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## RESEARCH ARTICLE

### INFLUENCES OF FASCIOLA INFECTION ON HEMATO-BIOCHEMICAL PARAMETERS IN EGYPTIAN BUFFALOES

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#### ABSTRACT

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

**Material & Method:** A total of 50 blood and serum samples were collected from infected as well 10 non-infected buffaloes during the ante-mortem examination. Animals were bled from the jugular vein into 10ml vacutainer tubes containing EDTA (Ethylene DiamineTetraacetic 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 Rbcs, 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 reduced in the values of total serum protein, AST/ SGOT, ALT/ SGPT and calcium

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

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#### INTRODUCTION

This study was carried out to reveal the Epidemicity of Fasciolosis infection in buffaloes in QENA Province and its effects on some blood physiological and biochemical aspects. Fasciolosis in the buffaloes cause extensive liver and bile duct damage by penetration of liver parenchyma and intestinal mucosal damage as immature stage. It causes extensive blood loss because of blood sucking habit of parasite and hemorrhage in liver and bile duct. By causing liver damage and blood loss it alters the haemato-biochemical parameters of the host (Behm and Sangster1999). Fasciolosis characterized by anemia due to liver damage caused by immature fluke tunneling through the liver parenchyma (Okaiyeto *et al.* 2012). Clinical examination of the Fasciola species 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, profuse diarrhea, weakness (Talukderet *al.* 2010), recumbancy and deaths. The infected buffaloes from unnoticed ill effects of the disease for a prolonged period before the disease is detected at a veterinary clinic and/or the abattoir (Edith *et al.*, 2010). Traditionally, Fasciola gigantica infection is diagnosed by fecal examination.

This has several disadvantages such as time consuming, requirement of the large volume of feces, inability to detect prepatent and ectopic infections and high false negative percentage in chronic infections due to intermittent shedding of eggs in feces. Furthermore, clinical disease can occur as early as 3-4 weeks post infection while fecal examination can confirm the diagnosis only after 13 weeks (Gupta *et al.* 2012). Serological tests are highly sensitive epidemiological tools for the detection of the disease, but their application is limited by cost and expertise in most developing countries like Egypt. Serum biochemistry of infected animals can be a good indication of the degree of damage to the host and the severity of infection (Otesile *et al.*, 1991).

#### MATERIALS AND METHODS

The study was carried out to unmask the haemato-biochemical alteration that take place because of Fasciola spp. infection in buffaloes at Qena governorate, Egypt. A total of 50 infected and 20 controls) blood and serum samples were collected during the anti-mortem examination. Animals were bled from the jugular vein into vacutainer tubes containing EDTA (Ethylene Diamine Tetra acetic Acid = prevents blood coagulation) for the estimation of various hematological parameters blood samples for the serum separation collected from the jugular vein in 10 ml capacity, serum clot activator tube without anticoagulant and were allowed to stand in

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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  $-20^{\circ}\text{C}$  in serum vials, which were properly capped and labelled. Kits supplied by Spectrum Diagnostics (Egyptian Co. For Biotechnology, Obour City Industrial Area, Cairo, Egypt), using a UV spectrophotometer.

### Sample processing

The haemato-biochemical parameters (total erythrocyte count (TEC), hemoglobin (Hb.), packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC), total protein, aspartate amino transferase (AST/ SGOT), alanine aminotransferase (ALT/ SGPT), calcium. Hematological parameters were analyzed by Automatic Analyzer 5010 spectrometers, and the kits from an Egyptian industry company. Results was entered into Microsoft excel 2010 and analyzed by SPSS statistical software version 20. Prevalence differences of study variables were analyzed by chi-square and descriptive statistics.  $P \leq 0.05$  was considered as statistically significant at 95% CI. The serum biochemical parameters infected groups were compared with those of the controls. The data as mean  $\pm$  standard error and range; one way ANOVA was used for multiple comparisons and to see their correlation.

### RESULTS

The hematological alterations shows that total erythrocyte count (TEC -  $5.15 \pm 0.19106/\mu\text{l}$ ), hemoglobin (Hb -  $7.29 \pm 0.21\text{g/dl}$ ), pack cell volume (PCV -  $24.82 \pm 0.88\%$ ) and lymphocyte ( $45.59 \pm 0.75\%$ ) counts were significantly decreased while total leukocyte count (TLC  $11.78 \pm 0.37 103/\mu\text{l}$ ), neutrophil ( $41.43 \pm 0.84\%$ ) and eosinophil ( $7.29 \pm 0.26\%$ ), Monocytes ( $2.78 \pm 0.16\%$ ), (Table 1).

**Table 1. Haematological values of control and Fasciola infected buffaloes**

Parameter	control buffaloes n=10	Infected buffaloe n=50
Total erythrocyte count (TEC) $10^6/\mu\text{l}$	$8.11 \pm 0.2$	$5.15 \pm 0.19^{**}$
Hemoglobin (Hb) g/dl	$11.39 \pm 0.20$	$7.29 \pm 0.21^{**}$
Packed cell volume (PCV) %	$36.08 \pm 0.1$	$24.82 \pm 0.88^{**}$
Total leukocyte count (TLC) $10^3/\mu\text{l}$	$10.39 \pm 0.36$	$11.78 \pm 0.37^{**}$
Neutrophils (%)	$31.04 \pm 0.85$	$41.43 \pm 0.84^{**}$
Lymphocyte (%)	$54.66 \pm 0.70$	$45.59 \pm 0.75^{**}$
Monocytes (%)	$2.05 \pm 0.15$	$2.78 \pm 0.16$
Eosinophils (%)	$4.12 \pm 0.22$	$7.29 \pm 0.26^{**}$
Basophiles (%)	$0.20 \pm 0.05$	$0.36 \pm 0.05^{**}$

\*significant at ( $P < 0.05$ ) \*\*significant at ( $P < 0.01$ )

**Table 2. Biochemical values of control and Fasciola infected buffaloes**

Parameter	control buffaloes n=10	Infected buffaloes n=50
Total protein(g/dl)	$8.18 \pm 0.12$	$7.37 \pm 0.09^{*}$
Calcium mg/dl	$10.39 \pm 0.20$	$8.29 \pm 0.21^{*}$
Aspartate aminotransferase (AST/ SGOT) IU/L	$200.53 \pm 5.00$	$315.82 \pm 13.40^{**}$
Alanine aminotransferase (ALT/ SGPT) IU/L	$58.85 \pm 2.60$	$89.25 \pm 2.14^{**}$

\*significant at ( $P < 0.05$ )\*\*significant at ( $P < 0.01$ )

The biochemical alterations shows that SGPT ( $89.25 \pm 2.14\text{IU/L}$ ), SGOT ( $315.82 \pm 13.40\text{IU/L}$ ), while Total Protein (TP-  $7.37 \pm 0.09\text{g/dl}$ ), and calcium ( $8.29 \pm 0.21 \text{mg/dl}$ ), (Table2).

### DISCUSSION

Fascioliasis is a very important disease in Qena governorate Egypt and outside because it causes heavy losses in milk production and put the animal under stress condition. In the present study, we encountered anemia, very low pack cell volume and hemoglobin and increased differential leukocyte counts in infected animals compared to non-infected ones. Similar findings were observed by (Taimur *et al.* 1993) who documented a total decline of TEC ( $4.92 \pm 0.59 \times 10^6/\text{mm}^3$ ), Hb. ( $6.87 \pm 0.67 \text{g} \%$ ) Value, PCV volume ( $24.68 \pm 1.20\%$ ) as compared with non-infected animals and their observed significantly ( $p < 0.01$ ) higher eosinophil ( $12.50 \pm 1.20 \%$ ) and neutrophil ( $31.80 \pm 1.10 \%$ ) counts and significantly ( $p < 0.01$ ) low lymphocyte count ( $54.18 \pm 2.40 \%$ ) in fasciola infected cattle in comparison with the non-infested. (Jatav *et al.* 2008) reported decreased PCV ( $36.4 \pm 2.01\%$ ), Hb. ( $14.7 \pm 1.20 \text{g/dl}$ ) 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 hemorrhage caused by extensive migration of the young fluke through the hepatic parenchyma and from the blood sucking activity of adult fluke, and this condition is responsible for decreasing TEC and Hb. Level in the animals (Pandya *et al.* 2015).

(Edith *et al.* 2012) who noticed eosinophilia ( $14.0 \pm 1.8\%$ ) and leucocytosis ( $9.5 \pm 0.2 \times 10^3/\text{cm}$ ) in Fasciola infected buffaloes, (Egbu *et al.* 2013) documented the decreased packed cell volume ( $24.13 \pm 0.71\%$ ), hemoglobin ( $7.42 \pm 0.20 \text{g/dl}$ ), RBC ( $3.86 \pm 0.13 \times 10^6/\mu\text{l}$ ), lymphocytes ( $44.05 \pm 2.61 \%$ ) and increased WBC ( $19.9 \pm 6.4 10^3/\mu\text{l}$ ), 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), (Molina *et al.* 2006)), (Haroun and Hussein 1975), in cattle and by (Doaa *et al.* 2007), (Waweru *et al.* 1999). Neutrophils are actively ameboid and phagocytes in nature. The phagocytic action of neutrophils may thus correlate with their increased numbers 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. The increased number of eosinophils in the blood might be an indication of parasitic infection (Ackerman *et al.* 1981). The net increase in the total leukocyte 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 parasites and the anemia developed resulting in the fall of PCV in the infected group of buffaloes. Reduction in the lymphocyte count in infected buffaloes might be due to the chronic infection of the Fasciola spp. On the other hand, significant increases was obtained in WBC, MCV and MCH of

the infected cattle, when compared with those of the control group. (Sykes *et al.*, 1980) and (Haroun and Hussein (1975). In addition to this we also detected heavy loss of total proteins from the animal body (Kumar *et al.* 1982). The changes in the differential counts may be a means of body defence against Fasciola obstructive effects are due to the toxin mediated lesion of the bone marrow. (Anita Ganguly *et al.*, 2016). Total protein level of buffaloes naturally infected with *F. gigantica* was significantly reduced compared with uninfected buffaloes (Swarup *et al.*, 1986), and level of AST and ALT were significantly elevated in infected animals, (Pal and Dasgupta 2006), (Sheikh *et al.* 2006), 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 vessel damage by the parasite the total protein (Pandya *et al.* 2015). The elevated AST and ALT levels declined significantly during the late prepatency and/or patency phase of the disease, yet these were throughout significantly higher than the healthy controls (Edith *et al.* 2010). Facials pp. causes the release of reactive oxygen species that produce damage to cell walls and hepatic tissue necrosis. These changes have an influence on biochemical parameters in serum including liver specific enzymes (Hodzic *et al.*, 2013). AST and ALT elevation may be due to parenchymal damage as a secondary effect of cholestasis (Salem and Hassan, 2011) (Deger *et al.*, 2008)). In large animals, serum concentrations of liver specific enzymes like transaminases are generally higher in acute liver disease than in chronic liver disease. (Diurnal and Yoseph 2014). The mineral estimation revealed a significant decrease in average car values in Fasciola infested bovine compared to healthy control (Imtiyaz Reshi *et al.* 2014). The values of calcium in infected buffaloes are lower than the controls due to disturbance in calcium metabolism and hepatic dysfunction, these agree with (Mbuh and Mbuye, 2005), (Hamilton and Bickle, 2006), (Sheikh *et al.*, 2006), (Okaiyeto *et al.*, 2012), (Kozat and Denizhan (2010).

## Conclusion

This study attempted to assess serum hematological, biochemical alteration that accompanies liver fluke infection in Egyptian buffaloes. The concentration of serum liver enzyme analysis was also used to evaluate liver fluke infection and indicated significant elevation of serum AST and ALT with serum hematologist and Serum Biochemical Study of Liver Fluke Infection in buffaloes.

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