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Hematological and biochemical parameters of sheep with mono and associative invasion by *Babesia ovis* and *Moniezia expansa* parasites

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ABSTRACT

Hematological and biochemical studies were conducted in the Guba-Khachmaz economic region on sheep infected with endoparasites (*Babesia ovis–Moniezia expansa*) mono- and associatively. For this purpose, blood samples from 45 sick and 10 healthy sheep were examined. Serological and biochemical studies were conducted on sheep infected with a monoinvasion of the primary blood parasite *Babesia ovis*. Studies were also conducted on sheep infected with an associative invasion of *Babesia ovis+Moniezia expansa*. For comparison, serological studies were conducted on healthy sheep. The average difference in hematological blood values in sheep with *Babesia ovis* monoinvasion was as follows: PCV, RBC, MCV, Hb, neutrophils, and monocytes decreased, while WBC, MCH, MCHC, and lymphocytes were relatively elevated. The average difference in hematological values in healthy and sick sheep with monoinvasion did not differ significantly for RBC, MCV, monocytes, WBC, MCH, MCHC, and lymphocytes ($p>0.05$). In sick sheep with associative invasion of *B. ovis+M. expansa* parasites, PCV, RBC, WBC, MCV, Hb, monocytes, neutrophils, and lymphocytes decreased sharply, while MCH, MCHC, and eosinophils increased sharply ($p<0.05$). The average difference in blood biochemical parameters in sheep with *Babesia ovis* monoinvasion was as follows: ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, and creatine were elevated, while albumin and total protein were decreased. Albumin, total protein, direct bilirubin, indirect bilirubin, and creatine did not change significantly ($p>0.05$). In sheep with associated *B. ovis+M. expansa* infection, the mean difference in ALT and AST levels was significantly increased, while albumin and total protein levels were significantly decreased ($p<0.05$). Total bilirubin, direct bilirubin, indirect bilirubin, and creatine were significantly elevated ($p>0.05$).

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INTRODUCTION

Small ruminants are a highly valued livestock species worldwide due to their meat, milk, and wool productivity. They are highly adaptable to extreme weather conditions and drought (Akinmoladun *et al.,* 2019). However, in tropical and subtropical regions, small ruminants face significant health challenges due to ticks and tick-borne diseases (Mabey, 2002; Demessie and Derso, 2015). Tick bites transmit protozoan parasites to animals, causing disease, reduced immunity, and decreased productivity (Eskezia and Desta, 2016).

Babesiosis in sheep, caused by several species of *Babesia*, is a serious tick-borne disease affecting sheep. *Babesia* species affecting sheep include the protozoan parasites *Babesia ovis, B. motasi, B. crassa, B. taylori, B. foliate, Babesia* sp. (Ceylan *et al.,* 2021; Schnittger *et al.,* 2022). Although eight *Babesia* species have been identified in sheep, *B. ovis* is considered the most pathogenic and potentially fatal (Firat *et al.,* 2024). There is extensive literature on the pathogenicity and widespread distribution of *Babesia ovis* in sheep. Cases of babesiosis infection in sheep have been reported in Turkey (Ceylan and Sevinc, 2020; Bozan *et al.,* 2024), Pakistan (Masih *et al.,* 2022), Iran (Gh *et al.,* 2020), Portugal (Horta *et al.,* 2014), Egypt (Hussein *et al.,* 2017), Israel (Pipano, 2022), Greece (Theodoropoulos *et al.,* 2006), Italy (Savini *et al.,* 1999), Nigeria (Adewumi *et al.,* 2022), and Tunisia (Rjeibi *et al.,* 2016), with infection accompanied by symptoms of fever, anemia, jaundice, hemoglobinuria, and causing mortality (Stevanović *et al.,* 2022). The main vector of the *B. ovis* parasite is the *Rhipicephalus bursa* tick. Although the larvae and nymphs of the *Rh. bursa* tick are infected with *B. ovis*, they are not capable of transmitting the parasite. Transmission occurs only at the imago stage of the tick (Erster *et al.,* 2016; Altay *et al.,* 2008). It has been noted that the *Rhipicephalus bursa* tick is widespread among sheep raised in various regions of Azerbaijan (Azizova, 2024).

Species of the genus *Moniezia*, belonging to the order Cyclophyllidea, family Anoplocephalidae, class Cestoda, are cosmopolitan species that localize in the intestines of ruminants and cause helminthiasis (Jalajakshi *et al.,* 2016; Shangaraev *et al.,* 2018; Diop *et al.,* 2015). *Moniezia* species in sheep continue to be a serious problem and cause significant economic damage to livestock farming (Choubisa and Jaroli, 2013; Sharma *et al.,* 2020). However, in cases of heavy infection, clinical signs such as growth retardation, diarrhea, anemia, intestinal pathology, reduced wool quality, and even death (Zhao *et al.,* 2009; Yan *et al.,* 2013). *Moniezia* is common in lambs under 1 year of age and rare in older sheep. Therefore, monieziasis is called the milk worm of sheep. It is more intensively observed in lambs during lactation. Since soil ticks are the intermediate host of the helminth, monieziosis is more common in sheep in areas with heavy rainfall (Sharma *et al.,* 2020).

M. expansa is distributed in farm animals all over the world with varying intensity. The invasion rate of sheep with this helminth is reported to be 16% in Iraq and 74% in Egypt, causing significant economic losses in sheep (Bashtar *et al.,* 2011; Alberfkani *et al.,* 2022). In Azerbaijan, *Moniezia expansa* species is intensively distributed in sheep and *M. benedeni* species is intensively distributed in goats and causing invasion (Azizova, 2024).

During the study of associative invasions of sheep, *Babesia ovis* was detected among primary blood parasites in the Guba-Khachmaz economic region, and the cestode species *Moniezia expansa* was detected among helminths. Considering the mono and associative spread of these parasites in the economic region and the fact that they cause serious pathology in sheep, the blood parameters of the animals were examined, and the results were analyzed.

MATERIALS AND METHODS

The research was conducted on livestock farms in the Siyazan, Shabran, Khachmaz, Guba, and Gusar districts, which are part of the Guba-Khachmaz economic region. During 2016-2023, peripheral blood smears from 3,435 sheep were examined in the economic region, and primary blood parasites were

found in 1,996 samples (I.E. 58.1%). The dominant species in the blood smears was the parasite *Babesia ovis*. In order to determine the associative invasion of helminths, a coprological study was conducted on 1,996 samples in which primary blood parasites were detected. Of the coprological samples, 1320 showed an associated invasion of *Moniezia expansa* (İ.E. 66.1%). Animals with positive peripheral blood smears underwent a clinical examination, a medical history was taken, and a serological sample was taken from the jugular vein. Serological samples of 20 sheep monoinvasion with the *Babesia ovis* parasite were subjected to hematological and biochemical examination. The examinations were carried out in the early stage of the disease. Serological samples of 25 sheep associated with *Babesia ovis+Moniezia expansa* parasites were subjected to hematological and biochemical examination. For comparison, 10 healthy sheep were examined. To identify the *Babesia ovis* parasite, they were stained with the Romanovsky-Giemsa staining method and microscopically examined at x1000 magnification (Giemsa, 1904; Kapustin, 1955). Coprological samples were examined for helminths using the Fulleborn and Vishnauskas methods. In the examinations, hematological parameters such as total erythrocyte count (TEC), hemoglobin concentration (Hb), packed cell volume (PCV), MCV, MCH, MCHC, platelet count, total leukocyte count (TLC), absolute granulocyte, lymphocyte and monocyte counts were evaluated using a hematological analyzer. Separated serological samples were evaluated and analyzed on a biochemical analyzer for aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, direct bilirubin (free), indirect bilirubin (conjugated), total protein, albumin and creatinine parameters.

Statistical analysis was applied using SPSS (Independent sample t-test), but statistically significant data were defined at a p-value ≤ 0.05 (IBM-SPSS, 2012).

RESULTS

Peripheral blood smear samples from 3435 sheep in the Guba-Khachmaz economic region were examined, and primary blood parasites were detected in 1996 samples (I,E. 58.1%). To determine the associative invasion with helminths, 1996 samples with primary blood parasites were subjected to coprological examination. Associative invasion with *M. expansa* was noted in 1320 of the coprological samples (I.E. 66.1%).

Serological samples from 20 sheep monoinvasion with *Babesia ovis* were subjected to hematological and biochemical examinations. Serological samples from 25 sheep coinfected with *B. ovis+M. expansa* were subjected to hematological and biochemical examinations. For comparison, 10 healthy sheep were examined.

The results of hematological examinations of healthy sheep and sick animals with mono and associative invasion were compared. In sick sheep with *Babesia ovis* monoinvasion, the mean difference in hematological values of blood parameters - PCV, RBC, MCV, Hb, Neutrophils, Monocyte decreased, WBC, MCH, MCHC, Lymphocyte, eosinophil was relatively elevated. In sick sheep with *B. ovis*, the mean difference in PCV, Hb, neutrophil values decreased sharply (p <0.05). Although no significant changes were observed in other indicators - RBC, MCV, Monocyte, WBC, MCH, MCHC, Lymphocyte (p>0.05), the mean difference in eosinophil values increased sharply (p<0.05). In sick sheep with *B.ovis+M.expansa* parasites, the mean difference in PCV, RBC, WBC, MCV, Hb, monocyte, neutrophil, lymphocyte decreased sharply, MCH, MCHC, eosinophil was significantly elevated (p <0.05) (Table 1).

In sick sheep with *Babesia ovis* monoinvasion, the mean difference in biochemical values of blood parameters – ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, creatinine increased, albumin, total protein decreased. In sick sheep with *B.ovis*, the mean difference in ALT, AST, total bilirubin values increased sharply (p<0.05). In other indicators- albumin, total protein, direct bilirubin, indirect bilirubin, creatinine, no

significant changes were observed (p>0.05). In sick sheep with *B. ovis+M. expansa* parasites, the mean difference in ALT, AST values increased sharply, albumin, total protein decreased sharply (p<0.05). Total bilirubin, direct bilirubin, indirect bilirubin, creatinine increased (p>0.05) (Table 2).

Table 1. Hematological parameters of sheep infected with mono and associative invasion

Parameters	Healthy (n=10) Mean ± SE	Monoinvasion (n=20) Mean ± SE	Associative invasion (n=25) Mean ±SE
PCV (%)	32.94±1.11	20.38±0.99	18.24±0.7
RBC (10^6/ul)	7.84±0.21	5.61±0.29	4.56±0.05
WBC (10^3 /ml)	9.29±0.90	6.9±0.30	5.41±0.07
Hb (g/dl)	10.32±0.37	5.81±0.26	3.66±0.19
MCV (fl)	39.52±0.78	37.2±1.75	34.6±1.32
MCH (pg)	10.43±0.20	13.17±0.33	18.45±1,12
MCHC (%)	28.64±0.66	31.35±0.64	35.66±1.88
Netrophils (%)	29.36±1.14	23.12±1.18	21.47±1.93
Lymphocyte (%)	43.08±0.69	39.11±0.84	36.7±1.72
Monocyte (%)	1.19±0.26	0.49±0.10	0.31±0.08
Eosinophil (%)	2.45±0.28	4.92±0.33	7.18±1.24

Table 2. Biochemical parameters of sheep infected with mono and associative invasion

Parameters	Healthy (n=10) Mean ± SE	Monoinvasion (n=20) Mean ±SE	Associative invasion (n=25) Mean ±SE
ALT(IU/L))	27.83±3.19	34.39±1.35	42.27±2.19
AST(IU/L))	141.38±10.68	196.71±18.73	242.4±19.27
Albumin (g/dl)	2.53±0.07	1.87±0.12	0.54±0.08
Total protein (g/dl)	6.42±0.17	4.83±0.33	2.81±0.62
Total Bilirubin (mg/dl)	0.32±0.19	2.68±0.25	3.14±0.49
Direct Bilirubin (mg/dl)	0.20±0.11	0.41±0.15	0.52±0.23
Indirect Bilirubin (mg/dl)	0.12±0.13	1.27±0.13	2.35±0.05
Creatinine (mg/dL)	0.68±0.02	0.89±0.46	1.77±0.32

Analysis of the results of the conducted research shows that changes in blood parameters during monoinvasion in sheep are more pronounced during associative invasion. Changes in these blood parameters lead to aggravation of the general physiological condition of the animal, complication of the pathological process, and ultimately to the death of the animal. The main factor affecting the changes in blood parameters of sick sheep with babesiosis and moniesiosis associated invasion is the result of the direct effect of both parasites on the cells of the reticuloendothelial system. In such an infection, the high intensity of parasites is important as the second main factor. The higher this indicator, the more changes occur in blood parameters. This is a criterion for assessing the parasite-host relationship.

DISCUSSION

Detailed studies have been conducted on the changes observed in blood parameters during babesiosis infection in sheep. Researchers note that the breakdown of erythrocytes during babesiosis leads to an increase in the level of total bilirubin. However, the increase in direct bilirubin is associated with impaired liver function (Sivajothi *et al.,* 2022). A significant increase in the number of total leukocytes and monocytes during babesiosis infection is associated with the phagocytosis process in which the broken erythrocytes are subjected. *Babesia* spp. invasion leads to the stimulation of the body's defense mechanism to produce antibodies against *Babesia* antigens. Leukocytes are produced in the bone marrow and partly in the lymph nodes, the increase or decrease of which is related to the intensity of infection of the animals with the parasite (Sevinc *et al.,* 2013). The increase in total leukocytes may be the result of active immunity, which increases the production of antibodies (Adua *et al.,* 2016).

Babesia parasites cause hemolysis, anoxia and inflammation of the mucous membranes. This leads to an increase in the level of AST, ALT and creatinine

in the liver and kidneys. As a result, cellular damage occurs in the animal's body (Ismail, 2012). A decrease in the albumin level is associated with a pathological process in the liver. Albumin excreted in the urine eventually causes anorexia, accompanied by fever in the animal (Ijaz *et al.,* 2013).

Changes in blood parameters during associative invasion of *B. ovis* and *M. expansa* in sheep have not been sufficiently studied.

Blood parameters were studied only in sheep with monieziasis. In sick animals, hematological parameters were observed: a significant decrease in the level of Hb, PCV and TEC, an increase in the number of neutrophils, eosinophils and the total number of leukocytes (TLC). Biochemical parameters: a significant decrease in total protein, a decrease in the level of albumins and a relative increase in the level of globulins. The activity of the enzymes AST, ALT and ALP was significantly increased (Sharma *et al.,* 2023).

The decrease in the level of total protein in sheep with monieziasis may be due to the inflammatory process caused by monieziosis in the localization organ – the intestine. Since at this time the absorption of protein metabolites in the intestine is impaired (Pandit *et al.,* 2009).

Researchers note that the infection rate of monieziosis in young sheep (up to 12 months) is higher than in older sheep (over 12 months). Young sheep are more susceptible to invasion, since their immunity is weaker than that of older animals (Squire *et al.,* 2019). Compared to age groups, lambs under one year are more susceptible not only to cestodes, but also to other helminths. Therefore, mortality in farms is more often observed in young animals (Azizova, 2024).

CONCLUSION

Studies conducted on associative invasions of sheep show that associative infection with endoparasites causes more serious pathological processes in animals. Sharp changes in hematological and biochemical parameters are pronounced and affect the productivity of animals. Associative invasion of sheep leads to serious losses, such as growth retardation, decreased weight gain and wool quality, as well as death of young animals.

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