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EFFECT OF EXPERIMENTAL INFECTION WITH *Theleria lestoqurdi* ON SOME HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN DESTERT EWES DURING PREGNANCY`

Aisha, A. Elsadig; Elmansoury, Y.H.A; Husna,M.Elbasheir;Saad,M.B.,Elhussein A.M,. and ,Babeker,E.A.

المستخلص

تهدف هذه الدراسة إلى تتبع أثر الإصابة بطفيل الثايلريا لستوكارده على بعض مكونات الدم والكيمياء الحيوية للنعاج الصحراوية بالسودان خلال فترة الحمل. تم تقسيم عشرون من هذه االنعاج إلى مجموعتين متساويتين. حقنت المجموعة (أ) بطفيل الثايلريا لستوكارده بينما بقيت المجموعة الثانية(ب) كشاهد. تم تنظيم دورة الشبق لكل الإناث وتركت لتحمل طبيعياً، جمعت عينات دم خلال فترة الحمل وتم فحصها واظهرت النتائج ان متوسط الهموغلوبين، حجم التكدس الخلوي، مجموع خلايا الدم البيضاء، البروتين والزلال قد شهد إنخفاضاً معنوياً ($P<0.05$) فى دم النعاج المصابة مقارنة مع الشاهد. كماأظهر متوسط الكرياتينين واليوريا إرتفاعاً معنوياً ($P<0.05$) فى دم الأغنام المصابة(أ) مقارنة مع الشاهد(ب) مما يدل على وجود أثر سالب لمرض الثايلريا على مكونات الدم وكيمياء الدم في النعاج بالسودان أثناء فترة الحمل.

Abstract

This study was designed to investigate the effects of experimental infection with *Theileria lestoquardi* on some biochemical and haematological pictures in Desert ewes during pregnancy. Twenty Sudanese desert ewes were divided equally into two groups of ten each. The first group(A) received infection with *T. lestoquardi*, while the second group(B) remained as an uninfected control. The estrous of the two groups were synchronized and allowed to mate. Some haematological and biochemical parameters were determined throughout the pregnancy period and compared in both group. The mean values of Hb, PCV,WBCs, serum total proteins, serum albumin and serum globulins were significantly ($P<0.05$) lower in the infected ewes(A) compared to the control ones(B). However, serum creatinine and serum urea were significantly higher ($P<0.05$) in the infected group(A) than in the control ones(B) thus reflecting the direct adverse effects of theileria infection.

Key words: *Theileria lestoquardi, pregnancy, sheep, haematology, serum biochemical.*

Introduction

Ovine theileriosis, is a tick-borne disease of sheep in the tropical and subtropical regions, caused by *Theileria hirci (T. lestoquardi*), *T. ovis (recondita), T. separate*, and transmitted by Hyalomma ticks, causing lethal infection and considerable mortality (Purnell, 1978). It is an economically important disease of small ruminants in tropics and subtropics (Guo *et al.*,2002)., *Theileria lestoquardi* causes malignant theileriosis in sheep and goats, causing fever, lymphadenopathy, wasting, anemia, and jaundice resulting in severe lymphoproliferative disease with high mortality and morbidity rates (Hooshmandrad and Hawa,1973). Acute form of the disease is more usual but subacute and chronic form also exists. The disease is enzootic from the North Africa through the Middle East to India (May and Hasso, 2002).however, theileriosis is economically important in small ruminants, particularly in sheep, it causes clinical illness and mortalities in the Middle East, west Asia, Indian subcontinent, parts of Africa and Mediterranean basin. (Rehman *et al.,* 2010). Haematological and biochemical studies on experimental *T. annulata* infection in crossbred calves (Sandhu *et al*., 1998) and *T. lestoquari* in sheep have been reviewed by Rehman *et al.,* (2010) and Rasheed and Al-Fetly (2012).

The present study was designed to investigate the effect of *T. lestoquardi* on some haematological and blood biochemical parameters during pregnancy period in Sudanese Desert ewes.

Materials and methods

Experimental animals: This study was carried out at the Department of Radioisotopes, Central Veterinary Research Laboratories, Soba west; Sudan, 25 Km South East of Khartoum state. The study was conducted on 20 Sudanese Desert ewes between 2-3 years of age , they were purchased from Abuzeid market west of Omdurman; Sudan . Prior to the commencement of the experiments the animals were screened parasitologically and serologically and selected as being free of ticks, internal parasitic infections, brucellosis, trichomniasis and any reproductive disorders or abnormalities. All animals were dosed with albendazol drange and repeated after 15days. The animals were fed on green forage and supplemented with a commercial pelleted diet. They were synchronized and allowed to mate, after conception they were randomly divided into two groups (A and B) ten each. Group (A) infected with *Theileria lestoquardi* whereas, Group (B) was kept as the uninfected control. The period of the study was divided into four pregnancy periods: pre pregnancy, early pregnancy, mid pregnancy and late pregnancy. Each period extends for six weeks.

Infection: Flat nymphs of *Hyalomma anatolicum* reared at the Department of entomology and Tick-borne diseases, CVRL, Khartoum were applied on a naturally infected ram to pick up the infection using ear bag technique according to the method described by Bailey (1960). Some of the emerging flat adult ticks were crushed and examined microscopically to insure their infection with theileria. Then

50 infected flat adults ticks were applied to feed on each ewe of Group (A). Establishment of infection was monitored daily by measuring rectal temperature and Geimsa stained smears till the presence of piroblasm and shizonts in the lymphocytes.

Collection of samples: After the appearance of *Theileria* in the blood of infected ewes, weekly whole blood samples (5 ml) were collected from the jugular vein of each animal in the two groups for Hb, PCV and WBCs determination. Similarlly10 ml of blood were withdrawn into plain vacutainers and allowed to clot at room temperature, collected sera were stored at -20^{o}C until analyzed for serum total proteins, albumin, creatinine and urea.

Oestrous synchronization and mating:

After infection, synchronization was performed so as to induce oestrus in ewes at the same time for the two groups. Synchronization was conducted by insertion of progesterone releasing intravaginal device (CIDR) that contains 0.3 gm slow release progesterone (Inter Ag, Hamilton, Netherlands). The CIDR remained in situ for 14 days (Ritar, *et. al.* 1984). At time of CIDR withdrawal, the ewes received an intramuscular injection of 400-500 IU of pregnant mare serum gonadotrophin (PMSG -intervet, UK). Two qualified rams sexually active and with good fertility record were introduced to each group 24 hours post PMSG, and ensuring that mating occurred at least 2-3 times for each ewe.

Statistical analysis: data were subjected to statistical analysis Using (SPSS) version 10.0 the student T-test was used and ,the differences were considered significant at $P<0.05$

Results

Haematological parameters:

The mean Haemoglobin levels were significantly ($P<0.05$) lower in ewes infected with *T. lestoquardi* (A) than in the control ewes (B) especially during the pre- and early pregnancy periods (table 1) The Hb level showed a decreasing trend with progress in pregnancy in both groups. Similarly, the PCV values are lower ($P<0.05$) in group A than B during early pregnancy stage however, the difference is insignificant at mid and late pregnancy. The infected group showed a significant decrease ($P<0.05$) of the WBCs counts compared to the control group at pre-, early and late pregnancy periods.

Table 1: Mean ± SD values of Hb concentrations (g/dl), PCV (%) and WBCs count in $mm^3(x10^3)$ during pregnancy of ewes in the control (GB) and infected (GA) groups

Parameter	Animal group	Pre pregnancy	Early pregnancy	Mid pregnancy	Late pregnancy
HB(g/dl)	B	9.3 ±1.4^{a,A}	7.6±1.6^{b,A}	6.9±1.5^{b,A}	6.6 ±1.61^{b,A}
	A	7.5±1.5^{a,B}	7.5 ±1.7^{a,A}	6.9 ±1.4^{b,A}	7.3 ±1.65^{a,A}
PCV (%)	B	26.0±2.8^{a,A}	24.7±3.3^{a,A}	19.3±3.6^{b,A}	18.1±3.9^{b,A}
	A	25.6±6.8^{a,A}	21.0±5.2^{b,B}	20.9±4.5^{b,A}	19.3±4.6^{b,A}
WBCs ($X10^3$)	B	8.8±2.3^{a,A}	9.2±3.9^{a,A}	8.7±3.2^{a,A}	7.5±2.7^{a,A}
	A	7.9±2.7^{a,A}	6.5±2.0^{b,B}	8.6±3.5^{a,A}	5.1±1.8^{b,A}

Values with different small superscripts within same rows were significantly different at P<0.05
Values with different capital superscripts within same columns were significantly different at P<0.05

Serum biochemical parameters

The mean values of serum total proteins in Group (B) and Group (A) showed a significant decrease during mid and late pregnancy (Table2). However, at the early stage of pregnancy this decrease was significant in group A compared to group B (P<0.05). on the other hand the albumin values in group A were lower than that in group B at any stage of pregnancy however, the differences is were significant (P<0.05) at the mid pregnancy (Table2). Concurrently, the values of serum globulins are slightly higher in the infected group A than B and the difference is significant (P<0.05) during early pregnancy period.

Serum creatinine concentrations in the infected group A were found to be higher (P<0.05) than the control group B (table 2). During pregnancy, the creatinine concentrations showed insignificant changes in GB (P>0.05) whereas, in A, a significant (P<0.05) increase was noticed in early pregnancy. However, the mean values of serum urea in A were significantly (P<0.05) higher than that of B especially during all pregnancy stages. Moreover, the values in GB showed significant (P<0.05) decrease during pregnancy with the lowest values at late pregnancy while, the decrease in the infected group A was insignificant (P>0.05).

Table 2: Mean ± SD values of some biochemical parameters during pregnancy of ewes in the control (GB) and the infected (GA) groups

Parameter	Animal groups	Pre-pregnancy	Early pregnancy	Mid pregnancy	Late pregnancy
Total proteins (g/dl)	B	7.38±0.40 ^{a,A}	7.27±0.48 ^{a,A}	6.15±1.35 ^{b,A}	5.14±1.74 ^{c,A}
	A	7.46±1.33 ^{a,A}	6.84±1.08 ^{a,B}	6.62±1.78 ^{a,A}	5.56±1.29 ^{b,A}
Albumin (g/dl)	B	3.35±0.32 ^{a,A}	3.19±0.34 ^{a,A}	3.05±0.52 ^{a,A}	2.90±0.48 ^{b,A}
	A	3.37±0.47 ^{a,A}	3.13±0.40 ^{a,A}	2.96±0.58 ^{ab,B}	2.72±0.49 ^{b,A}
Globulins (g/dl)	B	4.03±0.64 ^{a,A}	4.07±0.65 ^{a,A}	3.21±1.19 ^{b,A}	2.32±1.73 ^{c,A}
	A	4.10±1.16 ^{a,A}	3.73±0.98 ^{a,B}	3.37±1.39 ^{a,A}	2.82±1.19 ^{b,A}
Creatinine (mg/dl)	B	5.36±4.80 ^{a,A}	6.49±5.51 ^{a,A}	6.87±5.01 ^{a,A}	6.45±3.09 ^{a,A}
	A	8.87±4.50 ^{ab,B}	10.07±4.38 ^{b,B}	7.59±3.90 ^{a,A}	7.55±3.55 ^{ab,A}
Urea (mg/dl)	B	33.74±4.47 ^{a,A}	30.87±3.75 ^{a,A}	30.95±19.53 ^{a,A}	15.85±7.14 ^{b,A}
	A	34.05±2.72 ^{a,B}	32.91±5.20 ^{a,A}	33.70±15.08 ^{a,B}	26.28±9.91 ^{a,B}

Values with different small superscripts within same rows were significantly different at $P<0.05$
Values with different capital superscripts within same columns were significantly different at $P<0.05$

Discussion

The present data showed that mean values of HB, PCV and WBCs in the infected sheep A are lower than the control B. This was mainly attributed to the infection and destruction of the infected blood cells by the theilarial piroplasm and schizonts. A significant difference ($p<0.05$) in HB values was observed by Rehman *et al*. (2010) between the studied groups of sheep with a mild to severe anemia in the infected animal which is in agreement with the Hooshmandard and Hawa (1973). In addition Nazifi *et al.*, (2011) showed that sever progressive anemia in ovine malignant thieleriosis indicate increase exposure of RBCs to oxidative damage. Grewal *et al*. (2005) indicated that Theileria infection lead to increased oxidative stress to the animals and even increased activities of antioxidant enzymes could not decreased this oxidative stress this may be cause of elevated erythrocyte fragility due to membrane lysis and lower haemoglobin concentration.

The involvement of WBCs throughout the infection may lead to an increase followed by a decrease in WBCs count. Osman and Al-Gaabary (2007) reported a marked difference in total leukocyte counts of healthy and diseased animals. Similarlly Rehman *et al.* (2010) in their study observed that a significant difference ($P<0.05$) was present among all the groups of infected and control sheep. Rasheed and Al-Fetly (2012) reported non-significant increase ($p<0.05$) in WBC counts in infected sheep compared with control group whereas, the decreases in RBC counts ,Hb concentration and PCV were significant in the diseased sheep compared with control group, They also showed that the MCV and MCH values were revealed significant increase ($P<0.05$) in the infected sheep compared with the healthy ones.

Throughout the gestation period of the sheep, the values of HB, PCV and WBCs showed a gradual decrease with advanced pregnancy this may be due to the stress of pregnancy and development of the fetus. These findings are contrary to that obtained by Getnet and Abebe (2005) they found that white blood cells, red blood cells, packed cell volume and haemoglobin are slightly higher in late pregnant camels compared with the values obtained for non-pregnant ones. An increase in the leukocytes picture that was observed for pregnant camels is comparable with the results reported by Coles (1986) and Jain (1986) for cattle. This may be attributed to the physiological increase of neutrophils count at late stage of pregnancy before parturition in cattle (Doxey, 1977).

The profile of total proteins in the infected A seems to be associated with an increase in the globulins level, which reflects the increase in immunoglobulins molecules in the serum following infection. The insignificant changes in serum total proteins, albumines or gloulines showed the same trends in infected and control groups of sheep under the study. Lower concentrations of total proteins and globulines were reported at mid and late pregnancy period in both groups. These findings were in agreement with that of Amna (2002) she reported a significant decrease in total proteins, albumin and globulins in late pregnancy in cross bred goats this may be

due to decrease dietary intake, diarrhea and decrease synthesis of proteins in the liver caused by direct or indirect effect of parasite on the liver.

Higher Serum creatinine and urea levels in A compared to that of group B are due to the infection with theileria where an induced immune complex may cause mechanical damage to the glomeruli resulting in renal disturbances during infection. The findings are in line with those obtained by Rasheed, and Al-Fetly (2012) who observed a significant increase ($P<0.05$) in creatinine and serum urea in infected sheep than in control ones.

Gradual decrease in both groups of sheep was observed with progress pregnancy contrary to Amna (2002) who showed that pregnancy did not affect the level of urea in cross bred goats.

Conclusions: The present data demonstrates that theileria infection adversely affects the health and production of sheep that needs further investigations on molecular epidemiology bases in the Sudan.

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