

دراسات كيميائية وسيتولوجية لمركبات الدم في الجاموس المصاب بالديدان الكبدية

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الملخص

استهدف هذا البحث دراسة صورة الدم وبعض مكوناته الكيميائية في الجاموس المصاب بالديدان الكبدية بغرض المساعدة في تشخيص المرض وتقدير درجة الإصابة به مما يترتب عليه تقرير امكانية العلاج من عدمه ، كذلك معرفة مدى فاعلية الدواء المقترح .

وقد تم فحص ستين أنثى جاموس مصابة بالديدان الكبدية فقط هذا بالإضافة الى ثمان حالات أخرى غير مصابة وتأكد ذلك بفحص الحيوانات قبل الذبح وبعده وكذلك فحص عينات البراز بالإضافة الى الفحص المجهرى لأنسجة الكبد .

وعن طريق الفحص الميكروسكوبى لأنسجة الكبد لمعرفة مدى تليف هذه الأنسجة قسمت الحيوانات المصابة الى ثلاث مجموعات طفيفة - معتدلة وشديدة الإصابة .

وتبين من النتائج أن المجموعة شديدة الإصابة تتميز بنقص كرات الدم الحمراء وكمية الهيموجلوبين ونسبة الهيماتوكريت وكذلك بزيادة سرعة ترسيب الدم وزيادة كرات الدم البيضاء وخلايا النيوتروفيل والبازوفيل والمونوسيت ونقص في خلايا الليمفوسيت . أما المجموعتين الطفيفة والمتوسطة الإصابة فيميزها زيادة معدل سرعة ترسيب الدم وزيادة خلايا الأيوزينوفيل .

أما الدراسات الكيميائية فقد أظهرت نقص في كمية الفوسفور غير العضوى في أفراد المجموعة الشديدة الإصابة . وقد نلاحظ أيضا نقص في الألبرومين وزيادة مقابلة في الجاماجاوبيلين في المجموعتين المتوسطة والشديدة الإصابة . أما المجموعة طفيفة الإصابة فقد تميزت بزيادة في نسبة الفاجلوبيلين .

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SOME HAEMATOLOGICAL AND BIOCHEMICAL STUDIES ON FASCIOLA INFECTED BUFFALOES*

(With 3 tables and 6 figures)

By

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Blood picture values, inorganic and organic blood constituents of 60 female buffaloes naturally infected with *Fasciola gigantica* were examined. In addition, 8 normal cases were investigated. The tested buffaloes, (after slaughtering), were grouped according to the degree of liver cirrhosis into slight, moderate and severe, classes. Results revealed that, erythrocytes, hemoglobin and the packed cell volume were decreased in the severely affected group., A normocytic normochromic anemia was observed. Leukocytosis basophilia, neutrophilia, monocytosis and lymphopenia were also seen in this group. Eosinophilia was noticed in the slight and moderate classes. All affected buffaloes showed acceleration of sedimentation rate. Calcium magnesium, sodium and potassium were not changed. A decrease of inorganic phosphorus was noticed in the severely affected group. There were no differences between serum total proteins in fasciola infected buffaloes and the non infected mates. Albumin was decreased in the moderate and severe groups, while gammaglobulins were elevated.

INTRODUCTION

Mammalian hosts are susceptible to many parasitic infections, but the susceptibility and the pathological changes caused by different parasites vary considerably from host to host. So an acute, subacute or chronic disease occurs. In some instances blood examinations may throw light on the tissue reaction which occurs due to the effect of the parasite on the host.

Fascioliasis, as a parasitic disease, causes remarkable changes in the blood elements. ROBERTS (1968) and REID *et al.* (1970) reported the occurrence of anemia; hypoalbuminemia and hyperglobulinemia were also recorded by ROSS *et al.* (1966) and VASIL'EV (1967). Moreover, in fascioliasis, the tissue reaction accompanied by the changes of blood constituents varies according to the different phases of infestation. During the migratory phase, for example, eosinophils showed striking elevation (SINCLAIR, 1962 and MOROSHKIN *et al.*, 1964). With the arrival of the flukes to the bile ducts and the occurrence of liver cirrhosis, hypoalbuminemia and hyperglobulinemia were demonstrated (NANSEN *et al.*, 1968).

* Part of a thesis submitted to the Faculty of Veterinary Medicine, Cairo University for the M.V. Sc. (Clinical Pathology, 1972).

As the buffaloes in Egypt are markedly susceptible to *Fasciola gigantica* infestation and the disease causes great losses in this animal species (Veterinary reports, Ministry of Agriculture, Egypt, 1962-1969), the present investigation is, therefore, planned to study the changes of some cytological and biochemical blood constituents in buffaloes naturally infested with liver flukes, aiming to aid the diagnosis of this disease and the assessment of the degree of infection, so a correct prognosis and thus a successful treatment could be decided or not. Such a method would be also useful in the study of the efficiency of any proposed treatment.

MATERIALS AND METHODS

Sixty-eight non pregnant female buffaloes (*Bos bubalus*) over 5 years old were selected from Cairo abattoir for studying their cytological and biochemical blood elements. The animals were clinically normal. Post-mortem inspection done afterwards revealed no apparent pathological lesions or parasitic infestations other than fascioliasis. From each buffalo, 2 blood samples, a faecal sample and liver specimens were collected. The 1st blood sample was taken from the jugular vein on EDTA anticoagulant for estimating the blood picture values adopting the technique described by SCHALM (1965). The 2nd sample of blood was used for serum separation and estimation of Ca (FALES, 1953); inorganic phosphorus (FISKE and SABBAROW, 1925); magnesium (DENIS, 1922) and sodium and potassium (KING, 1961). Total serum proteins and the electrophoretic pattern of the individual proteins were also determined (KING and WOOTTON, 1959).

The obtained faecal samples were examined for the presence of eggs of fasciola or other worms. After slaughtering, liver specimens were collected for histopathological examination. Sections were prepared and stained with Hematoxylin and Eosin and Van Gieson's stains.

RESULTS

From the 68 inspected animals in the abattoir, 60 cases were proved to suffer from liver flukes based on the macroscopic and microscopic examination of the liver. The rest 8 buffaloes were found parasitic free and served as the control. Depending on the histopathological examination, the affected animals were classified according to the degree of liver cirrhosis into slight, moderate and severe groups. In the slightly affected group (25 buffaloes), there was an increase of the connective tissue elements within Glisson's capsule (Fig. 1). In the moderate cases (25), the connective tissue was, moreover, increase around the individual lobules of the liver (Fig. 2). Specimens from severely affected animals (10 cases) showed pericellular cirrhosis beside the marked thickening of the perilobular connective tissue (Fig. 3). The increased pericellular C.T. sometimes substitute the liver cells (Fig. 4). Newly formed bile ducts within the markedly increased C.T. of Glisson's capsule were also seen (Fig. 5).

Results of the hematological and biochemical studies of normal and fasciola infected buffaloes are shown in Tables 1, 2 and 3. The electrophoretic pattern of the serum proteins of a case from each examined group is illustrated in Fig. 6.

DISCUSSION

The effect of fascioliasis on liver parenchyma as seen by the histopathological examination, is a more reliable method for estimation of the degree of infestation, thus it is confirmative. Egg count, on the other hand, may not give an exact picture (PECHUER, 1967; DARSKI, 1969 and MATHUR, 1970). In the present investigation it was noticed that some of the examined buffaloes, although few flukes were seen in the liver, eggs could not be detected in their faecal droppings, while the examination of their blood gave significant changes compared with normal. Such observations may be supported by the comments given by the previous authors already mentioned.

Records concerned with the studies of blood constituents of buffaloes infested with fasciola is scarce. Therefore, studies dealing with the same changes in other animals will be considered.

The decrease of erythrocytes, packed cell volume and hemoglobin observed in the severely affected group was previously reported in cows, buffaloes and sheep (SINCLAIR, 1962; MOROSHKIN *et al.*, 1964 and SOLIMAN and ZAKI, 1964). However, the previous authors did not mention the severity of infestation in their examined animals. The present investigation revealed a normocytic normochromic anemia in severely affected buffaloes. This was in accordance with BITAKARAMIRE AND BWANGAMOI (1966) in experimentally infected calves and SINCLAIR (1962) and ROBERTS (1968) who reported normocytic anemia in sheep. However, a macrocytic hypochromic anemia was recorded by REID *et al.* (1970) in naturally affected sheep.

The anemia produced by fasciola infestation was explained by many investigators. HOLMES *et al.* (1967) and SEWELL *et al.* (1968) were of the opinion that the flukes cause loss of blood in the bile, while SYMONS and BORAY (1967) and (1968) suggested that anemia occurs due to consumption of blood by these flukes. Another explanation was offered by SINCLAIR (1956) who stated that anemia due to fascioliasis is secondary to a disorder of the reticulo-endothelial function which leads to decrease erythrocyte production and increase red cell destruction. The normocytic normochromic anemia observed in our Severely affected buffaloes is probably a secondary anemia with selective depression of erythropoiesis as a result of chronic fascioliasis.

TABLE 1. Comparative statistical analysis of the blood picture values in normal and fasciola infected buffaloes.

		RBC 10 ⁶ /cmm	PCV %	Hb gm %	MCV cu	MCH uu gm	MCHC %	SR mm/hr	
Mean values together with their standard deviation	Normal-	7.25 ±0.72	1.33 ±3.54	12.81 ±1.48	52.99 ±1.07	17.69 ±2.06	55.38 ±1.35	66.00 ±38.62	
	Infected	Slight-	6.27 ±1.06	34.90 ±4.43	11.44 ±1.35	56.33 ±6.47	18.51 ±2.26	32.86 ±1.74	99.50 ±7.47
		Moderate-	6.32 ±1.57	35.84 ±6.57	11.44 ±2.20	58.36 ±10.49	18.68 ±3.73	30.64 ±6.64	93.08 ±28.57
	Severe-	2.86 ±0.43	14.00 ±2.07	4.60 ±1.04	51.29 ±6.03	16.20 ±2.04	30.89 ±1.44	156.00 ±9.00	
Difference of means and significance of "t" test	a — b	0.98	3.41	1.37	3.34	0.82	0.53	33.50+	
	a — c	0.93	2.47	1.37	5.36	0.99	2.75	27.80+	
	a — d	4.39+	24.31	+ 8.21	+ 1.70	1.49	2.49	99.00+	
	b — c	0.05	0.94	0.00	2.03	0.17	2.22	6.42	
	b — d	3.41+	20.90	+ 6.84	+ 5.04	2.31	1.97	56.50+	
		3.46+	21.84	+ 6.84	+ 7.07	2.48	0.25	62.92+	

+ = Significantly different at 0.05 level of probability.

TABLE 1. (Contd.)

Differential leukocytic count/cmm in absolute numbers

		WBC 10 ⁹ /cmm	Direct eosinophils	Eosinophils	Basophils	Neutrophils	Lymphocytes	Monocyte
Mean values together with their standard de- viation	Normal ^a	6.78 ± 3.77	122.57 ± 100.00	307.37 ± 275.50	54.50 ± 52.52	3231.75 ± 1574.20	1834.57 ± 985.44	88.00 ± 59.16
	Slight ^b	5.52 ± 2.34	323.63 ± 247.08	383.43 ± 270.15	82.52 ± 77.85	3204.88 ± 1802.21	1327.68 ± 898.61	74.80 ± 89.49
	Moderate ^c	6.36 ± 5.16	660.47 ± 384.59	725.17 ± 432.33	76.28 ± 72.04	26.5852 ± 1377.00	1419.56 ± 849.30	101.56 ± 87.80
	Served ^d	10.77 ± 1.07	129.30 ± 105.97	171.90 ± 116.62	99.50 ± 106.91	84.50.20 ± 1316.40	633.30 ± 202.73	349.20 ± 190.74
Difference of means and significance of "t" test	a-b	1.27	201.06+	76.05	28.02	26.87	506.89	13.20
	a-c	0.43	537.9 +	417.79+	21.78	546.23	415.01	13.55
	a-d	3.99+	6.73+	135.47	45.00+	5218.45+	12.01.27+	261.20+
	b-c	0.84	336.85+	341.74+	6.24	519.36	91.88	26.76+
	b-d	5.25+	194.33+	211.53+	16.98	5245.32+	694.38+	274.40+
c-d	4.41+	531.17+	553.27+	23.22	5774.68+	786.26+	247.64	

+ — Significantly different at 0.05 level of probability.

TABLE 2. Comparative statistical analysis of inorganic constituents in normal and fasciola infected buffaloes

		Calcium mg %	Inorganic phosphorus mg%	Magnesium mg %	Sodium mEq/litre	Potassium mEq./l
Mean values together with their standard deviation	Normal ^a	12.00 ± 1.85	8.03 ± 1.92	2.67 ± 0.30	180.71 ± 5.14	6.43 ± 0.68
	Slight ^b	12.58 ± 2.07	7.50 ± 1.93	2.76 ± 0.68	177.37 ± 11.94	6.84 ± 1.06
	Moderate ^c	13.08 ± 1.38	7.98 ± 1.38	2.70 ± 0.47	180.60 ± 7.78	7.30 ± 1.18
	Severe ^d	10.80 ± 1.84	4.40 ± 1.34	2.24 ± 0.26	178.70 ± 9.12	7.25 ± 0.31
Difference of means and significance of "t" test	a-b	0.58	0.53	0.09	3.34	0.40
	a-c	1.08	0.05	0.03	0.11	0.86
	a-d	1.20	3.63 +	0.43	2.01	0.82
	b-c	0.43	0.43	0.06	3.23	0.46
	b-d	1.78	3.10 +	0.52	1.33	0.41
c-d	2.28	3.58 +	0.46	1.90	0.50	

+ × Significantly different at 0.05 level of probability.

TABLE 3. Comparative statistical analysis of total proteins and serum protein fractions in normal and fasciola infected buffaloes

	Total proteins gm %	Relative percentages			
		Albumin	Globulin	B—Globulin	Y—globulin
Mean values together their with their standard deviation	Normal ^a	40.75 ± 4.32	18.00 ± 2.77	14.62 ± 2.61	26.62 ± 2.50
	Slight ^b	30.20 ± 6.85	23.30 ± 2.32	17.00 ± 3.35	28.90 ± 4.51
	Moderate ^c	24.80 ± 3.74	18.90 ± 1.70	17.50 ± 4.19	38.80 ± 4.49
	Severe ^d	13.70 ± 1.18	20.60 ± 2.49	17.20 ± 1.09	48.50 ± 2.72
Difference of means and significance of "t" test		10.55	5.30 +	2.98	2.28
	a—b	15.95 +	0.90	2.88	12.18 +
	a—c	27.05 +	2.60	2.58	21.88 +
	a—d	5.40	4.40 +	0.10	9.90 +
	16.50 +	2.70	0.40	19.60 +	
	11.10 +	1.70	0.30	9.70 +	

+ = Significantly different at 0.05 level of probability

Fasciola infestation resulted in acceleration of sedimentation rate in all infected buffaloes. The elevation was parallel to the degree of liver damage and cirrhosis.

As far as the total leucocytic count, neutrophils, eosinophils and lymphocytes are concerned, the picture of these cells in the severely infected buffaloes suggested the phenomenon of stress. Previous cells, however, showed no alterations in the slightly and moderately diseased animals with the exception of eosinophils which observed a significant increase specially in moderately affected buffaloes. Eosinophilia was similarly reported by SINCLAIR (1962), SOLIMAN and ZAKI (1964) and ROBERTS (1968) in infested cows, buffaloes and sheep. Buffaloes suffering severe fascioliasis demonstrated also basophilia and monocytosis. The increase of monocytes reflects the chronicity of the condition (SCHALM, 1965).

Examinations performed revealed no significant variations in calcium, magnesium, sodium and potassium. However, a decrease of inorganic phosphorus was noticed in severely affected buffaloes. Controversy to our findings reported HAIBA *et al.* (1964) a reduction in the calcium content and no change in the inorganic phosphorus in affected buffaloes.

The unchanged total plasma proteins of buffaloes observed in this work agree with NANSEN *et al.* (1968), and ROBERTS (1968) in affected sheep. However, it differ from the fall recorded by HAIBA and SELIM (1960) and KONRAD (1968). Increased formation of plasma proteins in fasciola infested animals which aid the substitution of the lost plasma proteins into the gastrointestinal tract as suggested by MacCLEAN *et al.* (1968) may explain the unchanged values of this component observed in the present work. The decrease of albumin and the increase of gammaglobulins recorded by KONA (1957); NANSEN *et al.* (1968) and ROBERST (1968) agree with the findings of this work as far as the moderately and severely affected buffaloes are concerned. Our findings may confirm the suggestion of DOBSON (1967) that there may be a compensatory synthesis of globulins to overcome the loss of albumin.

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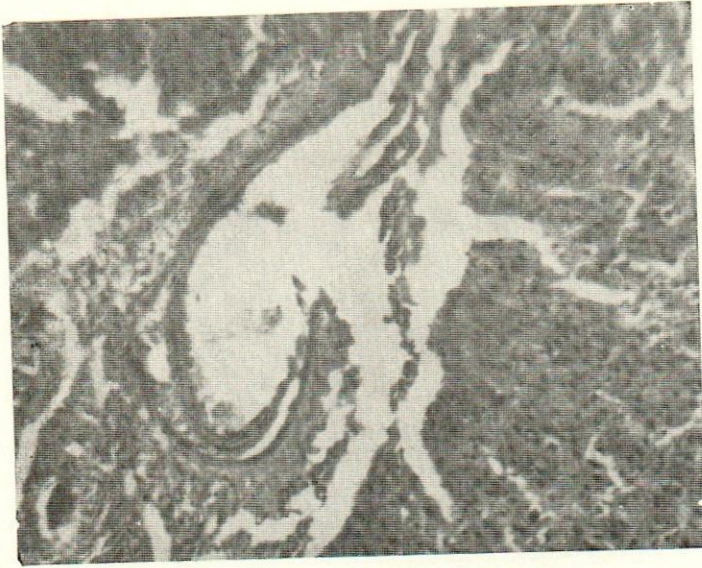


Fig. 1.—Slight fascioliasis. The connective tissue of the Glisson's capsule is increased (H and E ; $\times 64$).

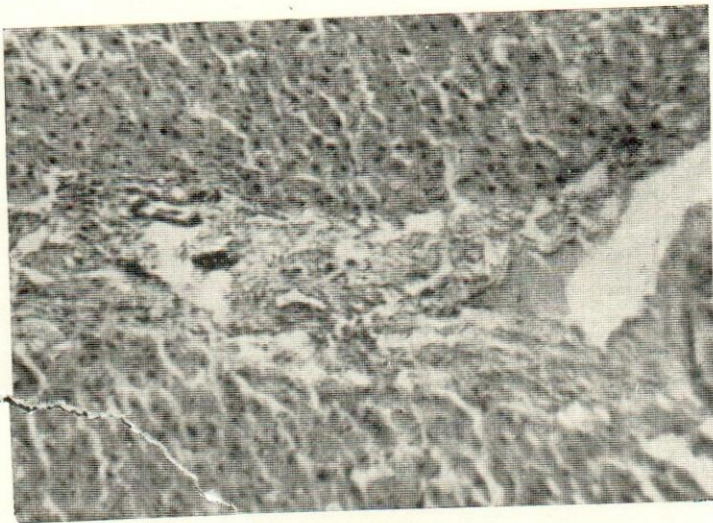


Fig. 2.—Moderate fasciola infection. The connective tissue markedly increased around the liver lobules (H and E ; $\times 64$).

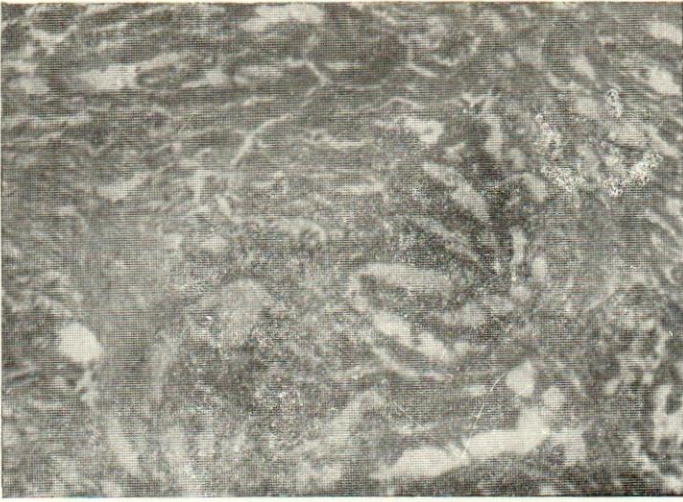


Fig. 3.—Severe Fasciola infection. Monolobular and pericellular cirrhosis (H and E; $\times 25$).

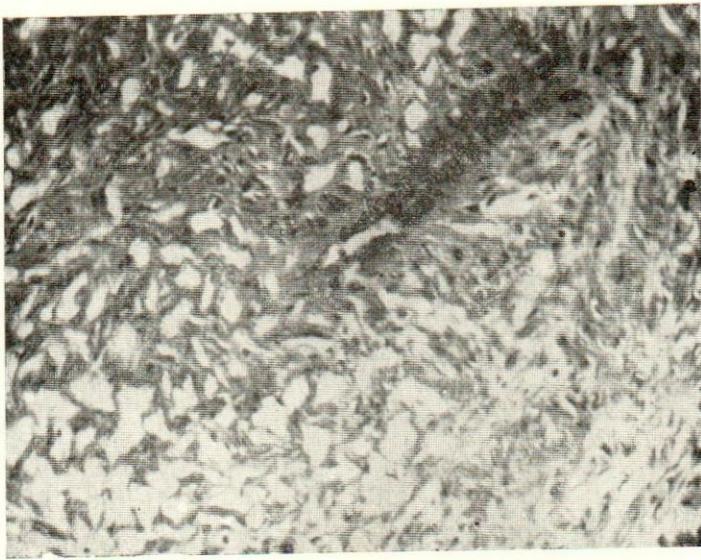


Fig. 4.—The increased connective tissue substitutes the liver cells in severe fascioliasis (H and E; $\times 25$).



Fig. 5.—Newly formed bile ducts in severe fascioliasis. (H and E ; $\times 64$).

N = normal
 S = slight
 M = moderate
 V = severe

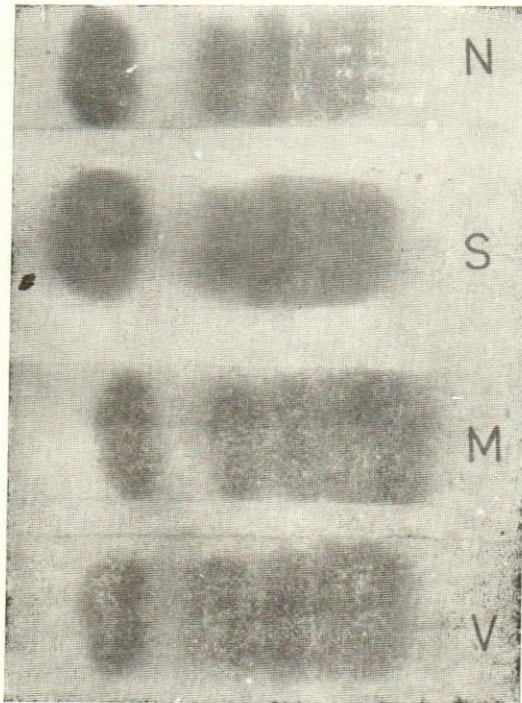


Fig. 6.—Showing the decrease of albumin and the increase of gamma-globulin in the moderate and severe stages of *Fasciola* infection.

