, execution, or interpretation of this research study. AI tools were not used to create, analyze, or modify any part of the manuscript. All content and intellectual input are solely the work of the authors.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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