احداث غرغرينا جلد وعضلات الفخذ بالفراخ باستخدام الكلوستريديم ولشباى نوع (١)

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أجريت محاولة احداث غرغرينا جلد وعضلات الفخذ بالفراخ باستخدام ميكروب الكلوستريديم ولشباى نوع (١) وقد قسمت الطيور المستخدمه بالبحث الى مجموعات مختلفة على حسب طريقة الحقن وكميته . وقد حقنت بعض الطيور فى العضلات والأخرى تحت الجلد وقد استخدمت طريقة التشريط فى الجلد وتنقيط الميكروب فى الأجزاء السابق تشريطها وقد لوحظت الفراخ بعض الحقن ودرست الأعراض الأكلينيكية واجريت الصفة التشريحية ودرست التغيرات المروفولوجية وكذا التغيرات الهستوباثولوجية ونوقشت نتائج البحث.

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EXPERIMENTAL GANGRENOUS DERMATOMYOSITIS HAEMORRHAGICAL IN BROILER CHICKS

(With One Table)

By

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SUMMARY

An attempt to develop gangrenous dermatomyositis in broiler chicks was carried out. The authors used an overnight cooked meat broth culture of Cl. welchii type A either alone or in addition to a 5% solution of calcium chloride. Different routes of inoculation were applied and the chicks were observed to record the clinical signs. Post-morteum examination was conducted on dead birds and attempts for isolation of the microorganism were done. Histopathologically the lesions were mainly gangrenous dermatomyositis haemorrhagica and was localised in the skin and in the inoculated muscles. The lesions were fully described and discussed.

INTRODUCTION

Anaerobic infections are considered to be from the most frequently encountered bacterial diseases in poultry. Natural and experimentaly avian entrotoxaemias due to Cl. welchii infection was reported by many workers allover the world, and recorded it as the second most frequent infectious disease threatening the industrial production of broilers, KOHLER et al. (1977).

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In the aviable literature, concerning with avian dermatomyosistis, neither constant aetiological agent nor complete pathological description could be found. Many synonyms for the disease were used as, vesicular dermatitis, avian malignant oedema, gas oedema, gangrenous dermatitis and wing rot, HOFFMAN, (1939), ROSSI, (1956), FRAZIER, PARIZEK and GARNER, (1964), FOWLER and HUSSUINI, (1975).

HOFFMAN, (1939) isolated Staphylococci from natural cases of vesicular dermatitis, while ROSSI, (1956) isolated Cl. welchii and referred it as the aetiological agent for such conditions. Synergistic relationship between Staph. aureus and Cl. septicum was reported by FRAZIER, PARIZEK and GARNER, (1964). BICKFORD, (1971) reported that gangrenous dermatitis in broiler chicks is usually associated with Cl. septicum, although Cl. perferingens and Cl. novyi had been also isolated by the author. Experimental gas oedema in chicks could be produced by Saunders and BICKFORD, (1965) and HINZ et al., (1973) using intramuscular and subcutaneous injection of Cl. septicum clultures.

In the present study the authors used Cl. welchii type A to study its effect on the thigh muscles of broiler chicks and to investigate if this organism has an inflammatory effect on the muscles as its effect on the alimentary tract, moreover the authors tried to throw some light on the pathogenesis, morphology and histomorphology of the disease in broiler chicks.

MATERIALS AND METHODS

Thirty eight, of six weeks old broiler chicks were used in this study. The chicks were obtained from Assiut Governerate Chicken Farm and kept during the experiment on a commercial ration prepared by the Manufacture of Assiut Governerate.

Before the experiment, five chicks were taken at random and subjected to post-morteus examinations, intestinal and caecal smears were examind for bacterial and parasitic infections.

An overnight cooked meat broth culture of Cl. welchii type A was used either alone or in addition to a 5% solution of sterlized calcium chloride in physiological saline as a deveitalizing agent. The standard strain of Cl. welchii type A, number 25 G, was obtained from Arabtirima, Institute Pendik, Istanbol, Turki. The cooked meat broth was prepared according to SMITH and HOLDMAN, (1968) and modified by adding glucose to aid growth and toxin production of the micororganism. The birds were classified into seven groups according to the route of injection, nature and amoutn of inoculum as shown in Table 1.

After inoculation the birds were kept under observation, the clinical signs, morphological findings, histopathological findings also were recorded attempt for isolation of the inoculated organism was carried out. For histological examinations small pieces of the inoculated muscles, as well as specimens from the liver and intestine were taken and fixed in 10% neutral buffered formalin. The specimens were processed and the haematoxylin and eosin stained sections were examined.

CLINICAL SIGNS

After inoculation, the birds showed depression, droopping of their wings and appeared with ruffled feathers. Locomotor disturbances as lamness was also observed. Moreover some birds were lying on one side and showed no ablity to move. The inoculation site appeared greenish black in colour and showed oedmatous swelling with puterified odour and cripitated under the finger.

MACROMORPHOLOGICAL FINDINGS

There was no evidence of gross pathological alterations in the muscles and internal organs examined from groups no. III and IV which were used as control groups.The other five groups showed varying degress of localized gangrenous and necrotizing dermatomyositis haemorrhagica.

The covering skin was greenish black to black in colour,moist and devoid from feathers. Serosanguinous infilterations of the skin, subcutaneous tissues and inbetween the muscles fibres could be detected. Muscles of the thigh at the site of injection were swollen, soft, daughy, usually odour.

On dissection the muscles appeared gray or white in colour, unless filled with blood, firm,dense and often somewhat depressed when compared with the surrounding muscles. In chickens which were injected intramuscularly or subcutaneously with the double dose, extension of the gangrenous necrotic reaction to the abdominal muscles was observed.

The degree of reaction described above, varied according to the method of inoculation and the amount of broth culture used. The most severe reaction was seen in group II and group VI. Less reacgion could be observed in group I and group V. Very milk reaction could be observed in group VII.

MICROMORPHOLOGICAL FINDINGS

No histological alterations neither in the thigh muscles nor in the internal organs of group III and group IV, which recieved the bacteria free inoculations could be detected. Otherwise all the sections examined from the other five groups showed nearly the picture of necrotic, gangrenous dermatomyositis haemorrhagica accompanied with inflammatory cellular infilterations,oedema and gas bubbles as well as feature of regeneration and mild fibroblastic proliferations. The degree of reaction varied from goup to group according to the route of injection, nature and amount of inoculum. The severe reaction was noticed by using the intramuscular and subcutaneous robtes of injections. The most severe one was observed by doubling the dose and the mildest one was observed by using the process of scarification and dropping of the inoculum.

At the site of injection the covering skin was completely necrosed and even in some cases no epidermis could be found. The subcutaneous

tissues showed gas bubbles, heavy infilterations with mononuclear inflammatory cells, rod shaped, bluish stained bacteria and areas of haemorrhages. The muscle fibres appeared histologically swollen,homogenous, hyaline in texture and showed features of coagulative necrosis. The cell outlines were to some extent still distinguished. The sarcoplasm appears strongly acidophlic and the myofibrils could not be seen. The nuclei appeared small in size and hyperchromatic (Fig. 1). In some muscle cells the sarcoplasm undergone fragmentation and sarcoplasmolysis. Other cells appeared atrophied and shrinked and their nuclei showed karyorrhexis and even karyolysis. Some necrosed areas showed gas bubbles which were recognized as irrigular empty spaces of various sizes (Fig. 2). These spaces have no wall of their own and tended to be spherical. In some areas myomalacia and complete softening of the sarcoplasm could be also seen (Fig. 3). The blood capillaries in the area showed mild to severe necrobiotic changes in their endothelial cell lining. Those cells showed well marked karyorrhetic changes in their nuclei. Most of the examined blood capillaries showed mild to severe perivascular lymphocytic cellular infilterations as well as activation and proliferation of the adventitial cells (Fig. 4). MOreover some capillaries were ruptured and the erythrocytes could be seen inbetween the muscel cells. In one case at the site of infection local acute suppurative myositis could be seen (Fig. 5).

DISCUSSION

In the present study,the lesions were mainly gangrenous necrotizing dermatomyositis haemorrhagica. The reaction obtained by intramuscular and subcutaneous routes of inoculations were more severe than those observed in birds infected by scarification of the skin and dropping of the culture. This could be attributed to the character of clostridial microorganisms which is known to be strictly anaerobic, so that it fails to grow in aireated superficial areas of the skin or respiratory passages. The lesions characterized by hyaline degeneration and necrosis of

muscle fibres associated with haemorrhages are possibly due to the liberation of the toxins in the area. Clostridial welchii toxin is an phospholipase enzyme, lecithinase, which capable to break down connective tissue materials, including collagen and cell membranes, (MIMS, 1976 and WILLIS, 1977). The effect on cell membranes may include the vascular endothelial membranes leading to the necrobiotic changes and the widespread haemorrhages observed in this study and or their rupture after thrombosis, BURNET and SCHUSTER, (1973).

Gas production, due to the fermentation of the carbohydrate content of the muscle cells after their death is probably the cause of the appearance of the gas bubbles in the tissue sectins of clostridially infected tissue, CHORS, (1962).

McCARTHY et al., (1963) indicated that small number of the bacteria is present in the normal tissue, 40-50% of the birds examined and that 61-74% of poultry bruises harbored relatively large number of both aerobic and anaerobic bacteria. These included Gram-positive and negative cocci, Gram-positive and negative rods and yeasts. Thirty six percent of Gram positive cocci were found to belong to the genus Staphylococci. Moreover the authrors established that the skin of birds is a source of these bacteria and a possible entery site to the traumatized areas. This may be the probable cause to the suggested mixed infections in the literature.

The changes occurring in traumatized tissues, espically in haemoglobin and the formation of its degradation products (hematin, biliverdin, bilirubin) may stimulate the gross of the microorganisms that may gain entrance into the contused areas, (McCARTHY et al., 1963, HAMDY and BARTON, 1965). The isolation of Staphylococci from naturally infected cases, (HOFFMAN, 1939, FRAZIER, PARIZEK, and GARNER, 1964) may, therefore, represent a secondery invador having no relation in inducing the primary lesion.

The Clostridium septicum may also induce similar lesions in broilers as stated by, FRAZIER, PARIZEK and GARNER, (1964) and BICKFORD,

(1971), is quite possible, since it is known to be associated with malignant oedema in animals and gas gangrene in man, both diseases are characterised by necrosis and oedema of the tissues which is frequently haemorrhagic, (SMITH and JONES, 1966 and JUBB and KENNEDY, 1970).

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Table 1

Showing route of injection, nature and amount of inoculum

Group	No. of animals	Route of infection	Nature of inoculum
I	6	I/M	1 ml culture + 0,2 ml cacl sol.
II	6	I/M	2 ml culture + 0,2 ml cacl sol
III	3	I/M	0,2 ml cacl sol
IV	3	I/M	1 ml sterile broth
			1 ml culture
V	6	S/C	1 ml culture
VI	3	S/C	2 ml culture
VII	6	Drop.	1-2 ml culture

I/M = Intramuscular S/C = Subcutaneous

Drop. = Scarification and dropping of culture

Cacl sol. = Calcium chloride solution

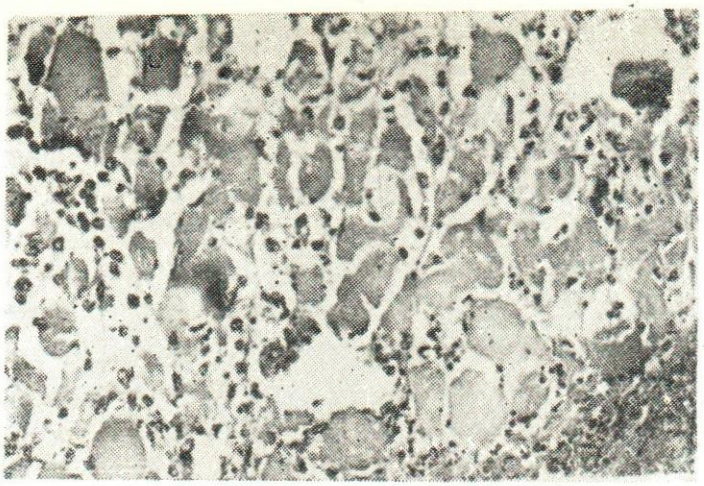
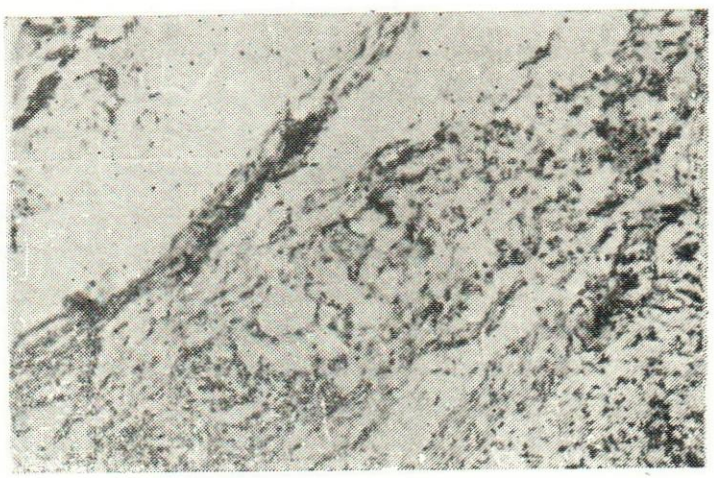


Fig. 1 Cross section in the muscle showing coagulative necresis. (H & E) (x 250).



Flg. 2 : Area of necrosis show gas bubbles (H & E) (x 100)

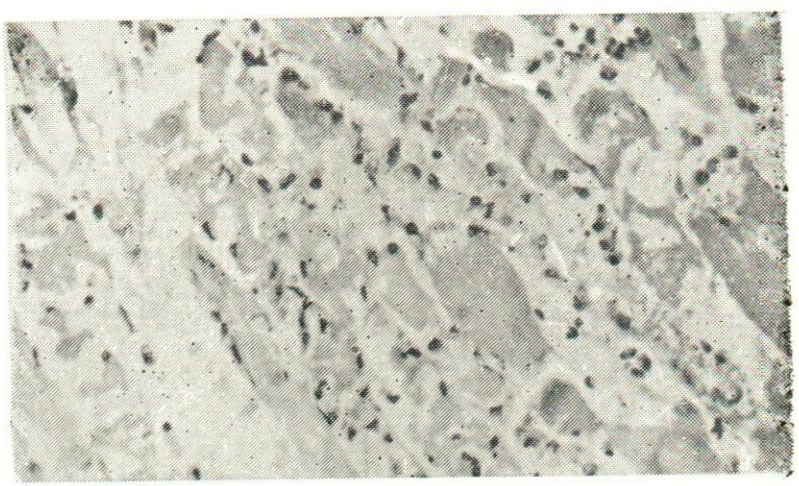


Fig 3 : Longitudenal section in the muscle showing myomalacia and softening of the sarcoplasm. (H & E) (x 400).

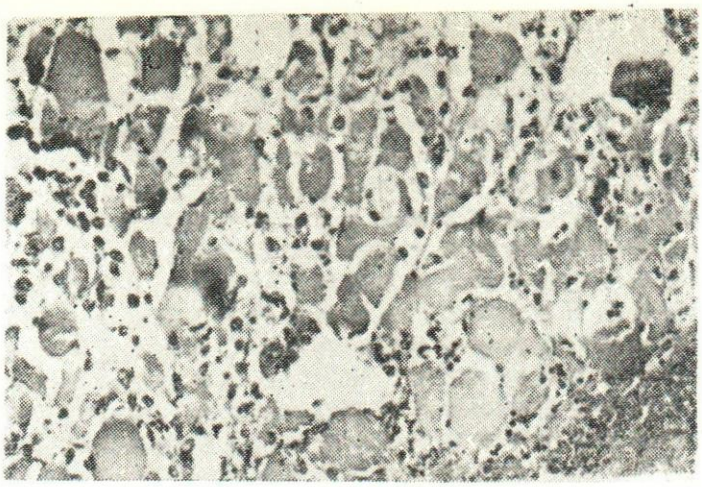
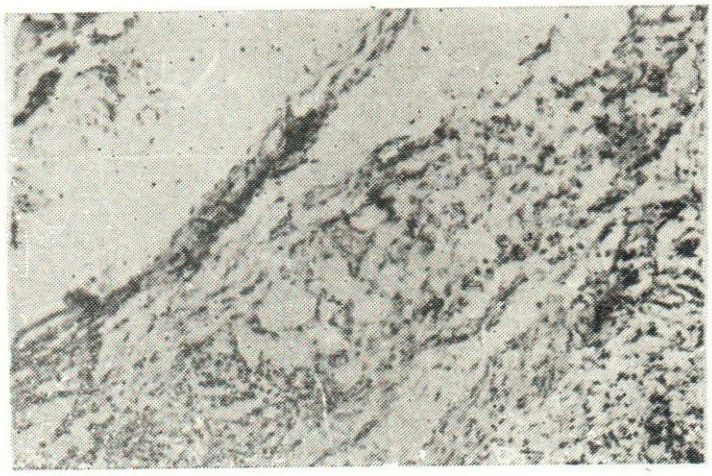
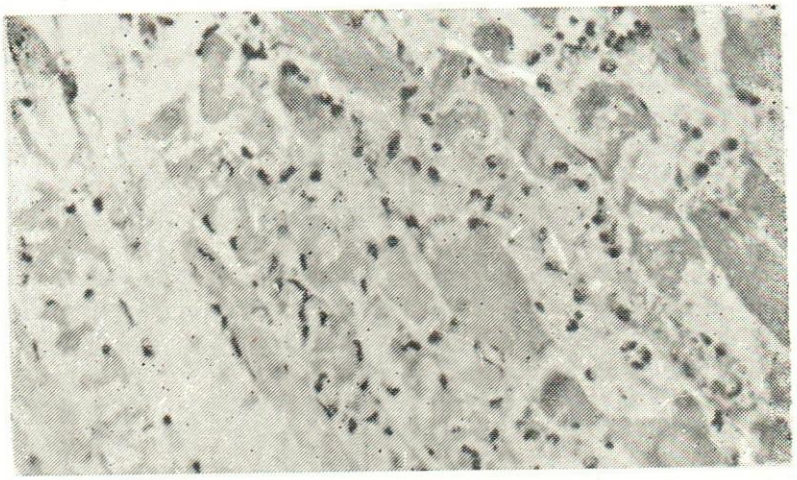


Fig. 1 Cross section in the muscle showing coagulative necresis. (H & E) (x 250).



Flg. 2 : Area of necrosis show gas bubbles (H & E) (x 100)



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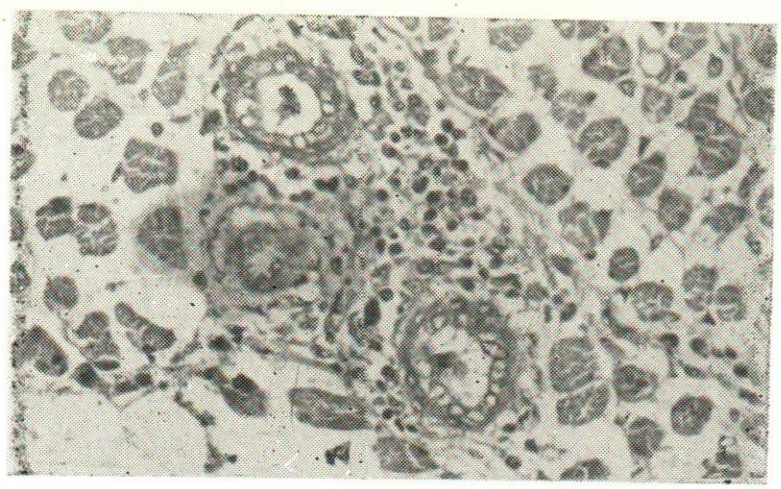

Fig. 4 : Blood vassels showing necrobiotic changes in their endothelium and perivascular lymphocytic infilteration (H & E) (x 250).

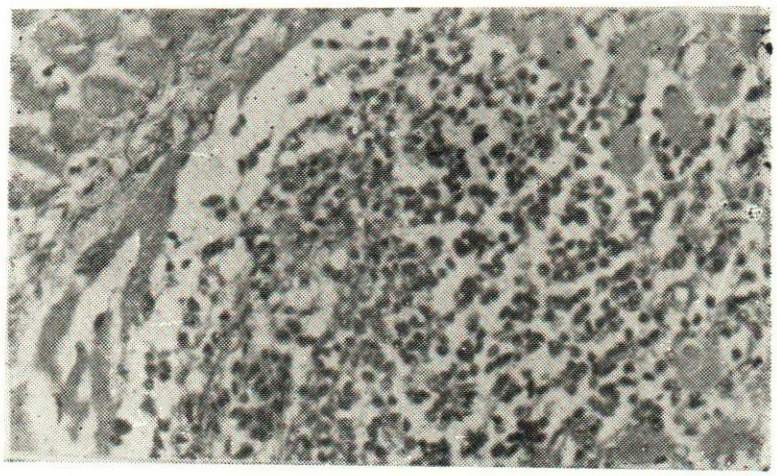

Fig. 5 : A section showing local acute suppurative myositis (H & E) (x 250).