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Investigation of some biochemical and haematological parameters in sheep infected with *Dicrocoelium dentriticum*

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ABSTRACT

Objective: The aim of this study was to investigate the changes in some biochemical and haematological parameters due to liver damage caused by parasites in sheep infected with *Dicrocoelium dendriticum*.

Material and Methods: The study was conducted on 10 healthy sheep and 60 sheep infected with *D. dendriticum*. After the blood was taken from the sheep brought to the slaughterhouse in Van Municipality, the liver and gall bladder were examined after the slaughter, and the blood of sheep infected with *D. dendriticum* was brought to the laboratory and examined for some haematological and biochemical parameters.

Results: As a result of the examination, some changes were observed in the biochemical and haematological parameters. In statistical analysis: Haematologically compared with the healthy group of sheep infected with *D. dentriticum*; WBC, Neu, Eo and Mon values were higher than the control group's values, while RBC, Hct, Hb and MCV values were lower and no significant change was found in the Lym level.

Results of biochemical parameters of sheep infected with *D. dentriticum* according to statistical analysis; ALT, AST and GGT values were higher than of the health group sheep, while serum TP, Alb and glucose values were found than lower.

Conclusion: As a result; WBC, Neu, Eo, Mon, ALT, AST and GGT levels in infected sheep due to dicrocoeliasis which cause important pathological disorders in liver that have functions such as synthesis, metabolism and detoxification in the organism were found to be significantly higher compared to the same values of the healthy group. It is predicted that these parameters may be useful in determining the degree and prognosis of liver damage and will shed light on the studies to be performed in this field.

Keywords: *Dicrocoeliosis, Sheep, Biochemical parameters, Haematologic parameters*

INTRODUCTION

Hepatic trematode infections in sheep cause significant economic losses due to slow growth, decreased meat, milk and wool yields, suppression of the immune system and death of animals in severe infections. *Dicrocoelium dentriticum* Rudolphi, 1819, one of the liver trematodes, causes dicrocoeliasis. Dicrocoeliasis is an important disease that causes liver damage in ruminants and causes significant economic losses in countries where sheep, goat, buffalo (Alvarez et al., 2009) and cattle breeding worldwide (Yang et al., 1998).

In dicrocoeliasis infections, it can cause severe infection in cases where the number of parasites is too high in the definitive host (Boch and Supperer, 2000; Akyol, 2001).

There is a direct relationship between the number of parasites and the symptoms that occur in animals

with dicrocoeliasis (Jithendran and Bhat, 1996). Experimentally, hepatic enzyme activities such as aspartate amino transferase (AST) and alanine amino transferase (ALT) were observed in lambs infected with 1000 and 3000 metacercariae of *D. dendriticum*. A significant correlation was found between increased hepatic enzyme activities and parasite density, and the highest activity was reported in lambs with the highest parasitic number (Manga-González and Ferreras, 2014).

Pathological injuries to the liver and gallbladder of recent hosts with dicrocoeliasis are probably caused by the toxic metabolites of the parasite and mechanical stimulation of the bile duct walls (Manga-González and Ferreras, 2014). *Dicrocoelium dendriticum,* AST, ALT, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin and total bilirubin levels increase. Infection has been reported to cause an increase in leukocyte and neutrophil counts and a decrease in lymphocyte count (Sanchez-Campos et al., 1999; Gonzalez-Lanza et al., 2000; Otranto and Traversa, 2002). In addition, it has been reported that malondialdehyde (MDA), an indicator of lipid peroxidation, increases and this causes oxidative stress (Şimşek et al., 2006).

Haematological and biochemical parameters in animal health are an important diagnostic tool for the assessment of disease by veterinarians and are also important for assessing the effectiveness of treatment in infected animals. Changes in enzyme activity in the blood are used in the clinical diagnosis of many diseases. Tissue damage in invasions caused by parasites such as *D. dendriticum* result in changes in serum enzyme activities. Especially in the toxic destruction of the liver, changing enzyme activities in the serum before shaping the clinical picture is of great importance in terms of early detection (Dağoğlu et al., 1995).

Dicrocoeliasis can also be diagnosed with ELISA, immunoelectrophoresis techniques, and changes in the liver's pathology in recent years, as well as the detection of typical trematode eggs in the stool (Ducháček and Lamka, 2003).

The aim of this study is to reveal the changes occurring in some haematological and biochemical parameters in naturally infected sheep with *D. dentriticum*, which causes significant pathological disorders in the liver.

MATERIALS and METHODS

After the anamnesis and general examinations of sheep brought to the slaughterhouse of Van Municipality, 5 ml blood samples were taken from Vena jugularis for haematological and biochemical analyses and transferred to anticoagulant and non-anticoagulant tubes. 30-50 grams of feces were taken from the rectum for stool examination. As a result of liver, gallbladder and fecal examinations, 60 sheep blood detected as infected by dicrocoeliasis were identified as infected group, while 10 sheep blood that were detected negative for dicrocoeliasis as a result of liver, gallbladder and stool examinations were determined as healthy group.

Fecal examination

After the liver and gall bladder were examined, the stool samples of the sheep detected to be infected were brought to Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Parasitology, and kept at –20 °C until necessary analyses were made. Benedek sedimentation method was used in the examination of stool samples (Toparlak and Tüzer, 1994).

Haematological analysis

Blood samples taken into tubes with anticoagulants were brought to Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Medicine on the same day and red blood cell (RBC), white blood cell (WBC), lymphocyte (Lym), monocytes (Mon), neutrophil (Neu), eosinophil (Eo), MCV (mean cell volume), hematocrit (Hct) and hemoglobin (Hb) haematological parameters were analysed by blood counting device (Veterinary MS4-S-Melet).

Biochemical analysis

Blood samples taken into anti-coagulated tubes were centrifuged at 3000 g for 5 minutes. The sera obtained were stored at -20 °C until biochemical parameters were analysed. ALT, AST, GGT, GLU (Glucose), albumin (Alb) and total protein (TP) levels were measured by commercial kits using auto analyser (BS-120 Vet-Mindray).

Statistical Analysis

Descriptive statistics for the characteristics of haematological and biochemical parameters of infected and healthy sheep with *Dicrocoelium dentriticum*; Mean, standard deviation were given as minimum and maximum values. Student's t test was used to compare the groups. Pearson correlation analysis was performed to determine

linear relationships between variables. Statistical significance level was accepted as 5% and SPSS (ver: 13) statistical program was used for all statistical calculations.

RESULTS

The results of haematological parameters of sheep infected with healthy sheep group and *D. dentriticum* are given in Table 1. In statistical analysis; WBC, Neu, Eo and Mon values of sheep infected with *D. dentriticum* were higher than those of the healthy sheep group, while RBC, Hct, Hb and MCV values were lower than the healthy sheep group.

Table 1. Haematological parameters of healthy and *Dicrocoelium dentriticum* infected sheep

Parameters	Healthy Group	Infected Sheep	p<
WBC (m/mm³)	8.60 [a]	14.12[b]	0.001
Lym (m/mm³)	3.64 [a]	4.31[a]	0.057
Mon (m/mm³)	0.57 [a]	0.69 [b]	0.001
Neu (m/mm³)	5.16 [a]	9.19 [b]	0.001
Eo (m/mm³)	0.39 [a]	0.74 [b]	0.001
RBC (m/mm³)	10.41 [a]	9.13 [b]	0.001
MCV (m/mm³)	27.91 [a]	23.93 [b]	0.001
Hct (%) (m/mm³)	26.33 [a]	22.38 [b]	0.001
Hb (g/dl) (m/mm³)	12.94 [a]	8.77 [b]	0.001

a, b: p<0.05 and a, a: p>0.05 were defined as statistical significance between the parameters on the same line and named with different letters

Table 2. Biochemical parameters of healthy and *Dicrocoelium dentriticum* infected sheep

Parameters	Healthy Group	Infected Sheep	p<
ALT (U/L)	17.17 [a]	32.08[b]	0.001
AST (U/L)	92.77 [a]	154.68 [b]	0.001
GGT (U/L)	52.24 [a]	81.21 [b]	0.001
TP (g/dl)	6.57 [a]	4.79 [b]	0.001
Alb (g/dl)	2.64 [a]	1.71 [b]	0.001
GLU (mg/dl)	71.75 [a]	59.18 [b]	0.004

a, b: p<0.05 was defined as the statistical significance between the parameters on the same line and named with different letters

Results of biochemical parameters of sheep infected with healthy sheep group and *D. dentriticum* are given in Table 2. According to statistical analysis, ALT, AST and GGT values of sheep infected with *D. dentriticum* were found to be high according to the same parameters of healthy sheep, while serum TP, Alb and glucose (GLU) values were found low.

DISCUSSION

Studies on haematological and biochemical parameters in sheep that are naturally infected with *D. dendriticum* are limited and little information is available. Changes in biochemical parameters of animals have been reported in parasitic infections (Değer et al., 1997; Şahin and Akgül, 2006). Experimentally, an increase in serum ALT and AST levels has been reported in mice infected with *D. dentriticum* (Sánchez-Campos et al., 1999). Yuksek et al. (2007), reported that there was an increase in serum ALT and AST levels in sheep infected with endoparasites. In *D. dentriticum* infected sheep, RBC, Lym, Hb and Hct values were significantly lower compared to the same parameters of healthy animals, WBC, neutrophil, eosinophil and MCV values were significantly higher than infection. Also, no significant changes were reported in MCH, monocyte and basophil counts. (Kaneko et al., 1997; Kramer, 2000; Matanović et al., 2007). In this study, in addition to the changes in serum Tp, Alb, GLU, AST, ALT and GGT levels in sheep infected with *D. dentriticum*; changes in WBC, Lym, Mon, Neu, Eo, RBC, MCV, Hc and Hb concentrations were examined. In the study, WBC, Neu, Eo and Mon levels of sheep infected with *D. dentriticum* were significantly higher than healthy sheep group. In addition, RBC, Hct, Hb and MCV of infected sheep were significantly lower than those of the healthy sheep group, but there was no significant change in Lym. In our study, haematologic data obtained from sheep infected with *D. dentriticum* were similar to those of the researchers (Kaneko et al., 1997; Kramer, 2000; Matanović et al., 2007).

D. dentriticum, which is the liver trematode of sheep, causes damage to the liver parenchymal tissue and fibrosis in the bile duct (Calleja et al., 2000). Serum AST and LDH activities reflect damage during the passage of young parasites throughout the liver parenchyma, while GGT increases provide information on the penetration of trematodes in the bile ducts (Gonzalo-Orden et al., 2003). Serum ALT is an enzyme normally found only in hepatocytes, while the AST enzyme is restricted to hepatocytes, red blood cells, cardiac and skeletal muscles; GGT is limited to hepatocytes; ALP bile ducts, bone and an intra hepatic enzyme found in the lining cells of the placenta. While serum ALT is normally found only in hepatocytes, AST enzyme is limited to hepatocytes in hepatocytes, red blood cells, cardiac and skeletal muscles, while GGT is limited to hepatocytes, while ALP and an intra-hepatic enzyme found in the lining cells of the placenta.

These enzymes are used as biological markers of hepatic disorders (Manga-González et al., 2004; Al-Hadithy et al., 2013). Serum glutamate dehydrogenase (GLDH) and gamma glutamyl transpeptidase (GGT) have been reported as the most sensitive markers of liver cell damage (Anderson et al., 1977). Some investigators have reported that plasma GLDH and GGT activities are more sensitive indicators of liver cell damage than AST activity in chronic and subclinical dicrocoeliasis (Sykes et al., 1980). However, GGT stability is reported to be a more important indicator in determining the level of damage in liver tissue due to the higher trematode infections (Blood et al., 1989; Gonzalo-Orden et al., 2003; Phiri et al., 2007; Raadsma et al., 2008). This decrease in plasma enzyme activities can be used to monitor the effectiveness of treatment in trematode infections (Gaasenbeek et al., 2001). In this study, ALT, AST and GGT values of sheep infected with *D. dentriticum* were found to be higher than those of healthy sheep. Increases in ALT, AST, and GGT concentrations of sheep infected with *D. dentriticum* have been investigated by researchers (Gaasenbeek et al., 2001; Gonzalo-Orden et al., 2003; Phiri et al., 2007; Severin et al., 2012).

Liver trematodes reduce glycogen reservoir in organ due to tissue damage both in hepatocytes and during migration of parenchymal tissue (Phiri et al., 2007). In studies on liver diseases, serum glucose levels are reported to be significantly lower than in healthy animals (Yadav and Sharma, 1986; Baghshani et al., 2012). In this study, the serum GLU levels of sheep infected with *D. dentriticum* were found to be lower than the healthy sheep group. It is thought that low GLU concentration in sheep with dicrocoeliasis may result from the use of GLU by *D. dentriticum* and the lack of sufficient glycogen reservoir due to hepatic dysfunction caused by *D. dentriticum* infection. The data obtained on the serum GLU levels of sheep infected with *D. dentriticum* support the data of the researchers (Yadav and Sharma, 1986; Phiri et al., 2007; Baghshani et al., 2012).

Hypoalbumin has been detected in hepatic infections and injuries and liver trematode infections (Thomas, 2000; Bosy-Westphal et al., 2001; Matanović et al., 2007). In studies, biochemical parameters in non-infected healthy sheep group were reported to be within the reference range (Kaneko et al., 1997; Kramer, 2000). In the study of sheep with dicrocoeliasis, serum total protein and albumin concentrations were reported to be lower than in healthy animals (Thomas, 2000; Matanović et al., 2007). In this study, total serum and albumin levels of sheep with dicrocoeliasis were found to be low, and it is in line with the results of the investigators (Thomas, 2000; Matanović et al., 2007).

CONCLUSION

D. dendriticum, which can be overlooked in organ aspects due to its morphological small size, causes both loss of yield in live animals and destruction of the liver due to the damage it causes in the liver, and in the case of infection with this parasite in animals, disturbances in the body's natural chemistry can occur. In this study, changes in some biochemical parameters in sheep with dicrocoeliasis were examined and evaluated statistically. In particular, increase in WBC, Neu, Eo and Mon values and decrease in RBC, Hct, Hb and MCV values were found to be statistically significant.

In the study, it has been determined that some haematological and biochemical parameters cause significant changes in sheep infected with *D. dentriticum* and we believe that it will shed light on the future studies.

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