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## Study on Haemato-Biochemical Alterations occurred in *Fasciola* spp. infected Buffaloes

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### Abstract

**Aim:** This study was performed to unmask the haematological and biochemical alterations occurred in blood and serum of infected buffaloes, respectively.

**Material & Method:** A total of 100 blood and serum samples were collected from infected as well as non-infected buffaloes during the ante-mortem examination. Animals were bled from the jugular vein into 10ml vacutainer tubes containing EDTA (Ethylene Diamine Tetraacetic Acid) for the estimation of various haematological parameters and 10 ml blood collected in serum clot activator tube to study biochemical parameters.

**Result:** The infected buffaloes showed a significant reduction in the mean Hb, PCV, and lymphocyte and increased TLC, neutrophil and eosinophil. The monocytes level decreased and basophils levels increased non-significantly in infected buffaloes. In the biochemical profile significantly reduction in the values of total serum protein, total albumin and A:G ratio and increased in AST/ SGOT, total globulin, ALT/ SGPT, acid phosphatase (ACPase), alkaline phosphatase (AKPase) and total bilirubin level reported.

**Conclusion:** Based on the results we got during study it can be conclude that *Fasciola* spp. infection cause heavy liver and muscle damage and cause heavy loss of blood from the body.

**Keywords:** AST, ALT, Biochemical, EDTA, haematological, PCV, TLC.

### 1. Introduction

Water buffaloes (*Bubalus bubalis*) are originally from Asia and they are mainly distributed in tropical and subtropical Asia [1]. The buffalo is the predominant domestic animal for milk and meat production. On average, buffaloes are about four times as productive as average indigenous cows in India [2]. Fasciolosis in buffaloes is asymptomatic, subclinical and/or chronic form of the disease, adversely affecting their reproductive cycle, weight gain, food conversion efficiency and productivity. In small ruminants like sheep and goats, it occurs in both acute and chronic form, whereas in large ruminants generally chronic form is common [3]. The frequency of outbreaks increases between October and May, but sporadic outbreaks continue throughout the year [4]. Fascioliasis is also a secondary zoonotic infection in man. The World Health Organization (WHO) listed it among human parasites of public health importance [5].

Fasciolosis in the buffaloes cause extensive liver and bile duct damage by penetration in liver parenchyma and intestinal mucosal damage as immature stage. It cause extensive blood loss because of blood sucking habit of parasite and haemorrhage in liver and bile duct. By causing liver damage and blood loss it alter the haemato-biochemical parameters of the host [6]. The disease is characterized by anemia due to severe liver damage caused by immature fluke tunneling through the liver parenchyma [7]. Clinical examination of the *F. gigantica* infected animal showed pale visible mucous membrane, bottle jaw syndrome which may occur occasionally when massive *Fasciola* infection causes sufficient liver destruction leading to cessation of protein synthesis [8].

This work is designed to monitor the haemato-biochemical alterations occurred in buffaloes because of liver and bile duct damage and extensive blood loss in the buffaloes.

### Materials and Methods:

#### Ethical approval:

Institutional Animal Ethics Committee of Veterinary College, Anand Agriculture University has accorded permission for the collection of blood from the slaughter houses for the study.

**Study area and sample collection:**

The study was carried out to unmasked the haemato-biochemical alteration that take place because of Fasciola spp. infection in buffaloes at Anand and Ahmedabad Districts' local slaughter houses of Gujarat. A total of 100 (infected=80, uninfected=20) blood and serum samples were collected during the anti-mortem examination. Animals were bled from the jugular vein into vacationer tubes containing EDTA (Ethylene Diamine Tetra acetic Acid = prevent blood coagulation) for the estimation of various haematological parameters. Blood samples for the serum separation collected from jugular vein in 10 ml capacity serum clot activator tube without anticoagulant and were allowed to stand in undisturbed in slanted position for about three to four hours at room temperature. Extreme care was taken to prevent hemolysis. The clot was retracted and the serum was separated out and the serum samples were centrifuged. The serum thus collected was stored in deep freeze at -20°C in serum vials, which were properly capped and labelled.

**Sample processing:**

The haemato-biochemical parameters viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total

leukocytes count (TLC), different leukocyte count (DLC), total protein (TP), albumin, globulin, A:G ratio, alkaline Phosphatase (AKP), acid Phosphatase (ACP), aspartate aminotransferase (AST/ SGOT ), alanine aminotransferase (ALT/ SGPT) and total bilirubin were studied. Hematological parameters were analyzed by Automatic Analyzer Hema-2062 manufactured by, Analytical Technologies Ltd. and biochemical parameters in serum were measured by Semi-automatic Analyzer NOVA-2021 manufactured by, Analytical Technologies Ltd.

Statistical analysis:

Data analysis were done as per standard method described by Snedecor and Cochran<sup>[9]</sup>.

**Results:**

The haematological alterations shows that total erythrocyte count (TEC -  $5.15 \pm 0.19$  10<sup>6</sup>/μl), haemoglobin (Hb -  $7.29 \pm 0.21$  g/dl), pack cell volme (PCV -  $24.82 \pm 0.88$  %) and lymphocyte ( $47.59 \pm 0.75$  %) counts were significantly decreased while total leucocyte count (TLC -  $12.78 \pm 0.37$  10<sup>3</sup>/ μl), neutrophil ( $42.43 \pm 0.84$  %) and eosinophil ( $8.29 \pm 0.26$  %) counts were significantly increased compared to non-infected buffaloes (Table 1).

**Table 1:** Haematological values of Fasciola infected buffaloes and non – infected buffaloes. (Mean ± SE)

S. no	Parameter	Non infected buffaloes n=20	Infected buffaloes n=80	P value	Stat. sign.
1	Total erythrocyte count (TEC) 10 <sup>6</sup> /μl	9.11 ± 0.19	5.15 ± 0.19	0.00	**
2	Haemoglobin (Hb) g/dl	12.39 ± 0.20	7.29 ± 0.21	0.00	**
3	Packed cell volume (PCV) %	38.08 ± 0.87	24.82 ± 0.88	0.00	**
4	Total leukocyte count (TLC) 10 <sup>3</sup> /μl	10.39 ± 0.36	12.78 ± 0.37	0.00	**
Differential Leukocytes count (%)					
1	Neutrophils (%)	33.05 ± 0.85	42.43 ± 0.84	0.00	**
2	Lymphocyte (%)	54.88 ± 0.76	47.59 ± 0.75	0.00	**
3	Monocytes (%)	2.85 ± 0.17	2.78 ± 0.16	0.78	--
4	Eosinophils (%)	4.14 ± 0.27	8.29 ± 0.26	0.00	**
5	Basophiles (%)	0.22 ± 0.05	0.38 ± 0.05	0.017	*
(**=P<0.05, ***=P<0.01.)					

The biochemical alterations shows that SGPT ( $79.25 \pm 2.24$  IU/L), SGOT ( $305.82 \pm 13.40$  IU/L), total bilirubin ( $1.93 \pm 0.13$  IU/L), level increased significantly because of heavy damage to the liver due migration of the immature stage of the fluke through liver parenchyma. AKPase ( $189.50 \pm 6.31$  IU/L) and ACPase ( $4.30 \pm 0.20$  IU/L) level also significantly

increased due to muscledamage by the fluke while Total Protein (TP-  $7.37 \pm 0.09$ g/dl), albumin ( $1.92 \pm 0.06$  g/dl) and A:G ( $0.36 \pm 0.02$ ) level significantly decreased due to lss of proteins in blood compared to non-infected buffaloes (Table 2).

**Table 2:** Biochemical values of Fasciola infected buffaloes and non-infected buffaloes (Mean ± SE)

S. no	Parameters	Infected buffaloes (n=80)	Non-infected buffaloes (n=20)	P value	Stat. sign.
1	Total protein (g/dl)	7.37 ± 0.09	8.18 ± 0.12	0.00	**
2	Albumin (g/dl)	1.92 ± 0.06	3.36 ± 0.09	.000	**
3	Globulin (g/dl)	5.44 ± 0.09	4.82 ± 0.09	0.00	**
4	A:G ratio	0.36 ± 0.02	0.71 ± 0.02	0.00	**
5	Aspartate aminotransferase (AST/ SGOT) IU/L	305.82 ± 13.40	210.53 ± 5.00	0.00	**
6	Alanine aminotransferase (ALT/ SGPT) IU/L	79.25 ± 2.14	48.85 ± 2.60	0.00	**
7	Acid phosphatase (ACP) IU/L	4.30 ± 0.20	2.38 ± 0.11	0.00	**
8	Alkaline phosphatase (AKP) IU/L	189.50 ± 6.31	113.48 ± 4.98	0.00	**
9	Total Bilirubin (mg/dl)	1.93 ± 0.13	0.92 ± 0.05	0.00	**
(**=P<0.05, ***=P<0.01.)					

**Discussion:**

Fasciolosis is very important disease in india and outside because it causes heavy loss in milk production and put animal under stress condition. In the present study we encountered anaemia, very low pack cell volume and hemoglobin and increased deferential leucocyte counts in infected animals compared to non-infected ones. Similar findings were

observed by Taimur *et al* <sup>[10]</sup> who documented total decline of TEC ( $4.92 \pm 0.59 \times 10^6$ mm<sup>3</sup>), Hb. ( $6.87 \pm 0.67$  g %) Value, PCV volume ( $24.68 \pm 1.20$ %) as compared with non-infected animals and they observed significantly (p<0.01) higher eosinophil ( $12.50 \pm 1.20$  %) and neutrophil ( $31.80 \pm 1.10$  %) counts and significantly (p<0.01) lower lymphocyte count ( $54.18 \pm 2.40$  %) in fasciola infected cattle in comparison with the non-infested

animals, Jatav *et al.* [2] reported decreased PCV ( $36.4 \pm 2.01\%$ ), Hb. ( $14.7 \pm 1.20$  g/dl) and increased neutrophil ( $23.2 \pm 2.45\%$ ) and no change in monocytes count ( $2.7 \pm 1.01\%$ ) and basophil count ( $1.4 \pm 0.24\%$ ) while they recorded increased lymphocyte count ( $64 \pm 2.21\%$ ) and normal eosinophil count ( $4.2 \pm 1.56\%$ ) as compared to present study.

The lower values of TEC and Hb. in infected buffaloes might be due to loss of blood due to resulting from severe haemorrhage caused by extensive migration of the young fluke through the hepatic parenchyma and from blood sucking activity of adult fluke, and this condition is responsible for decreasing TEC and Hb. level in the animals. Other scientists also in accordance with the present findings viz, Chandra *et al.* [3] who found anemia and increased eosinophil (9-38 %) counts, Edith *et al.* [11] who noticed eosinophilia ( $14.0 \pm 1.8\%$ ) and leucocytosis ( $9.5 \pm 0.2 \times 10^3/\text{cmm}$ ) in *Fasciola* infected buffaloes, Egbu *et al.* [12] documented the decreased packed cell volume ( $24.13 \pm 0.71\%$ ), haemoglobin ( $7.42 \pm 0.20$  g/dl), RBC ( $3.86 \pm 0.13 \times 10^6/\mu\text{l}$ ), lymphocytes ( $44.05 \pm 2.61\%$ ) and increased WBC ( $19.9 \pm 6.4 \times 10^3/\mu\text{l}$ ), neutrophils ( $43.15 \pm 1.27\%$ ), eosinophils ( $4.00 \pm 0.40\%$ ) and no change in monocytes ( $1.69 \pm 0.17\%$ ) counts. Above all results were in line with the present study.

Neutrophils are actively amoeboid and phagocytic in nature. The phagocytic action of neutrophils may thus correlate with their increased number as a first line of defense of the host in the present study. Reduction in the lymphocyte count in infected buffaloes might be due to the chronic infection of the *Fasciola spp.* The lymphocytes are the second line of cellular defense and in chronic infection there is high demand. However the production of these cells does not meet the demand, so the total count is reduced. Increased number of eosinophils in the blood might be an indication of parasitic infection. Net increase in the total leukocytes count may also be due to the increase in neutrophils and eosinophils. PCV may have decreased due to the lower concentration of erythrocytes per unit volume of blood as a result of destruction of small blood vessels by parasite and the anemia developed resultant in the fall of PCV in the infected group of buffaloes.

Biochemical or enzymatic changes take place in two stages in the Fasciolosis. The first stage changes caused by immature stage of flukes and second stage changes caused by adult stage of parasites. In the present study we found increased AST, ALT, AKPase and ACPase level. Acid phosphatase is destructive enzyme so increased when tissue damage occurs and alkaline phosphatase is healing enzyme so increased in response to the tissue damage for the healing while AST and ALT are liver specific enzymes. In addition to this we also detected heavy loss of total proteins from the animal body.

Kumar *et al.* [13] who documented significantly higher values for AST, ALT but no significant changes were recorded in total protein, albumin and globulin values while in our study the total protein and albumin level were significantly decreased and globulin level increased slightly compared to non-infected animals. Swarup *et al.* [14] assayed the biochemical indices in goats naturally infected with *F. gigantica* and compared with uninfected goats and found that the infected goats had significantly lower level of albumin ( $3.1 \pm 0.1$  g/dl) and reduced level of A:G ratio ( $1.1 \pm 0.1$ ) and AKP level noted high in the infected goats as noticed in the buffaloes in present study. Chaudhri *et al.* [15] made biochemical observation in buffaloes naturally infected with *F. gigantica*. Total protein level of buffaloes naturally infected with *F. gigantica* were significantly reduced compared with uninfected buffaloes and level of AST and ALT were

significantly elevated in infected animals, Pal and Dasgupta [16] also encountered hypoproteinaemia, hypoalbuminaemia, hyperglobulinemia, increased levels of total serum bilirubin, AST, ALT and ALP level and Sheikh *et al.* [17] encountered fasciola infected cattle had significantly low serum protein ( $5.10 \pm 0.18$ ) and albumin ( $2.26 \pm 0.27$ ) and significantly high values of aspartate aminotransferase ( $150.63 \pm 0.93$ ) and alanine aminotransferase ( $21.7 \pm 0.70$ ). These results are also completely in line with the present study.

In the Fasciolosis fluke migrates in the liver parenchyma and cause traumatic hepatitis and because of this hypoalbuminaemia, loss of total protein, increased AST and ALT level occur. AST and ALT are indicator enzymes of liver damage. After flukes attains maturity and entered into the bile ducts the AST level declines and AKP level increased significantly. The present study in which the AST, ALT, total bilirubin level increased significantly and total protein and albumin level decreased significantly (Figure 2) which correlates with the study of Edith *et al.* [18] who recorded remarkably elevated AST (40.8%), ALT (140.0%) and alkaline phosphatase (107.9%) level. Gupta *et al.* [19], Raval *et al.* [20] and Swarup and Pachauri [21] also reported higher values of AST, ALT and total bilirubin which were in agreement with the present findings.

These elevated AST, ALT and total protein level of enzymes suggestive of bile duct hyperplasia, cholangitis, periportal fibrosis, and biliary obstruction. Because of biliary obstruction and blood vessels damage by the parasite the total protein and albumin level decreased and hypoalbuminemia and hypoproteinemia occurs in the animal body. The increase in serum ACP level might be due to stressed condition of the animal and haemolysis of red blood cell by parasite, it increase the level of ACP as red blood cell is composed of acid phosphatase. Increased level of AKP in the present study might be due to the recovery of tissue damaged by parasites.

#### Summary:

This study revealed TEC, PCV and lymphocyte counts were significantly decreased while TLC, neutrophil and eosinophil were significantly increased due to the chronic infection of the fluke. The biochemical alterations shows increased ALT, AST, AKPase and total bilirubin level while Total protein, Albumin and A:G ratio were significantly decreased due to damaged liver, bile duct and muscles. Infected buffaloes shows anemia and edematous condition because of loss of erythrocyte and total protein.

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