Clinical and biochemical alterations in Afshari sheep naturally infected with piroplasm parasites

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Abstract

Piroplasmosis, caused by Theileria and Babesia species, is a major tick-borne disease affecting sheep health and productivity in Iran. This study evaluated alterations in the lipid profile, fat-soluble vitamins, and hematological and hepatic parameters of Afshari sheep naturally infected with piroplasm parasites. A cross-sectional survey was conducted in 2024 on 48 Afshari sheep from Kerman, Quchan, and Birjand regions. Animals showing clinical signs consistent with theileriosis or babesiosis and confirmed microscopically by Giemsa-stained blood smears were included. Blood samples were collected from the jugular vein for hematological and biochemical analysis. Complete blood counts, liver enzyme activities, lipid profiles, and concentrations of vitamins A, E, and K were determined. Data were analyzed using SPSS and R software, with significance set at $p<0.05$. Among the examined animals, 26 were infected and 22 served as uninfected controls. Infected sheep showed significant reductions in hematological indices (RBC, Hb, and WBC counts), total protein, triglycerides, and fat-soluble vitamin levels, accompanied by elevated liver enzyme activities ($p<0.05$). These alterations indicate anemia, hepatic dysfunction, and metabolic disturbances associated with piroplasmosis. Routine monitoring of liver enzymes and biochemical markers is recommended for early detection and management of infection in sheep flocks.

Keywords: Afshari sheep, piroplasmosis, hematology, lipid profile, liver enzymes

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Introduction

The Afshari sheep is recognized as a significant indigenous livestock breed in Iran, valued for its large body size and high meat production potential. This breed is well adapted to cold climates and is predominantly found in the mountainous regions of the country (Mohammadi *et al*., 2011, Ghafouri-Kesbi and Eskandarinasab, 2018). Afshari sheep are highly valued for their meat production and also demonstrate commendable performance in milk yield. They possess notable genetic potential, including larger lamb size and accelerated growth rates, which contribute significantly to their overall economic importance. (Pourtahmasebian Ahrabi *et al*., 2021).

Theileriosis and piroplasmosis are major tick-borne parasitic diseases affecting sheep in Iran, caused by protozoan species of the genera *Theileria* and *Babesia*, respectively (Soosaraei *et al*., 2018a; Gh *et al*., 2020). These diseases contribute substantially to economic losses in sheep farming, primarily due to their detrimental effects on animal health, including anemia, fever, reduced productivity, and, in severe cases, mortality (Hasheminasab *et al*., 2018). In Iran, approximately 23% of sheep are infected with theileriosis, with notably higher prevalence rates reported in regions such as Sistan and Baluchestan (up to 71%) and Razavi Khorasan (55.6%) (Soosaraei *et al*., 2018b). Piroplasmosis is also widespread, with infection rates averaging around 49%, and reaching up to 54% in certain areas (Hashemzadeh-Farhang and Akbari-Shahkhosravi, 2024). The distribution and prevalence of these diseases vary by region and season, influenced by tick activity and climatic conditions (Irfan *et al*., 2023). These findings underscore the significant impact of theileriosis and piroplasmosis on sheep health and productivity in Iran.

Monitoring the health status of sheep is essential for livestock producers, particularly through the assessment of lipid profiles and fat-soluble vitamins. These parameters serve as important indicators of the animal's physiological condition, nutritional status, and immune competence. Abnormal lipid levels such as altered concentrations of cholesterol, triglycerides, and lipoproteins are associated with various health disorders, compromised immune responses, and reduced productivity in sheep. (Kurmaeva *et al*., 2023). For example, analyzing blood parameters during pregnancy can reveal early physiological changes associated with gestation, thereby enhancing reproductive management and enabling better prediction of health outcomes in ewes (Goldansaz *et al*., 2022). Vitamins A, E, and K play crucial roles in supporting immune function, protecting the body against external stressors, and maintaining metabolic balance. Monitoring their serum concentrations is essential, as both deficiencies and excesses can negatively affect overall health and impair the animal’s ability to resist disease..

Hematological and hepatic indicators serve as critical diagnostic tools in identifying parasitic infections in sheep.

Parasitic organisms often alter key blood parameters, such as reducing hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts—hallmarks of anemia. Conversely, elevated white blood cell (WBC) counts, particularly increases in lymphocytes, eosinophils, and monocytes, reflect the host's immune response to parasitic invasion. Notably, eosinophilia is a common and specific marker associated with parasitic diseases (Allu *et al*., 2025). Liver biomarkers, such as elevated levels of aspartate aminotransferase (AST), are commonly observed in response to hepatic damage caused by parasitic migration or bile duct disturbances. Additionally, decreased serum albumin concentrations are frequently reported, likely resulting from impaired liver function or protein loss associated with parasitic infections (Samadie *et al*., 2016).

To date, no comprehensive study has investigated the biochemical and hematological alterations in Afshari sheep affected by theileriosis or piroplasmosis. Existing research has primarily focused on molecular diagnostics and pathogen identification. Considering the economic and agricultural significance of the Afshari breed in Iran and the substantial impact of these parasitic diseases on their health, productivity, and survival there is a critical need to understand the physiological changes induced by such infections. Detailed knowledge of blood and biochemical markers is essential for early diagnosis, effective clinical management, and the development of targeted disease control strategies. Therefore, the present study aimed to evaluate lipid profiles, fat-soluble vitamins, hematological parameters, and liver function indicators in Afshari sheep naturally infected with Theileria or Babesia

Material and methods

Source of animals and samples

This study was conducted in 2024 across three Iranian towns—Kerman, Quchan, and Birjand—where sheep diseases are prevalent. A total of 48 Afshari sheep were selected based on clinical signs such as high fever, pulmonary edema, respiratory distress, pale mucous membranes, and jaundice. Among them, 26 sheep diagnosed with theileriosis or piroplasmosis were chosen from the three locations, based on disease prevalence and other relevant factors. Additionally, 22 clinically healthy sheep from the same farms were included as controls. The sample distribution consisted of 5 infected and 5 healthy sheep from Birjand, 8 infected and 4 healthy sheep from Kerman, and 13 infected alongside 13 healthy sheep from Quchan.

Sampling and microscopic examination procedures

Prior to sampling, sheep were clinically evaluated for parameters including heart rate, pulse strength, and the coloration of the sclera and gingival mucosa. Blood samples were aseptically collected from the jugular vein following proper restraint of each animal. Approximately

4–5 mL of blood was drawn into tubes containing EDTA as an anticoagulant, gently mixed to prevent clot formation, and immediately transported on ice to the laboratory. Serum was separated by centrifugation at 1000 rpm for 10 minutes.

Thin blood smears were prepared from each sample, fixed with absolute methanol for 5 minutes, and stained using a 10% Giemsa solution for 30 minutes. The stained slides were examined under oil immersion at 1000× magnification to detect Theileria and Piroplasma parasites. A minimum of 10 microscopic fields per slide were evaluated, and samples exhibiting parasitic forms were recorded as positive.

Hematological and biochemical analysis methods

Blood samples were analyzed within 4 to 5 hours of collection. A complete blood count (CBC) was performed to assess parameters including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and differential leukocyte counts (neutrophils, lymphocytes, monocytes, and eosinophils). Serum albumin and total protein levels were measured using standard laboratory protocols and Schroederman diagnostic tools. Liver enzyme activity, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), was also evaluated to assess hepatic function (Altug *et al*.), alkaline phosphatase (Sacco *et al*.), Liver enzymes, including lactate dehydrogenase (LDH), were measured using automated analyzers and commercial Zellbio diagnostic kits. Additionally, serum concentrations of triglycerides, cholesterol, and fat-soluble vitamins, vitamin E, vitamin A, and vitamin K were assessed. Triglyceride and cholesterol levels were determined spectrophotometrically using Zellbio kits and standard biochemical assay protocols.

Statistical analysis method

Data were analyzed using SPSS version 22 software. To compare mean values, either the independent t-test or the non-parametric Mann-Whitney test was applied. A significance level of 95% was set for all tests, with p-values of less than 0.05 considered statistically significant.

Results

The results of this study showed that Afshari sheep with theileriosis or piroplasmosis exhibited significant alterations across hematological, biochemical, and nutritional parameters compared to uninfected controls.

Hematological parameters

The infected group showed significantly reduced levels in various hematological parameters. Mean WBC reduced from 2.97±1.64 in uninfected sheep to 1.25±9.29 in infected sheep (p=0.020). RBC values were 2.04±1.11 in non-infected animals and 0.86±0.46 in infected ones (p=0.021). Hgb decreased

from 4.28±1.78 in healthy sheep to 1.58±0.61 in the infected group (p<0.001). Additional parameters, including Hct, MCV, MCH, PLT, and MCHC, demonstrated notable decreases (all p<0.05) (Table 1).

Table1: Hematological parameters in uninfected versus infected Afshari sheep.

Hematological factors	Uninfected sheep	Infected sheep	P-V	Change(%)
WBC (dL)	2.97±15.64	1.25±9.2	0.020	-57.91
RBC (Millions/misrol)	2.04±11.13	.83±4.96	<0.001	-59.31
Hgb (dL)	2.31±8.7	.85±5.65	<0.001	-63.20
Hct (%)	49±39.41	3.45±18.1	<0.001	-92.96
MCV (fL)	2.29±28.57	1.19±23.67	<0.001	-48.03
MCH (pg)	.72±8.75	.68±7.1	0.040	-5.56
PLT (*1000/Microl)	172.02±143.72	35.28±215.71	0.190	-49.49
MCHC (g/dL)	47.55±9.25	40.64±8.83	0.120	-14.53
Lym (%)	12.86±50.33	7.27±52.28	0.185	-43.47
NEU (%)	13.27±45.77	7.74±38.42	0.136	41.67-
Mon (%)	1.73±2.05	1.73±3	<0.001	0.00
Eos (%)	.69±6.85	.99±1.94	0.147	43.48

Biochemical liver indicators

Infected sheep had lower serum enzymes and proteins: AST dropped from 28.58±13.55 to 9.87±26.14 (p<0.05), ALT from 22.16±8.61 to 13.16±2.90 (p<0.05), ALP from 97.15±15.03 to 63.06±7.91 (p<0.05), albumin from 3.97±0.67 to 2.96±1.12 (p<0.05), LDH from 1012±65 to 298±24 (p<0.05), and total protein from 7.26±1.11 to 4.65±1.63 (p<0.05) (Table 2).

Table 2: Serum liver enzyme and protein profile in uninfected and infected Afshari sheep.

Biochemical factors	Infected sheep	Uninfected sheep	P-V	Change(%)
AST (u/L)	28.58±135.33	9.87±286.14	<0.05	-65.47
ALT (U/L)	13.71±47.67	8.44±81.28	<0.05	-38.44
ALP (U/L)	58.11±208	37.73±358	<0.05	-35.07
Alb (g/dL)	.22±3.5	.25±2.43	0.175	13.64
LDH (U/L)	154.36±661	45.7±101	<0.05	-70.39
TP (g/dL)	.46±7.63	.72±6.11	0.162	56.52

Lipid profile and fat-soluble vitamins

Significant reductions were seen in triglycerides, cholesterol, and all measured vitamins. Triglycerides decreased from 6.29±45.75 (uninfected) to 1.70±23.75 (infected), cholesterol from 6.40±4.34 to 5.04±4.80, vitamin E from 0.60±1.25 to 0.11±1.25, vitamin K from 12.12±2.96 to 4.33±4.70, and vitamin A from 12.12±2.96 to 4.33±4.70 (all p<0.05) (Table 3).

Table 3: Lipid profile and fat-soluble vitamin levels in uninfected versus infected Afshari sheep

Parameter	Uninfected sheep	Infected sheep	P-V	Change(%)
Triglyceride (mg/dL)	6.29±45.75	1.70±23.75	<0.05	-72.97
Cholesterol (mg/dL)	6.40±34.75	5.04±62.50	<0.05	-21.25
Vitamin E	.16±1.50	15.49±.02	<0.05	-87.50
Vitamin K	.60±1.25	.05±.13	<0.05	-91.67
Vitamin A	12.12±2.96	4.83±44.70	<0.05	-60.15

Discussion

The results of this study indicate that sheep infected with theileriosis or piroplasmosis exhibit significant reductions in key hematological parameters, including red blood cell (RBC) count, hemoglobin concentration, hematocrit (HCT), and other erythrocyte indices, compared to healthy controls. In contrast, liver enzyme activities—such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) —were markedly elevated in infected animals, suggesting hepatic involvement. Additionally, affected sheep showed decreased serum levels of fat-soluble vitamins (A, E, and K) and reduced lipid profiles, which may reflect compromised nutritional status. These findings underscore the substantial impact of theileriosis and piroplasmosis on the physiological health of sheep.

Consistent with previous studies by Khaki *et al*. (2015) and Villanueva *et al*. (2022) our findings confirm that theileriosis is associated with pronounced declines in RBC count, hemoglobin levels, and hematocrit, reinforcing the link between parasitic infection and hematological disruption (Khaki *et al*., 2015; Villanueva-Saz *et al*., 2022). This anemia may be due to the bug invading and destroying red blood cells, along with limiting bone marrow work, which means fewer new red blood cells are made. The seen changes in red cell numbers point to a large pale type of anemia, showing the same sickness method in small animals infected by Theileria (Yang *et al*., 2022). Supportive therapy for sheep with theileriosis-related anemia focuses on vitamin B complex, especially vitamin B12, to stimulate red blood cell production and aid recovery, while preventive measures center on regular tick control using acaricides and good management practices to reduce infection risk. These combined approaches help manage hematological declines and prevent new infections effectively (Satbige and Patil, 2020; Arif *et al*., 2024).

The rise of liver enzymes like AST, ALT, ALP, and LDH in sheep that have theileriosis or piroplasmosis shows the liver is involved because of the illness. Along with these results, Abdullah and Oleiwi saw higher liver enzyme levels in sheep sick with Theileria, which means there is harm to liver cells caused by parasite toxins and swelling (OLEIWI *et al*., 2020; Abdullah., 2022). The damage to the liver can be understood by cell death from parasites and body-wide inflammation in affected animals (Akhter *et al*., 2017). To reduce these effects, early detection and good

antiparasite care are very important. Also, careful watching of the liver work is suggested.

Sheep affected by theileriosis or piroplasmosis show lower amounts of vitamins A, K, and E, which points to a weaker food and body working condition. Similar results have been noted by Nuri Altug *et al*. (2004) who saw lower serum levels of vitamin B12 and helpful vitamins in cows sick with theileriosis, linking it with anemia and stress from too many free radicals; (Altug *et al*., 2014) however, these results clash with ones from Nazifi *et al*. (2013) who found no major differences in fat-friendly vitamin levels between sick and healthy sheep (Nazifi *et al*., 2013). This difference can be linked to changes in sheep types, and also variations in how bad the infections are among the groups looked at. Oxidative stress from Theileria infections lowers antioxidant vitamins and throws off metabolic balance in sheep and cows (Caglar *et al*., 2024). Molecular studies show that Babesia and Theileria cause blood breakdown and metabolic problems, making nutrient shortages and immune issues worse in animals that chew cud (Almazán *et al*., 2022; Kuibagarov *et al*., 2023). So, food that helps particularly with antioxidants is important to fix body balance and boost the immune system during and after sickness from parasites.

In our study, sheep sick with theileriosis or piroplasmosis showed lower fat amounts, especially lower cholesterol and triglyceride levels. While Akhter *et al* (2017) noted a drop in triglycerides in sick sheep, they said there were higher cholesterol levels (Akhter *et al*., 2017). Conversely, Abdalla *et al*. (2020) observed elevated triglyceride and cholesterol levels in infected sheep, findings that contrast with our results (Abdalla *et al*., 2020). Variations in findings could be attributed to differences in parasite species or strains, infection stages, host breed, and metabolic conditions, as well as environmental or nutritional factors. For instance, increased cholesterol levels may arise from acute-phase responses and liver dysfunction that alter lipid metabolism, while decreased levels likely reflect heightened lipid consumption by the immune system and parasite survival mechanisms (Hosny *et al*., 2010). Additionally, methodological differences in sample collection timing relative to disease progression and co-infections may influence lipid measurements, contributing to inconsistent results. Therefore, alongside specific antiparasitic treatments, nutritional interventions aiming to restore lipid profiles, such as lipid-rich supplements and antioxidants, are recommended to support metabolic health and promote convalescence in infected sheep.

Conclusion

The findings of this study demonstrate that theileriosis and piroplasmosis exert a profound negative impact on sheep health, primarily through the induction of anemia, hepatic dysfunction, and disruptions in nutritional and metabolic processes. Infected sheep exhibited

significantly reduced red blood cell counts and serum levels of essential fat-soluble vitamins (A, E, and K), alongside elevated liver enzyme activities and diminished lipid profiles. These physiological alterations reflect the systemic burden imposed by these parasitic infections. To mitigate disease transmission and severity, it is recommended to adopt integrated tick control strategies, including the routine use of acaricides and effective pasture management. Early diagnosis through vigilant clinical monitoring and timely administration of appropriate antiparasitic treatments is crucial. Furthermore, nutritional support particularly supplementation with vitamins A, E, and K, along with lipid-enriched diets may enhance recovery and strengthen immune responses. Regular evaluation of liver function and biochemical markers is also advised to detect complications early and maintain overall flock health.

Limitations

This study has several limitations that should be considered when interpreting the findings:

Cross-sectional design

The study provides only a snapshot of hematological and biochemical changes, without capturing the progression of infection or recovery over time.

Biological variability

Differences in factors such as age, sex, nutritional status, and the presence of concurrent illnesses among the sampled sheep may have influenced the measured parameters.

Environmental factors

Climatic and ecological conditions affecting the flocks were not controlled, which could have impacted physiological responses and clinical signs.

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Ethics

The study design was approved by the ethics committee of the Islamic Azad University, Kerman, Iran.

Authors' contribution

Study concept and design: A. A.F

Acquisition of data: M. M.S.

Analysis and interpretation of data: A. A.

Drafting of the manuscript: F.S, B.SH, M.M

Critical revision of the manuscript for important intellectual content: A. A

Statistical analysis: M. M.S

Administrative, technical, and material support: A.A

Conflict of interest

The authors certify that they have no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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