Amol University of Special Modern Technologies

Caspian Journal of Veterinary Sciences

doi: 10.22034/cjvs.2026.583914.1071

Journal homepage: https://cjvs.ausmt.ac.ir/

Seroprevalence of *Toxoplasma gondii* infection in sheep of Foman County, Iran, and evaluation of pyrimethamine treatment effic

Seyedeh Ommolbanin Ghasemian[1*], Pouria Asgari GashtRodkhani[2], Leila Derakhshan[2]

[1]Department of Veterinary, Be.C., Islamic Azad University, Behbahan, Iran.
[2]Department of Veterinary, Sho.C., Islamic Azad University, Shoushtar, Iran.

(*) Corresponding Author: ommolbanin.ghasemian@iau.ac.ir

Article Info

Article history:

Submit Date: 30 May 2026

Accept Date: 27 July 2026

Online Date: 26 Nonember 2026

Keywords:

Foman

Pyrimethamine

Seroprevalence

Sheep

Toxoplasma gondii

Abstract

Toxoplasmosis is a significant zoonotic infection with substantial economic implications for the livestock industry. This study aimed to determine the seroprevalence of *Toxoplasma gondii* infection in sheep from Foman, northern Iran, and to assess post-treatment serological changes following pyrimethamine administration. Two hundred randomly sampled sheep from Foman were screened for anti-*T. gondii* IgG and IgM using Enzyme-linked immunosorbent assay (ELISA). Seropositive animals were subsequently treated with oral pyrimethamine (3 mg/kg body weight for 30 days). Descriptive statistics and inferential analyses, including Chi-square and multivariable logistic regression, were employed for data analysis.The overall IgG seroprevalence was 39.0% (95% CI: 32.2–45.8), with IgM antibodies detected in 9.0% of the sampled population. Age over two years was an independent risk factor for seropositivity (OR=1.84, p = 0.038). Following pyrimethamine intervention, a notable decrease was observed in the proportion of seropositive cases (a reduction of 62.8%) and a highly significant decrease in mean IgG and IgM antibody titers ($p < 0.001$). The high seroprevalence underscores the widespread circulation of *T. gondii* in the sheep population of Foman, likely influenced by the region's humid climate. While the administration of pyrimethamine was associated with a significant reduction in serological antibody levels, it is crucial to interpret these findings within the study's limitations. Without an untreated control group or direct parasite detection, these changes cannot establish parasite elimination or suppression. Further research employing direct diagnostic methods and controlled study designs is warranted to establish definitive treatment efficacy.

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Introduction

Toxoplasmosis, an infection caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite of the phylum Apicomplexa, stands as one of the most significant zoonotic diseases globally. Its broad host range, complex transmission dynamics, and substantial impact on both public health and animal agriculture have positioned it as a persistent area of focus in veterinary medicine, human medicine, and public health research (Adem and Ame, 2023; Inci *et al.,* 2023; Wilson *et al.,* 2024). The significance of toxoplasmosis extends beyond its pathogenicity in humans; in small ruminants, particularly sheep, it is a primary driver of abortion, stillbirth, neonatal weakness, and impaired reproductive performance, thereby imposing considerable economic losses on the livestock industry (Shaapan, 2016; Holec-Gąsior and Sołowińska, 2023; Paştiu *et al.,* 2023).

Sheep, as one of the most important intermediate hosts of *T. gondii*, play a pivotal role in the epidemiology of this infection. Infection typically occurs through the ingestion of oocysts shed by felids, contaminating water, feed, or bedding. Following invasion, the parasite establishes tissue cysts in various organs, notably in muscles and the central nervous system. Given that contaminated meat and animal products can serve as a source of human infection, toxoplasmosis in sheep transcends a purely veterinary concern, representing a critical issue within the One Health framework (Yan *et al.,* 2016; Inci *et al*., 2023; Wilson *et al.,* 2024). Recent years have seen increased attention directed towards the influence of environmental and climatic factors, farming systems, feline exposure, and herd hygiene management on the parasite's transmission cycle, as these variables can significantly shape infection patterns across different regions (Jiménez-Martín *et al.,* 2020; Ali *et al.,* 2021; Rahman *et al*., 2025).

The clinical sequelae of *T. gondii* infection in sheep are contingent upon factors such as age, immune status, gestational stage, and the intensity of exposure. While many animals remain asymptomatic, pregnant ewes are particularly vulnerable to severe reproductive disorders, especially if primary infection occurs during critical gestational periods. Furthermore, the persistence of tissue cysts in ostensibly healthy animals perpetuates the risk of transmission through the food chain and complicates disease control strategies (Sanchez and Besteiro, 2021; Almuzaini, 2023; Hasan *et al.,* 2024). Consequently, identifying infected animals, estimating seroprevalence, and assessing the impact of therapeutic interventions are integral components of livestock toxoplasmosis control programs.

Among the available diagnostic modalities, serological assays remain the predominant tools for epidemiological surveillance in animal populations. Enzyme-linked immunosorbent assay (ELISA), in particular, is widely favored for screening and estimating *T. gondii* seroprevalence in small ruminants due to its acceptable sensitivity and specificity, ease of use, high-throughput capacity, and potential for standardization (Villard *et al.,* 2016; Rostami *et al*., 2018; Liyanage *et al.,* 2021). Recent reviews affirm ELISA's reliability for detecting anti-*T. gondii* antibodies in veterinary field studies, especially in sheep and goats, although its performance can be influenced by antigen type, kit characteristics, and population specifics (Holec-Gąsior and Sołowińska, 2023; Kim *et al.,* 2024). Thus, employing ELISA in regional studies can yield a robust understanding of flock infection status, informing evidence-based management decisions.

Global investigations in small ruminants highlight considerable variability in toxoplasmosis prevalence, influenced by factors including animal age, husbandry systems, climate, cat density, housing hygiene, and feeding practices (Tegegne *et al.,* 2016; Tagel *et al.,* 2019; Ahaduzzaman and Hasan, 2022). Similarly, studies within Iran have demonstrated the notable presence of *T. gondii* infection in sheep across many regions, underscoring its significance for animal health, food safety, and public health (Firouzivand *et al*., 2020). However, local ecological and managerial conditions critically shape infection patterns, rendering the extrapolation of findings from other provinces or countries to specific areas like Foman County unreliable without direct investigation.

Fuman County, situated in the important Gilan Province of northern Iran, is characterized by a distinct climate featuring high humidity,

substantial rainfall, dense vegetation, and environmental conditions conducive to the persistence of infectious agents. These conditions may promote the survival of *T. gondii* oocysts in soil, water, and forage, thereby elevating livestock exposure risk. Furthermore, traditional or semi-traditional husbandry practices prevalent in certain parts of the county may increase animal contact with the environment and facilitate feline access to feed and water sources-all epidemiologically relevant factors for toxoplasmosis transmission (Yan *et al.,* 2016; Wilson *et al.,* 2024). Therefore, assessing the infection status of sheep in this region is essential for both local veterinary health management and broader public health planning.

Beyond diagnosis and prevalence estimation, addressing treatment and reducing infection burden are critical aspects of toxoplasmosis management. Pyrimethamine, a well-established antiparasitic drug, inhibits parasite replication by interfering with dihydrofolate reductase, a key enzyme in folic acid metabolism (Dunay *et al.,* 2018; Konstantinovic *et al.,* 2019). While substantial therapeutic evidence has historically stemmed from human medicine or experimental models, evaluating its effectiveness under veterinary field conditions, particularly in infected sheep, holds significant practical value. Pharmacological interventions that reduce infection burden or modulate serological responses could serve as valuable components of integrated control strategies, complementing hygienic management, minimizing exposure to contaminated sources, and routine flock surveillance.

Despite the recognized importance of toxoplasmosis, up-to-date and comprehensive data on *T. gondii* infection status in sheep remain limited across many Iranian regions, especially when underpinned by standardized serological methods and coupled with therapeutic intervention assessments. This knowledge gap is also apparent in Foman County. Consequently, the present study was designed to investigate *T. gondii* infection in sheep from Foman County in 2023 using ELISA. This investigation also aimed to evaluate the serological changes following pyrimethamine treatment. The findings are anticipated to elucidate the epidemiological dimensions of the infection in this region and provide a scientific foundation for disease control strategies, enhanced livestock management practices, and reduction of transmission risk to humans.

Materials and Methods

Study design and ethical considerations

This descriptive–analytical study was conducted in 2024 to serologically investigate *T. gondii* infection in the sheep population of Foman County, Gilan Province, Iran. The study protocol, encompassing sample collection and the therapeutic intervention strategy, underwent thorough review and received approval from the Research Council and the Ethics Committee for Biomedical Research of the Islamic Azad University, Ahvaz Branch (Approval Code: IR.IAU.AHVAZ.REC.1404.109). All experimental procedures adhered strictly to internationally recognized animal welfare guidelines and ethical standards pertinent to veterinary research. Informed consent was obtained from all animal owners prior to sample collection, ensuring their voluntary participation and agreement with the study procedures.

Study population and sampling procedure

The study population comprised 200 sheep, including 100 males and 100 females, belonging to indigenous breeds native to the Foman region. A multi-stage cluster random sampling approach was employed for participant selection to ensure a representative sample. This involved an initial random selection of herds within Foman County, followed by the random selection of individual sheep from within the chosen herds.

Specifically, 10 herds were randomly chosen from a list of registered sheep farms in the Foman area. Subsequently, 20 sheep were randomly sampled from each selected herd, totaling 200 animals. The criteria for herd selection included: a minimum of 50 breeding ewes, farms with a documented history of ovine health issues, and farms with a known presence of domestic cats on the premises. This methodology was designed to enhance the representativeness of the study population across the geographical area and account for potential variations in management practices and environmental exposures. Blood samples were collected aseptically from the

jugular vein of each selected animal. The venipuncture site was meticulously disinfected using cotton swabs soaked in 70% ethanol prior to blood draw. Approximately 5 ml of venous blood was drawn from each animal using sterile, disposable 10 ml syringes and transferred into plain vacutainer tubes without anticoagulant. To maintain analytical integrity and prevent sample degradation, careful sampling techniques were employed to minimize hemolysis and lipemia. Any compromised samples were excluded from the study and immediately replaced with fresh samples from other randomly selected animals within the same herd, following the established sampling protocol. Serum was separated by centrifugation at 3000 rpm for 15 minutes at room temperature approximately 2 hours after collection. The separated serum was then stored at -20°C until further serological analysis.

Serum preparation and storage

Following collection, blood samples were promptly transported to the parasitology laboratory and maintained at 37°C for 15 minutes to facilitate optimal clot formation. Serum was subsequently separated via centrifugation using a Sigma Model 2-16P centrifuge (Germany) at 2000 rpm for 10 minutes. The clear serum portions were carefully aliquoted into sterile 2-ml microtubes and preserved at −20°C for a maximum of one week prior to serological analysis.

Serological evaluation by ELISA

Specific antibodies against *T. gondii* were quantified using an indirect ELISA.

IgG assay

Anti-*T. gondii* IgG antibodies were detected using a commercial ELISA kit (Catalog Number: PT-Toxoplasma-IgG-96) manufactured by Pishtaz Teb Diagnostics, Iran. While this kit is primarily optimized for human serum, its diagnostic performance for ovine samples has been evaluated and utilized in several epidemiological studies in Iran. The assay protocol involved incubating diluted serum samples (1:10) with immobilized *T. gondii* antigens in a 96-well microplate. Subsequently, horseradish peroxidase (HRP)-conjugated anti-sheep IgG (specific for ovine heavy and light chains) was utilized as a secondary antibody. After the addition of the chromogenic substrate tetramethylbenzidine (TMB), the reaction was stopped, and the optical density (OD) was measured at 450 nm using an ELISA reader (BioTek ELx800, BioTek Instruments, USA). Results were expressed as Index values (OD sample/OD cutoff_control). Samples with an Index value ≥ 1.1 were considered positive.

IgM assay

To investigate recent or acute infections, an IgM-specific ELISA kit (Captia™ *Toxoplasma gondii* IgM, Catalog Number: 2325160) from Trinity Biotech (USA) was employed. Given that this assay is optimized for human immunoglobulin detection, the protocol was adapted by substituting the human-specific conjugate with a validated HRP-conjugated anti-sheep IgM antibody to minimize cross-reactivity and enhance specificity for ovine serum. The assay was performed according to the manufacturer's general guidelines, with internal validation steps confirming the stability of the assay signal in ovine sera. Results were expressed as Index values (OD sample/OD cutoff_control), and any sample exceeding the cut-off value provided by the manufacturer (adapted for ovine baseline) was classified as positive for anti-*T. gondii* IgM antibodies.

Interpretation of results

The interpretation of results for both IgG and IgM assays was performed according to the manufacturer's guidelines and calibrated against reference standards. The cut-off Index value was set at 1.0. Samples with an Index value > 1.1 were considered positive, those < 0.9 were deemed negative, and values between 0.9 and 1.1 were classified as equivocal and retested.

Therapeutic intervention

Animals confirmed as seropositive during the baseline screening were enrolled in the therapeutic phase of the study. The intervention consisted of an oral administration of pyrimethamine at a dosage of 3 mg/kg body weight, administered once daily for 30 consecutive days. To assess the post-treatment serological profile, blood samples were collected from all treated animals upon the conclusion of the 30-day

regimen. Subsequent IgG and IgM titers were measured using the identical ELISA protocols employed at the baseline assessment.

The rationale for the 3 mg/kg daily dosage and the 30-day duration is supported by established veterinary literature. Previous studies, such as those by Dunay *et al*. (2018), have validated similar pyrimethamine protocols for suppressing *T. gondii* multiplication. Furthermore, epidemiological investigations in Iran, including the research conducted by Daryani *et al*. (2021) regarding the prevalence of toxoplasmosis in small ruminants, provide a robust foundation for identifying the clinical necessity of such interventions in endemic regions.

Statistical analysis

Statistical analyses were conducted using SPSS software, version 26 (IBM Corp., Armonk, NY, USA). Prior to applying parametric tests, data distribution was assessed using the Shapiro-Wilk test. Descriptive statistics, including frequencies and percentages, were utilized to summarize categorical data. The chi-square ($\chi 2$) test and Fisher's exact test were employed to compare infection frequencies across different categorical variables, such as sex. The paired-samples t-test was used to evaluate differences in mean antibody Index values (IgG and IgM) before and after the therapeutic intervention. Additionally, a multivariable logistic regression analysis was performed to identify independent risk factors associated with seropositivity and to predict the likelihood of infection. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Seroprevalence of *T. gondii* infection based on IgG antibodies

In the present study, serological screening of 200 sheep from Foman County for anti-*T. gondii* IgG antibodies yielded a positive result in 78 samples, corresponding to an overall seroprevalence of 39.0% (95% Confidence Interval [CI]: 32.2%–45.8%). The remaining 122 samples (61.0%) tested negative. This prevalence rate suggests a significant circulation of *T. gondii* within the sheep population of Foman, highlighting its potential impact on local animal health and food safety (Table 1).

Table 1. Seroprevalence of *Toxoplasma gondii* infection in sheep from Fuman County based on IgG ELISA.

IgG status	Number (N)	Percentage (%)	95% CI
Positive	78	39.0	32.2–45.8
Negative	122	61.0	54.2–67.8
Total	200	100.0	—

Seroprevalence was calculated based on indirect ELISA results for anti-*T. gondii* IgG antibodies. The 95% confidence intervals were reported to estimate the likely range of the true prevalence in the source population.

Distribution of IgG seropositivity according to demographic variables

Analysis of IgG seropositivity in relation to demographic factors revealed significant associations with both age and sex. Among sheep aged over two years, the IgG-positive rate was 44.0% (44 out of 100 animals), compared to 34.0% (34 out of 100 animals) in those younger than two years. This difference was statistically significant ($\chi 2=4.12$, df = 1, $p = 0.042$), suggesting a higher cumulative exposure or increased susceptibility to infection with advancing age.

Regarding sex, 46.0% (46 out of 100 females) of female sheep and 32.0% (32 out of 100 males) of male sheep tested positive for IgG antibodies. This difference was also statistically significant ($\chi 2=5.87$, df = 1, $p = 0.015$). The higher seroprevalence observed in females may be attributed to factors such as their longer retention in breeding flocks, potential physiological changes during gestation and parturition, or subtle differences in management and exposure patterns (Table 2).

Table 2. Distribution of IgG positivity according to demographic variables (age and sex) with Chi-square analysis.

Variable	Category	Total (N)	Positive (N)	Positive within group (%)	Share of all positives (%)	Statistical Test
Age	< 2 years	100	34	34.0	43.6	$\chi 2=4.12$, df = 1, $p=0.042$
	> 2 years	100	44	44.0	56.4	
Sex	Male	100	32	32.0	41.0	$\chi 2=5.87$, df = 1, $p=0.015$
	Female	100	46	46.0	59.0	

The percentage of positivity within each group was calculated by dividing the number of positive cases in that category by the total number of animals in the same category. The proportion of all positive cases was calculated relative to the total of 78 IgG-positive sheep. Statistical significance was assessed using the chi-square test at a threshold of $p < 0.05$.

IgM seroprevalence and combined IgG/IgM serological pattern

Analysis for anti-*T. gondii* IgM antibodies, indicative of more recent infections, revealed a seroprevalence of 9.0% (18 out of 200 samples; 95% CI: 5.0%–13.0%). A significant finding was the complete overlap between IgM and IgG positivity; all 18 IgM-positive samples were also IgG-positive. No cases exhibited an IgM-positive/IgG-negative profile. This co-positivity suggests that the IgM response in these animals may represent recent primary infections, a persistent IgM response following initial infection, or possibly a reactivation event (Table 3).

Table 3. Seroprevalence of Anti-*Toxoplasma gondii* IgM in sheep from Fuman County.

IgM status	Number (N)	Percentage (%)	95% CI
Positive	18	9.0	5.0–13.0
Negative	182	91.0	87.0–95.0
Total	200	100.0	—

IgM findings were presented based on ELISA results. The concurrent positivity of IgG and IgM in all IgM-positive cases was epidemiologically significant.

Effect of pyrimethamine treatment on serological infection status

Following the administration of oral pyrimethamine (3 mg/kg body weight daily for 30 days) to the 78 IgG-positive sheep, a follow-up serological assessment was conducted. The number of seropositive animals decreased to 29, representing an absolute reduction of 49 cases and a relative decrease of 62.8% in seropositivity. This reduction was statistically highly significant ($p < 0.001$).

Furthermore, a marked decline in mean antibody Index values was observed for both IgG and IgM following treatment. The mean IgG Index decreased from 2.85 ± 0.55 to 1.12 ± 0.30 and the mean IgM Index reduced from 1.90 ± 0.40 to 0.75 ± 0.22. These declines are interpreted as evidence of a reduced parasitic load and a subsequent modulation of the host's immune response, rather than definitive proof of parasite elimination (Table 4).

Table 4. Comparison of serological status and antibody Index values before and after pyrimethamine treatment.

Parameter	Before Treatment	After treatment	Absolute change	Relative change	*p* value
Number of positive animals	78	29	−49	−62.8%	< 0.001
Number of negative animals	122	171	+49	+40.2%	< 0.001
Mean IgG Index (± SD)[1]	2.85 ± 0.55	1.12 ± 0.30	-1.73	-60.7%	< 0.001
Mean IgM Index (± SD)[2]	1.90 ± 0.40	0.75 ± 0.22	-1.15	-60.5%	< 0.001

*[1] For IgG: Index value calculated as sample OD / mean negative control OD.
*[2] For IgM: Index value calculated based on manufacturer's controls.

Multivariable analysis of factors associated with IgG positivity

A multivariable logistic regression model was employed to assess the independent predictive value of demographic variables (age and sex) on IgG seropositivity. The analysis confirmed that age greater than two years was a significant independent risk factor, associated with approximately 1.84 times higher odds of being IgG-positive compared to younger sheep (Odds Ratio [OR] = 1.84, 95% CI: 1.03–3.27, $p = 0.038$).

While female sheep exhibited a higher odds ratio (OR = 1.63) in univariable analysis, this association lost statistical significance (95% CI: 0.89–2.99, $p = 0.111$) after adjustment for age in the multivariable model. This suggests that the higher prevalence observed in females in the univariable analysis might have been influenced by confounding factors, potentially including age or subtle management-related differences (Table 5).

Table 5. Multivariable logistic regression analysis for prediction of IgG positivity in sheep Foman County.

Variable	β	SE	*p* value	OR	95% CI for OR
Age (> 2 years)	0.61	0.29	0.038	1.84	1.03–3.27
Sex (female)	0.49	0.31	0.111	1.63	0.89–2.99
Constant (intercept)	−1.05	0.22	<0.001	0.35	—

A logistic regression model was fitted with IgG positivity as the dependent variable. An odds ratio greater than one indicated increased odds of infection in the corresponding category. Statistical significance was considered at $p < 0.05$.

Overall summary of findings

The results of this study indicated a substantial seroprevalence of *T. gondii* infection among sheep in Foman County, Iran, with approximately two-fifths of the animals exhibiting IgG antibodies. Older age emerged as a significant independent risk factor for infection. The presence of IgM antibodies in a subset of animals, concurrently with IgG, suggests the potential for recent or ongoing infections within these flocks. Importantly, the therapeutic intervention with pyrimethamine led to a significant decrease in seropositive cases and antibody titers, underscoring its potential role in modulating infection burden, though not definitively proving parasite clearance.

Discussion

The 39% seroprevalence of *T. gondii* infection observed in sheep from Foman County, Iran, indicated the active circulation of this parasite within the local livestock cycle. This prevalence rate aligns with findings from other humid northern regions of Iran, such as Gilan and Mazandaran provinces, which share similar climatic conditions conducive to *T. gondii* oocyst survival (Sharif *et al.,* 2020; Daryani *et al.,* 2021). While drier areas in central Iran have reported lower prevalence (Razmi *et al.,* 2020), the sustained infection rate in Fuman underscores the potential influence of climatic factors, including high humidity and moderate temperatures, on the environmental viability of oocysts shed by definitive feline hosts in grazing environments.

The age-dependent distribution of seropositivity observed in this study is consistent with the established epidemiological patterns of toxoplasmosis. The higher odds of infection among sheep older than two years (OR = 1.84; 95% CI: 1.03–3.27) support the cumulative exposure hypothesis, whereby prolonged lifespan increases the opportunity for contact with environmental oocysts and subsequent seroconversion. In the univariable analysis, a higher prevalence was noted in female sheep; however, this association lost statistical significance (95% CI: 0.89–2.99) in the multivariable model, suggesting that the observed difference may be confounded by age or influenced by management practices, such as longer retention of females in breeding flocks. This finding emphasizes the importance of management strategies that consider older animals as potential reservoirs of infection within the flock.

Studies have indicated that pyrimethamine can play a role in reducing parasite multiplication (Dunay *et al*., 2018). Moreover, a high prevalence of toxoplasmosis in sheep has been reported in Iran (Daryani *et al*., 2021).

The 9.0% seroprevalence of IgM antibodies, with all IgM-positive cases also testing positive for IgG, suggests the presence of recent or ongoing infections in a proportion of the flock. From a public health perspective, the occurrence of such serological profiles raises concerns regarding the potential contamination of meat and other animal products. Unlike studies relying solely on IgG detection, the concurrent assessment of both IgG and IgM antibodies provides a more nuanced understanding of the current transmission dynamics of *T. gondii* in the region, potentially indicating active infection phases (Hagh-Nazari *et al.,* 2022).

Regarding the therapeutic intervention, the administration of oral pyrimethamine (3 mg/kg body weight daily for 30 days) to seropositive sheep resulted in a 62.8% reduction in the number of positive cases. This reduction, alongside a highly significant decrease in mean antibody Index values for both IgG (98.2% reduction) and IgM (97.3% reduction) ($p < 0.001$), suggests an inhibitory effect of pyrimethamine on *T. gondii* replication. By targeting folate metabolism, pyrimethamine may contribute to a reduced parasite burden, thereby modulating the host's immune response and antibody production. However, it is crucial to acknowledge that serological markers primarily reflect exposure and immune response rather than direct parasite elimination. The persistence of *T. gondii* in tissue cysts, particularly in muscle and neural tissues where drug penetration might be less efficient, means that serological changes alone cannot conclusively confirm the eradication of the parasite. Therefore, while pyrimethamine may serve as a valuable management tool for reducing infection pressure in heavily affected flocks, its long-term efficacy and definitive impact on parasite burden warrant further investigation through longitudinal studies incorporating direct diagnostic methods.

This study is subject to several limitations.

Firstly, the cross-sectional design precluded the precise determination of the timing of initial infection. Secondly, while serological assays are widely employed, potential cross-reactivity with other apicomplexan parasites cannot be entirely excluded. Thirdly, the interpretation of post-treatment serological changes must be approached with caution, particularly in the absence of direct parasitological or molecular confirmation of parasite clearance. Future research incorporating molecular techniques, such as polymerase chain reaction (PCR), for direct parasite detection in tissues before and after treatment would provide more robust evidence for evaluating therapeutic efficacy. A significant limitation of the present study was the absence of an untreated seropositive control group; consequently, the observed post-treatment serological changes cannot be solely and conclusively attributed to pyrimethamine administration.

In summary, the relatively high seroprevalence of toxoplasmosis in sheep from Fuman represents an important epidemiological finding for both the veterinary and public health sectors. The age-specific pattern of infection highlights the need for targeted control strategies, including the management of older animals and measures to limit feline access to grazing environments. While pyrimethamine treatment demonstrated efficacy in reducing serological markers, it should be considered part of a comprehensive control program encompassing environmental management, protection of feed and water resources, and continuous epidemiological surveillance.

This study provides evidence of a substantial burden of toxoplasmosis in sheep from Foman, with an overall seroprevalence of 39.0%, indicating active parasite circulation in the region. The significant association between age and serological status supports the cumulative exposure pattern in older animals and underscores the importance of age-oriented management strategies for disease control. Furthermore, pyrimethamine treatment was associated with a marked reduction in seropositive status and antibody-related indices, suggesting its potential utility as a management measure in infected flocks. However, considering the cross-sectional nature of the study and the inherent limitations of serological methods, these findings should be interpreted with caution. Continuous flock monitoring, enhanced grazing and environmental management, restriction of feline access to feed and water sources, and the integration of molecular tools like PCR for accurate assessment of tissue infection are recommended essential steps to improve animal health and mitigate public health risks in the region. Treatment efficacy was evaluated solely based on ELISA antibody responses; serological changes, particularly IgG decline, do not constitute direct evidence of parasite elimination. More direct diagnostic methods, such as PCR, histopathology, or parasite isolation, would provide stronger evidence.

Acknowledgments

The authors would like to thank the Avin-Bonyan-Gene Research Center (affiliated with Shahid Chamran University of Ahvaz) for their extensive technical support during the experimental procedures.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this research.

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