

HEMATO-BIOCHEMICAL PROFILES OF SHEEP INFECTED WITH FASCIOLOSIS IN COMPARISON WITH HEALTHY CONTROLS

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✉ Supporting Information

ABSTRACT: Fasciolosis can cause considerable change in hematological parameters and liver driven serum enzymes. Hence, the aim of this research is to assess the hematological and biochemical changes in sheep infected with Fasciolosis in comparison with healthy controls. A total of 52 local breed (26 from naturally infected and 26 from healthy control group) matched with sex and age were enrolled in the study. Five ml blood using EDTA vacutainer tube for hematology and another 5 ml blood by serum separating tubes for serum biochemical profiles were taken and analyzed using Sysmex automated hematological analyzer and Vegasys chemistry analyzer respectively. The hematological analysis indicated there were significant mean reductions ($P < 0.001$) in tRBC, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC) in case group (5.62 ± 1.15 , 8.92 ± 1.66 , 20.12 ± 5.78 , 26.05 ± 4.16 , 9.444 ± 2.00 and 29.72 ± 2.55) than control group (10.27 ± 1.36 , 12.68 ± 1.66 , 38.9 ± 6.05 , 40.50 ± 3.35 , 13.100 ± 1.35 and 34.327 ± 3.06) respectively. On the contrary the mean value of tWBC and eosinophil were significantly higher in the infected group (11.27 ± 2.7 and 7.19 ± 2.81) than the flock free of Fasciolosis (7.12 ± 2.61 and 2.82 ± 1.07) respectively. Regarding biochemical parameters, the significant mean elevation ($P < 0.001$) in serum ALT (125.92 ± 20.71) and AST (34.73 ± 11.97) in the infected sheep were observed than ALT (77.04 ± 13.96) and AST (23.69 ± 7.37) of the control groups. Whereas, the serum total protein and glucose level (5.17 ± 1.05 and 21.15 ± 11.08) in Fasciola infected sheep were significantly lower ($P < 0.001$) than healthy control (7.33 ± 1.06 and 32.62 ± 9.48 , respectively). The alteration in hemato-biochemical profile suggested a great impact of Fasciolosis on liver physiology leading to loss of production and productivity in sheep industry.

Keywords: Biochemical Profile, Fasciola, Hematology, Sheep.

INTRODUCTION

The liver plays a central role in the body of organisms. Hepatic pathology may result in significant disturbances and changes in blood components, carbohydrate and protein metabolisms as well as bile flow and composition. Certain hemato-biochemical changes are essential feature hepatocytes infection (Grunwaldt et al., 2005).

In Fasciolosis, the metabolic processes of the liver are gradually reduced (Doaa et al., 2007). As a result, the biochemical molecules like blood glucose, liver derived serum enzyme, serum proteins, and others are altered following injury and damage of hepatocytes (Behm and Sangster, 2006).

Liver enzymes are known to have intracellular action and their levels in the blood are very low under normal condition. Any increments in the systemic circulation are evidence of enzyme release due to tissues damage (Grunwaldt et al., 2005). The migration of larvae in the liver parenchyma is the primary cause for hemorrhage and severe tissue damage responsible for alteration of hematological and biochemical parameter related to the Fasciolosis (Mas-Coma et al., 2009). Consequently, exposed animals are predisposed to secondary bacterial infections (Anosike et al., 2005).

The aim of the study was to assess the hematological and biochemical changes in sheep naturally infected with Fasciolosis in comparison with health control groups.

MATERIALS AND METHODS

Study population

Fifty-two local breed sheep above 2 years of age from both sex reared under extensive farming system enrolled to this study. Sheep naturally infected with Fasciolosis were considered as case group while animals which were free of Fasciolosis during the study period were taken as healthy control group.

RESEARCH ARTICLE
 PII: S222877012000010-10
 Received: Aug 29, 2019
 Revised: Mar 20, 2020

Sampling methods

The study animals were purposively selected by faecal sedimentation techniques to confirm the presence/ absence of *Fasciola's* eggs. Sheep with the EPG (Egg count per gram of faeces) above 200 were selected as infected group and sheep which were negative at the time of examination were considered as health control group with additional treatment with single dose of Triclabendazole 10 mg/kg to avoid infection with immature stage. Both groups were kept under similar diet and management system.

Analysis of hematological parameters

Five milliliters of venous blood were taken from the jugular vein into Ethylene diamine tetra acetic acid (EDTA) (HiMedia Laboratories LTD®, Mumbai, India) tubes and stored at +4 °C. The CBC (complete blood count) were measured using an automated Sysmex hematology blood analyzer.

Analysis of biochemical parameters

Another five milliliters of blood were drawn from jugular vein into serum separating tubes and allowed to stand in undisturbed and slanted position for three to four hours. The clot was retracted to separate the serum. The collected serum was stored at -20°C in serum vials, which were properly capped and labelled. Serum level aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and glucose were measured according to standard procedures by Vegasys Clinical chemistry analyzer using enzymatic methods at 37 °C.

Data analysis

Data analysis was done by STATA software ver.12. Significant differences on the hematological and biochemical parameters between infected and control group were determined by t-test. Results are expressed as mean ±SD (standard deviation). Differences were considered as significant when P<0.05.

Ethical statement

Ethical clearance was obtained from the Research and Ethical Committee of University of Gondar. Permission approval was also obtained from the district and verbal consent from each owners during selection of the animals. The infected animals were ethically euthanized and the health control group was transferred to Anatomy department for teaching learning process.

RESULTS AND DISCUSSION

Hematological profile

Fasciolosis causes hematological changes on the host that harbor the parasites. The current study indicated the change in hematological values of RBC and its components such as total red blood cell (tRBC), hemoglobin (Hb) and hemotocrite (Hct). The infected sheep showed significant mean reduction (P<0.001) in tRBC ($5.62 \pm 1.15 \times 10^6/\mu\text{l}$) compared to the control group ($10.27 \pm 1.36 \times 10^6/\mu\text{l}$). Accordingly, the mean value in Hb and Hct were significantly reduced (P<0.001) in *Fasciola* infected sheep than non-infected groups. There was significant mean variation in Hb and Hct in infected group (8.92 ± 1.66 g/dL, 12.68 ± 1.66 %) and health controls (20.12 ± 5.78 g/dL, 38.9 ± 6.05 %) respectively (Table 1). Fasciolosis were also induced significant change in all RBC indexes. The infected sheep demonstrated significant mean reduction (P<0.001) in MCV, MCH and MCHC (26.05 ± 4.16 fl, 9.444 ± 2.00 pg and 29.716 ± 2.55 g/dL) compared to the non-infected sheep (40.50 ± 3.35 fl, 13.100 ± 1.35 pg and 34.327 ± 3.06 g/dL, respectively) (Table 2). Regarding to leukocytes, the total white blood cells (tWBC) and eosinophils count were significantly elevated (P<0.001) in case groups than controls. The mean count of tWBC was significantly raised from 7.12 ± 2.61 to $11.27 \pm 2.7 \times 10^3/\mu\text{l}$ and eosinophils from 2.82 ± 1.07 to 7.19 ± 2.81 % in non-infected group and infected groups respectively. However, there were no significant changes in neutrophils, lymphocytes, basophils and monocytes count between the infected and non-infected group (Table 3).

Table 1 - Erythrocyte values of *Fasciola* infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean±SD	Control group (n=26) Mean±SD	P-value
1	tRBC (/10 ⁶ /μl)	5.62±1.15	10.27±1.36	0.001
2	Hb(g/dl)	8.92±1.66	12.68±1.66	0.001
3	Hct (%)	20.12±5.78	38.9±6.05	0.001

Table 2 - Value of RBC indexes in *Fasciola* infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean±SD	Control group (n=26) Mean±SD	P-value
1	MCV (fl)	26.05±4.16	40.50±3.35	0.001
2	MCH (pg)	9.444±2.00	13.100±1.35	0.001
3	MCHC (g/dL)	29.716±2.55	34.327±3.06	0.001

Biochemical test results

The biochemical analysis of the present study showed the serum level of AST and ALT were significantly increased ($P < 0.001$) in sheep naturally infected with Fasciolosis than the control group. The mean value of serum AST and ALT in animals infected with Fasciolosis were $(125.92 \pm 20.71$ and 34.73 ± 11.97 IU/L) indicated higher elevation compared to the mean value in health controls (77.04 ± 13.96 and 23.69 ± 7.37 IU/L) respectively (Table 4). In contrast, the total protein and glucose determination revealed there were significant mean reduction ($P < 0.001$) in total protein and glucose in case group as compared to the controls. The mean reduction in total protein value from 7.33 ± 1.06 to 5.17 ± 1.05 g/dL and glucose from 32.62 ± 9.48 to 21.15 ± 11.08 mg/dL were observed in comparison between health control and infected groups respectively (Table 4).

Table 3 - Leukocytes values of Fasciola infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	WBC ($10^3/\mu$ l)	11.27 \pm 2.7	7.12 \pm 2.61	0.001
2	Neutrophils %	65.65 \pm 6.33	68.64 \pm 3.78	0.73
3	Lymphocytes %	21.03 \pm 4.81	20.43 \pm 3.67	0.61
4	Basophils %	0.77 \pm 0.48	0.77 \pm 0.60	0.98
5	Eosinophil %	5.16 \pm 1.22	1.82 \pm 1.07	0.001
6	Monocytes %	7.19 \pm 2.81	8.34 \pm 0.90	0.97

Table 4 - Biochemical profiles of Fasciola infected and control sheep (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	AST(IU/L)	125.92 \pm 20.71	77.04 \pm 13.96	0.001
2	ALT(IU/L)	34.73 \pm 11.97	23.69 \pm 7.37	0.001
3	Total protein (g/dl)	5.17 \pm 1.05	7.33 \pm 1.06	0.001
4	Glucose (mg/dl)	21.15 \pm 11.08	32.62 \pm 9.48	0.001

DISCUSSION

Liver fluke infestation in ruminants has important implications on animal health and welfare, farming economics and food production all over the world. It causes heavy loss in production and productivity since the animals became under stress condition.

The current study indicated various changes in hematological parameters. The tRBC, Hb, Hct, showed significant mean reduction in infected groups than non-infected groups. This result is supported by other study conducted by Pandya et al. (2015) who reported severe anemia in sheep infected with Fasciolosis. The lower mean values of total erythrocytes, Hct and Hb in infected sheep might be due to hemorrhage following severe damage by extensive migration of young fluke and blood sucking activity of the adult fluke (Radostits et al., 2007). Furthermore, the continuous drainage of iron stores and depression of erythropoiesis due to chronic inflammation of liver were thought to be responsible for reduction in total red blood cell and related components (Berry and Dagie, 1978; Chandra et al., 2011).

The infected sheep demonstrated significant mean reduction in MCV, MCH and MCHC compared to those of control. The finding is in line with the result of Pandya et al., (2015) who recorded microcytic and hypochromic anemia in Fasciola infected animals. However, the current result contrasts with the study conducted by Egbu et al. (2013) who reported high level of MCV, MCH and MCHC in the infected group. The variation could be due to ecological and nutritional differences that greatly affect the hematological profiles. The possible reason for microcytic and hypochromic anemia is related to depletion of iron stores due to damage of liver parenchyma (Beesley et al., 2018).

The eosinophilia observed in the present study supported by various researchers Egbu et al. (2013); Pandya et al. (2015) and Matanovic et al. (2007) who had similar observation. The elevation of eosinophils is an indication of body defense mechanism against parasitic infection (Duffus et al., 1980). This is due to inflammation and reaction by secretory antigenic substances released by fluke and cell mediated immunity (Radostits et al., 2007).

In the present study, the presence of leukocytosis is supported by previous findings reported by Sykes et al. (1980) and Zhang et al. (2005). The net increase in total leukocytes count (WBC) in Fasciola infection may also be due to marked eosinophilia which is associated with parasitic infection. The present study also revealed that there were certain biochemical and enzymatic change related to Fasciolosis. The serum level of liver enzyme (ALT and AST) significantly increased in case group than the control. The findings are in line with corresponding studies conducted by Pandya et al. (2015); Edith et al. (2012); Yasuda (1988) and Sheikh et al. (2006) who mentioned significant increase in mean value of

AST and ALT in Fasciola infected animals. The possible cause is that Fasciola can release reactive oxygen species that can produce damage to cell wall and hepatic tissue which is responsible to the release of intracellular enzyme to the circulation that can increase their serum level (Hodzic et al., 2013).

The significant mean reduction of serum glucose and protein level in sheep infected with Fasciolosis was observed than the flock free of Fasciolosis in this study. This finding agreed with the report of Phiri et al. (2007), who revealed serum glucose and protein values were lower in infected animals than normal. The lower glucose level could be because of inhibition of hepatic glucogenic pathways due to the migration of flukes that causes severe liver pathology and death of hepatocytes (Sharon, 2013).

Similarly, the reduction in total protein level attributed to the damage of hepatocytes by the parasite. The flukes reside in the liver disruption the protein synthesis leading to the development of hypoproteinemia (Martina and Jozica, 2012).

DECLARATIONS

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Authors' contribution

All the five authors reviewed the paper and contributed in developing the content.

Availability of data

The data can be availed to the journal upon request.

Consent to publish

Not applicable

Conflict of interest

The authors declare they have no competing of interests.

Acknowledgement

The authors would wish to acknowledge University of Gondar, Office of Vice President for Research and Community service and College of Veterinary Medicine and Animal Sciences for their support through the whole process of developing this publication.

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