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Blood picture and hepatic changes in rabbits experimentally infected with *Trypanosoma evansi.* Iraqi strain

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Abstract

Seven adult male rabbits (infected group) aged 8-10 months, were infected with 10^5 of *T. evansi* isolated from Iraqi camels. The parasites were inoculated into the lateral ear vein at different time intervals. The infection induced clinical symptoms of disease, presenting as acute and chronic phases depending upon the duration of infection. Thick and thin blood smears were made daily until the end of the experiment for detection of parasites and description of blood cells, respectively. Differential leukocyte count (DLC) was also done. The parasite was observed in the blood during the acute phase only. Leucocytosis due to marked lymphocytosis was recognized in the acute phase, followed by leucopenia during the chronic phase. The main changes in the erythrocytes were the presence of macrocytes, Howell-Jolly bodies, target cells, stomatocytes and Burr cells; significant platelet deficiency was also observed. Liver slices revealed fatty degeneration, hepatic necrosis and inflammatory reaction extending through the liver parenchyma. The reported results were compared with the other seven rabbits (control group).

INTRODUCTION

Trypanosomes *(T)* are a wide range of blood parasites, which cause trypanosomiasis in both human and animals such as *T.rhodesiense*, *T.vivaxs*, *T.bruce T.gambiense, T.congolense* and *T. evansi*. *Trypanozoon evansi* was first diagnosed and named by 'Griffith Evans' in 1880 in Punjab/India from infected camels and horses, causing a disease known as 'Surra'. In Iraq *T. evansi* was first diagnosed by Major Chadwick (1938) in dogs[1] and in camels as an enzootic disease. *T. evansi* was also noted to affect cattle and buffalo[2]. Trypanosomiasis in humans is known as 'African sleeping disease'. The main mechanism for spread of these parasites is by mechanical transmission. Some form of haematophagous insects such as *Tobanid* flies can transfer the infected blood to other healthy organisms. Trypanosome species are carried by *Tobanid* flies. *T. evansi* remains monomorphic throughout its life cycle, while *T. brucei* subspecies present in different forms during different points of its life cycle[3]**.** The present study deals with the clinical, hematological and pathological changes in blood and liver of adult male rabbits experimentally infected with the Iraqi strain of *T.evansi*.

MATERIALS AND METHODS

Fourteen adult male rabbits aged 8-10 months old, of New Zealand white strain were used for this experiment. The animals were maintained under the same period of daylight and 25°C temperature; they received adequate green food and water throughout the period of the experiment. The rabbits were randomly assigned into two groups: one included seven animals (an infected group), the other one a control. Each rabbit of the infected group received 10^5 of *T. evansi* strain that infects Iraqi camels *(Camelus dromedaus)* intra-venous injection via the lateral ear vein. Thick and thin blood smears were made daily until the end of the sixth week from both groups[4]. At the end of experiment, all of the rabbits were terminated by sodium phenobarbitone injection in their lateral ear vein. All rabbits had collection of their livers, which were then dissected longitudinally and transversely, and finally fixed by Bouin's fixative solution [5.6]. The liver pieces were washed thoroughly by water, and processed by automatic tissues processor then embedded in paraffin wax. Liver sections were then sliced by microtome to about 3-4µm thickness. The slices were then fixed on glass slides and stained by Harris heamatoxylin-eosin stain[5]. All of the stained slides were examined microscopically to observe pathological changes due to infection with *T.evansi*. Blood smears were stained by Leishmania stain and examined for detection of parasites, differential leucocytes count (DLC) and for description of morphological changes in the blood cells.

RESULTS

Clinical signs of infected animals were approximately the same during the first three days of the early acute stage: a rise in temperature, loss of appetite, reduced food consumption, emaciation due to loss of body weight, progressive weakness, dullness, pale mucous membranes and anaemia. The observed clinical signs in the chronic stage were a roughened hair coat, more dulled recumbence and fluctuating pyrexia. Some animals showed signs of corneal opacity and blindness, and these remained until the end of the experiment.

Trypanosomes were detected in the blood smears made from the infected rabbits group 10-14 days after infection. Leucocytosis due to lymphocytosis was diagnosed in the late acute stage. Over later weeks, trypanosomes disappeared from the blood films (chronic stage). These smears still showed anisocytosis and pokilocytosis; leucopenia was the predominant feature in this phase. The main morphological changes in erythrocytes were the presence of Howell-Jolly bodies (inclusion bodies), hypochromic cells (Fig.1) and stomatocytes. A great deficiency or disappearance of platelets (Fig.2) Burr cells, target cells, macrocytes, and microcytes were also observed in the blood smear examination (Fig.3).

Histological examination of liver sections revealed fatty changes and progressive destruction of hepatic parenchyma. The inflammatory reaction extended from the portal tract to the parenchyma, causing hepatic necrosis. A bridging formation between the portal area and central area was observed. The results compared with that of the control group

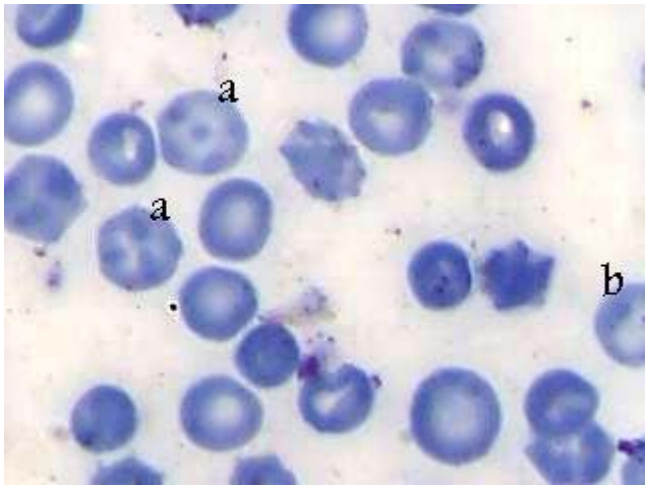

Figure (1) Blood film: a. Howell-Jolly bodies.
b. hypochromic cell

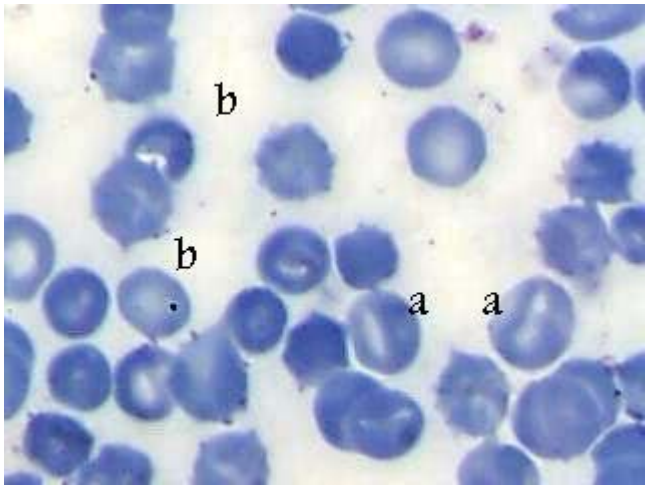

Figure (2) Blood film: a. stomatocyte cells
b. deficiency or disappearance of platelet

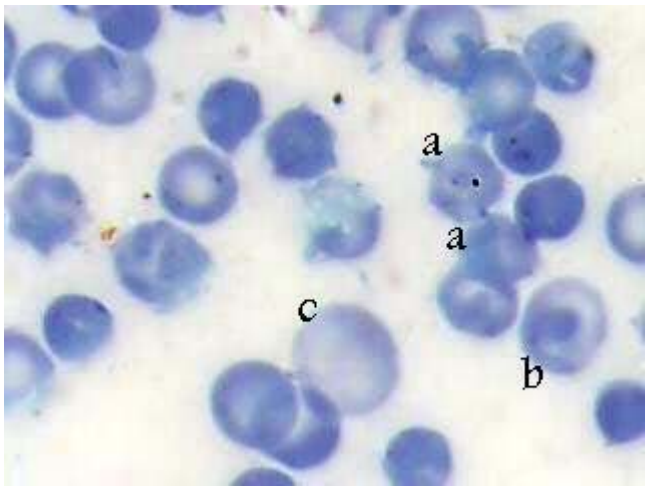

Figure (3) Blood film: a. Burr cell. b. target cell.
c. macrocyte cel

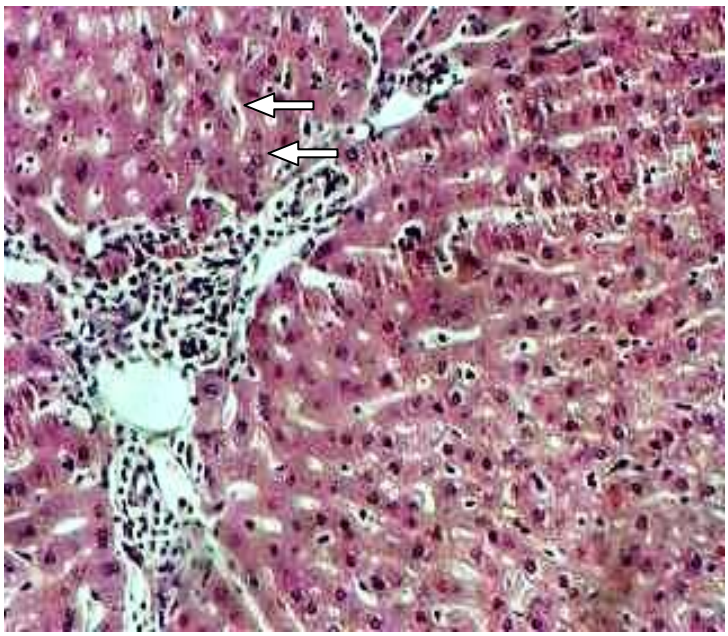

Figure (4) Liver (40 x) - Fatty change (arrows)
- Necrosis of hepatocytes
- Inflammatory cells in portal area extend into the liver parenchyma

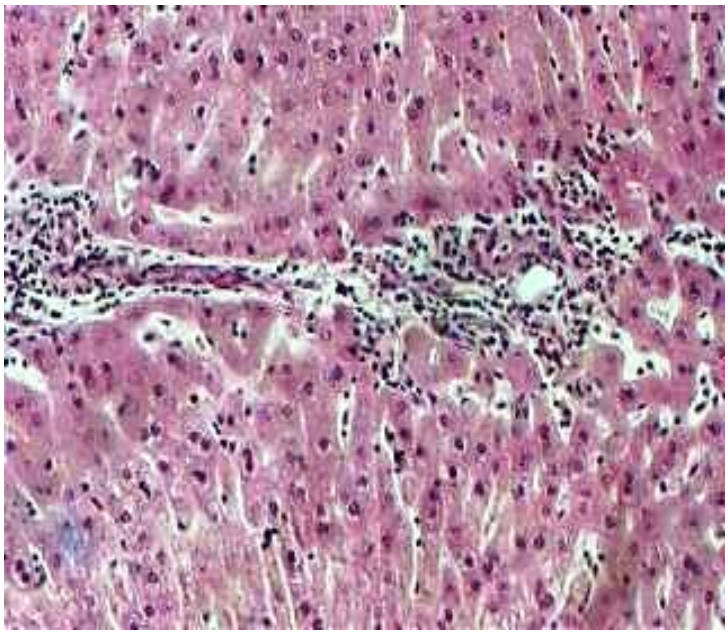

Figure (5): Liver (40 x) - formation of bridge (inflammatory reaction) between portal area and central vein

Discussion

The observed clinical signs in rabbits of this study included a rise in temperature during the first three days after infection, in appetite, progressive emaciation, a refusal to mobilize due to recumbence, depression, conjunctivitis, corneal opacity and anaemia in most of the infected rabbits. Dargantes *et al* [7]described similar signs in goats, though these changes were not pathognamonic in the absence of parasite in the blood. Audu *et al* [8] recognized the same signs in sheep. Damayanti *et al* [9] reported them in Indonesian buffalo infected with *T.evansi*. Silva *et al*[10] observed these symptoms in Brazelian Pantanal due to *T. vivax* infection. Anemia was the most distinct feature of disease[8] in most of the experimental animals and it varied from moderate to severe. Herrera *et al* [11] and Masaka [12] observed anaemia in goats and cattle infected with *T.vivax*.

Trypanosoma evansi produces parasitemic waves observed three days post inoculation in rabbits. The parasite was detected in the blood films during daily routine examination of infected blood films (acute phase). More than two weeks later, the trypanosomes disappeared from the blood (chronic phase). Sheep infected with *T.evansi* were also positive for the parasite during the prepatent period which varied between 3-6 days, and two distinct forms of disease were produced in sheep, namely acute (4-14 days post infection) and chronic (43-59 days post infection), the fluctuating pyrexia coincided with rise in parasitemia, as observed by Audu *et al* [8]**.** The parasite was also detected in goats during parasitemia [7]. Only Herrera *et al*[11] observed that parasitemia extends to the end of experimental period, on coati of South America infected with *T.evansi* infection. A significant increase in the total number of leucocytes was observed in the acute phase of infection because of lymphocytosis; lymphopenia was subsequently observed in the chronic phase. These results coincide with other findings of experiments done previously in buffalo calves, bovine [13.10]**,** sheep [14]**,** ewes[15] and rabbits [16] infected with *T.evansi, T.vivax, T. evansi* and *T. bruci* and *T.b gambiense* respectively. Another experiment on goats opposed the mentioned results and stated that leucocytosis was not a reliable indicator of infection[7]**.** In infected camels[17]**,** a significant decrease in lymphocyte with a visible increase in leucocytes and neutrophils was noted. In other experimental infection of Norwegian lemmings with *T. lemmi,* the leukocyte counts remained the same[18].

Morphological changes of erythrocytes showed the presence of anisocytosis, pokilocytosis, target cells, macrocytes, Howell-Jolly bodies, Burr cells and stomatocytes, as well as deficient haemoglobin in erythrocytes that appeared hypochromic. These changes occurred due to liver disease. The deficiencies of essential elements such as ferrous, vitamin B12 and folates resulted from a loss of appetite. The presence of Burr cells was an indicator of renal failure. Above all, many of the experiment results mentioned a significant decline in haemoglobin percentage and the total erythrocyte count was under its normal level [8.9.11.13.15]**.** The presence of macrocytes was confirmed by many results observed by Silva *et al*[10]**,** Ogunsanmi *et al*[15] andWiqer[18]. Their studies supported our results and denoted that the presence of macrocytic hypochromic cells in the acute stage and normocytic hypochromic cells in chronic stage[19]. This observation has been challenged by Emeribe[16]**,** where it has been stated that the macrocytic cells shifted terminally to microcytic hypochromic cells, with evidence of a moderate anisocytosis and pokilocytosis of erythrocytes.

Hepatic changes may occur due to trypanosome infection or their products. Biswas *et al* [20] observed predominantly histopathological changes such as pseudo-lobule formation, necrosis and hemorrhage within the sinusoids of the liver, with fatty degeneration in hepatic cells of the bandicoat rat infected with *T. evansi*. The changes were destructive and irreversible. Hepatomegaly was seen by Dargantes *et al* [21], and Damayanti *et al* [9] noticed congestion in the liver following necropsy in goat and buffalo infected with *T.evansi*. Necrotic foci in the liver and destruction of hepatocytes with infiltration by inflammatory cells in the liver of goats were observed by Ngeranwa *et al* [22]. Losos and Ikede[23] reported that *T.bruci* localized in the connective tissues of dermis and sub cutis of ears, lips, nose, eyelids and also the connective tissues of the nasal mucous membranes in the rabbit.

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ألخلاصه

خضعت سبعة أرانب ذكور (مجموعة الاصابه) بالغه بعمر 8-10 شهور إلى الاصابه بمثقبات ايفا نسي (*T.evansi*) المعزولة من الجمال العراقية وبجرعة قدرها 10^5 من الطفيلي عن طريق الوريد الاذني الحافي (lateral ear vein).إن ألاصابه أدت الى ظهور المرض السريري بنوعيه الحاد والمزمن اعتمادا على طول فترة المرض ونوع الخلايا الالتهابية.

عملت المسح الدموية السميكة والرقيقة يوميا ولحين انتهاء التجربة لغرض الكشف عن الطفيلي ولعمل توصيف لكريات الدم الحمراء والصفائح الدموية إضافة لإجراء العد التفريقي للكريات الدموية البيضاء(DLC). تم تشخيص طفيلي *T.evansi* في المسحة الدموية السميكة من فترة الاصابه الحادة فقط.وأن زيادة الكريات الدموية البيضاء الناتجة عن الزيادة الملحوظة في عدد الخلايا اللمفاوية في الفترة الحادة من الاصابه تبعتها قله عدد الكريات الدموية البيضاء في الفترة المزمنة من المرض.

عملت الشرائح الكبدية فى نهاية التجربة بعد قتل جميع الأرانب بزرق مادة الصوديوم فينوباربيتون عن طريق الوريد الاذني الحافي. وقد شملت التغيرات المرضية النسيجية على التنكز ألدهني في الخلايا الكبدية أضافه إلى التنخر الحاصل في تلك الخلايا. كما لوحظت الخلايا الالتهابية(نوع لمفوسايت)ممتدة مابين المنطقة ألبوابيه باتجاه الوريد الكبدي المركزي مكونه ما يشبه الجسر وان هذه النتائج سجلت بالمقارنة مع مجموعة السيطرة.