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PATHOLOGY OF RABBIT VIRAL HAEMORRHAGIC DISEASE: HEPATIC AND PULMONARY LESIONS

(With 10 Figures)

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النزف الدموي الفيروسي في الأرانب: الآفات الباثولوجية في الكبد و الرئة

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تم تشخيص وباء مرض النزف الدموى الفيروس في محافظة أسيوط وقد اعتمد التشـــخيص المرضى على تاريخ المرض، أعراضه، الآفات الباثولوجيـــة، التغـــيرات الميكروســـكوبية. وصلت نسبة الوفيات في هذا الوباء الى ٦٠% في الأرانب المفطومة حديثـــا و ٨٠% فـــي الكبار. وكانت أهم الأعراض الموت المفاجئ وأنزفة الأنف وقد أظهر التشريح المرضـــى وجود أنزفة بالرئة وتتكرز بالكبد. وقد أوضحت الصورة الهستوباثولوجية تتكرز تجلطـــي وكذلك تتكرز تحللي في الكبد بالإضافة إلى الأنزفة الكبدية والأجسام التجميعية. في الرئـــة كان وجود النزيف في الحويصلات الهوائية ووجود الخلايا الإلتهامية بالحويصلات الهوائيـــة والأوعية الدموية في كل الشرائح التي فحصت.

SUMMARY

An outbreak of rabbit haemorrhagic disease (RHD) is diagnosed in Assuit governorate. Diagnosis is based on history, clinical symptoms, post mortem lesions, histopathological lesions, virus isolation, and haemoagglutination and haemoagglutination inhibition test. Mortality rate reached 60% in recently weaned youngs and 80% in adults. Sudden death with epistaxis was characteristic clinically. On dissection pulmonary congestion and haemorrhages were consistent findings. Coagulative and lytic necrosis, parynchymal and portal haemorrhages in

addition to the presence of inclusion bodies were the main hepatic lesions.

Key words: *Rabbit viral Haemorrhagic Disease.*

INTRODUCTION

The disease is caused by genus cailcivirus, Meyers et al. (1991), Park and Itakura (1992) and studdert (1994). The virus like particles were reported to be present in the nucleus or cytoplasm of hepatocytes or other cells, Marcato et al. (1988) while Park et al. (1992) and Park and Itakura (1992) reported that the virus has a predilction for hepatocytes and replicates in their cytoplasm.

Peracute, acute and subacute forms were described. Sudden death without any symptoms in the peracute form. High fever, depression, foamy bloody discharge from external openings and convulsions before death were reported in the acute and subacute forms, (Cao et al., 1986, Lee et al., 1990, Sammim et al., 1995 and Sandford 1996).

At necropsy peticheal and ecchymotic haemorrhages in subcutaneous tissues, in the respiratory organs, liver and occasionally in the kidneys were reported. In addition swollen, pale, friable liver with necrotic foci was frequently described, Xu and Chen (1989), Salem and El-Ballal (1992) and Collery et al. (1995).

Xu et al. (1985), Nowatony et al. (1992) described the histopatho logical findings of RHD as necrotic hepatitis with slight polymorph nuclear leucocytic infiltration, pulmonary congestion and haemorrhages and non-suppurative encephalitis. The presence of pulmonary intravascular macrophages was added by Carrasco et al. (1991). Hepatocytic acidophilic intracytoplasmic inclusions were described by Stoerckle Berger et al. (1992) and Abdel Aziz et al. (1995), while Nawwar et al. (1996) and Aly (1998) described intranuclear inclusion bodies in intact hepatocytes. Some authors described the disease with neither intracytoplasmic nor intranuclear inclusions, Boucher (1989).

In Egypt the disease causes great economic losses (Ghanem and Ismail 1991, Salem and El-Ballal 1992, El Zanaty 1994, Abdel Aziz et al., 1995 and Aly 1998).

The objectives of this work were directed to identify the causative agent of the outbreak and study in details the gross and histopathological findings in the livers and lungs of the naturally

infected rabbits. The pathological changes in the other organs will be published later.

MATERIALS and METHODS

During this winter season (1999-2000), an outbreak of rabbit haemorrhagic disease (RHD) is diagnosed in Assuit Governorate. Eight out of ten adult rabbits and twelve out of twenty recently weaned young rabbits died. The case, history and clinical symptoms were recorded. The post mortem examination was performed on all dead animals. Samples from livers and lungs were taken from freshly dead rabbits as a material for virus demonstration Haemoagglutination , Haemoagglutination inhibition tests and histopathological examination. For the latter the samples were fixed in 10% neutral buffered formaline. The routine histopathological technique was performed and paraffine sections were stained with haematoxylin and eosin (Bancroft, 1982). For bacteriological isolations smears from liver and heart blood were cultured on nutrient agar, blood agar and cooked meat media.

For virus isolation the method of Liu et al. (1984) and Chen 1986 was conducted. Haemagglutination test and haemagglutination inhibition test were done after Pu et al. (1985).

RESULTS

Aetiological results:

Smears from liver and heart blood were negative for bacterial isolation. Tissue suspension from liver and lungs gave positive HA using 1% washed avian RBCs. Virus inoculation via allantoic sac of chicken embryos gave no embryonic deaths or embryonic lesions. The embryonic fluid and liver suspension gave negative HA using avian RBCs.

Clinical symptoms: Sudden deaths without any symptoms were observed in most of cases. Some of the affected rabbits showed high fever, depression, foamy bloody discharge from external openings, in coordination and convulsions before death. The morbidity rate was 100% but the mortalities ranged from 60% in youngs to 80% in adults.

Post mortem examination: Post mortem examination revealed severe hyperaemia of the subcutaneous blood vessels. Haemorrhages and oedema in the body cavities were of variable amounts. In all the examined rabbits, the lungs were voluminous, darkly congested and

rather firmer than normal. Irregularly sized patches of haemorrhages were detectable. On cut section blood oozes freely. In all the dissected cases the livers were enlarged friable and pale in colour. The hepatopathic lesions were obvious grossly as irregularly distributed tiny pale or less often dark foci. The foci were sharply delineated from the adjacent parenchyma. The size of such foci was variable, ranging from small to several centimeters in diameter. Near the gall bladder, the liver was slightly greenish discoloured by bile pigment. Variably sized dark red foci of haemorrhages ranging from petichae to ecchymosis were seen especially at the margin of the liver. Congested livers oozed blood on cut section.

Histopathological findings: All the examined liver sections showed various degenerative changes, included proteinous dystrophy, hydropic degenration and fatty change. Among the examined cases such changes differ in intensity but were diffuse and constant in all cases. The hepatocytes were mostly dissociated and the cords were disrupted. Advanced changes were focally seen. Foci of necrobiosis revealed acidophilic coagulated cytoplasm. The nuclei were pyknotic and karyorrhetic (Fig1). Complete sharply delinated foci of coagulative necrosis were seen (Fig 2). In these areas partial loss of hepatic architecture was observed. Severe atrophy and necrosis of hepatic cords was seen in some sections, (Fig 3). The hepatic cords were thin , thread like discontinuous and completely compressed and atrophoied. Many of their cells loose their nuclei. In between the cords oedema, haemorrhages and few inflammatory cells could be seen.

In three out of the twenty examined cases, the hepatopathic alterations were slightly different. In addition to the necrobiotic and necrotic changes the foci of hepatic necrosis were associated with mild atrophy of cords. In between the cords the sinusoids were dilated and of various shapes. These dilated sinusoids contained polymorphnuclear cells with karyorrhetic nuclei. (Fig 4) Some hepatocytes revealed cytoplasmic and nuclear features of necrosis. Pyknosis and fragmentation of nuclear chromatin were prominent. In others the nuclei were completely lost. In some areas the necrosis reached to complete lysis and only the hepatic framework could be seen.

Variable sized foci of haemorrhages were seen in all the examined liver sections. These haemorrhages were either parenchymal (Fig 5) or in the portal triads (Fig 6). The parenchymal extravasions were seen either in place of necrotic and lytic hepatic cells or in between

the atrophied and expanded cords. The portal haemorrhages were seen perivascularly.

The vascular walls showed necrosis, hyalinization and separation of their layers. The extravasations were seen in between the blood vessel laminae. In the intact hepatocytes, the nuclei were mostly altered, (Fig 7 a,b,c,d). They appeared either with karyorrhetic nuclei or with marginated chromatin and enlarged nucleolus and some of them revealed acidophilic or slightly basophilic inclusion-like bodies. Such bodies were surrounded by hallow empty zone.

The intrahepatic bile ducts showed inward hyperplastic papillary projections from the epithelial lining (Fig 8). In some cases intraepithelial developmental Eimerial stages or intraluminal ocytes could be seen, but in most of cases the minimal hyperplastic changes in epithelium without any indication of protozoal infection.

All the lungs of the examined rabbits revealed focal intraalveolar haemorrhages (Fig 9). The neighbouring alveoli were more expanded and showed partial or complete rupture of their walls. In some alveoli alveolar macrophages were seen. In the vascular ramifications dystrophic changes in their walls were prominent. Thrombosis, endothelial degeneration, and vacuolation of the smooth muscle cells of the media were detectable. Intraluminally monocytes, polymorphnuclear cells and desquameted endothelial cells could be seen (Fig (10 a, b, c).

DISCUSSION

In the present investigation RHD could be diagnosed at Assuit Governorate. Several sporadic outbreaks were previously reported in many rabbitaries in Egypt. Ghanem and Ismail (1991), El Zanaty (1994) and Aly (1998).

In many epornitics of the disease in different countries, high mortality rates and great economic losses were reported Xu et al. (1985) and Cao et al (1986). Similar findings were also reported in Egypt, Abdel Aziz et al. (1995) and Aly (1998). In the present paper the mortality rate reached to 80% in adults and 60% in recently weaned youngs. Such high mortality rates and severity of lesions are probably due to the absence or deficiency of RHD viral-antibodies in the infected rabbits.

Sudden death and haemorrhages from external openings which were prevalent clinical features in this outbreak were observed also by Salem and El Ballal (1992) and Nawar et al. (1996).

On post mortem examination, the lesions observed were as described by (Sammin et al., 1995 and Sandford, 1996; Belemezov et al., 1989; Salem and El-Ballal 1992 and Nawar et al., 1996). These lesions included severe hyperaemia of the subcutaneous blood vessels, abdominal haemorrhages, frothy exudate and haemorrhages in the lungs. The liver was enlarged, pale in color and friable. The lesions in the liver were sharply delinated necrotic foci in addition to areas of haemorrhages. Collery et al. (1995) added haemorrhages in the myocardium, haemorrhagic fluid in the body cavities and enlargement of the spleen. In the present paper all the examined liver sections revealed various degenerative changes. These changes were, similarly reported by Gregg and House (1989), Marcato et al. (1992), and Aly (1998). In our opinion these dystrophic changes could be probably assumed either to the tropism and multiplication of the virus in the cytoplasm of hepatocytes or even to the angiopathic alterations which reached to complete endothelial destruction and thrombosis of some vessels.

The parenchymal focal haemorrhages seen in the present paper could probably be attributed to the severe lytic necrotic changes in he hepatic cords. In addition thrombosis of blood vessels noticed in the present paper may lead to capillary rupture and necrotic hepatitis. Similar thrombotic lesions were also noticed by Xu and Cxen (1989) and Gregg et al. (1991).

The portal haemorrhages observed in our results could be related to the vascular destructive changes which were either due to the direct effect of the Viraemia and destruction of blood vessels or even due to immuno-complexes formed during the course of the diseases. Plassiart et al. (1992) reported the probability of the virus induced immuno complexes inspite of the short incubation period and rapid course of the disease. In addition to the probability of virus tropism to the vascular endothelium could not be neglected. The hepatocytic intranuclear inclusion bodies which probably support the diagnosis of the diseases were also reported by Nawar et al. (1996) and Aly (1998) while Stoerckle Berger et al. (1992) and Abdel Aziz et al. (1995) described the intracyotplasmic location of the inclusions. Stoerckle-Berger et al. (1992) described granular intracytoplasmic reaction in an

immunohistochemical study. Salem and El-Ballal (1992), demonstrated the viral particles ultrastructurally.

Absence and presence of inclusion bodies in cytoplasm or nuclus of hepatocytes could probably be related to the strains of the virus and their tropism for multiplication.

The mild hyperplastic epithelial changes of the bile ducts seen in the present paper were also described by Fuchs and Weissenbock (1992).

The pulmonary changes in the present paper included circulatory disturbances, the interalveolar capillaries were congested. Microthrombi were seen in some vessels and intravascular macrophages were also noticed. Corrasco et al. (1991) explained that the stimulation of pulmonary macrophages occurs by congestion oedema and haemorrhages and they play an important role in retention and removal of the viral or particles of bacteria and cell debris from the blood stream, as also stated by Rogers (1958), and Mathisaon and Ulevitch (1979). Haemorrhages occurred within and inbetween the alveoli. The pulmonary circulatory disorders were also reported by (Xu and Chen, 1989; Park et al., 1992; Salem and El-Ballah, 1992; Ghanem and Ali 1993 and Abdel Aziz et al., 1995 and Aly, 1998). The presence of haemorrhagic pneumonia, desquamation of alveolar and bronchialar epithelium was described by Fuchs and Weissenback (1992), while hyperplasia of bronchial epithelium and peribronchial lymphocytic aggregations were also described by Ghanem and Ali (1993). The presence overinflated alveoli with partially or Completely ruptured wall could be considered as emphysmatous changes to compansate the function of the blood obliterated alveoli. It could be concluded that RHD is one of the serious diseases of rabbitary and the deaths could be attributed to the general circulatory disorders, the severe necrotic hepatitis and the extensive pulmonary haemorrhages. Tropism and cytopathic effect of the virus on endothelial cells needs experimental investigation. The detailed gross and microscopic lesions in other organs will be discussed later.

While hyperplasia of bronchial epithelium and peribronchial lymphocytic aggregations were also described by Ghanem and Ali (1993).

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LEGEND OF FIGURES

Fig. 1: Liver showing disrupted cords. Hepatocytes with coagulated acidophilic cytoplasm and pyknotic or karyorrhtic nuclei (H.& E. X 250).

Fig. 2: Liver showing sharply delinated foci of coagulative necrosis (H. & E. X 400).

Fig. 3: Liver showing severe atrophy of hepatic cords (H. & E. X 400).

Fig. 4: Liver showing dilated sinusoids containing polymorphnuclear leucocytes (H. & E. X 250).

Fig. 5: Liver showing parenchymal hemorrhages. (H. & E. X 400).

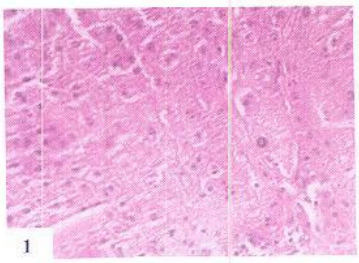
Fig. 6: Liver showing portal hemorrhages. (H. & E. X 250).

Fig. 7 a,b,c,d: Hepatocytic nuclei showing margination of chromatin, chromatorhexis and inclusion like bodies. Some nuclei with enlarged nucleolus. (H. & E. X 1000).

Fig. 8: Bile duct showing epithelial hyperplastic changes (H. & E. X 250).

Fig. 9: Lung showing focal intra-alveolar hemorrhages (H. & E. X 250).

Fig. 10: a,b,c: Pulmonary blood vessels showing degenerative changes (H. & E. X 400).



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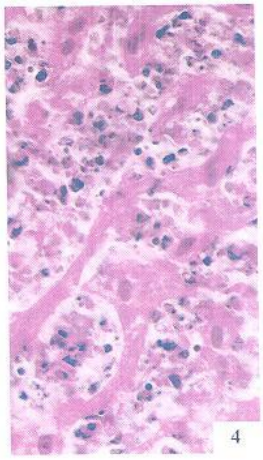

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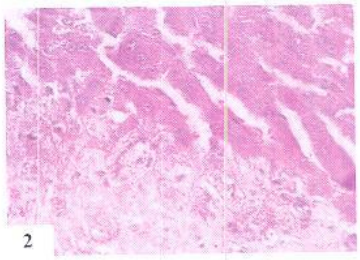

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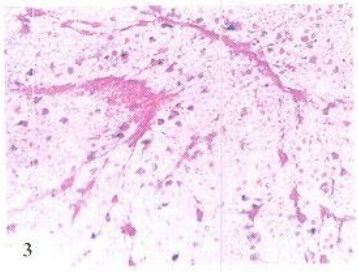

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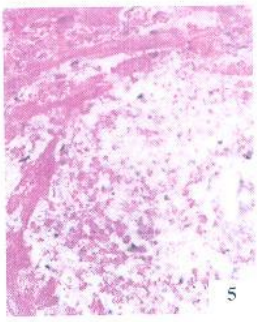

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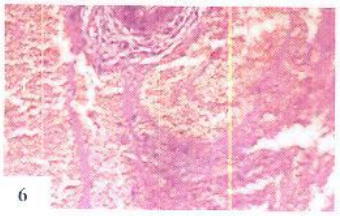

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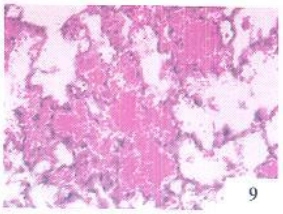

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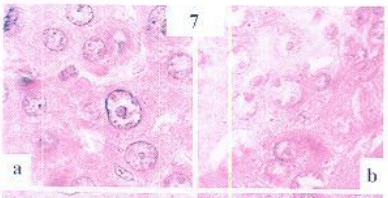

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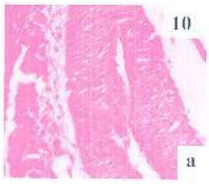

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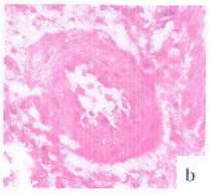

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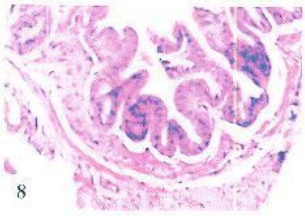

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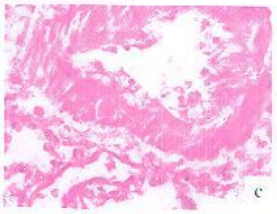

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