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Evaluation of oxidant, antioxidant, interleukin, and biochemical parameters in sheep with cystic echinococcosis and dicrocoeliosis

FATMA ERTAŞ[1], AHMET UFUK KÖMÜROĞLU[2], ÖZLEM ORUNÇ KILINÇ[3], YILDIRAY BAŞBUĞAN[4], NAZMI YÜKSEK[4]

[1]Department of Medical Services and Techniques, Tuzluca Vocational School, Iğdır University, Iğdır, Türkiye
[2]Health Service Vocational School, Van Yüzüncü Yıl University, Van, Türkiye
[3]Department of Medical Services and Techniques, Özalp Vocational School, Van Yüzüncü Yıl University, Van, Türkiye
[4]Department of Internal Medicine, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van 65100, Türkiye

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Ertaş F., Kömüroğlu A. U., Kilinç Ö. O., Başbuğan Y., Yüksek N.

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Summary

Aim: Endoparasitic infections have long posed a significant threat to the sheep industry, leading to substantial economic losses. Among these parasites, *Dicrocoelium dendriticum* and, particularly, *Echinococcus granulosus* are globally important zoonotic parasites. This study aimed to investigate the levels of oxidative stress markers, antioxidant parameters, interleukins, and biochemical indicators, as well as their correlations, in sheep affected by cystic echinococcosis and dicrocoeliosis.

Methods: In this study, various biochemical and immune-related parameters were assessed. Serum samples collected from infected sheep were analyzed using ELISA to measure interleukin-18 (IL-18), interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-4 (IL-4), NGAL, neopterin, myeloperoxidase, SOD, GPX, catalase, GSH, and MDA. Additionally, spectrometric analysis was performed to determine the levels of AST, ALT, GGT, albumin, globulin, total protein, ALP, and iron.

Results: Serum MDA levels were found to be significantly elevated in infected sheep compared to the healthy group. In contrast, serum SOD and GSH levels in infected sheep were significantly lower than those in healthy sheep. Additionally, serum AST and ALT activities were markedly higher in the infected group. Interleukin levels showed no significant differences between the two groups. Overall, markers of liver function and oxidative stress were notably increased in infected sheep.

Conclusion: It was concluded that oxidative stress and liver damage occurred in the infected group, and their immune system was actively involved in the response. This study not only adds to academic knowledge, but should also help veterinarians and breeders working in the field to make more accurate decisions. Thus it is expected to contribute directly to herd health, animal welfare, economic efficiency, and public health. Correlation data may also be useful in guiding the treatment process.

Keywords: antioxidant, oxidant, *Dicrocoelium* spp., *Echinococcus* spp., interleukin

Endoparasites have long posed a significant threat to sheep farming, leading to substantial financial losses (2, 7, 15). Among these parasitic diseases, echinococcosis and dicrocoeliosis present important challenges to the sheep industry (2, 16). Dicrocoeliosis is caused by *Dicrocoelium dendriticum*, which primarily invades the gallbladder and bile ducts of sheep, goats, cattle, and occasionally humans. Infection occurs when ants carrying metacercaria are ingested with food. Cystic echinococcosis (CE) is the larval stage of the zoonotic parasite *Echinococcus granulosus*, whose definitive hosts are carnivores. Many domestic and wild animals, including humans, act as intermediate hosts (16, 37). Sheep infected with CE and *D. dendriticum* exhibit signs such as weakness, loss of appetite, depression, weight loss, and reduced milk production. These diseases result in significant economic losses in certain countries (15, 31, 44).

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Cytokines are small molecular proteins that facilitate intracellular communication (12). They play a crucial role in coordinating defense responses and maintaining homeostasis within the central nervous system (31). Interleukins (IL), a key subgroup of cytokines, are specialized proteins released by leukocytes that stimulate the immune system (12). Cystic echinococcosis (CE) affects primarily visceral organs, particularly the liver and lungs, triggering cell-mediated immune responses. During CE infection, Th1 and Th2 cells actively secrete distinct cytokines from T helper cells, contributing to the immune response (1, 31, 42, 43).

Th1-type cytokines, including IFN-γ, IL-2, and IL-1, are released predominantly during the initial immune response, followed by the production of Th2-type cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13. An experimental study on mice infected with *E. granulosus* reported a significant release of interleukins, indicating an active immune response. Additionally, in sheep with dicrocoeliosis, both humoral and cellular immune responses were triggered, leading to changes in IL levels (17).

Neopterin is a nonspecific marker of activated cell-mediated immunity, associated with the release of interferon-gamma. It may serve as a valuable indicator for assessing disease severity and making a more accure prognosis. However, studies exploring its role in parasitic diseases remain limited (6).

Parasites elevate free radical levels in host cells, triggering lipid peroxidation and oxidative stress, which ultimately leads to cell and tissue damage in the affected organs (11). Malondialdehyde (MDA), a key biomarker of this damage, is widely used to assess oxidative stress. In response, host cells activate antioxidant mechanisms to counteract oxidative stress (1). These mechanisms involve molecules that aim to prevent or minimize the damage caused by free radicals to proteins, lipids, and nucleic acids (30). Key antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX or GSH-Px), and glutathione reductase (GR) (5). Among them, SOD, GSH, and CAT interact with free radicals, preventing their transformation into more harmful forms and inhibiting the formation of new radicals. Additionally, these antioxidants help regulate the transfer of free radicals between cells, further mitigating oxidative damage (3).

Enzyme activity plays a crucial role in diagnosing diseases, assessing their severity, and making prognoses (46). Elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) are commonly associated with liver diseases (14). Myeloperoxidase (MPO), another essential enzyme, serves as a key antimicrobial catalyst in mammalian neutrophils (35). It plays a vital role in the immune system by utilizing hydrogen peroxide and chloride ions to generate hypochlorous acid (HOCl), which effectively eliminates bacteria and pathogens. However, excessive MPO activity has been linked to inflammatory diseases, as it contributes to oxidative stress and tissue damage (29, 35).

Iğdır province of Türkiye is a region where parasitic infections are highly prevalent. However, research on this topic remains limited. Additionally, no studies have been found in the literature that examine the levels of antioxidants, interleukins, and biochemical markers, along with their correlations, in cases of combined parasitic infections. This study aimed to investigate the relationship between interleukins and various biochemical parameters in sheep naturally infected with echinococcosis and dicrocoeliosis. In addition, this study is hoped to produce improvements in early diagnosis and clinical follow-up, detection of infected animals without clinical signs, treatment and protection strategies, understanding the effect of parasitic infections on herd productivity, preventive medicine and vaccination studies, as well as indirect benefits in terms of public health.

Material and methods

This study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Decision No. 2020/07-08 of July 28, 2020). During the study, the ear tag numbers of animals brought to the slaughterhouse were recorded, general health assessments were conducted, and blood samples were collected. Blood was duly collected from the vena jugularis of the sheep and brought to the laboratory in cold chain and centrifuged at 3000 rpm for 10 min. Serum-separated blood was stored in Eppendorf tubes at –20°C until counted.

Common parasites that settle in the liver of sheep include *Fasciola* spp., *Dicrocoelium dendriticum*, and CE, while those that inhabit the gastrointestinal system include nematodes (*Haemonchus contortus*, *Trichostrongylus* spp., *Ostertagia* spp., *Nematodirus* spp.), cestodes (*Taenia* spp., *Moniezia* spp.), and protozoa (*Eimeria* spp., *Giardia* spp.). In this study, 1,500 samples from sheep were examined for these common parasites. The presence of parasite eggs or cysts was diagnosed under a microscope, using native, flotation, and sedimentation techniques for fecal samples. Additionally, the presence of cysts detected through macroscopic examination of the liver and lungs of slaughtered sheep, along with the migration paths of the parasites, was used to support and strengthen the parasite diagnoses.

As a result of these examinations, four groups were established for the study: Healthy, CE (Cystic Echinococcosis), Dicrocoeliosis, and CE + Dicrocoeliosis. Each group consisted of 15 samples, with a total of 60 sheep included in the study. The experiment was conducted in a randomized manner, and the group distribution is presented in Table 1.

Tab. 1. Grouping of sheep based on their infection status

1st group (n = 15)	2nd group (n = 15)	3rd group (n = 15)	4th group (n = 15)
Healthy	CE	Dicrocoeliosis	CE + Dicrocoeliosis

Laboratory diagnostic methods

Diagnostic methods. Healthy group: This group consisted of samples in which no parasites were detected through macroscopic and microscopic analyses.

CE group: During postmortem veterinary inspection of animals slaughtered at the abattoir, cyst fluid was aspirated with a syringe from cysts macroscopically detected in the livers of sheep, and samples in which protoscoleces were observed were considered positive (9). Samples that tested negative for the other parasites mentioned above were also included in this group.

Dicrocoeliosis group: The parasite was diagnosed by identifying the parasite in liver and bile duct sections after slaughter, as well as by detecting its eggs in fecal analysis, and these samples were included in this group (33). Additionally, samples that tested negative for the other parasites mentioned above were also included in this group.

CE + Dicrocoeliosis group: Samples that tested positive for both CE and Dicrocoeliosis, but negative for the other parasites mentioned above, were included in this group.

Biochemical analysis. For serum biochemical analysis, the following parameters were measured by the ELISA method: interleukin-18 (IL-18, catalog number: E0213Sh), IL-6 (catalog number: E0072Sh), IL-10 (catalog number: E0053Sh), IL-4 (catalog number: E0079Sh), NGAL (catalog number: E0059Sh), neopterin (catalog number: E0020Sh), myeloperoxidase (MPO, catalog number: E0005Sh), superoxide dismutase (SOD, catalog number: E0119Sh), glutathione peroxidase (GPX, catalog number: E0120Sh), catalase (CAT, catalog number: E0128Sh), glutathione (GSH, catalog number: E0093Sh), and malondialdehyde (MDA, catalog number: E0039Sh). The serum was obtained and analyzed in the laboratory (BT Lab). Additionally, AST, ALT, GGT, albumin, globulin, total protein, ALP, iron, and iron binding capacity were analyzed using an atomic absorption spectrophotometer (Archem Diagnostics, AR-99B).

Statistical analysis. Experimental data were analyzed using one-way ANOVA, and mean differences were assessed using Duncan's multiple range test at a 5% significance level $\alpha = 0.05$ in SPSS 22. Results were expressed as mean ± standard deviation ($\overline{x} \pm SD$).

To further explore associations between parameters, heat map clustering was applied for visualization (ClustVis online). Principal component analysis (PCA) and correlation analysis were conducted using the PAST and JASP software.

Results and discussion

Biochemical results. The results of this study indicated that the serum SOD activity in sheep infected with *Dicrocoelium dendriticum* was significantly lower than in the healthy group ($p < 0.05$, Tab. 2). However, neither individual nor combined infections resulted in significant variations in SOD levels ($p > 0.05$). Although GPx activity was lower in the healthy group compared to the infected groups, the difference was not statistically significant ($p > 0.05$). Similarly, catalase activity was higher in the healthy group than in the infected groups, but this difference was not significant either ($p > 0.05$). The catalase levels in sheep infected with *Echinococcus granulosus* were lower than those in sheep with dicrocoeliosis or co-infection (CE + Dicrocoeliosis), but this difference was not significant ($p > 0.05$). Like SOD levels, the serum GSH level in sheep infected with *D. dendriticum* was significantly lower than in the healthy group ($p < 0.05$). However, neither individual nor combined infections led to significant differences in GSH levels ($p > 0.05$). Among the non-enzymatic antioxidants, albumin levels were significantly lower in infected sheep compared to the healthy group ($p < 0.05$). However, no significant differences were observed between the infected groups ($p > 0.05$, Tab. 2).

The serum MDA level in the healthy group was significantly lower than in the infected groups ($p < 0.05$). Moreover, the serum MDA levels in sheep infected with CE and CE + Dicrocoeliosis were significantly higher than those in the healthy group ($p < 0.05$, Tab. 3).

Among the biochemical parameters, AST, ALT, and GGT levels were significantly higher in infected sheep compared to the healthy group ($p < 0.05$). In contrast, total protein and globulin levels were significantly lower in infected sheep

Tab. 2. Analysis of antioxidant parameters in the study groups

Parameters	Groups			
	Healthy	CE	Dicrocoeliosis	CE + Dicrocoeliosis
Enzymatic Antioxidants				
SOD	14.93 ± 4.04^{a}	11.93 ± 4.39^{a,b}	10.69 ± 4.10^{b}	12.98 ± 3.19^{a,b}
GPx	34.52 ± 18.55^{a}	50.77 ± 20.47^{a}	41.16 ± 15.98^{a}	42.90 ± 25.42^{a}
CAT	1051.85 ± 66.53^{a}	985.93 ± 195.38^{a}	1012.84 ± 155.86^{a}	1042.98 ± 58.73^{a}
Non-Enzymatic Antioxidants				
GSH	13.27 ± 5.70^{a}	11.10 ± 3.50^{a,b}	8.85 ± 3.46^{b}	10.29 ± 2.05^{a,b}
Albumin	3.48 ± 0.47^{a}	2.18 ± 0.21^{b}	2.34 ± 0.26^{b}	2.23 ± 0.24^{b}

Explanations: SOD – superoxide dismutase; GPx – glutathione peroxidase; CAT – catalase; GSH – glutathione; AL – albumin. In statistical analyses, different letters (a, b) indicate significant differences between groups ($p < 0.05$); a, b – no difference between groups with same letter

Tab. 3. Comparison of MDA levels in the study groups

Parameters	Groups			
	Healthy	CE	Dicrocoeliosis	CE + Dicrocoeliosis
MDA	0.77 ± 0.07^{b}	0.90 ± 0.05^{a}	0.86 ± 0.03^{b}	0.086 ± 0.04^{b}

Explanations: In statistical analyses, different letters (a, b) indicate significant differences between groups ($p < 0.05$); a, b – no difference between groups with same letter

Tab. 4. Comparison of biochemical parameters in the study groups

Parameters	Groups			
	Healthy	CE	Dicrocoeliosis	CE + Dicrocoeliosis
AST	69.43 ± 8.16[b]	113.44 ± 10.60[a]	105.01 ± 8.82[b]	111.90 ± 12.26[a, b]
ALT	29.25 ± 5.23[b]	44.55 ± 5.40[b]	49.60 ± 8.00[a, b]	51.81 ± 8.95[a]
GGT	18.68 ± 3.89[b]	24.72 ± 4.76[b]	25.73 ± 4.83[b]	31.00 ± 4.02[a]
TP	6.66 ± 0.94[a]	4.09 ± 0.48[b]	4.42 ± 0.52[b]	4.19 ± 0.53[b]
Fe	59.00 ± 7.67[ns]	60.11 ± 7.02[ns]	56.46 ± 7.59[ns]	56.45 ± 7.78[ns]
Globulin	3.18 ± 0.46[a]	1.91 ± 0.28[b]	2.08 ± 0.26[b]	1.96 ± 0.28[b]
NGAL	38.11 ± 20.37[ns]	48.62 ± 22.70[ns]	41.52 ± 24.65[ns]	40.28 ± 17.82[ns]

Explanations: AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma glutamyl transferase; TP – total protein; Fe – iron; NGAL – neutrophil gelatinase-associated lipocalin. Different letters indicate significant difference ($p < 0.05$); ns – non-significant

Tab. 5. Comparison of immune markers (interleukins, neopterin, and MPO) in the study groups

Parameters	Groups			
	Healthy	CE	Dicrocoeliosis	CE + Dicrocoeliosis
IL-4	66.12 ± 24.33[ns]	61.76 ± 17.03[ns]	58.51 ± 24.17[ns]	66.78 ± 20.07[ns]
IL-6	41.14 ± 17.40[ns]	50.02 ± 21.05[ns]	51.29 ± 21.26[ns]	47.62 ± 19.88[ns]
IL-10	120.84 ± 42.55[ns]	119.20 ± 49.78[ns]	107.74 ± 57.17[ns]	132.48 ± 32.47[ns]
IL-18	33.27 ± 17.38[ns]	31.77 ± 15.58[ns]	31.77 ± 15.58[ns]	35.78 ± 18.45[ns]
MPO	7.07 ± 3.58[b]	9.76 ± 4.63[a, b]	12.54 ± 4.97[a]	8.60 ± 3.18[b]
Neopterin	9.14 ± 3.11[a, b]	8.24 ± 2.15[b]	10.65 ± 3.03[a]	9.03 ± 2.11[a, b]

Explanations: IL – interleukin; MPO – myeloperoxidase. Different letters indicate significant difference ($p < 0.05$); ns – non-significant

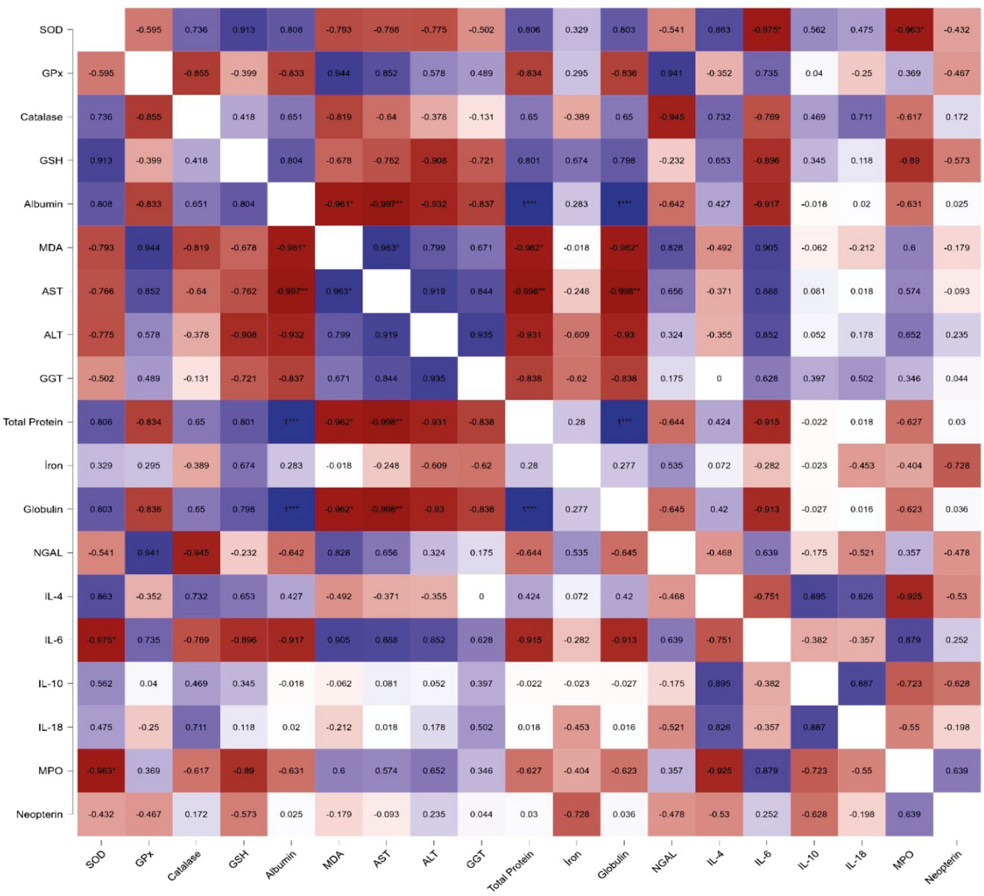

Fig. 1. Correlation matrix for parameters measured in the study groups

($p < 0.05$). However, no significant differences were observed in iron (Fe) content and NGAL levels between healthy and infected sheep ($p > 0.05$, Tab. 4).

There were no significant differences in IL-4, IL-18, IL-10, and IL-6 levels between the groups. However, MPO and neopterin levels were highest in sheep infected with dicrocoeliosis. (Tab. 5).

Multivariate analysis of parameters and treatments. In addition to one-way analysis of variance (ANOVA), the mean values of our data were subjected to a series of statistical analyses to reduce dimensionality, assess correlations, visualize relationships, and clarify the distribution of parameters among the experimental groups. First, a correlation analysis was performed (Fig. 1). According to correlation coefficients, SOD showed a positive correlation with GSH, albumin, MDA, total protein, and globulin ($p < 0.05$). These positive correlations may be attributed to the role of SOD in antioxidant defense, protein synthesis, and lipid peroxidation. However, SOD was negatively correlated with AST ($r = -0.994$; $p < 0.05$), suggesting a potential link between oxidative stress and liver function impairment. A heat map was generated to cluster the experimental groups (Fig. 2). Two main clusters were identified. The first cluster included the healthy group, and the second cluster comprised the infected groups, which were further divided into two sub-clusters. The first subset included CE and Dicrocoeliosis. The second subset consisted of CE + Dicrocoeliosis, indicating a distinct biochemical profile for co-infected sheep. These clustering patterns highlight clear differences between

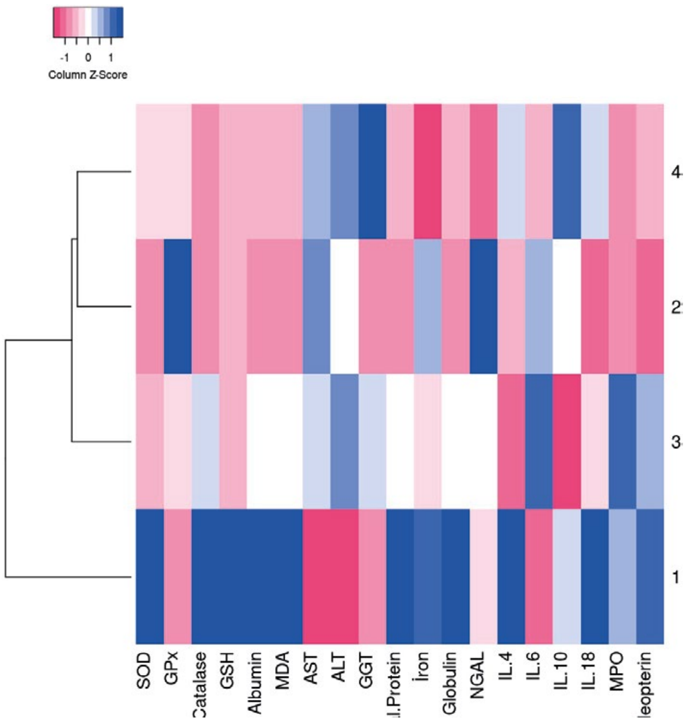

Fig. 2. Heat map-clustering analysis of the antioxidant, biochemical and immune parameters

healthy and infected animals, with co-infected sheep showing unique oxidative stress and biochemical response patterns.

Dicrocoeliosis and cystic echinococcosis (CE) are two significant parasitic diseases affecting both humans and domestic animals. Notably, CE is an important public health concern, prompting WHO to issue warnings regarding its impact. Understanding the pathogenesis of these diseases requires a comprehensive analysis of the host's immune and biochemical responses to parasitic infections. As with all infections, host responses tend to be more aggressive in cases of mixed parasitic infections. Numerous studies have demonstrated that oxidative stress plays a key role in animals infected with parasites (39, 41). This is attributed primarily to increased inflammatory markers and reduced antioxidant levels during parasitic infections (10). In this study, we assessed the inflammatory response, oxidative stress levels, and their correlations with routine biochemical parameters in sheep infected with two liver-damaging parasites, providing insights into their impact on the host's metabolism and immune function.

According to a previous study, serum SOD levels in cattle with cystic echinococcosis (CE) are significantly lower than those in healthy animals (19). This decline in SOD activity has been attributed to the conversion of O_2^- (superoxide anion) into hydrogen peroxide, driven by the excessive production of superoxide anions associated with oxidative stress (27). Similarly, reduced SOD activity has been reported in humans with echinococcosis (25) and fasciolosis (24) and in sheep with dystomosis (13). The decrease in antioxidant capacity due to reduced SOD levels can lead to increased tissue damage and is believed to further enhance free radical production (27). As presented in Table 2, the serum SOD activity in sheep with dicrocoeliosis was significantly lower than in the healthy group ($p < 0.05$). Although serum SOD activity was also lower in sheep with CE and CE + Dicrocoeliosis compared to the healthy group ($p > 0.05$), the difference was not statistically significant. However, SOD levels in the CE and CE + Dicrocoeliosis groups were higher than those in the *Dicrocoelium* spp. group ($p > 0.05$), though the difference was not significant. These findings align with previous studies, underscoring the role of oxidative stress and impaired antioxidant defense in parasitic infections.

A previous study found that GSH levels were significantly reduced in hamsters infected with *Dicrocoelium dendriticum* compared to the healthy group (39). In the current study, GSH levels in sheep with dicrocoeliosis were also significantly lower than those in the healthy group ($p < 0.05$). Although GSH levels were also lower in CE and CE + dicrocoeliosis groups, the difference was not statistically significant ($p > 0.05$). This reduction in GSH levels is thought to be linked to the toxic accumulation of lipid peroxidation products, such as MDA, as observed in the correlation analysis ($r = -0.994$; $p < 0.05$). The depletion of GSH, a key antioxidant, may contribute to increased oxidative stress and further tissue damage in infected animals. Studies have reported both increases and decreases in antioxidant enzyme levels in various diseases (36). One such study observed increased glutathione peroxidase (GPx) activity in the erythrocytes of cattle with CE (19). Similarly, another study reported an increase in GPx activity in hamsters infected with *Dicrocoelium dendriticum* (41). Parallel GPx elevation trends have also been noted in sheep with fasciolosis (8) and dystomatosis (13). However, conflicting findings exist. For example, a study found that erythrocyte GPx activity in camels with CE was significantly lower in cases of liver infection (22), while no significant difference in GPx levels was observed between infected and healthy sheep (21). Moreover, decreased GPx activity has been reported in humans infected with CE (25, 28). The increase in GPx levels may be attributed to oxidative stress parameters stimulating the gene expression of antioxidant-responsive elements (30). Elevated GPx activity is often associated with potential liver damage, reflecting the liver's adaptive response to scavenge excess reactive oxygen species (ROS) and mitigate oxidative stress (13). This adaptive increase in GPx activity has been previously reported in various conditions associated with oxidative damage, such as acute exercise or liver infections (23). Therefore, it can be suggested that the elevated serum GPx activity observed in infected sheep serves as a protective defense mechanism against oxidative damage (22). In the present study, GPx activity in the healthy (uninfected) group was lower than in the infected groups, although the difference was not statistically significant ($p > 0.05$). This trend may indicate an adaptive response in infected animals to mitigate the effects of oxidative stress induced by parasitic infections.

Previous studies have reported that albumin levels were significantly lower in sheep with cystic echinococcosis (CE) compared to healthy sheep. It has been suggested that during oxidative stress, the liver's ability to synthesize this free radical-scavenging antioxidant may be impaired (20). Similarly, Samadieh et al. (40) and Manga-González et al. (32) found that albumin levels in sheep with dicrocoeliosis were significantly lower than those in healthy animals. In the present study, serum albumin levels were also significantly lower in the infected group compared to the uninfected group, a finding that aligns with previous research. This reduction is attributed to liver damage and oxidative stress caused by parasitic infections. Among oxidative stress markers, MDA has been widely recognized as a biomarker of oxidative stress severity (16). Increased MDA concentrations have been reported in the chronic phase of fascioliasis in rats (32), humans (24), and sheep (16). Additionally, Samadieh et al. (40) demonstrated that serum MDA levels were significantly higher in sheep with dicrocoeliosis compared to the healthy group. Consistent with these findings, the present study (Tab. 3) revealed that serum MDA levels in the healthy group were significantly lower ($p < 0.05$) than in sheep infected with CE, dicrocoeliosis, or mixed infections. These results reinforce the conclusion that oxidative stress and tissue damage are key pathological processes occurring during parasitic infections, in agreement with previous research.

Liver tissue and bile duct damage have been reported to influence the levels of certain liver enzymes (16). Consistent with these findings, Kolodziejczyk et al. (26) and Ertaş & Kırmızıgül (16) reported that an increased lipid peroxidation rate in infected sheep was associated with elevated serum AST and ALT activities, both of which are key markers of liver damage. It has been suggested that the elevated levels of these enzymes may result from a pathogenic mechanism linked to metabolites released by the parasite, which are directly hepatotoxic (26). In the present study (Tab. 4), serum AST and ALT activities in the healthy group were significantly lower than in the infected groups. The higher enzyme levels in infected sheep suggest the presence of liver tissue damage, reinforcing the association between parasitic infections and liver dysfunction. Additionally, Ertaş & Kırmızıgül (16) reported that GGT enzyme levels were significantly higher in sheep with fasciolosis compared to healthy animals, indicating damage to the bile ducts. This further supports the notion that liver and biliary injury play a crucial role in the pathophysiology of parasitic infections.

It is well established that interleukin levels vary across different diseases (34, 48). In echinococcosis, an immune response is not typically activated during the acute phase. However, in the chronic phase, a Th2 response is triggered, leading to an increase in IFN-gamma, IL-4, and IL-6 levels (48). According to one study, an early increase in Th1 interleukins, such as IL-2, IFN-gamma, IL-15, and TNF-alpha, was observed in mouse models with CE. These interleukins play a lethal role against the parasite. However, as the infection progresses, the Th1 response gradually shifts to a Th2-type response, which has been reported to stimulate the expression of anti-inflammatory interleukins, including IL-4, IL-10, IL-5, IL-6, and IL-13. This shift is believed to favor the survival of parasites by promoting immune tolerance and reducing the host's resistance (34).

Similarly, another study demonstrated that IL-4 and IL-10 levels were elevated in infections with *Echinococcus granulosus* in both humans (47) and experimental mouse models (38). In the present study, serum interleukin levels were found to be lower in the healthy group compared to the mixed infection group, while remaining similar to the levels observed in the other infected groups. However, these differences were not statistically significant (Tab. 5). Despite the absence of a significant difference between groups, the findings suggest that mixed infections may induce a more intense immune response, potentially due to the synergistic impact of the two parasites on the host's immunity.

Gaafar et al. (18) investigated myeloperoxidase (MPO) levels and reported that this parameter increases during the early stages of helminth infections. Similarly, Tischendorf et al. (45) observed alterations in neutrophil and/or monocyte-macrophage activity, suggesting an immune system response to infection. Supporting these findings, Atlıhan et al. (4) reported elevated serum MPO activity in mice infected with *Echinococcus granulosus*. They suggested that this increase reflects the functional activity of the leukopoiesis system, which plays a crucial role in host defense mechanisms against helminth infections. Consistent with these studies, the present study (Tab. 5) found that serum MPO levels in the healthy group were significantly lower than those in the *Dicrocoelium* spp. and CE + *Dicrocoelium dentriticum* groups ($p < 0.05$). This increase in MPO is likely due to increased production of reactive oxygen species (ROS) and hypochlorous acid (HOCl), resulting from neutrophil degranulation in response to liver tissue damage caused by the parasitic infections.

According to correlation analyses performed in this study, there was a negative correlation between SOD and AST. This relationship may be attributed to the role of SOD in antioxidant defense. SOD helps reduce oxidative stress, thereby minimizing liver and heart damage, which in turn is reflected in lower AST levels. Catalase (CAT) showed a positive and significant correlation with albumin, MDA, globulin, and neopterin, but no significant correlation with other parameters. This suggests that catalase plays a key role in antioxidant defense and in the regulation of oxidative stress markers in the body. Additionally, GSH was negatively correlated with AST and positively correlated with total protein ($p < 0.05$), indicating its protective role in liver function and protein metabolism. Albumin was positively correlated with MDA, total protein, and globulin, but negatively correlated with AST, which highlights its function in antioxidant defense and protein homeostasis. MDA showed a negative correlation with AST, but a positive correlation with total protein and iron, suggesting that lipid peroxidation (indicated by MDA) is associated with increased protein metabolism and iron utilization. A negative correlation was observed between AST, total protein, and globulin, likely due to the role of AST as a marker of liver and heart damage, which can impair protein synthesis in the body. Furthermore, GGT and iron showed a negative correlation, which may be explained by the pro-oxidant activity of GGT in the presence of iron, the influence of dietary factors, and the relationship between GGT levels and liver damage. Lastly, a positive correlation was found between total protein and globulin, which confirms their interconnected roles in immune function and protein metabolism.

The analysis of correlations between interleukins revealed a negative correlation between IL-4 and IL-6. This relationship may be explained by the inhibitory effects of IL-6 on Th2 cell polarization and IL-4 production, as well as the antagonistic interaction between IL-6 and IL-12 in the regulation of immune responses. IL-6 is known to promote inflammatory Th1 responses, while IL-4 plays a key role in Th2-mediated immunity, suggesting a balancing mechanism between pro-inflammatory and anti-inflammatory pathways in the immune response.

In this study, SOD, GSH, albumin, AST, ALT, MDA, and MPO levels were found to be significantly lower in infected sheep compared to the healthy group. Although interleukin levels were higher in the mixed infection group, the differences were not statistically significant. These findings suggest that oxidative stress develops in infected sheep, characterized by low antioxidant levels and high oxidant parameters. The changes found in liver-related biochemical markers indicate potential liver injury, while alterations in interleukin and MPO levels suggest a significant impact on immune balance. Identification of relationships between biochemical, oxidative stress, and immune parameters may simplify the diagnostic process and increase the accuracy of disease assessment and management. This study not only adds to academic knowledge, but should also help veterinarians and breeders working in the field to make more accurate decisions. Thus it is expected to contribute directly to herd health, animal welfare, economic efficiency, and public health.

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Corresponding author: Fatma Ertaş; e-mail: fatma.ertas@igdir.edu.tr