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BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES ASSOCIATED WITH MUSCULAR DYSTROPHY IN NEWLY BORN LAMBS

(With 4 Tables and 10 Figures)

By

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التغيرات البيوكيميائية والهستولوجية المرتبطة مع استحالات العضلات فى الحملان حديثى الولادة

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يهـدف هذا البحث إلى دراسة تشخيص الموت المفاجيء فى الأغنام حديثة الولادة والوقوف لمعـرفة سبب الوفاة حتى نتلاشى مثل هذه الظاهرة وذلك بواسطة الإختبارات البيوكيميائية المخـتلفة هذا بالإضافة إلى دراسة الصفة التشريحية والتغيرات الهستوباثولوجية. واشتملت هذه الدراسة على إجراء اختبارات تشخيصية على مجموعتين الأولى تضم ١٣ (ثلاث عشر) حـيوان رضيع مصاب بعرج وعدم انتظام فى الحركة – أعراض تنفسية اسهال والمجموعة الثانـية تضم (٣) ثلاث حيوان رضيع لا يوجد عليها أية أعراض وتعتبر كضابطة للتجربة. وكانـت أهـم الملاحظات التشخيصية على الحيوانات المريضة هـى: ١- ارتفاع مستوى انـزيم اوكسـالو اسـيتك ترانس امينيز والبيروفيك استيك ترانس امينيز زانزيم الكرياتينين فوسـفوكينيز وانزيم اللكينك دهيدروجينيز فى المصل. ٢- انخفاض مستوى فيتامين هـ فى المصل. ٣- انخفاض مستوى أملاح السيلينيوم فى المصل. ٤- انخفاض مستوى الصوديوم والكالسـيوم والفسـفور فى مصل الدم. ٥- زيادة فى مستوى البولينا والكرياتينين وحمض البولـيك والبوتاسـيوم فـى المصل. ٦- يوجد نقص ملحوظ فى كرات الدم الحمراء ونسبة الهيموجلوبين وكذلك PCV ممـا يـؤدى إلى ظهـور الميكروسيتـك ميكروكوميك انيميا. ٧- وجود تنكرز وستحالة بعضلات القلب والعضلات الهيكلية مع وجود تغيرات باثولوجية واضـحة بالكبد والرقبة والكليتين. مما سبق يتضح أن انخفاض نسبة السيلينيوم مع فيتامين هـ يؤدى إلى ظهور هذه الأعراض فى الأغنام الرضيعة وبسبب نقص فى جميع العناصر المخـتلفة مـع زيادة إنزيمات الكبد والكلى والعضلات مما يؤدى إلى ضمورها وينتج عنه نفوق هذه الحملان.

SUMMARY

This study was performed to diagnose Nutritional Muscular Dystrophy (NMD) in newly born lambs using biochemical, haematological and histopathological methods. Two groups of animals were used in this investigation, the first group included 13 lambs suffering from lameness, insufficiency of movement, stiff gait, emaciation and weakness. The second group included 3 apparently healthy lambs used as control. Blood and serum samples were collected from each animal for haematological and biochemical investigation. Three affected lambs were slaughtered for postmortem and histopathological examination. Specimens from skeletal muscles, lung, heart, spleen, liver and kidneys were examined histopathologically. The obtained results revealed: 1) Elevation in ALT, AST, CK and LDH activities. 2) Elevation of urea, creatinine and uric acid levels. 3) Decrease in serum vitamin E level (α-tocopherol). 4) Decrease in serum selenium level. 5) Decrease in serum sodium, total calcium and inorganic phosphorus and increase potassium levels. 6) Microcytic hypochromic anaemia characterized by low MCV and MCH. 7) Vacuolar degeneration of cardiac muscles. 8) Hyalinization of skeletal muscles. 9) Coagulative necrosis in the epithelial cells lining the proximal convoluted tubules of kidney. 10) Focal necrosis of hepatic tissue with kupffer cell proliferation. 11) Hyperplastic activation in the lining epithelial cells of the lung bronchioles. This study indicated that the nutritional muscular dystrophy occurs mostly in young lambs less than one month causing high mortality rate due to cardiac myopathy and respiratory failure.

Key words: Muscular dystrophy, newly born lambs

INTRODUCTION

Several diseases are caused by or associated with deficiency of either selenium or vitamin E or both which usually predisposes by some uncertain conditions such as an increase in dietary unsaturated fatty acids, unaccustomed exercise and rapid growth in young animals (Meschy, 2000). Natural vitamin E in feed stuffs is unstable in the presence of heat, oxygen and moisture as these conditions promote oxidation. Also, activity of vitamin E decreases during processing and storage of feed stuffs as well as during manufacture and storage of finished animal feeds (Coelho, 1991). Consequently animal

supplementation with vitamin E is recommended, particularly for high producing or rapidly growing animals or those under stressful environmental condition (Valentino *et al.*, 2000).

Nutritional muscular dystrophy is principally a disease of herbivorous which is characterized by degenerative myopathy of variable severity. It causes clinical syndrome of muscular stiffness and disability in the early weeks of life (Rue and Brintional, 1982).

In young lambs, muscular dystrophy is characterized by generalized weakness, stiffness and deterioration of muscles (Greig and Hunter, 1980). The lesions of the affected muscles are usually of non progressive character and the affected tissues have a remarkable capacity for complete regeneration (Ramirez *et al.*, 2001). Alteration in enzymatic activities due to nutritional muscular dystrophy were recorded by many investigators (Rue and Brintional, 1982; Fiy *et al.*, 1991 and Bickhardt, 1999). The present study was undertaken to estimate the sero-biochemical and histopathological changes in newly born lambs suffering from muscular dystrophy associated with high mortality rate.

MATERIAL and METHODS

Animals: In a private farm in Sakha (Animal Production Unit), about thirteen lambs out of fifty were suffering from lameness, insufficiency of movement, stiff gait, weakness, and emaciation. The rest were apparently normal. They were all about 2-3 weeks old. Three of them were slaughtered for post-mortem and histopathological examination.

Samples: Blood samples were obtained from all of 13 diseased and 3 control lambs. Two blood samples were taken in clean and dry test tubes. One of them contains heparin as anticoagulant (1-2 IU/ml) to be used for haematological examination. The other sample was left to clot and centrifuged for separation of clear serum to be used for estimation of different biochemical parameters as shown in table (1). The heparinized samples were used for erythrocytic and leucocytic counts using improved Neubouer haemocytometer and calculated according to Richard *et al.*, (1999).

Pathological examination: Postmortem examination was done immediately after slaughter and tissue specimens from lung, heart, liver, spleen, kidney and skeletal muscles were taken and fixed in 10% neutral buffered formalin, then processed for paraffin embedding. Sections of 4-5 micron were prepared and stained with haematoxylin and eosin Bancroft *et al.* (1994).

Biochemical analysis:

Table 1: procedures adopted for serum biochemical analysis.

Parameter	Author
Selenium	Bunriel and Ramirez (1957)
Vitamin E (α tocopherol)	Quaife (1947)
Creatine kinase CK	Szase and Busch (1979)
Lactate dehydrogenase (LDH)	Caboud and Worblewski (1958)
Alanine aminotransferase (ALT)	Reitman and Frankel (1957)
Aspartate aminotransferase (AST)	Reitman and Frankel (1957)
Urea	Coulombe and Fevreau (1963)
Creatinine	Husdan and Rapaport (1968)
Total calcium	Glinder and King (1972)
Inorganic phosphorus	Klichling and Freiburg (1951)
Total magnesium	Neil and Neely (1956)
Sodium and potassium	Buriel and Ramirez (1957)
Uric acid	Arliss and Entvistle (1981)

Statistical analysis: the obtained results were statistically analyzed using student-t test according to Petrie and Watson (1999).

RESULTS

Results are tabulated in four tables and ten figures.

DISCUSSION

The main clinical feature of the affected lambs were lameness, insufficiency of movement, stiff gait, emaciation and weakness that indicating nutritional muscular dystrophy. Similar clinical picture in the early weeks of life was recorded by Rue and Brintional, (1982) and Pord *et al.* (1995).

Postmortem findings: Muscles were pale in colour oedematous and moist. Examination showed congestion of the internal organs (lungs, liver, kidneys and heart with hydropericardium). There were emphysema and petichial haemorrages scattered on the surfaces of the lungs. Pale yellowish patches were seen on the surface of liver. Similar findings were recorded by Rue *et al.*, (2000).

Haematological results: Microcytic hypochromic anaemia with decrease in RBCs, Hb, PCV and decrease in MCV were reported in the affected animals compared to the apparently healthy lambs as shown in table (2). The obtained results are in consistence with that reported by Salah (2000).

These results, revealed generally a coexistence of clinical marginal anaemia with clinical laboratory selenium and vitamin E deficiency. These obtained results were in agreement with results reported by Salah (2000) who mentioned that the selenium is necessary for normal haemoglobin formation, where the normal selenium nutrition is essential for iron absorption and its transportation to the liver and reticulo-endothelial system.

Serum biochemical analysis: Results shown in table (3) indicated significant increase in the activities of ALT, AST, CK and LDH enzymes. This can be due to enzyme release from damaged musculature as reported by Martin (1983) and Fry *et al.*, (1994). As CK and LDH enzymes are present in high concentration in the heart and skeletal muscles of sheep Brown and Wanger (1968). Similar findings were reported by Fry *et al.*, (1999) who mentioned that serum CK activities which have reference range < 500 IU/L in healthy lambs may reach 5.000-10.000 IU/L in the affected animals, also AST activity may be elevated 3-10 fold above normal in nutritional muscular dystrophy. The same was recorded by Bickhardt *et al.* (1999) who recommended the estimation of plasma ALT and CK with α tocopherol for diagnosis of NMD. On the other hand Rue and Brintional (1982) reported increase in lactate dehydrogenase enzymes (LDH) due to muscular damage of affected sheep by selenium and Vit. E deficiency. Results in Table (3) also indicated significant elevation in serum urea and creatinine in the affected lambs accompanied with histopathological changes in kidneys represented by coagulative necrosis in the epithelial lining of proximal and distal convoluted tubules and focal leucocytic infiltration as shown in (Figs., 5 & 6), which reflect kidney inflammation. Similar findings were observed by Letoublon *et al.*, (1971), Ali (1992) and Rue *et al.*, (2000) in NMD affected lambs. The recorded results in table (3) showed significant decrease in serum selenium and α tocopherol (vitamin E) in affected lambs compared with apparently healthy ones, these finding together with that of increase enzyme activities indicate nutritional muscular dystrophy. Results of serum total calcium, total magnesium, inorganic phosphorus potassium and sodium levels in affected and apparently healthy lambs were presented in table (4). The tabulated results showed significant decrease in serum total calcium, total magnesium, inorganic phosphorus and sodium levels in the affected animals with significant increase in serum potassium level. Blood and Hunderson (1974) and Ali (1992) reported similar decrease in calcium level of lambs affected with NMD. Also Kursa (1974) and Ramirez *et*

al., (2001) reported decrease in calcium, inorganic phosphorus, magnesium and sodium levels in young lambs affected by NMD. The marked decrease in serum sodium with the significant increase in serum potassium levels also reflects the renal failure together with the pathological changes reported in kidneys due to deficiency in vitamin E and selenium in lambs. These results resembled that reported by Ali (1992) and Salah (2000).

On the other hand Kursa (1974) and Ramirez *et al.*, (2001) found decrease in serum potassium level during the course of the disease due to degenerative change in proximal and distal renal tubules.

Histopathological findings: histopathological examination of skeletal muscles revealed focal infiltration of mononuclear leucocytic inflammatory cells in between the muscle bundles which appeared hyalinized, highly eosinphilic and lost their striation as shown in Figs. (1 & 2). The cardiac muscle also showed intrasarcoplasmic vacuolar degenerative changes in focal manner as seen in Fig. (3). The observed myocardial degeneration may be due to rapid accumulation of calcium in damaged myocardial cell which interfere with mitochondrial activity and as mitochondria bind more calcium, their ability for respiration and phosphorlation decreases as recorded by Cheema and Gilani (1978). Martin (1983) reported that the histopathological characters of skeletal and cardiac muscles in case of selenium and vitamin E deficiency were degenerative and necrotic alterations. Also, Greig and Hunter (1980) observed the deterioration of muscles in young lambs suffering from NMD. Diffuse lymphoid depletion was observed allover the white pulp in spleen as shown in Fig. (4). These results resembled those reported by Martin (1983). There was coagulative necrosis in the epithelial cells lining the proximal and distal convoluted tubules associated with focal mononuclear leucocytic inflammatory cells infiltration (Figs., 5 & 6). There was hyperplastic activation in the lining epithelial cells of the lung bronchioles forming a finger like projections protruded in the bronchial lumen with sever dilatation of the peribronchiolar blood vessels and oedema (Fig, 7) the same was seen by Rue *et al.*, (2000).

On the other hand there was diffuse proliferation of kupffer cells allover the hepatic tissue (Fig., 8). Focal necrotic area in the hepatic tissue associated with an increased proliferation of kupffer cells were noticed (Figs., 9 and 10). The same was seen by Rue and Brintional (1982), Martin (1983), Bickhardt *et al.* (1999) and Rue *et al.*, (2000). From this study, it can be concluded that deficiency of vitamin E (α tocopherol) and selenium in newly born lambs causing NMD

(Nutritional Muscular Dystrophy), characterized by degenerative myopathy and necrosis of skeletal and cardiac muscles and highly elevated AST, ALT, CK and LDH, with microcytic hypochromic anemia. These serobiochemical and histopathological changes is a good evidence of deficiency of tochoperol and selenium which is the main cause of high mortality in young lambs.

Table 2: Haematological findings in affected and apparently healthy lambs.

Haematological parameters		Apparently healthy animals (n=3)	Affected animals (n=13)
RBCs (10^6/ul)		9.82 ± 0.46	6.64 ± 0.45**
Hb (gm/dl)		12.82 ± 0.49	9.04 ± 0.37**
PCV (%)		38.64 ± 1.02	31.14 ± 0.62***
MCV f_l		4403 ± 1.99	48.0 ± 2.17**
MCH (pg/cell)		13.22 ± 0.53	13.48 ± 1.01
MCHC (gm/dl)		33.61 ± 1.13	28.35 ± 1.07**
TLC (10^3/ul)		9.50 ± 0.37	9.0 ± 0.14
Absolute differential leucocytic count (10^3/ul)	E	0.45 ± 0.07	0.52 ± 0.04
	B	0.008 ± 0.0002	0.52 ± 0.04
	M	0.60 ± 0.01	0.64 ± 0.025
	L	4.20 ± 0.18	4.45 ± 0.25
	N	3.85 ± 0.06	4.12 ± 0.21

N = number of animals.
* P < 0.05 = significant.
** P < 0.01 = more significant.
*** P < 0.001 = highly significant.

Table 3: Serum biochemical parameters of the affected and apparently healthy lambs.

Parameter group	Apparently healthy	Diseased animals
ALT (U/L)	58.5 ± 2.1	77.3 ± 3.22**
AST (U/L)	15.3 ± 0.32	114.6 ± 1.2***
CK (IU/L)	170 ± 6.25	2130 ± 94.3***
LDH (IU/L)	1250 ± 90.1	3670 ± 112.6***
Selenium mg/dl	8.28 ± 0.45	0.08 ± 0.003***
Vitamin E µg/d	3.97 ± 0.28	0.2 ± 0.01***
Urea mg/dl	38.4 ± 12	51.6 ± 1.44**
Creatinine mg/dl	0.92 ± 0.04	1.84 ± 0.06**
Uric acid mg/dl	7.1 ± 0.35	8.4 ± 0.44*

Table 4: Serum analysis of some electrolytes in affected and apparently healthy lambs.

Electrolytes	Apparently healthy	Diseased animals
Total calcium (mg/dl)	2.75 ± 0.06	1.92 ± 0.03*
Inorganic phosphorus (mg/dl)	1.65 ± 0.07	1.02 ± 0.04*
Total magnesium (mg/dl)	1.16 ± 0.1	0.92 ± 0.1*
Sodium (mg/dl)	141 ± 2.1	130 ± 1.98*
Potassium (mg/dl)	3.1 ± 0.31	5.1 ± 0.4**

Fig. 1: Skeletal muscle of affected lamb showing mononuclear leucocytic inflammatory cell infiltrations inbetween the hyalinized muscle (H & E X 40).

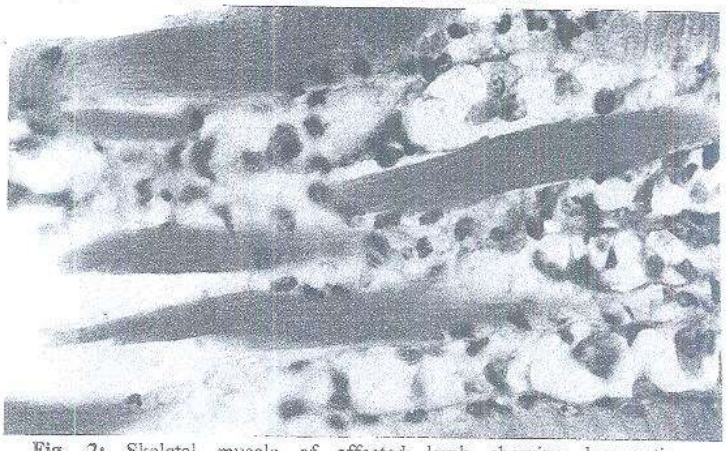

Fig. 2: Skeletal muscle of affected lamb showing leucocytic inflammatory cells inbetween hyalinized skeletal muscle bundles by higher magnification (H & E X 160).

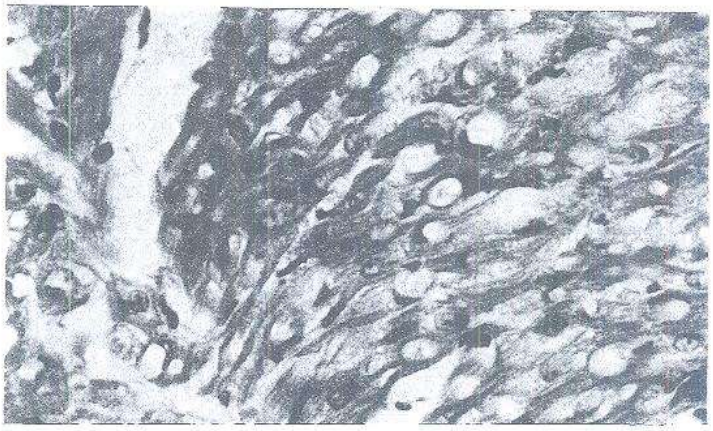

Fig. 3: Heart of the affected lamb showing vacuolar degeneration in the sarcoplasma of the myocardial muscle (H & E X 160).

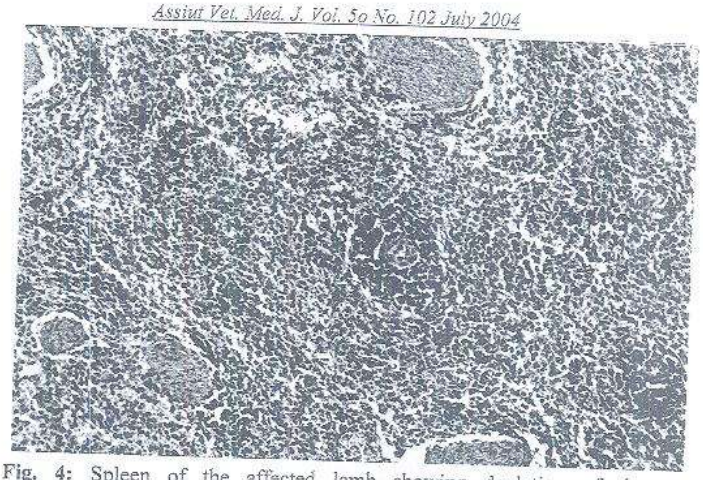

Fig. 4: Spleen of the affected lamb showing depletion of the lymphocytes in the white pulps (H & E X 40).

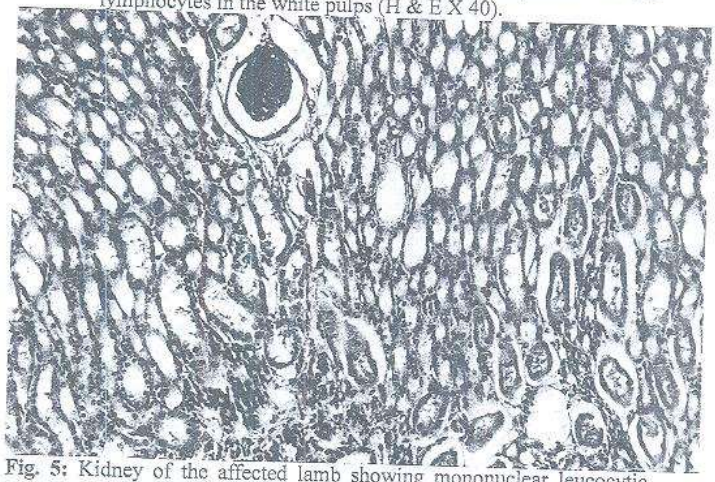

Fig. 5: Kidney of the affected lamb showing mononuclear leucocytic inflammatory cell infiltrations inbetween the degenerated and necrosed renal tubules (H & E X 40).

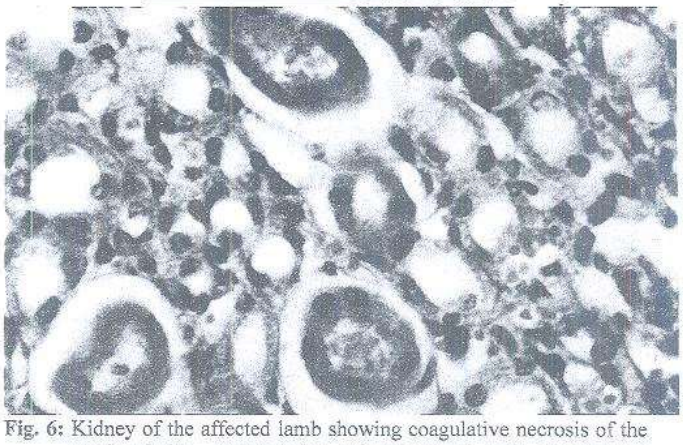

Fig. 6: Kidney of the affected lamb showing coagulative necrosis of the renal tubules, extravasation of red blood cells as well as leucocytic inflammatory cell infiltrations by higher magnification (H & E X 160).

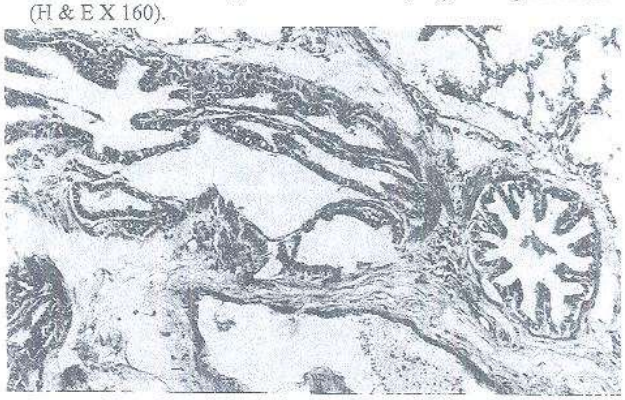

Fig. 7: Lung of the affected lamb showing hyperplastic proliferation in the lining epithelial cells of the lung bronchioles forming finger like projections protruded in the bronchial lumen with sever dilatation of the peribronchiolar blood vessels and oedema (H & E X 40).

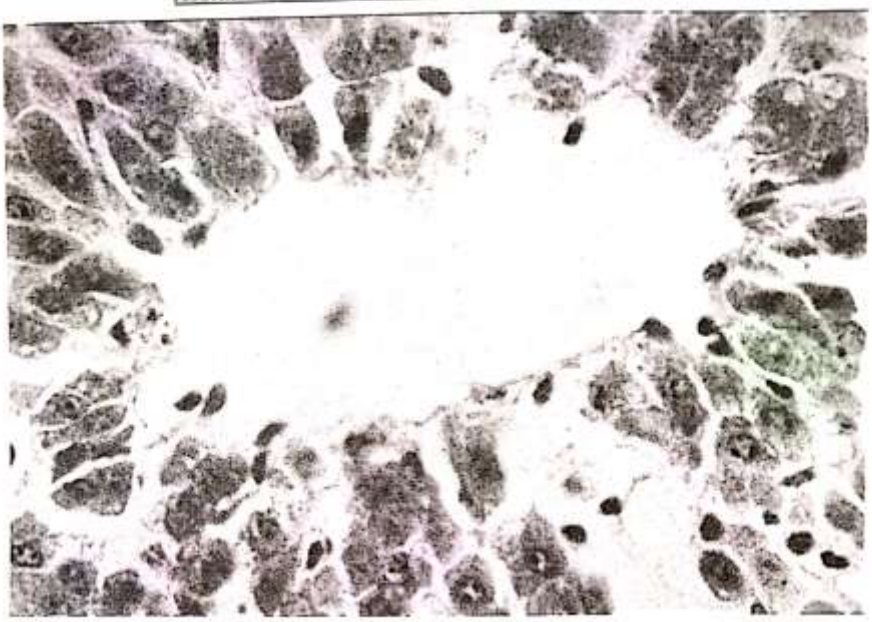

Fig. 8: Liver of the affected lamb showing kupffer cell proliferation (H & E X 160).

Fig. 9: Liver of the affected lamb showing focal necrotic area (H & E X 40).

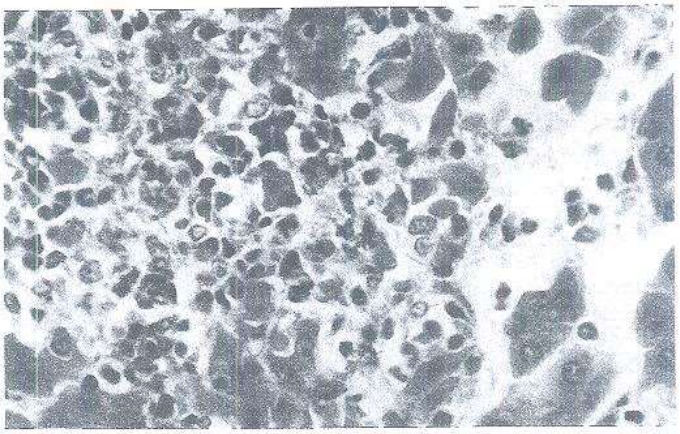

Fig. 10: Liver of the affected lamb showing focal necrosed area (H & E X 160).

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