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Research Article

HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN OVINE FASCIOLOSIS

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ABSTRACT

Fasciolosis, caused by *Fasciola gigantica*, is one of the most important helminthic diseases of ruminants. The immature *F. gigantica* flukes cause disruption of liver tissues and haemorrhages during migration thus resulting into significant haematological and biochemical changes. Two groups of sheep were included in the present study. Group I (n=6), initially showing pale conjunctiva, prostration, partial anorexia etc., later died and their post mortem examination revealed immature *Fasciola* in pale and degenerated liver with a worm burden ranging from 110 to 193. In group II, 10 healthy sheep were included as control. The examination of faecal samples collected from sheep of groups I and II did not reveal parasitic eggs/oocysts/cysts. However, immature *Fasciola* spp. was recovered from the degenerated liver of dead sheep from group I. Group I showed significantly ($p<0.001$) lowered values of total erythrocyte counts, haemoglobin and packed cell volume and lymphocytes than those of group II sheep indicating haemorrhagic anaemia. Sheep of group I also showed significantly higher values of total leucocyte count, neutrophil count and non-significant ($p>0.05$) increase in eosinophil counts. Serum samples of group I showed significantly lowered ($p<0.01$) values of calcium, cholesterol, high density lipoprotein than that of group II. Significantly ($p<0.01$) increased activities of alanine transaminase, aspartate transaminase and gamma glutamyl transpeptidase were observed in sheep of group I as compared to group II. Haematological and biochemical alterations, as observed in the present study, are good indicators of severity of disease and are considered to be critical for the diagnosis, prognosis and effective therapy.

Key Words: Fasciolosis, *Fasciola gigantica*, haemato-biochemical changes, sheep

One of the most important helminthic diseases of ruminants is fasciolosis. It is caused by both immature and mature *Fasciola gigantica* (*F. gigantica*) and having a cosmopolitan distribution. The World Health Organization listed it among human parasites of public health importance (WHO, 2007). Studies have shown that human fasciolosis has increased significantly in 51 countries worldwide since 1980 with several geographical areas being highly endemic (Mas-Coma *et al.,* 1999). This disease has been observed in acute, sub acute and chronic forms in animals. Acute infections result into high mortality, whereas sub acute and chronic infections cause reduction in production of meat, milk and wool and decrease in fertility (Mason, 2004). The immature *F. gigantica* flukes cause disruption of liver tissues and haemorrhages during migration thus resulting into significant haematological and biochemical changes. Severe anaemia is resulted when mature helminths feed on the blood of final host (Wiedosari *et al.,* 2006). The adult flukes are localised in bile ducts of domestic and wild ruminants and cause subclinical and chronic disease. The severity of the disease is directly proportional to the adult parasitic load in bile ducts.

Determination of the concentration of blood parameters provides useful information that serves as the basis for the diagnosis, treatment and prognosis of specific diseases affecting animals (Yokus and Cakir, 2006). These changes are more severe in small ruminants like sheep where ratio of mass of the parasites to liver is much smaller. The diagnosis of this disease in sheep, as in other ruminants has been solely by the detection of *Fasciola* eggs in the faeces of infected animals (Boray, 1985). Although the procedure is simple and confirmatory, it is, however, not a useful diagnostic tool at low levels of adult fluke burden. Hence the need for methods other than faecal examination for the diagnosis of infection with fasciolosis has been obvious for decades (Ahmed *et al.,* 2006). Present study was done to evaluate the haematological and biochemical changes and find out the biological indicator for detecting fasciolosis in sheep naturally infected with *F. gigantica.*

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MATERIALS AND METHODS

Six adult sheep (3-6 years old) were brought to LUVAS Regional Centre, Uchani Karnal (Haryana) in the month of January, 2015 showing pale conjunctiva, prostration, partial anorexia etc. As per history, the sheep flock to which these dead animals belonged were being grazed on the sides of water channel infested with *Lymnaea auricularia* (intermediate host of *Fasciola*). Post mortem examination revealed immature *Fasciola* in pale and degenerated liver. Examination of livers as per technique of Ministry of Agriculture, Fisheries and Food (1977) yielded a worm burden ranging from 110 to 193. These sheep were designated as infected group (Group

I). Ten adult sheep from *Fasciola* free region and showing no parasitic infection in faecal and blood samples were included as apparently healthy control group (Group II). The faecal samples from sheep of groups I and II were collected, processed and examined for the presence of parasitic eggs/oocysts/cysts by the routine techniques of simple floatation and sedimentation methods. The blood samples of groups I (collected before death) and II were also collected with or without anticoagulant (EDTA). Blood smears were prepared immediately, stained with Giemsa stain and examined microscopically for the presence of haemoprotozoan infections. The blood collected in anticoagulant vials was also examined for hematological parameters including haemoglobin (Hb; gm/dL), packed cell volume (PCV; %), total erythrocyte count (TEC; $x10^6$ per µL), total leukocyte count (TLC; $x10^3$ per µL) and differential leukocyte count (DLC; %) as per the method of Schalm *et al.* (1975). The coagulated blood samples were centrifuged at 5000 rpm for 15 min and the serum was collected for biochemical estimations including total serum protein (TSP), glucose (Glu), calcium (Ca), phosphorus (P), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (Cr), total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG), total bilirubin (TBIL) and direct bilirubin (DBIL) by using commercial kits (Erba Diagnostics Ltd., India) with semi autoanalyzer (3000 Evolution, Biochemical Systems International, Italy).

The student t-test was used to analyze the significant differences between the haematological and biochemical parameters of the *Fasciola*-infected and the non-infected samples. Values of $P \leq 0.05$ were considered significant. Results were expressed as means±SE.

RESULTS AND DISCUSSION

The examination of faecal samples collected from sheep of groups I and II did not reveal parasitic eggs/oocysts/cysts. However, immature *Fasciola* spp. was recovered from the degenerated liver of dead sheep in group I. Further processing and staining of recovered flukes with Borax carmine revealed that these flukes were of *F. gigantica* species as per key provided by Soulsby (1986).

The haematological values of sheep in groups I and II have been presented in Table 1. Sheep of group I showed significantly ($P \leq 0.001$) lowered values of TEC, Hb, PCV and lymphocytes than those of group II sheep indicating anaemia. This anaemia was haemorrhagic (blood loss anaemia), which was associated with abnormal iron metabolism due to chronic invasion, migration of immature

Table 1
Haematological parameters of *Fasciola* infected sheep

Parameters	Infected sheep (n=6; Mean±SE)	Healthy sheep (n=10; Mean±SE)	Reference values of normal sheep¥	P value
Hb (gm/dL)	6.2±0.207**	10.55±0.28	9-15	<0.00001
PCV (%)	19.5±0.84**	32.9±0.87	24-46	<0.00001
MCV (fL)	40.15^{NS}±2.74	35.08±1.05	28-40	0.061
MCH (pg)	13.5±0.48 ^{NS}	11.24±0.34	8-12	0.051
MCHC (%)	36.7±0.84 ^{NS}	32.07±0.27	31-34	0.865
TEC ($\times10^6$/µL)	4.97±0.43**	9.43±0.28	9-15	<0.00001
TLC ($\times10^3$/µL)	18.62±1.71**	8.44±0.786	4-12	0.00002
Lymphocyte (%)	16±3.57**	52.7±3.94	40-75	0.00002
Monocyte (%)	$1.33±0.20^{NS}$	1±0.21	0-6	0.288053
Neutrophil (%)	79.33±3.7**	44.1±3.77	10-50	0.00002
Eosinophil (%)	3.33±0.55 ^{NS}	2.6±0.30	0-10	0.227

Hb=Haemoglobin; PCV=Packed cell volume; TEC=Total erythrocyte count; MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration; TLC=Total leukocyte count.
¥Reference range adopted from Jain (1993)
*Significant at P<0.05, **Significant at P<0.01, ^{NS}Non significant at P<0.05

flukes inside the liver parenchyma. The severity of anaemia depends on number of flukes inside the liver (Egbu *et al.,* 2013). On the other hand, there was non significant increase in MCV, MCHC and MCH values in sheep of group I as compared to group II. Egbu *et al.* (2013) also observed higher MCV and MCH in fasciola infected animals. Sheep of group I also showed a significant increase in TLC, neutrophil counts and a non-significant ($p>0.05$) increase in eosinophil counts. The changes in the differential counts may be a means of body defence against *Fasciola* obstructive effects or due to the toxin mediated lesion of the bone marrow.

Group I showed significantly lowered ($p<0.01$) values of Ca, cholesterol, HDL and non significant decreased level of LDL than that of group II (Table 2). These findings are in conformity with those in the reports of Kozat and Denizhan (2010) in fasciolosis in sheep. The liver has a central role in various aspects of lipid metabolism. In fasciolosis, the migrating flukes cause the death of the hepatocytes and the consequent severe pathology, which could result in the disturbance of lipids and lipoproteins in their serum levels. A significant increase in the activity of ALT, AST, GGT and high total bilirubin level was also observed in group I (Table 2). *Fasciola* species lead to the release of reactive oxygen species causing damage of cell wall resulting into hepatic tissue necrosis (Deger *et al.,* 2008). These changes influence biochemical parameters in serum and thus determination of specific liver enzymes is very valuable tool for diagnosis

Table 2
Biochemical parameters of *Fasciola* infected sheep

Parameters	Infected sheep (n=6; Mean±SE)	Healthy control (n=10; Mean±SE)	Reference value of normal sheep#		P Value
			Mean±SE	Range	
Ca (mg/dL)	8.27±0.35**	9.66±0.18	12.16±0.28	11.5-12.8	0.0007
P (mg/dL)	4.53±0.26^{NS}	4.92±0.17	6.4±0.2	5.0-7.3	0.2185
ALT (U/L)	81.66±9.66**	21.4±3.47	30±4	-	<0.00001
AST (U/L)	261.83±19.54**	70.4±2.73	207±43	60-280	<0.00001
GGT (U/L)	158.33±16.48**	27.9±1.62	33.5±4.3	20.0-52.0	<0.00001
Glucose (gm/dL)	56.6±4.07^{NS}	55.2±3.2*	68.4±6.0	50-80	0.7828
TSP (g/dL)	6.8±0.345^{NS}	6.2±0.112	7.2±0.52	6.0-7.9	0.6674
Albumin	3.65±0.314^{NS}	3.01±0.18	2.7±0.19	2.4-3.0	0.0837
BUN (mg/dL)	30.03±1.83**	15.67±1.58	17.38±1.18	8-20	0.00004
Cr (mg/dL)	1.28±0.12^{NS}	1.45±0.11	0.73±0.19	1.2-1.9	0.3578
TBIL (mg/dL)	1.4±0.16*	0.99±0.05	0.23±0.10	0.1-0.5	0.0122
DBIL (mg/dL)	0.28±0.07^{NS}	0.32±0.04	0.12±0.02	0-0.27	0.6665
TG (mg/dL)	44±2.71*	30.6±2.22	-	-	0.01156
Cholesterol (mg/dL)	66.83±4.51**	108.4±4.44	64±12	52-76	0.00001
HDL (mg/dL)	19.83±2.21**	50±3.04	-	-	0.00001
LDL (mg/dL)	38.2±5.24^{NS}	52.28 ±4.04	-	-	0.05169

#Reference range adopted from Kaneko (1997); Ca=Calcium; P=Phosphorus; TSP=Total serum protein; ALT=Alanine transaminase; AST=Aspartate transaminase; GGT=Gamma-glutamyl transpeptidase; BUN=Blood urea nitrogen; Cr=Creatinine; TBIL=total bilirubin; DBIL=direct bilirubin; TC=Total cholesterol, HDL=High density lipoprotein, LDL=Low density lipoprotein; TG=Triglycerides

®Friedewald *et al.* (1972) Formula: LDL=TC-HDL-TG/5.0 (mg/dL),

*Significant at P<0.05, **Significant at P<0.01, ^{NS}Non significant at P<0.05

of hepato-biliary diseases. Physiologically, normal levels of enzymes in cells or serum are maintained by constant synthesis, simultaneous degradation, inactivation and elimination of enzymes (Lee *et al.,* 2005). However, due to disruption of hepatocellular integrity, enzymes from damaged cells are released into the blood and their concentration increases above the physiological values. Increased values of serum transaminases (ALT, AST) at the early stage of the infection could be related to the hepatocellular necrosis and degenerative changes produced by migration of juvenile flukes through the liver parenchyma (Boone *et al.,* 2005; Taleb *et al.,* 2007; Deger *et al.,* 2008; Hodzic *et al.,* 2013). Higher levels of AST observed in infected sheep suggest hepatocellular damage and are indicative of chronic fasciolosis (biliary phase), regenerative changes in the parenchyma and the normalization of the liver function. Serum GGT values in group I were significantly higher than that in group II (Table 2). Few authors (Taleb *et al.,* 2007; Matanovic *et al.,* 2007; Hodzic *et al.,* 2013) reported that elevation in GGT level is an indicator of chronic changes, cholestasis and epithelial damage in bile ducts caused by the presence of adult flukes in biliary tract. Mert *et al.* (2006) detected statistically significant elevation of GGT and AST in sheep with chronic fasciolosis. Matanovic *et al.* (2007) have found elevated level of GGT in organically farmed sheep naturally infected with *F. hepatica*. Haematological and biochemical alterations, as observed in the present study, are good indicators of severity of disease and are considered to be crucial for the diagnosis, prognosis for effective therapy.

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