دراسة بثالوجية لتأثير عدوى البابيزيا باى جيمينا التجريبية على عجول البقر والجاموس المصرى قبل وبعد ازالة الطحال

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الملخص

درس تأثير العدوى التجريبية للبابيزيا باى جيمينا على عجول البقر والجاموس بعد وقبل أزالة الطحال . وجدت تغيرات بثالوجية فى المخ والرئتين والقلب والكليتين والغدد الليمفاوية والطحال وقد كانت صور التغيرات البثالوجية فى الأعضاء بعد الوفاة ، وكذلك التغيرات البثالوجية الميكروسكوبية فى الأنسجة تدل على أن سبب الوفاة هو نقص الأكسجين وتحلل كرات الدم الحمراء .

وكان طور المرض أكثر حدة فى العجول البقرى عن العجول الجاموسى كما دلت على ذلك درجة الاستحالات المرضية فى الأنسجة وكذلك درجة اصابة الأوعية الدموية وشدة تحلل الدم . كما أن العجول الجاموسى عاشت المرض مدة أكبر من العجول البقرى .

وقد ثبت أن ازالة الطحال تهدم مقاومة الحيوان للمرض كما دلت على ذلك شدة التغيرات البثالوجية . وقد وجد أن زيادة نمو الغدد الليمفاوية قد يكون ذو طبيعة دفاعية لعدوى البابيزيا كما هو ذو طبيعة تعويضية لازالة الطحال .

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PATHOLOGICAL STUDY ON THE EFFECT OF BABESIA BIGEMINA EXPERIMENTAL INFECTION IN SPLENECTOMISED AND NONSPLENECTOMISED EGYPTIAN COW AND BUFFALO CALVES

(4 Figures)

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SUMMARY

The effect of experimental Babesia bigemina infection was studied in splenectomised and nonsplenectomised cow and buffalo claves. Pathological changes were found in the brain, lungs, heart, kidneys, lymph nodes, and spleen (in nonsplenectomised animals). The post-mortem and histopathological pictures were that of anoxia and haemolysis. The course of the disease was more acute in cow calves than buffalo claves ; as indicated by the severity of the degenerative changes, degree of damage to blood vessels, and pronounced haemolysis. Buffalo calves survived the disease longer period than cow calves. Also the severity of the mentioned indices indicated that splenectomy breaks the animal resistance. Hyperplasia of the lymph follicales may be more or less of reactive nature to Babesia infection as to be a compensation to splenectomy, as found in the control animals.

INTRODUCTION

Although the pathological picture of natural and experimental Babesia infection in cattle and dogs was studied by many authors : ZOLTNIK (1953) ; MAEGRAITH (1957)); WRIGHT (1958) ; MALHERBE (1965, 1966) and ROGERS (1971), certain questions still up till now a matter of controversy as no conclusion could be made of their final results.

What is the histological defensive reaction to Babesia infection ? What are the organs affected and the degree of its involvement ? ZLOTNIK (1953, CALLOW and Mc GAVIN (1963), wrote on cerebral Babesiosis, MALHERBE (1966) searched kidney involvement. The role of the spleen was observed by WRITE (1958).

Do the parasite secreats toxins and toxins and toxemia is the cause of death as NEITES (1938) observations ? or anemia resulted from haemolysis of the parasitized R.B.Cs is the main cause of death as stated by ROGERS (1971).

The aim of this study is to try to answer these questions and to describe in detail the pathological picture in our native buffalo and cow calves.

MATERIAL AND METHODS

The effect of Babesia bigemina infection was studied on splenectomized and nonsplenectomized cow and buffalo calves.

Eight calves were included in this experiment. The calves were proved to be normal by clinical and haematological examinations. Six out of these were infected with 10 ml. infected blood, previously gained from a naturally diseased cow. The infected animals were devided into three groups :

I : The first group composed of two splenectomized and infected cow calves, (No. 1 & 2).

II : The second group composed of two splenectomized and infected buffalo calves (No. 3 &4).

III : The third group composed of two experimentally infected calves with intact spleen. One buffalo calf (No. 5) and one cow calf (No. 6). Treatment was applied to this group, after the 7th day from infection in buffalo calf, using Acaprin 5% solution. and after the 4th day from infection in cow calf, using Pirovan 5% solution. Both solutions were used in a dose of 1 ml./100 Kg body weight on two successive days.

IV : A fourth, non-infected control group composed of two calves : cow calf (No. 7) and buffalo calf (No. 8) were splenectomized, left without infection and slaughtered after one month.

Technique : Leucocytic differential counts were performed. Post-mortem examination on the dead and slaughtered calves were done. Any deviation from normal was recorded. Tissue slices not more than 1.5 × 1 × 0.5 CMs in thickness were cut from the cerebrum, cerebellum, heart, lung, liver, kidney, spleen (from non-splenectomized calves) and skeletal lymph nodes.

These slices were fixed in neutral buffer formalin and embedded in paraffin. Tissue sections were stained by haematoxylin and eosin for histopathological examinations.

RESULTS

In the first infected group of splenectomized cow calves, animal No. 1 died after 4 days from infection and animal No. 2 died after 9 days from infection.

The post-mortem examination revealed the following picture in the first animals the internal organs were congested and degenerated. The vessels of the meninges and choroid plexus were dilated and hyperaemic. There was increased amount of gelatinous blood stained fluid in the pericardial sac. Petechial haemorrhages were detected on the pericardial and coronary fat. The lung was congested. The liver was congested and the gall bladder was distended with dark green bile. The kidneys were congested and showed subcapsular petechial heamorrhages. The urinary bladder was normal, but distended with about 2-2,5 litres of coffee-coloured urine. The skeletal lymph nodes were congested and some of them demonestrated heamorrhagic areas.

In animal No. 2 the post-mortem picture was the same with exception that the lung was normal and the subcapsular petechial heamorrhages on the kidneys was not demonstrated.

Histopathologically, in animal number one the cerebrum and cerebellum showed mild degree of capillary hyperaemia and haemorrhages (Fig. 1) with dilatation of the Virchow rubin spaces. Slight neuronal degeneration were evidenced by chromatolysis of few population of neurons. A focus of ground substance disintegration were detected in the grey matter of the cerebrum.

The heart showed mild degree of granular proteinous dystrophy. The lungs were slightly hyperaemic. The liver cells suffered hydropic proteinous dystrophy. Few polymorphnuclear leucocytes were detected in the sinusoids. The disse spaces were dilated and filled with plasma. Some small foci showed lysis of liver cells. The portal veins were dilated and filled with plasma.

The kidneys showed glomerular changes manifested by hyperaemia of the tufts and dilatation of the Bowmans spaces, which were filled in some of them with acidophilic granular fluid. Hyperaemia of irregular distribution was demonstrated in the capillaries of the cortex and meddulla. The epithelium of majority of the convoluted tubles demonestrated the picture of hyaline droplet proteinous dystrophy (Fig. 2). Proteinous and haemoglobin casts could be seen in the luminae. The rest of the tubules showed granular and hydropic proteinous dystrophy.

The skeletal lymph nodes were congested, in some of them extravasation of R.B.Cs were seen. There was no more than evidence of slight reticular proliferation with tendency to macrophage development.

In animals No. 2 the histopathological picture was more or less the same with the following differences. The lungs were normal. In the liver, beside the dystrophic changes of the parenchyma, there were activation of R.E. cells with phagocytized haemosiderin pigments. The kidneys showed beside the above mentioned changes a few small foci of nonsuppurative interstitial nephrites. Haemosiderin pigment was detected in the phagocytes of lymph nodes.

In the second group of splenectomized buffalo calves, death occurred after 2 weeks in animal (No. 3) and the other animals (No. 4) was slaughtered one month from infection. The post-mortem picture revealed the following:

In animal (No. 3) the meningeal vessels were congested. The heart chambers were filled with clotted blood. The lungs were aneamic. The liver was enlarged and congested. Pale yellow areas were observed in cut section. The kidneys showed greyish discoloration with exhausted perineal fat. The lymph nodes were slightly congested and enlarged.

In animal (No. 4), the post-mortem picture was the same with the following differences. The lungs were congested and oedematous. The small intestines showed features of serous enteritis. The gluteal muscles of the right hind quarter showed features of muscular dystrophy.

Microscopically, in animal (No. 3) the cerebrum and cerebellum showed no more than capillary hyperaemia. The heart showed insignificant degree of granular dystrophy. The lungs showed collapsed capillaries. The liver cells were swollen, with granular cytoplasm. Small foci of few cells showed necrobiotic changes. Large areas of necrosis was demonsrated near the portal triads. The sinusoids were dilated and hyperaemic. The kupffer cells were reacting with phagocytosis of haemosiderin pigments. The histocytic elements of the portal triads were also active (Fig. 3).

The bidneys showed focal interstitial inflammation in the cortex around, necrobiosed and destroyed tubules. There was accumulation of round cell infilteration. Fibrosis of some foci of the glomerular capsule in the vicinity, and cystic dilatation of some tubules were observed. The rest of the parenchyma showed proteinous dystrophy.

The lymph nodes showed hyperplasia of the lymph follicles together with sinus catarrh (Fig. 4).

In animal (No. 4), the post-mortem picture was more or less the same with the following differences. The heart showed no significant changes. The lungs were congested and oedematous. The liver paranchymal damage was of milder degree. The small intestines demonestrated serous enteritis. Muscular proteinous dystrophy was observed in the skeletal muscles. Extravasation of R.B.Cs. were detected.

The third group was composed of nonsplenectomized cow and buffalo calves. The cow calf (No. 6) was slaughtered one month from treatment and complete recovery. The only detectable postmortem change was slight enlargement of the spleen. The buffalo calf (No. 5) died suddenly 20 days after treatment without recovery. The post-mortem picture revealed the following : The brain was congested. There was slight swelling and congestion of the spleen. The liver showed multiple necrotic foci which appeared on the surface and extended deep in the parenchyma. These foci are irregular in shape and of different sizes. Some of them showed congested redened centers, others surrounded by haemorrhagic areas.

Histopathologically, in the cow calf (No. 6), the internal organs were nearly unchanged except the spleen, where lymph follicles showed evidence of hyperplasia and the red pulp was congested.

The histological picture of buffalo calf (No. 5) was as follows:

The cerebrum showed capillary hyperaemia, together with dilatation of perivascular spaces, perivascular haemorrhages were observed around the capillaries. Neuronal degeneration was evidenced by chromatolysis, nuclear changes and satellitosis. In the cerebellum, the hyperaemia was severe; haemorrhage was observed together with a focus of ground substance disintegration.

The heart showed severe picture of proteinous dystrophy. The liver revealed large areas of coagulative necrosis infected with large bacilli. Hyperaemia was prominant specially in the necrotic boundary zone. The rest of the parenchyma suffered from proteinous dystrophy.

The kidneys showed irregular foci of heamorrhages and capillary hyperamia. The parenchymatous cells manifested picture of proteinous dystrophy. There was focal interstitial reaction varying from round cell infilteration around destroyed tubules up to complete fibrosis.

The spleen and lymph nodes showed evidence of follicular hyperplasia.

In the fourth control group, the two splenectomized cow (No. 7) and buffalo (No. 8) calves were slaughtered one month after splenectomy. The inspection of the carcusses did not demonestrate any detectable gross lesions except in buffalo calf (No. 8) which showed few irregularly scattered foci of haemorrhages in the lower part of the apical and cardiac lobes of both left and right lungs :

Histopathologically, examination revealed that the brain and heart were nearly normal. The lungs in one case (No. 7) showed areas of emphysema. In the other case of buffalo calf (No. 8), foci of aspirated blood were detected. Small foci of interstitial reaction were detected in the kidneys. The most significant changes were follicular hyperplasia of the skeletal lymph nodes.

DISCUSSION

The post-mortem picture of experimental Babesia infection in cow and buffalo calves was that of anoxia and haemolysis.

The signs of anoxia observed were passive congestion of the internal organs, haemorrhages on the serous cardiac membranes, lymph nodes, and degenerative changes of the internal organs.

The haemolysis was demonstrated by haemoglobin-urea and the presence of haemoglobin in the kidney tubules. Haemosiderosis was observed in generalised manner in the reticulo-endothelial systems of the liver, spleen in non-splenectomized animals and lymph node macrophages. Over distention of the gall bladder with greenish oily bile, indicated excessive secrations. The presence of bloody stained transudate in the pericardium was also characteristic for haemolysis.

The picture in general was interpretted as that of toxaemia. Some writers, like WRIGHT (1958) was of the opinion that the destruction of parasitized erythrocytes and release of toxins produce the characteristic symptoms of pyrexia and haemoglobin urea and anemia.

In the literature, there is little support for the production of toxins by Babesia. Such view of toxin production arises from the observation that in chronic piroplasmosis, where parasites are rare, the anaemia may be marked. Such animals respond to treatment and the blood picture then returns to normal (NEITEZ, 1938).

REUSSE (1954), MAEGRAITH (1957), MALHERBE (1965) and ROGERS (1971) stated that anoxia as result of parasitic destructive haemolysis of R.B.Cs is the main cause for the generalised capillary hyperamia and degenerative changes of the internal organs.

The organs commonly affected in this experimental Babesia infection were brain, lungs, heart, kidneys, lymph nodes and spleen (in nonsplenectomized animals).

The changes involved the cerebrum and cerebellum were hyperaemia, dilatation of the perivascular spaces, perivascular haemorrhages, neuronal degenerations and foci of ground substance disintegration. These are all the changes found and could be incriminated for the responsibility of the nervous symptoms that appear on animals. ZLOTNIK (1953) described dilatation of the brain capillaries which are studied with erythrocytes as the only change found in cerebral piroplasmosis of cattle.

CALLOW and COWORKER (1963) described the histological changes in cerebral Babesia Argantina experimental infection in cattle. The changes were congestion of the capillaries, dilatation of the perivascular and perineural spaces, interstitial oedema and in one case, perivascular haemorrhage.

PIERCY (1947) in his discussion on the cause of death in peracute cases of natural Babesia infection in dog did not see the congestion and degenerative changes of the internal organs as sufficient causes of death. He also denied the production of toxins. In his opinion the answer for the cause of death lies in the brain lesions. ZLOTNIK (1953) was of the same opinion and stated that blocking of the brain capillaries by parasitized red corpuscles was found to be an important contributary cause of death. On the other hand RESUSSE (1954) stated that cerebral changes were unimportant in the pathogenesis of infection with B. canis and that they occurred only in the final stage when an acute oxygen deficiency due to anaemia prevailed. But CALLOW and COWORKER (1963) was against that opinion and stated that the nervous syndrome appears in affected cattle which was still strong and there was no evidence that the animal being severely anaemic.

The heart showed hyperaemia and mild degree of granular dystrophy in the first and third groups of splenectomized cow calves and non-splenectomized buffalo calf. But in the group of buffalo calves no significant changes were found.

The changes in the lungs varied from hyperaemia to oedema. The liver changes varied from proteinous hydropic dystrophy up to focal necrosis. The R.E. cells reacted and the majority of them were laden by haemosiderin pigments. Hyperaemia was a prominent feature. In our opinion these dystrophic and congestive changes were due to anoxia resulting from the destructive haemolysis of the parasitized R.B.Cs.

MALHERBE (1965) examined livers from dogs with Babesiosis at various stages and he observed that the development of cellular dystrophy progresses centrifugally from the central veins in the lobules and the lobules tend to be diffusely affected. He concluded that the anoxia is responsible for these unspecific changes.

MAEGRAITH (1957) doubted the role of swollen Kupffer cells and parasitized R.B.Cs or swollen parenchymal cells as mechanical impairment of the circulation in the liver and underlined the role of anoxia as a causative agent for the degenerative changes.

The kidneys showed either granular proteinous dystrophy or hyaline droplet dystrophy. Haemoglobin casts were demonstrated in the tubules. Few foci of interstitial reaction were also observed Congestion was a prominent feature.

Although the picture of proteinous dystrophy in the kidneys was not specific and could be regarded as a result of generalized anoxia, but the appearance of hyaline droplet dystrophy can be regarded with special significance. DAVYDOVSKY (1971) consider the pathogenesis of hyaline droplet dystrophy in the kidneys as a result from reabsorption of denaturated proteins in the kidney tubules. The presence of haemoglobin casts in the lumen of tubules and droplet hyaline in its epithelium may be considered as evidence of release of haemoglobin, but an abnormal fraction of globulin which was denaturated by the parasites.

MALHERBE (1966) in his histopathological observation on natural cases of Babesia canis infection, found that kidney involvement with subsequent nitrogen retention is an important feature of Babesisasis. MAEGRAITH (1967) described the kidney gross and histopathological changes in Babesia canis infection and he found that the gross changes were congestion of the medulla, sometimes the whole organ, with great prominence of the vessels at the cortico-medullary junctions. The microscopical changes were shrunken glomeriolar tufts. The epithelium was degenerated with shedding of debris into the lumen. Haemoglobin or its derivatives could be differentiated in

granular from in the tubules, cells and in the casts in cases manifesting haemoglobin urea.

The presence of focal interstitial reaction in our opinion is not specific for experimental Babesia infections as these foci were demonestrated in the normal control calves.

The spleen in nonsplenectomized infected animals showed congestion and follicular hyperplasia. The hyperplastic reaction of the lymphoid follicles was also, evidenced in the skeletal lymph nodes. These consolidates with the facts that there was increase in the lymphocytes in the blood as was indicated by differential leucocytic counts.

The question is whether hyperplasia of the lymph nodes and lymphocytosis are reactions to splenectomy ? (as hyperplasia of the lymph nodes was demonestrated in the splenectomized control group). As follicular hyperplasia was demonestrated in the spleen of the nonsplenectomized infected animals, so hyperplasia of the lymph follicles in our opinion is more or less of a reactive nature as a compensatory one.

This view is supported by the observation of WRIGHT (1958) that splenectomy breaks to state of premunity of previously infected animals and which precipitates a relapse suggestive that the cells of the reticulo-endothelial system prevent the increase in number of the parasites during the state of immunity.

In our opinion the hyperplastic reaction of the lymph follicles playes a definite role in the process of immunity. This is supported by our findings that, in the treated cow calf, alhtough all the internal organs retained its normal appearance, the reaction of the lymph follicles did not subside but remained even after complete recovery.

Comparing the course of the disease and pathological alterations in experimental Babesia infection in the group of cow calves and buffalo calves, the following differences were found :

1. The course of the disease was more acute in cows than in buffaloes. In the group of cow calves death occured after 4 to 9 days. While in the group of buffalo calves, one died 2 weeks after infection and the other survived to one month when it was slaughtered.

2. Haemolysis as indicated by generalized haemosiderosis and haemoglobin-urea was severer and more prominent in the group of cow claves.

3. The damage of the blood vessels was severer in cow calves as indicated by peticheal haemorrhages on the serous membranes and lymph nodes and perivascular haemorrhages in the brain.

4. The degenerative changes were more advanced in the internal organs of cow calves.

These results agree with the work of MAROTAIN (1966) on his experimental infection of Babesia bigemina on both buffaloes and cattle. The auther's conclusion was, although buffaloes like cows are susceptible to Babesia infection in natural and experimental conditions, but the degree of clinical and patho-anatomical changes were feebly expressed in buffaloes than in cattle. In his observations latent form of Babesia infection predominates and are more common in buffaloes than the clinical form of the disease.

The treated buffalo calf was clinically normal after treatment for a period of 20 days, but without complete recovery. The congestion and degenerative changes of the internal organs can be regarded as patho-anatomical evidences of infection. The internal organs of the treated cow calf were normal. Only the reaction of the lymph follicles did not subside, but remained even after complete recovery. As mention above, this may prove the role played by follicular hyperplasia in the mechanism of immunity in Babesia infection.

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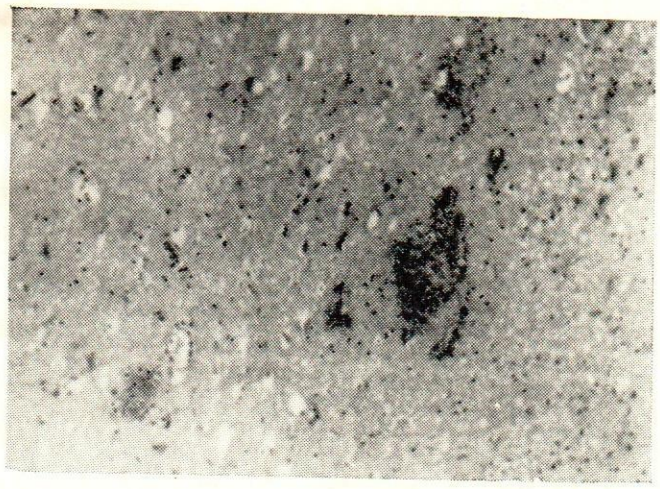

Fig. 1.—Cerebrum showing hyperaemia and haemorrhages. H. and E. (12.5 × 20).

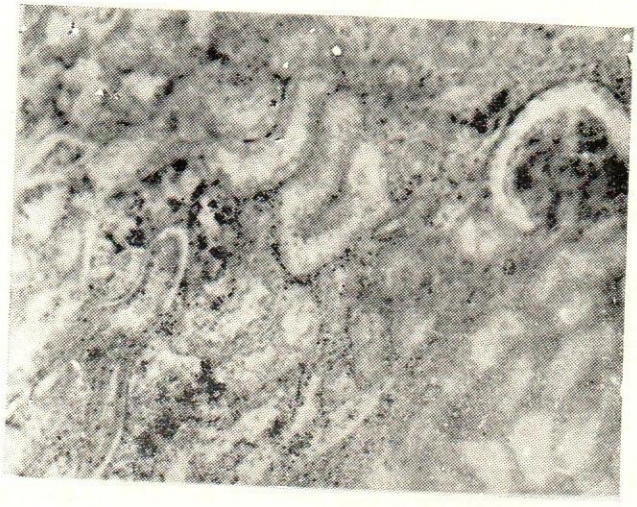

Fig. 2.—Kidneys showing hyaline droplet dystrophy and haemoglobin deposition. H. and E. (12.5 × 20).

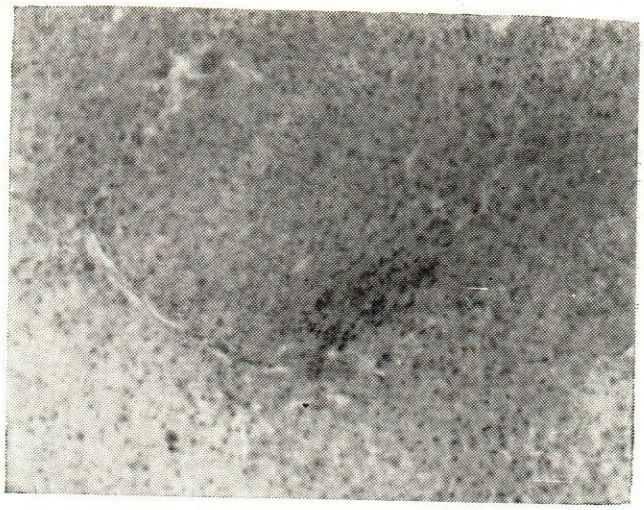

Fig. 3.—Liver showing reaction of kupffer cells with dystrophy and cell necrobioses near the portal tract. H. and E. (12.5 × 20).

Fig. 4.—Follicular hyperplasia, as a reaction to Babesia infection. H. and E. (12.5 × 40).