



## Clinicopathological Responses to Experimental Infection with *Ehrlichia ruminantium* in Desert Goat Kids

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### Abstract

Heartwater (cowdriosis) is a rickettsial infection caused by *Ehrlichia ruminantium*, affecting wild and domestic ruminants and is transmitted by ticks of the genus *Amblyomma*. The present study was conducted to investigate the clinical and pathological responses following experimental infection of desert kids with *Ehrlichia ruminantium*. Experimental kids were allotted into three groups according to their average body weights. The first group (n = 4) served as uninfected control. The second group (n = 4) was injected intravenously with 5 ml of blood collected from the donor goat infected with *E. ruminantium* to represent a low dose (Ld) of infection. The third group (n = 5) was injected with 7 ml of blood from the infected donor goat with *E. ruminantium* to represent the high dose (Hd) of infection. The experiment extended for a period of 36 days. The infected animals demonstrated the susceptibility of Desert kids to different levels of infective dose. Clinical signs were manifested by fluctuated fever, followed by inappetence, depression, mild incoordination and respiratory distress and death. Pathological alterations in *Ehrlichia*-infected animals were mainly reflected in the form of moderate effusion of body cavities, hydrothorax and hydropericardium, Flabbiness and congestion of heart muscles were reported. The lungs and brain were congested and oedematous. Crushed smears made from brain cortex showed presence of *E. ruminantium* colonies. Other lesions included oedema of the mediastinum and associated lymph nodes. The histopathological changes in various organs of *E. ruminantium* infected goats were similar. The heart showed congestion of blood vessels. Muscle cells appeared thin with a focal proliferation of lymphocytes. The lungs showed areas of collapse, focal oedema, emphysema, haemorrhage and massive congestion of blood vessels and capillaries. A mild to moderate brain oedema, foci of necrosis and perivascular spaces were seen, the kidney showed Moderate dilatation of proximal, distal and collecting tubules were observed and Glomerular tuft appeared lobulated with widened Bowman's space. The spleen showed prominent white pulp with germinal centres and marked congestion.

**Key words:** *Amblyomma varegatum*, *Ehrlichia ruminantium*, Pathology, Desert kids, Sudan.

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### Introduction

Heartwater or cowdriosis is tick borne-infection caused by *Ehrlichia ruminantium*, affecting wide range of domestic ruminants,

and *Amblyomma varegatum* is responsible for its transmission (Andrew *et al.*, 1989). The disease occurs throughout most of sub-Saharan Africa and it is considered as an

important tick-borne infection (Provost and Bezuidenhout, 1987). In West Africa, *A. variegatum* is the main tick species infesting cattle (Koney *et al.*, 1994), and it is the major vector of *E. ruminantium* (Gueye *et al.*, 1993). In Senegal, high mortality rate due to heartwater was observed in indigenous small ruminants (Gueye *et al.*, 1984). While in Gambia, mortality due to cowdriosis was recently reported in cattle undergoing an experimental trypanosome infection and exposed to natural tick challenge (Mattioli *et al.*, 1994).

Despite the socio-economical role of small ruminants in Darfur, little attention has been drawn towards their diseases in general and particularly tick-borne infections. The earliest recognized disease of small ruminants in Sudan was babesiosis which has been shown to cause serious losses (Karrar, 1960). Although there is inadequate information on its actual economic impact on livestock production, it is generally accepted that heartwater is either the most or second most important tick-borne disease in Africa. Depending on the area, heartwater ranks either second or third amongst diseases such as East Coast Fever (Provost and Bezuidenhout 1987), tsetse-transmitted Trypanosomosis, Rinderpest and perhaps also Schistosomosis. Heartwater is a major obstacle with regard to the introduction of highly producing animals intended for the upgrading of local breeds (Uilenberg, 1982). Furthermore, it remains a real threat to areas such as Sudan where potential vectors are present (Abel Wahab *et al.*, 1998; Mohammed and Hassan, 2007). The present work was then directed to establish an experimental infection in susceptible desert kids with *E. ruminantium* under laboratory conditions in Nyala Town and to investigate its clinical and pathological sequelae.

## Materials And Methods

### Study area:

This study was conducted in Nyala town, South Darfur state, Western Sudan between the latitude 8° 30"-13° 30" north and longitude 23° 15"- 28° east. The climate in south Darfur state is savannah type with clay sandy soil in the south, while the north is semi desert with sandy soil, during the period from March 2006 to February 2007.

### Experimental animals:

Experimental desert goats (13 kids) for this trial were obtained from Nyala livestock market and brought to Nyala University farm, they were (6-9 month-old) weighing 13-19 kg. The primary selection criteria of kids were based on their absence from tick infestation and not suffering from increased temperature or any nervous symptoms. Animals were kept in clean pens. They were ear-tagged. All animals were thoroughly checked for clinical fitness and absence of internal parasites by faecal floatation and centrifugation examinations. In addition they received prophylactic doses of Oxytetracycline (Avico, Jordan) and Levamisole (Avico, Jordan) and allowed a three-week period of adaptation before beginning of the experiment. Sorghum hay and water were provided *ad libitum*.

### Preparations of tick inoculums:

For preparation of infective material containing *E. ruminantium*, *Amblyomma variegatum* ticks were collected from cattle presented to Nyala slaughter house for domestic consumption. The ticks were kept alive and identified according (Walker *et al.*, 2003) method, cleaned and thoroughly washed by cool Phosphate Buffered Saline (PBS). According to Birnie *et al.* (1985) method, a hundred of these ticks (50 males and 50 females) were ground up using pestle and mortar after adding 50 ml of cool phosphate buffer saline (PBS, pH 7.0) as diluent to a volume of 0.5 ml per tick. The tick homogenate was allowed to stand in a sterile glass cylinder for 10 minutes at room

temperature, the supernatant was then removed into a glass flask and the Cryopreservant Dismethyl Sulphoxide (DMSO) was slowly added to give a final concentration of 10%. A volume of 5 ml of the supernatant was inoculated intravenously into one male-goat (donor) 13 days before the beginning of the experiment. The animal was kept under thorough observation; the establishment of infection was judged by recording daily temperature and by observation of any clinical symptoms indicative for heartwater.

#### **Experimental design:**

Experimental kids were allotted into three groups according to their average body weights. The first group (n = 4) served as uninfected control. The second group (n = 4) was injected intravenously with 5 ml of blood collected from the donor goat infected with *E. ruminantium* to represent a low dose (Ld) of infection. The third group (n = 5) was injected with 7 ml of blood from the infected donor goat with *E. ruminantium* to represent the high dose (Hd) of infection. The experiment extended for a period of 36 days. A clinical observation was followed throughout the observation period for all experimental animals and clinical symptoms were recorded following the guidelines of Kelly (1986). During this period rectal temperature of infected and control animals was recorded on daily basis and every other day respectively, body weights were recorded weekly

#### **Pathological techniques:**

Postmortem examinations were done on moribund as well as dying kids for detection of gross lesions. Tissue samples of the brain, heart, liver and kidney were collected and immediately preserved in 10% formal saline for routine histopathological processing and staining with haematoxylin and eosin (H&E).

#### **Statistical analysis**

The analysis of variance was used to analyze the results using CCS programme in a personal computer and P values less than 0.05 were considered significant.

#### **Results**

##### **Clinical observations:**

*Ehrlichia ruminantium* infected goats developed mild clinical changes a few days after inoculation. These were manifested by fluctuated fever, followed by inappetence, depression, mild incoordination and respiratory distress. The course of symptoms and duration of clinical signs of the disease were considerably variable within individual animals, the rise of body temperature over 40°C was observed on day 18 for low infected group and on day 24 post infection for highly infected group. No significant changes were recorded between infected groups (Figure 1). Figure (2) illustrates the effect of *E. ruminantium* on mean live body weights of infected goats, although slight decrease was recorded for low and high dosed infected animals, no significant changes were found between infected animals.

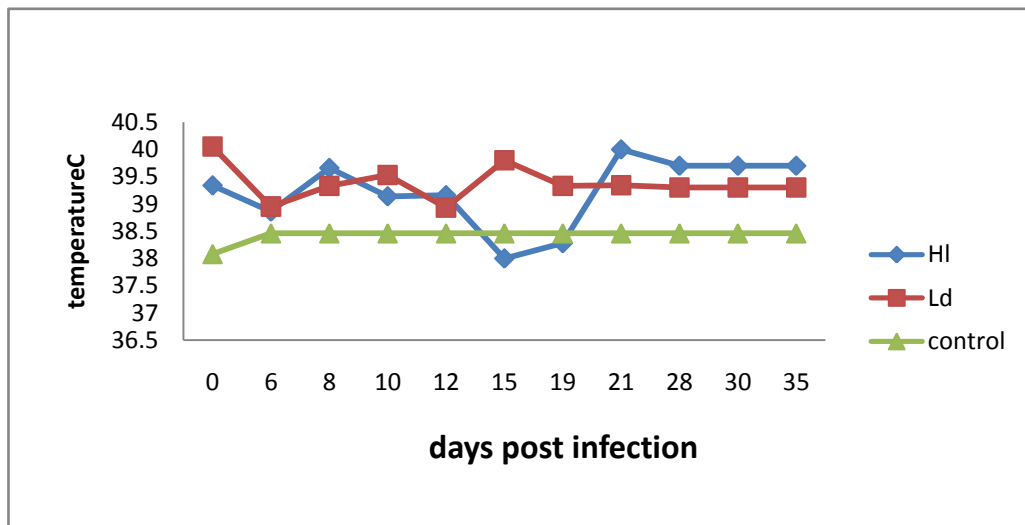


Figure 1: Mean temperature of infected and control goats during the experimental period

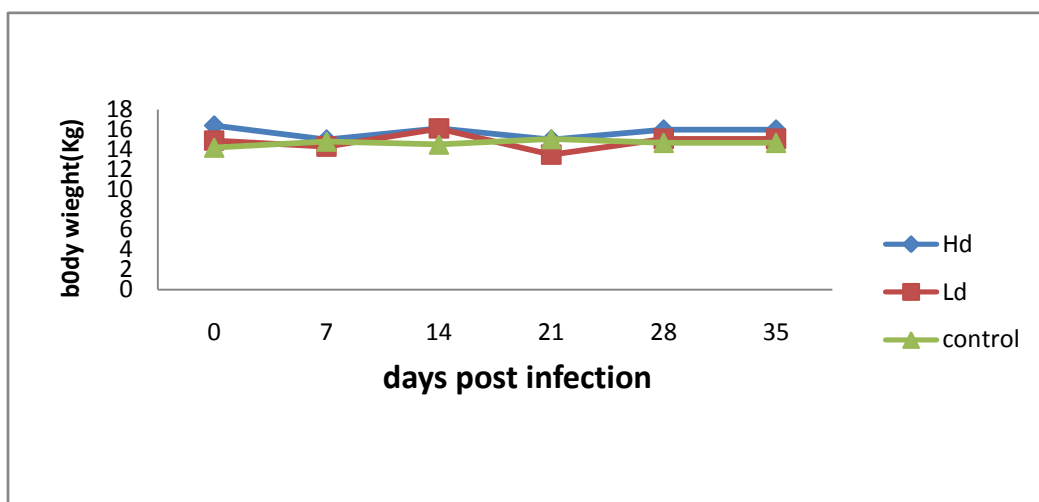
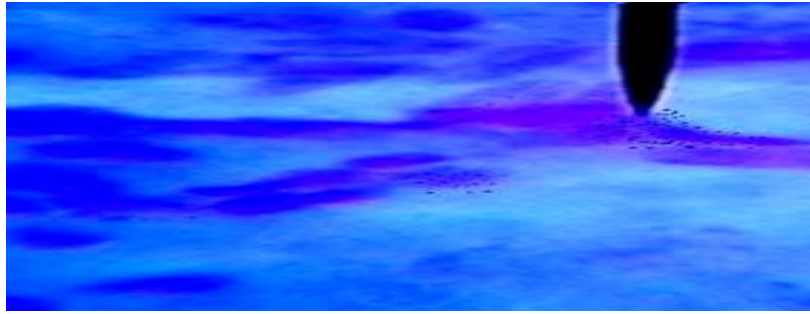


Figure 2: Mean live body weight of infected and control goats during the experimental period

**Post mortem findings:** Post mortem examination of died kids showed that most of *E. ruminantium* infected goats showed moderate effusion of body cavities, hydrothorax and hydropericardium. The lungs were congested, oedematous. Other lesions included oedema of the mediastinum and associated lymph nodes. Crushed smears made from brain cortex showed

presence of *E. ruminantium* colonies (Figure 3). The brain was congested and oedematous (Figure 4). Flabbiness and congestion of heart muscles was observed (Figure 5). A mild to moderate splenomegaly, hepatomegaly and extension of bladder (Figure 6) and slight swelling of kidneys were also evident.



**Figure 3: Crushed smear of brain cortex colonies (arrowhead). Showing *E. ruminantium* (Giemsa's stain) X 1000**



**Figure 4: Flabbiness and congestion of heart muscles in goat Goats Infected with *E. ruminantium*.**



**Figure 5: Hepatomegaly and extension of gall bladder infected with *E. ruminantium* infected goat**

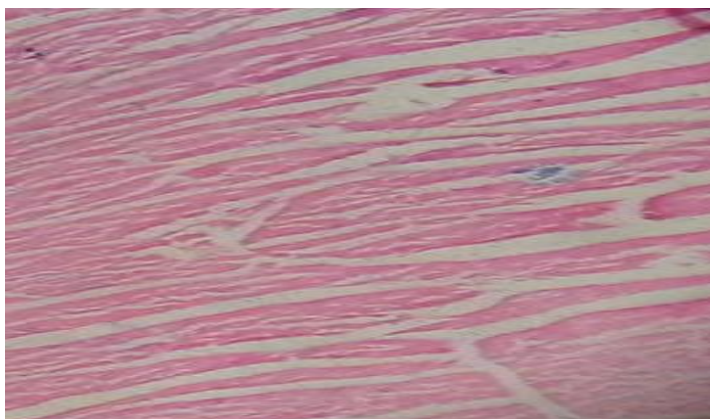


**Figure 6: Congested brain with oedema in *E. ruminantium* infected goats**

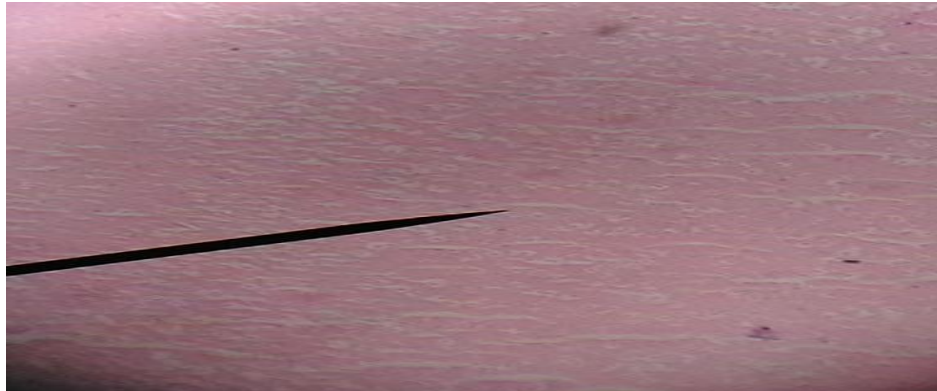
### **Histopathological findings**

The histopathological alterations in various organs of *E. ruminantium* infected goats were similar. The heart showed congestion of blood vessels. Muscle cells appeared thin with a focal proliferation of lymphocytes (Figure 7). The lungs showed areas of collapse, focal oedema, emphysema, haemorrhage and massive congestion of blood vessels and capillaries. A mild to moderate brain oedema, foci of necrosis and perivascular spaces were seen (Figure 8) the kidney showed Moderate dilatation of proximal, distal and collecting tubules were

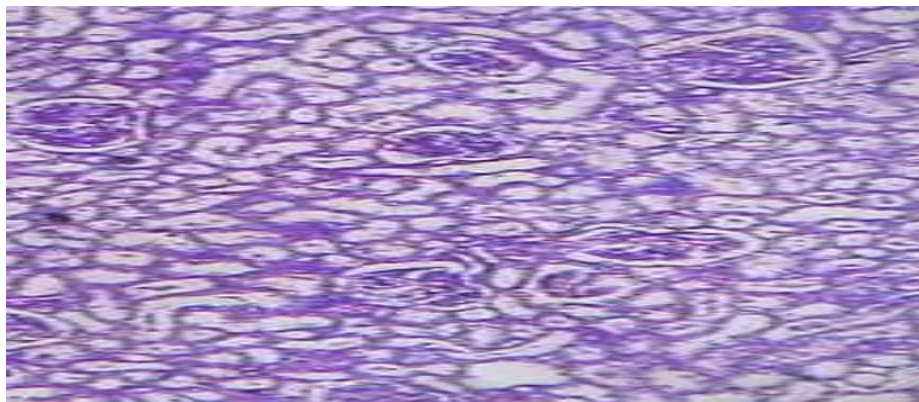
observed and Glomerular tuft appeared lobulated with widened Boman's space (Figure 9). The spleen showed prominent white pulp with germinal centres and marked congestion. The lymph nodes were widened with medullary sinuses containing large macrophages, lymphocytes and erythrocytes. Prominent lymphoid follicles with germinal cells, perivascular oedema and thickened cellular of medullary cords were observed. Hepatocytes appeared swollen with disintegrated cytoplasm and widened sinusoides (Figure 10).



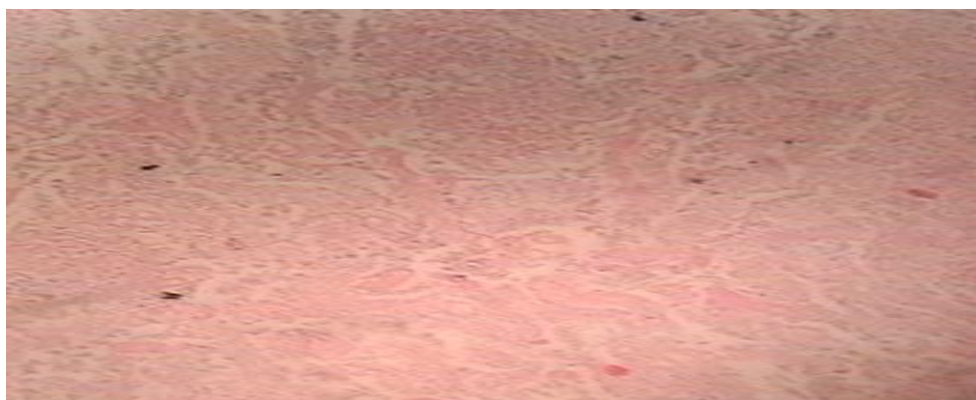
**Figure 7: Heart of infected goat showing myocardial atrophy with focal proliferation of interstitial cells. (H&E) X 250.**



**Figure 8: Brain of goat Note widened perivascular spaces, foci of necrosis in the cerebellar cortex (H&E) 100.**



**Figure 9: Kidney of infected goat with moderate dilatation of convoluted tubules widened, collecting ducts and Boman's spaces and lobulated glomerular tuft (H&E) X 100.**



**Figure 10: Liver of goat infected showing swollen hepatocytes with disintegrated cytoplasm and widened sinusoids. (H&E) X 250.**

## Discussion

Ticks and tick-borne diseases represent an economically important constraint to livestock production in the Sudan. Among the most important of the tick-borne diseases of livestock in this country is heartwater, which is caused by *Eriechia ruminantium* (formerly *Cowdria ruminantium*) and transmitted by ticks of the genus *Amblyomma*. Until very recently little has been known about the epidemiology of heartwater. As stated by Hoogstraal, (1956), the Sudanese soil, in general, constitutes the most favourable environment for tick growth and development. In fact, numerous species were identified in various parts of the country, particularly the Blue and White Nile areas (Jongejan *et al.*, 1987; Mohammed and Hassan, 2007) and Kordofan (Osman *et al.*, 1982). However, little information is currently available on the status of tick infestation in Darfur state, in which a several severe outbreaks of tick borne infections occurred (Tageldin *et al.*, 1992; Musa *et al.*, 1996; Abdel Wahab *et al.*, 1998). Among these fatal tick borne infections is cowdriosis which is transmitted biologically by ticks of the genus *Amblyomma* (Andrew *et al.*, 1989). In the Sudan, heart water was diagnosed early by Karrar (1960) in sheep and goats in eastern Sudan and later in White Nile area (Karrar, 1965). The presence of *Amblyomma lepidum* has been detected in sheep from Blue Nile State (Mohammed and Hassan, 2007). Early experimental work has been achieved by Fawi *et al.* (1977).

Experimental research on tick-borne diseases requires breeding and rearing the vector tick under laboratory condition. A field isolated infected *Amblyomma variegatum* from cattle was used in the present work to provide a source of *E. ruminantium* isolate for transmission of the bacteria to experimental animals. The experimental isolation of *E. ruminantium* was carried out by injecting a homogenate of *Amblyomma variegatum*

infected ticks on two naive kids. The establishment of infection was judged by recording daily temperature and by observation of any clinical symptoms indicative for heart water. The developmental behaviour of inoculated kids was almost similar to those previously described for the same species (Prozesky, 1987c; Camus *et al.*, 1996; Mohammed, 2004).

Inoculated experimental goats developed typical signs of cowdriosis similar to those previously reported in field cases (Karrar, 1965; Uilenberg, 1983; Mohammed, 2004). These were characterized by fluctuated fever, followed by inappetence, depression, mild in coordination and respiratory distress. The course of symptoms and duration of clinical signs of the disease were considerably variable within individual animals, the rise of body temperature over 40° C was observed on day 18 for low infected group and on day 24PI for high infected group. No significant changes were recorded between infected groups. Death occurred only in one animal received the low infective dose. *E. ruminantium* is well established and electron microscopy study (Peinaar, 1970) has demonstrated that the organism replicates mainly by binary fission of small and intermediate forms which constitute the basic elements of cowdriosis reaction in infected animals. The magnitude of the reaction is essentially related to the specific strain of the infective organism and the nature of the susceptible host. Moderate to high levels of infections were consistently observed with *E. ruminantium* (Camus *et al.*, 1996; Mohammed, 2004). The organism used in the present study was isolated from cattle coming from endemic area with cowdriosis (Rehaid Elbirdi strain). On the other hand, an outbreak of cowdriosis in South Darfur province was found to be associated with much higher percentages of *E. ruminantium* in crushed smears (Musa *et al.*, 1996). Further research



is therefore required in order to determine the extent of cowdriosis reaction with different *E. ruminantium* strains obtained from various parts of the State.

In the present study, the gross pathological findings in *E. ruminantium* infected goats were rather non-specific as manifested by moderate effusion of body cavities, hydrothorax and hydropericardium. The lungs were congested, oedematous. Other lesions included oedema of the mediastinum and associated lymph nodes. Crushed smears made from brain cortex showed presence of *E. ruminantium* colonies. The brain was congested and oedematous. Flabbiness and congestion of heart muscles was observed. A mild to moderate splenomegaly, hepatomegaly and extension of bladder and slight swelling of kidneys were evident. These changes were similar to those previously reported for natural and experimental cowdriosis in sheep (Mohammed, 2004) and goats (Prozesky, 1987b; Camus *et al.*, 1996).

The histopathological alterations in various organs of *E. ruminantium* infected goats with different levels of infection were similar. The heart showed congestion of blood vessels and muscle cells appeared thin with a focal proliferation of lymphocytes. Similar lesions were observed by (Pienaar *et al.*, 1966). Oedema of the blood vessel walls which is not reported here is infrequently seen (Prozesky and Du Plessis, 1985). The lungs showed areas of collapse, focal oedema, emphysema, haemorrhage and massive congestion of blood vessels and capillaries. The lesions detected in the brain confirmed well with findings reported by Prozesky (1987b) and Camus *et al.*, (1996) which included a mild to moderate brain oedema, foci of necrosis in the cerebellar cortex, necrotic degeneration of neuroglia, swollen axons and haemorrhages affecting mainly the mid brain. Mohammed (2004) reported similar lesions in sheep infected

with *E. ruminantium*. Oedema of the brain is responsible for the nervous signs while hydropericardium contributes to cardiac dysfunction during the terminal stages of the disease and progressive pulmonary oedema and hydrothorax result in asphyxia (Camus *et al.*, 1996).

The histopathological changes in kidneys of goats' kidney were moderate dilatation of proximal, distal and collecting tubules and glomerular tuft appeared lobulated with widened Bowman's space. Nephrotic changes of varying degree are a common feature reported in many works (Mohammed, 2004). Changes in the spleen were mainly reflected in form of congestion. Likewise, the hepatic lesions were concentrated on swelling of the hepatocytes, and widening of the sinusoids. In most animals that die of heart water, the hepatic changes are inconspicuous and congestion is the only splenic change (Prozesky, 1987b; Camus *et al.*, 1996). The lymph nodes were widened with medullary sinuses containing large macrophages, lymphocytes and erythrocytes. Prominent lymphoid follicles with germinal cells, perivascular oedema and thickened cellular of medullary cords were also observed.

In conclusion, It was clearly evident from the present investigation that naeive goats in the study area are highly susceptible to *E. ruminantium* infection. Despite the relatively low infective dose used in this study, the organism managed to establish a frank disease in susceptible kids. The disease was characterized by severe clinical signs with extremely pathological and biochemical changes. The previously reported natural outbreaks were equally serious. It is very fortunate that the vector tick has a narrow spectrum in the study area. The disease therefore poses a real threat for small ruminants industry and exportation.

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## الإستجابة المرضية السريرية لصغار الماعز الصحراوي المصابة تجريبيا بالكاودريا منناتم

ناهد مصطفى سليمان<sup>1</sup> و آدم داوود بكر<sup>2</sup> و أحمد الحاج بشار<sup>1</sup> و التجاني أحمد أصيل<sup>3</sup> و محمد سيد محمد<sup>4</sup>

- 1) قسم الطفيليات / كلية العلوم البيطرية / جامعة نيالا.
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- 5) كلية الزراعة والثروة الحيوانية / قسم الطب البيطري / جامعة القصيم.

### المستخلص:

تحدث الجرثومة ارليخيا رومينتشيوم مرض موه القلب أو الخدر في المجترات الأليفة والمتوحشة ، وينقل المرض بواسطة القراد الصلب من جنس أمبليوما، ويحدث المرض في نطاق واسع من المنطقة المدارية ويعد من أمراض القراد المنقولة ذات الأثر الأقتصادي الكبير. أجريت الدراسة الحالية للتقصي عن الإستجابة المرضية و السريرية المحدثة بالإصابة التجريبية لصغار الماعز الصحراوي بالجرثومة كاودريا منناتم وقد أثبتت العدوى التجريبية القابلة للعالية للإصابة بالبكتيريا في وسط الحيوانات المصابة عند إستخدام مستويات مختلفة من الجرعة المعدية. إنحصرت العلامات السريرية في حمى متذبذبة وإختلال الشهية والكآبة والإحتلاج الحركي بالإضافة إلى عسر التنفس والنفوق . شملت التغييرات المرضية العيانية في الحيوانات النافقة تراكم السوائل في تجاويف الجسم وموه الصدر والتأمور. كما بدت عضلات القلب رخوه ومحتقنة وظهرت الإحتقان والتورم على الرئة والدماغ ، وأظهرت العينات التي تم تحضيرها من الدماغ مستعمرات البكتيريا المسببة للمرض. شملت التغييرات الخلوية النسيجية إحتقان الأوعية الدموية إضافة إلى تكاثر الخلايا للمفاوية بالعضلات القلبية و الوهط و الوذمة الموضعية و النفاخ الرئوي والنزف و الإحتقان بالشعيرات الدموية الرئوية. تم تسجيل تكون الو نم الدماغى و النخر البؤري إضافة إلى توسع الأنابيب القاصية و الدانية والأنابيب الجامعة .