



Determine the Prevalence of *Theileria* spp. Infection and Associated Biochemical Changes in Naturally Infected Sheep in Al-Kharj City, Saudi Arabia

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10.18805/IJAR.BF-2136

ABSTRACT

Background: Theileriosis is a tick-borne hemoparasitic disease affecting both domestic and wild small ruminants and is widely distributed in tropical and subtropical regions. It is primarily transmitted by ticks of the genera *Hyalomma* and *Haemaphysalis*, leading to considerable economic burdens due to reduced productivity, increased treatment costs and mortality.

Methods: This study aimed to determine the prevalence of *Theileria* spp. infection and its associated biochemical alterations in sheep in Al-Kharj, Saudi Arabia. The study was conducted from July to October 2025 and included 280 sheep (92 males and 188 females) from 26 randomly selected herds. Blood samples were aseptically collected via jugular venipuncture for serum separation and biochemical analysis. Detection of *Theileria* infection was performed microscopically using Giemsa-stained blood smears.

Result: The overall prevalence of *Theileria* spp. infection was 13.9%. Infected sheep showed clinical signs such as pale mucous membranes, anorexia and tick infestation, with slightly higher infection rates observed in younger sheep (≤ 2 years) and females. Biochemical findings revealed significantly elevated liver enzymes (ALT, AST and ALP) (independent samples t-test, $p < 0.001$) and decreased total protein levels, indicating hepatic dysfunction and impaired protein metabolism. Additionally, significant mineral imbalances were observed, including reduced calcium and increased phosphorus levels ($p < 0.001$), while significantly elevated creatinine and urea concentrations ($p < 0.001$) suggested renal involvement. These results demonstrate the systemic effects of *Theileria* infection on sheep health in the study area.

Key words: Biochemical alterations, Microscopic examination, Protozoan diseases, Sheep, *Theileria*, Ticks.

INTRODUCTION

Livestock farming is an important source of high-quality nutrition and economic development worldwide; however, its productivity is often challenged by pests and disease infestations, leading to reduced productivity, increased animal mortality and significant economic losses for farmers and nations (Lawal-Adebawale, 2020). Small ruminants, particularly sheep and goats, represent a major component of livestock production due to their substantial contribution to meat, milk and skin production, as well as their socio-economic and cultural importance (Sargison, 2020). Moreover, several studies indicate that small ruminant production provides both food and non-food products, creates diverse market opportunities and forms an integral part of the nomadic culture in the Middle East, including Saudi Arabia (Salami *et al.*, 2019). Sheep and goats are susceptible to various pathogens and parasites, with gastrointestinal parasitic infections being the most common. These include helminths, protozoan diseases such as coccidiosis and haemoparasites (Estrada-Reyes *et al.*, 2019). Haemoprotozoan parasites such as *Trypanosoma*, *Theileria*, *Babesia* and *Anaplasma* are widely distributed among ruminants and represent the most prevalent blood parasites in sheep and goats. These pathogens are transmitted by arthropod vectors and typically cause destruction of red blood cells, leading to

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How to cite this article: Mares, M.M., Murshed, M., Hailan, W.A., Matar, A.M. and Al-Quraishy, S. (2026). Determine the Prevalence of *Theileria* spp. Infection and Associated Biochemical Changes in Naturally Infected Sheep in Al-Kharj City, Saudi Arabia. *Indian Journal of Animal Research*. **60(9)**: 1665-1671. doi: 10.18805/IJAR.BF-2136.

Submitted: 26-04-2026 **Accepted:** 15-07-2026 **Online:** 27-07-2026

anemia, anorexia, weight loss, jaundice, infertility and, in severe cases, high morbidity and mortality (Sharifi *et al.*, 2016). Assessing the prevalence of *Theileria* infection and the associated hematological and biochemical alterations provides valuable information about the severity of the disease and serves as an important tool for diagnosis, prognosis and evaluation of therapeutic interventions (Col and Uslu, 2007). To the best of our knowledge, limited studies have investigated the prevalence of *Theileria* infection and the associated biochemical alterations in sheep. Therefore, the present study was conducted to

determine the prevalence of *Theileria* parasites in Al-Kharj city, Saudi Arabia and to evaluate the pattern of changes in selected blood biochemical parameters in sheep naturally infected with theileriosis. The findings of this study may contribute to a better understanding of the pathogenesis of the disease and provide supportive information for the diagnosis of ovine theileriosis.

MATERIALS AND METHODS

Study area, animals and sample collection

This study was conducted from July to October 2025 in Al-Kharj city, Saudi Arabia. Al-Kharj is located southeast of Riyadh and is administratively part of the Riyadh Region. A total of 280 sheep (92 males and 188 females) belonging to 26 randomly selected herds were included in the study, with the average herd size typically ranging between 80 and 220 animals. Seventy samples were collected monthly and for each sampled animal, the date of sampling as well as sex and age information were recorded. No data indicated that any antiparasitic treatment had been previously administered to the animals. Blood samples were collected aseptically via jugular venipuncture using sterile 5 mL disposable syringes. The collected blood was transferred into plain tubes for serum separation to be used in biochemical analysis. All samples were transported to the laboratory under refrigerated conditions and processed within four hours of collection to ensure sample integrity. Ethical approval for this study was granted by the Research Ethics Committee (REC) at King Saud University (KSU), under Ethical Reference No. KSU-SE-23-73.

Microscopic detection of *Theileria* in blood smears

Theileria infection was detected microscopically following the protocol described by Huber *et al.* (2017). Fresh blood smears were prepared by placing a drop of blood on a clean glass slide and spreading it using a second slide held at a 45° angle. The prepared smears were then fixed in 100% ethanol and subsequently stained with Giemsa stain. Finally, the slides were examined under a high-

magnification oil immersion lens using a light microscope (Olympus CX41, Tokyo, Japan) at 100× magnification.

Analysis of biochemical parameters

Serum samples were prepared by centrifugation of whole blood at 3,000 rpm for 10 minutes, followed by biochemical evaluation. Commercial veterinary diagnostic kits Equine Panel kit (Skyla Corporation, Hsinchu, Taiwan), validated for livestock use, were employed to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), urea, creatinine, phosphorus and calcium. All procedures were carried out in strict accordance with the manufacturer's guidelines and quality control standards.

Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS), version 27. Differences between the mean biochemical indicators of the infected and non-infected groups were assessed using an independent samples t-test, with statistical significance set at $p \leq 0.05$. Effect size (Cohen's d) was calculated using Microsoft Excel and graphical representations with standard error bars were generated to compare the two groups.

RESULTS AND DISCUSSION

Direct microscopic examination of Giemsa-stained blood smears showed that 39 (13.9%) of the examined sheep were positive for theileriosis (Fig 1). The present study therefore recorded an overall prevalence of 13.9% for *Theileria* infection in sheep from Al-Kharj city. This rate is lower than that reported in Riyadh Province, where microscopic examination detected 33.2% infection and PCR revealed an even higher prevalence of 46% (Alanazi *et al.*, 2019). Similarly, higher rates were reported in Madina and Tabuk, where microscopy identified 6% while PCR-based methods increased detection to 18.6% (Almahallawi *et al.*, 2024), indicating regional variability within Saudi Arabia. In agreement with the present findings, a study

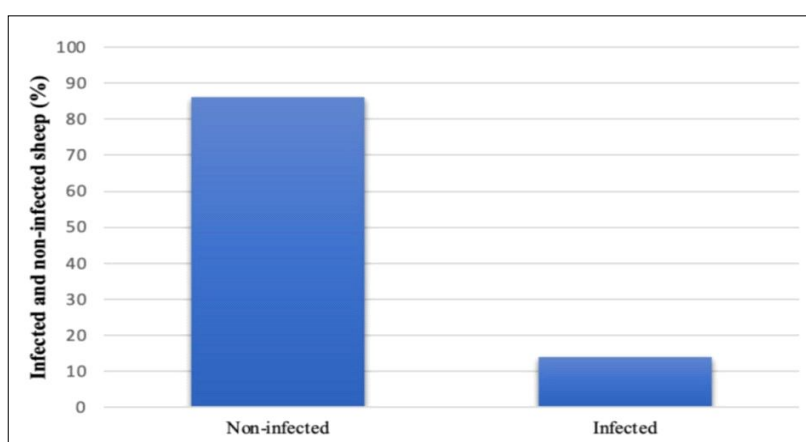


Fig 1: The infection rate of *Theileria* spp. in sheep.

from India confirmed *Theileria luwenshuni* infection in sheep by microscopy and PCR and reported clinical manifestations such as anemia, fever and weakness in affected animals (Dhaygude *et al.*, 2021). When compared internationally, the prevalence observed in this study is slightly lower than that reported in Egypt (17.08%) (Hegab *et al.*, 2016) and Pakistan (16.5%) (Shabbir *et al.*, 2010), but is comparable to findings from Lahore, Pakistan (13.8%) (Naz *et al.*, 2012), western Iran (12.85%) (Bahrami *et al.*, 2013) and Iran (11.9%) (Razmi *et al.*, 2006). These differences in prevalence are likely influenced by geographic location, environmental conditions and climatic factors that affect tick distribution, seasonal activity and transmission dynamics of *Theileria* (Ahmed *et al.*, 2002). In agreement with this, Durrani *et al.* (2012) reported that geographical and ecological factors significantly influence the epidemiology of theileriosis. Overall, the moderate prevalence observed in Al-Kharj suggests that local environmental conditions play an important role in shaping the distribution of *Theileria* infection.

The infected samples showed various intra-erythrocytic forms morphologically consistent with *Theileria* (Fig 2).

Clinically, sheep infected with *Theileria* showed several noticeable signs, including pale ocular mucous membranes, loss of appetite and the presence of ticks in the ears of the affected animals. These clinical manifestations may be associated with the parasitic infection and its impact on the general health status of the infected sheep (Fig 3).

Regarding age, a higher prevalence of theileriosis was observed in sheep aged ≤ 2 years compared to those older than 2 years. Among 208 sheep aged ≤ 2 years, 30 (14.4%) were infected, whereas 9 out of 72 sheep older than 2 years (12.5%) tested positive. This indicates that younger sheep were slightly more susceptible to *Theileria* infection than older animals (Table 1).

A higher prevalence of theileriosis was also observed in female sheep compared to males. Out of 188 examined females, 28 (14.8%) were found to be infected, whereas 11 out of 92 males (11.9%) tested positive for the infection. This finding indicates that females showed a slightly greater susceptibility to *Theileria* infection than males in the examined population (Table 2).

The biochemical analysis revealed marked alterations in liver function parameters between infected and non-infected sheep. Infected animals showed significant elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) compared with healthy controls, while total protein and albumin levels were significantly reduced (Table 3; Fig 4-5). These changes suggest hepatic dysfunction and impaired protein metabolism associated with *Theileria* infection.

Table 1: Infestation percentage according to the age groups of animals.

Species	Age	No. of examined animals	No. of infested (%)
Sheep	≤ 2	208	30 (14.4%)
	> 2	72	9 (12.5%)
Total		280	39 (13.9%)

Table 2: Infestation percentage according to the sex groups of animals.

Species	Sex	No. of examined animals	No. of infested (%)
Sheep	Male	92	11 (11.9%)
	Female	188	28 (14.8%)
Total		280	39 (13.9%)

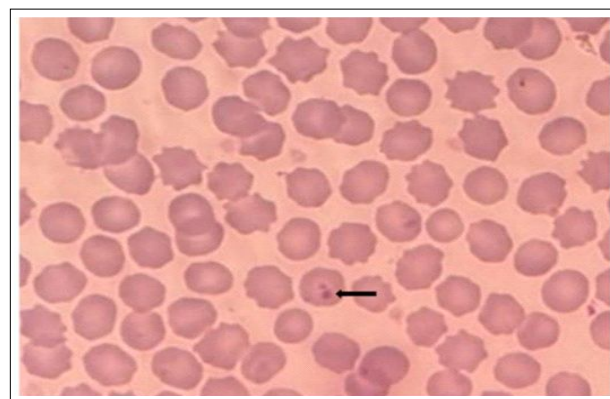


Fig 2: Giemsa-stained thin blood smear (100x magnification) showing *Theileria* species within sheep red blood cells (RBCs).

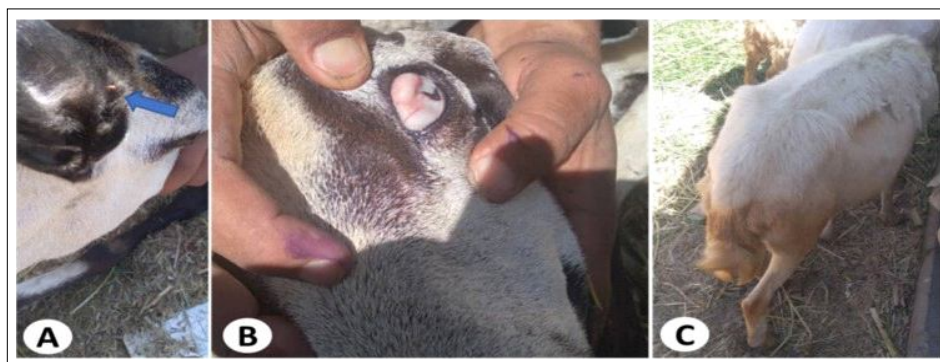


Fig 3: (A) Tick infestation in the ear of sheep, (B) Pale mucous membranes in sheep infected with *Theileria* and (C) General weakness in animals infected with *Theileria* and ticks.

The observed increase in AST, ALT and ALP indicates liver involvement and hepatocellular damage caused by the parasitic infection. Similar findings have been reported in previous studies, where Baghshani *et al.* (2012) observed elevated AST and ALT levels in sheep with theileriosis and Sandhu *et al.* (1998) reported significant increases in AST, ALT and ALP in calves experimentally infected with *Theileria annulata*, particularly from day 16 post-infection. In addition, Al-Obaidi and AlSaad (2004) documented elevated ALT and AST levels in sheep infected with *Theileria hirci*. Similarly, infections caused by *Theileria* spp. and *Babesia* spp. in sheep have been associated with clinical signs of anemia

and tick infestation, accompanied by biochemical disturbances indicative of systemic organ involvement (Haq *et al.*, 2021). These enzymatic elevations are generally attributed to hepatic stress and leakage of intracellular enzymes into the bloodstream due to tissue damage induced by the parasite. In parallel, the significant reduction in total protein and albumin levels in infected sheep further supports the presence of liver dysfunction and metabolic disturbances. Comparable results were reported by Bilgiç *et al.* (2017) and Kachara *et al.* (2025), who associated hypoproteinemia and hypoalbuminemia in *Theileria* infected animals with impaired hepatic synthesis,

Table 3: Effect of *Theileria* spp. infection on biochemical parameters in infected and non-infected sheep.

		Mean	Std. deviation	Std. error	T	<i>p</i>	Cohen's d
ALT U/L	Infected	21.06	7.31	1.77	3.84	0.001	1.317
	Noninfected	13.50	3.53	0.86			
ALP U/L	Infected	208.53	15.68	3.80	9.47	0.000	3.247
	Noninfected	120.82	34.84	8.45			
AST U/L	Infected	205.24	28.99	7.03	5.21	0.000	1.787
	Noninfected	155.53	26.59	6.45			
Total protein g/dL	Infected	3.03	1.22	0.29	-13.74	0.000	-4.714
	Noninfected	7.40	0.49	0.12			
Albumin g/dL	Infected	3.35	0.37	0.09	-7.64	0.000	-2.620
	Noninfected	4.12	0.19	0.05			
Creatinine mg/dL	Infected	1.21	0.47	0.11	4.00	0.001	1.372
	Noninfected	0.75	0.09	0.02			
Urea mg/dL	Infected	38.99	19.88	4.82	2.76	0.013	0.946
	Noninfected	25.36	4.44	1.08			
Calcium mg/dL	Infected	6.31	1.95	0.47	-6.54	0.000	-2.244
	Noninfected	9.60	0.71	0.17			
Phosphorus mg/dL	Infected	10.34	1.76	0.43	6.26	0.000	2.147
	Noninfected	7.03	1.29	0.31			

Statistically significant differences in biochemical parameters between infected and non-infected sheep affected by *Theileria* spp., as determined by the independent samples t-test.

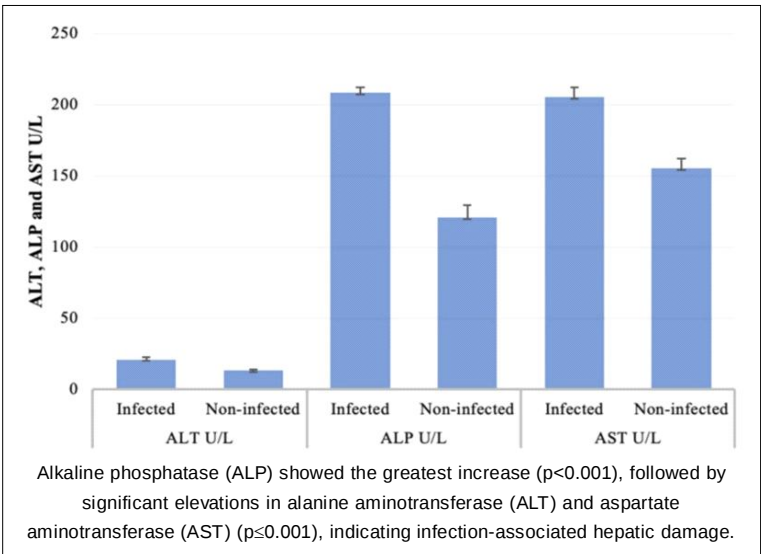


Fig 4: Liver enzyme levels were significantly elevated in infected animals compared to non-infected.

increased protein catabolism and altered protein metabolism during infection. Moreover, hypoalbuminemia in parasitic diseases is often linked to hepatic insufficiency or protein loss through inflamed gastrointestinal tissues (Miller *et al.*, 2010). Overall, these findings collectively highlight the significant impact of *Theileria* infection on liver function and protein homeostasis in affected sheep.

The analysis of mineral parameters revealed significant alterations between infected and non-infected sheep. Infected animals showed a marked increase in serum phosphorus (P) levels accompanied by a significant decrease in calcium (Ca) levels compared with healthy controls, indicating that *Theileria* infection may disrupt mineral homeostasis and metabolic processes (Table 3; Fig 6). These findings are consistent with previous studies reporting similar disturbances in mineral balance in *Theileria* infected animals (Zintl *et al.*, 2003; Mohamed, 2017; Yaghfoori *et al.*, 2017). The observed hypocalcemia may be related to the close physiological association between calcium and albumin metabolism, particularly in

conditions of reduced albumin synthesis during infection. On the other hand, the increase in serum phosphorus levels could be attributed to multiple pathological mechanisms, including hemolysis, tissue damage, impaired renal function, as well as reduced feed intake and dehydration. These factors collectively contribute to the release and accumulation of phosphorus in the circulation. Overall, these results indicate that *Theileria* infection is associated with significant disturbances in mineral metabolism in affected sheep.

The renal function parameters revealed significant differences between infected and non-infected sheep. Infected animals exhibited elevated serum levels of urea and creatinine compared with healthy controls, suggesting that *Theileria* infection may impair renal function and disrupt normal metabolic waste excretion (Table 3; Fig 7-8). These findings are in agreement with previous studies reporting similar alterations in renal biomarkers. Al-Obaidi and AlSaad (2004) and Baghshani *et al.* (2012) documented increased urea levels in sheep infected with *Theileria*, while Col and Uslu (2007) reported elevated creatinine concentrations

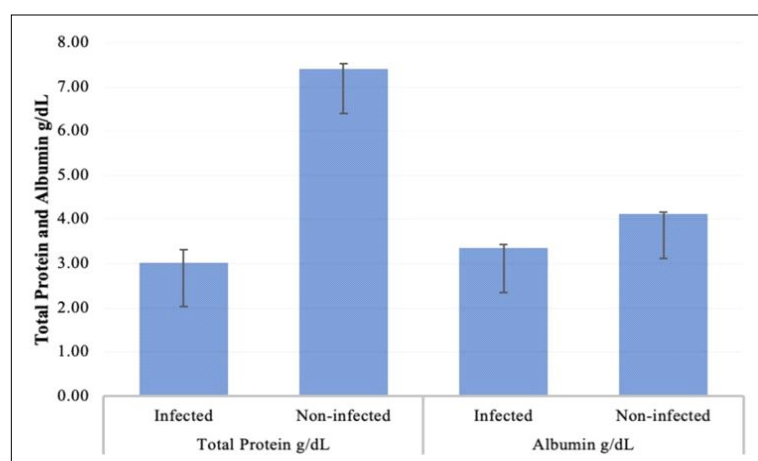


Fig 5: Total protein and albumin levels were significantly reduced in infected animals compared to non-infected ($p < 0.001$), with a very large effect size for total protein, indicating severe protein loss or infection-associated malnutrition.

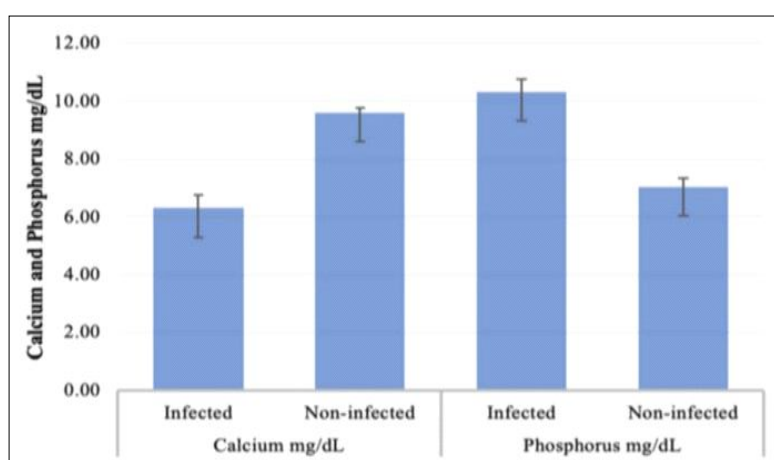


Fig 6: Calcium levels were significantly decreased, whereas phosphorus levels were significantly increased in infected animals compared to non-infected ($p < 0.001$), indicating marked disruption of mineral metabolism associated with infection.

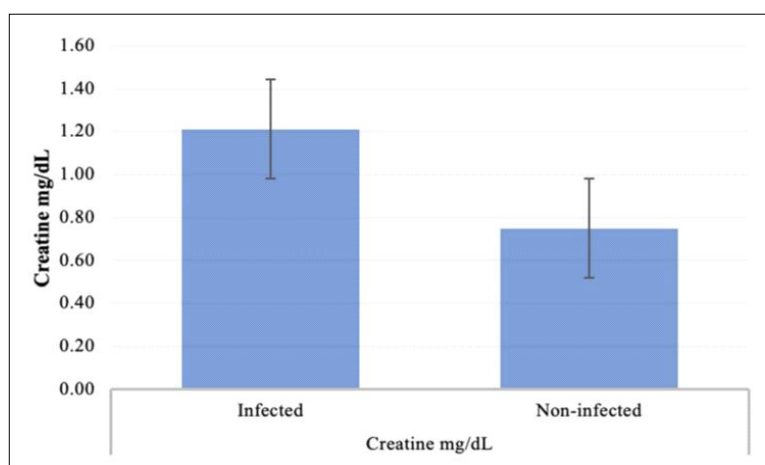


Fig 7: Creatinine levels were significantly elevated in infected samples compared to non-infected ($p < 0.001$), indicating impaired renal function associated with infection.

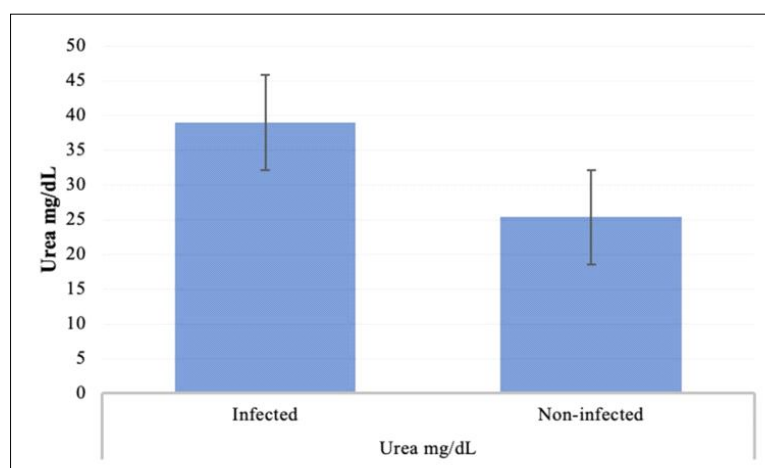


Fig 8: Urea levels were significantly elevated in infected samples compared to non-infected ($p < 0.001$), indicating impaired renal function associated with infection.

in cattle naturally infected with *Theileria annulata*. The increase in these parameters is generally indicative of possible renal impairment, which may result from infection-associated kidney damage or functional disturbances affecting the excretory capacity of the kidneys.

CONCLUSION

Theileriosis in sheep is often not detected during the early stages of infection and usually becomes apparent only after several biological indicators in the affected animals have been altered. Although treatment can save infected animals even at advanced stages of the disease, they may remain debilitated for several months after recovery. Therefore, from both economic and animal welfare perspectives, prevention of infection is preferable to treatment.

ACKNOWLEDGEMENT

This work was funded by Ongoing Research Funding Program, (ORF-2026-1084), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that they have no conflicts of interest related to this study.

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