Investigation of serum Amyloid A, haptoglobin and fibrinogen levels in sheep infected with *Fasciola hepatica*

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Abstract

The present study aimed to determine the changes in serum haptoglobin, serum amyloid A and fibrinogen levels in pathological disorders of sheep liver with *Fasciola hepatica*. Sixty sheep (50 with fascioliasis and 10 healthy) were used in the present study. Statistical analysis results demonstrated that SAA, Hp and fibrinogen concentration levels of the sheep infected with Fasciola hepatica were higher when compared to SAA, Hp and fibrinogen concentration levels in healthy sheep ($P < 0.05$). While ALT, AST and GGT values of sheep infected with *Fasciola hepatica* were found to be higher than those of the healthy sheep, serum TP, ALB and glucose levels were lower. In statistical analysis, it was found that WBC, Neu and Eo values of sheep infected with *Fasciola hepatica* were higher when compared to WBC, Neu and Eo values in the control group. In conclusion, serum haptoglobin, serum amyloid A, and fibrinogen levels were significantly higher in sheep infected with Fasciola hepatica when compared to the healthy group. Due to the fact that increases in serum haptoglobin, serum amyloid A and fibrinogen levels in sheep with fasciolosis were similar to those observed in liver disorders, it was suggested that these parameters could be useful in determination of the liver damage levels and prognosis and in studies on liver disorders.

Keywords: sheep; *Fasciola hepatica;* haptoglobin; serum Amyloid A; fibrinogen

Introduction

Livestock industry is an important and traditional sector in the agricultural economy of Turkey (Tuncer *et al* 2018, Tuncer 2019). Fasciolosis is a liver disease caused by trematodes of the *Fasciolidae* family, especially by the *Fasciola hepatica* and *F. gigantica* species (Tınar and Korkmaz, 2003; Zhang *et al.,* 2005). Turkey is suitable for propagation of *Fasciola* species because of its climatic and ecological factors, but only *F. hepatica* and *F. gigantica* have been reported in Turkey. Fasciolosis leads to significant pathological disorders in the liver (Toparlak and Gül, 1988; Gargılı *et a.,l* 1999).
Acute-phase proteins are produced by liver (Ceciliani *et al.,* 2012). The function of these proteins is to protect the organism from further injuries, to eliminate infectious agents, to remove harmful molecules and residues for the organism, to activate a repair process necessary for the organism to recover its normal functions, and to maintain homeostasis (Pfeffer *et al.,* 1993; Ulutas and Ozpinar, 2006). Several studies have been conducted on acute-phase protein levels in various diseases in veterinary science (Ganheim *et al.,* 2007), and it was reported that serum amyloid A (SAA) was one of the initial acute-phase proteins in *Theileria annulata* induced infections in cattle and could be used as an indicator of inflammatory reaction. Biochemical parameter levels are used as significant reference in the assessment of animal health and diagnosis of disease (Kragsbjerg *et al.,* 1995).

Fibrinogenis a substrate promoter for fibrin formation and has tissue-production, restorative and clot-forming tasks that mediate the migration of inflammatory cells to the site of inflammation (Gruys *et al.,* 2005). Fibrinogen is synthesized by the liver in reaction to the acute-phase response (Cray *et al.,* 2009) and its levels in healthy cattle vary between 200 and 700 mg/dl (Gruys *et al.,* 1994). Fibrinogen levels generally increase in infectious, purulent, traumatic, and neoplastic diseases and are used as a nonspecific marker for the determination of tissue damage and inflammation when calculated along with the erythrocyte sedimentation rate (Gruys *et al.,* 2005). Fibrinogen has been used as an indicator of inflammatory disease in cattle for several years (Eckersall and Conner, 1988). Albumin is responsible for 75% of the plasma osmotic pressure and is the main amino acid source that can be used by the animal when necessary (Cerón *et al.,* 2005). It has been reported that it can be used to evaluate the effect of collection and transportation stress on animals infected with experimental *Dictyocaulus viviparus* (Ganheim *et al.,* 2004).

Although a specific elevation in liver enzymes and changes in other serum metabolites due to common liver parasite infections (*Fasciola hepatica*) have been extensively investigated in ruminants, there are no studies on serum haptoglobin, serum amyloid A, and fibrinogen levels in sheep. The present study aims to determine the changes in serum haptoglobin, serum amyloid A, and fibrinogen levels in sheep with fasciolosis that leads to important pathological disorders in the liver which is responsible for synthesis, metabolism, and detoxification.

Material and Methods

The present study was conducted on a total of 60 sheep from four sheep flocks belonging to different private farms. Fifty sheep were naturally infected with *Fasciola hepatica* and *Fasciola hepatica* antibodies were present in serum samples obtained from blood samples determined by using ELISA kit. Besides 10 clinically healthy sheep were used as control group. According to ELISA tests, *Fasciola hepatica* was negative in control group. After determining Fasciolosis and control groups of the study. Stool samples of approximately 30-50 g were collected from the rectum of sheep with probable fascioliasis into stool collection containers. Samples were numbered based on pedigree information. The samples were transported to the laboratory and kept at -20 °C until the time of the tests. The Benedek sedimentation method was used in the examination of the stool samples (Toparlak and Tüzer 1994). This research was approved (28/12/2017 and 27552122-604.01.02-E.91877) by the Animal Research Ethics Committee of Van Yuzuncu Yil University in Van, Turkey.

Hematological analysis

Blood samples were taken from the jugular vein into EDTA tubes and stored at +4°C. Samples were analyzed within 4 h. Total white blood cells (WBC), lymphocyte (Lym), monocytes (Mon), neutrophil (Neu), eosinophil (Eo) red blood cells (RBC), mean corpuscular volume (MCV), hematocrit (Hct), and hemoglobin (Hb). Hematologic parameters were determined by using a blood count device (Veterinary MS4-S-Melet Schloesing Laboratories in France).

Biochemical analysis

Blood was drawn from jugular vein into anti-coagulant free tubes. Blood samples in the anticoagulant-free tubes were centrifuged at 1400 × g for 10 min (Rotofix32®-Hettich), and their serums were extracted. Sera were frozen in Eppendorf tubes at −20°C. Serum haptoglobulin (Bovine Haptoglobin ELISA Kit, Catalog No: 201-04-0121), Serum amyloid A (Bovine SAA ELISA Kit, Catalog No: 201-04-0126), and fibrinogen levels (Bovine Fibrinogen, ELISA Kit, Catalog No: 201-04-0086) were determined with ELISA device (ELISA reader®-DAS) based on the method specified in commercial test kits. Serum alanine transaminase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), total protein (TP), albumin (Alb) and glucose (GLU) levels were measured with auto analyzer (BS-120 Vet-Mindray).

Statistical Analysis

Descriptive statistics on biochemical parameters for the sheep with fascioliasis and healthy sheep were expressed as Mean, Standard Deviation, Minimum and Maximum values. Kruskal-Wallis test was used to compare the biochemical parameters between the groups. Duncan multiple comparison test was used to identify differences between the groups. Spearman correlation coefficient was calculated separately for the groups to determine the correlation between these variables. The statistical significance level was accepted as 5% and the SPSS statistical software (ver 2.0) was used for the calculations.

Results

Hematological parameters are summarized in Table 1. In statistical analysis, significantly lower number of RBC, Hb, Hct, and MCV were recorded in sheep from the infected with *Fasciola hepatica*, whereas WBC, Neu, and eosinophils were significantly higher than in the control group. There were no significant changes in lymphocytes, and monocyte count between the infected and non-infected group. Biochemical results are presented in Table 2. In sheep sera from the infected with *Fasciola hepatica* had significantly higher ALT, AST and GGT levels, serum total protein, albumin and glucose values were significantly lower than in control group. Serum amyloid A, Hp and fibrinogen concentration levels of sheep infected with *Fasciola hepatica* were higher than control group ($P < 0.05$).

Table 1. Hematological parameters in sheep infected with *Fasciola hepatica* and healthy sheep

Parameter	Control $\bar{x} \pm SD$	*F. hepatica* $\bar{x} \pm SD$
WBC ($10^3/mm^3$)	8.65±0.69[a]	14.59±2.84[b]
Lym(10^3/mm3)	2.33±0.53[a]	3.45±2.31[a]
Mon.($10^3/mm^3$)	0.69±0.20[a]	0.64±.19[a]
Neu($10^3/mm^3$)	5.40±0.71[a]	8.96±2.25[b]
Eo($10^3/mm^3$)	0.37±0.23[a]	0.75±0.10[b]
RBC($10^6/mm^3$)	10.31±1.52[a]	9.37±0.44[b]
MCV	27.73±1.28[a]	27.15±2.01[b]
Hct (%)	28.96±4.61[a]	24.40±2.13[b]
Hb (g/dl)	11.26±1.98[a]	9.10±0.93[b]

$\bar{X} \pm SD$: mean ± standart deviation; **a, b:** Different lower cases in the same column represent statistically significant differences ($P<0.05$).

Table 2. Biochemical parameters in sheep infected *Fasciola hepatica* and healthy sheep

Parameter	Control $\bar{x} \pm SD$	*F. hepatica* $\bar{x} \pm SD$
ALT (U/L)	18.64±3.44[a]	32.45±6.77[b]
AST(U/L)	105.36±9.88[a]	167.79±65.36[b]
GGT(U/L)	53.58±5.91[a]	87.07±23.13[b]
TP (g/dl)	6.36±1.32[a]	4.73±.88[b]
Alb (g/dl)	2.61±0.79[a]	1.62±0.64[b]
GLU (mg/dl)	60.65±13.10[a]	46.41±12.16[b]
Serum Amyloid A (ng/L)	2.68± 0.21[a]	16.29±8.18[b]
Fibrinogen (ng/L)	7.80±3.13[a]	16.65±5.80 [b]
Haptoglobin (ng/L)	76.08±39.99[a]	185.86±91.40 [b]

Discussion

The aim of the present study was to determine the changes in serum haptoglobin, serum amyloid A, and fibrinogen levels in sheep with fasciolosis that lead to significant pathological disorders in liver which has functions such as synthesis, metabolism, and detoxification.

Fasciolosis is diagnosed with clinical and autopsy findings, stool examination methods, biochemical analyzes, imaging techniques and serologic methods in animals (Tınar and Korkmaz, 2003). Furthermore, it was reported that the changes in serum AST, GLDH and GGT activities are important in the assessment of the different stages of *F. hepatica*

infection (Benchaoui *et al.,* 1993; Yang *et al.,* 1998). In another study, it was reported that the increases in GLDH and GGT activities were sensitive markers for liver damage due to migration of developing trematodes (Vengušt *et al*., 2003), while it is known that AST is a less sensitive marker for liver injury (Sykes *et al.,* 1980; Kozat and Denizhan, 2010). Ferre *et al* (1994) reported that the increases in AST and GLDH enzyme activities occurred during the migration of young trematodes through the liver parenchyma, while GGT increased during the penetration of trematodes into the bile ducts. In the present study, AST, ALT and GGT serum activities in sheep infected with *F. hepatica* were significantly higher when compared to the control group. Increases in serum AST, ALT, and GGT activities in sheep infected with *F. hepatica* were consistent with the previous study findings (Sykes *et al.,* 1980; Benchaoui *et al.,* 1993; Ferre *et al.,* 1994; Yang *et al.,* 1998; Vengušt *et al.,* 2003; Kozat *et al.,* 2006; Mert *et al.,* 2006; Kozat and Denizhan, 2010).

Cellular immune response against *F. hepatica* infection is widely used in various animals (Mert *et al.,* 2006). It was reported that peripheral blood eosinophil count increases in sheep infected with *F. hepatica* and *F. gigantica* (Zhang *et al.,* 2005). In another study, it was reported that Hb and Hct levels decreased in sheep infected with *F. hepatica* (Hawkins 1984). In the present study, it was determined that WBC, Neu and Eo values were higher in sheep infected with *Fasciola hepatica* when compared to the control group WBC, Neu and Eo values. Hematological findings of the present study were consistent with the findings reported in the literature (Hawkins, 1984, Zhang *et al.,* 2005, Mert *et al.,* 2006).

Acute phase proteins are used as parameters in various inflammation, infection and trauma cases, in the evaluation of the immune system response, and in the prognosis and diagnosis of several diseases (Gruys *et al.,* 1994, Kent 1992). Several studies reported that serum amyloid A, serum haptoglobin, ceruloplasmin, and fibrinogen concentrations increased due to several infections and inflammatory cases in ruminants (Conner and Eckersall 1988; Conner *et al.,* 1989; Pfeffer *et al.,* 1993; Ganheim *et al.,* 2003; Wells *et al.,* 2013). In an experimental study, it was reported that serum Hp and SAA levels did not increase during common gastrointestinal nematode infections in sheep, while serum Hp and SAA concentrations increased in liver trematode infection and Johns disease (Ganheim *et al.,* 2004). In another study, it was reported that SAA was one of the first emerging acute phase proteins in *Theileria annulata* infections in cows and it could be used as an indicator of inflammatory reaction (Glass *et al.,* 2003; Nazifi *et al.,* 2008; Başbuğ and Gül, 2011). It was reported that calves infected with *Dictyocaulus viviparus* are reported to increase in acute phase proteins (APP) haptoglobin, serum amyloid A (SAA) and fibrinogen levels (Ganheim *et al.,* 2004). In the present study, it was found that SAA, Hp and fibrinogen concentrations were higher in sheep infected with *Fasciola hepatica* compared to SAA, Hp and fibrinogen concentrations in healthy sheep ($P < 0.05$). The increased SAA, Hp, and fibrinogen concentrations determined in the present study in sheep infected with *Fasciola hepatica* were consistent with other studies (Conner and Eckersall, 1988; Conner *et al.,* 1989, Pfeffer *et al.,* 1993; Ganheim *et al.,* 2003; Ganheim *et al.,* 2004; Wells *et al.,* 2013).

Serum albumin is a negative acute phase protein (Thomas, 2000, Tothova *et al.,* 2014) synthesized in the liver (Kozat and Sepehrizadeh, 2017), and it is the most important extracellular antioxidant due to the thiol group content (Halliwell, 1988). The albumin synthesis is decreased in liver infections and injuries (Thomas 2000). In the present study, it was found that the total protein and albumin levels in *F hepatica* infected sheep were lower when compared to the control group (Halliwell, 1988; Thomas, 2000). It was also found that serum haptoglobin, amyloid A, and fibrinogen levels were higher in sheep infected with *Fasciola hepatica* than those of the healthy sheep, whereas serum total protein and albumin levels were lower compared to the control group. Increased acute phase protein levels in sheep with fasciolosis suggested that the decrease in total protein and albumin levels could be caused by pathological liver disorders.

In conclusion, the increases in serum haptoglobin, serum amyloid A, and fibrinogen levels in sheep with fasciolosis were similar to liver disorder findings and it is suggested that these parameters could be useful in the evaluating degree and prognosis of liver damage and could shed light on future studies on liver disorders.

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