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Effects of *Theileria lestoquardi* Infection on Haematological and Biochemical Parameters in Experimentally Infected Desert Ewes

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Abstract

In an experimental infection of sheep with *Theileria lestoquardi* some biochemical and haematological parameters were studied in ten Sudanese desert ewes together with 10 clinically healthy ewes. The obtained results showed a significant (P<0.05) decrease in haemoglobin, packed cell volume and white blood cells counts compared to the control group. The decrease in Hb concentrations and PCV% was observed 7-10 weeks after tick application whereas; the decrease in WBCs occurred at week 5 and week 6 after tick application. There was also a significant decrease (P<0.05) in serum total proteins, and serum globulins values of the infected group at week 3- week7 after tick application. A significant increase (P<0.05) in serum urea and serum creatinine values in the infected group was noticed during the first 7-8 weeks in the infected group compared to the control one's.

Key words: *Theileria lestoquardi*, Sheep, Haematological, Serobiochemical, Sudan

1. Introduction

Tick- borne diseases of small ruminant are of highly economic importance in many countries. Malignant theileriosis of sheep and goats caused by *Theileria lestoquardi* is considered among the most important small ruminant diseases and constituted an obstacle to the industry in countries like the Sudan (Bakheit *et. al*., 2006). When the infested sheep develop theileriosis, *Theileria* schizonts are demonstrated in liver, spleen, lung, kidney lymph node and peripheral blood (Hong *et. al*., 2003).

Theileria lestoquardi, was shown to be widely distributed in main sheep grazing areas in Sudan where 16.3% of sheep surveyed showed reactive antibodies in IFAT (Salih *et. al.*, 2003). Biochemical investigations were very important to understand the host parasite relationship and to study the metabolism of the parasite, which may be helpful in the diagnosis and therapeutic processes (Yadav *et. al*., 1986).

2. Materials and Methods

2.1. Experimental Animals

This study was carried out at the Department of Radioisotopes, Central Veterinary Research Laboratories, Khartoum, Sudan. It was conducted on Sudanese desert ewes. The ewes were clinically healthy, free of ticks, parasitic infections including theileriosis and *Brucellosis.*

Before the commencement of the experiments, the animals were kept in the premises of the Veterinary Research Administration for five weeks to adapt themselves to the new environment and to be examined for any clinical detectable diseases such as brucellosis, trichomoniasis, theileriosis and any reproductive disorders or abnormalities. All animals were dosed with broad spectrum antihelmenthic and anti coccidial. The animals were fed on green forage and supplemented with a commercial pelleted diet. Ewes were randomly divided into two groups (A and C) of ten each. Each group was housed separately at the Department of radioisotopes barns of 20 square meters each. They were provided with metal feeding troughs and plastic containers for water. Ewes of group A were experimentally infected with *Theileria lestoquardi*, whereas; ewes in group C remained as the uninfected control.

2.2. Infection with Theileria lestoquardi

A ram which was naturally infected with Theileria lestoquardi was brought from Atbara, North Sudan by the aid of the regional veterinary research laboratory in Atbara. This ram was particularly brought as it was proved to carry 10% parasitaemia the parasitaemia was further confirmed in the department of ticks at Soba. This sheep was used as the source of infection.

Flat nymphs of *Hyalomma anatolicum* were applied on this ram to pick up the infection using ear bags according to

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the method described by Bailey (1960). Engorged nymphs collected and kept in the laboratory to the second stage (flat adult). Infected flat adults emerging from the nymphs were allowed to feed again on the experimental ewes.

Establishment of infection was monitored daily by measuring rectal temperature. Thin blood smears from the ear vein were prepared daily, fixed with absolute methanol and then stained with fresh Giemsa stain.

2.3. Collection of Samples

Weekly Blood samples were collected from the jugular vein into plain and heparinized vacutainer tubes from each ewe throughout the study. Sera were separated and kept at -20 ^{0}C until analyzed. All the haematological parameters were estimated by the methods described by Schalm (1965).

Total protein was determined using Biurret reagent as described by King and Wooton (1965), Albumin was determined according to Bartholmew and Delany (1966) while, Urea level was determined according to the method outlined by Evans (1968) using Randox commercial kits (Diamond Road ,Crumlin,Co.Antrim,United Kingdom, BT29 4QY).

2.4. Statistical Analysis

Using (SPSS) version 10.0 data were analyzed using student T-test analysis, differences were considered significant at $P<0.05$ level.

3. Results

3.1. Haematological Parameters

3.1.1. Haemoglodin concentration

The weekly mean Hb concentration, PCV and total white blood counts were monitored for 10 weeks in both groups of sheep post tick application. The starting concentrations of Hb at the first week was >8.0 g/dL then gradual decrease was noticed in the infected group which showed lower Hb values compared to the control group (Table 1), however, the difference was significant ($P<0.05$) in weeks 7-10 reaching lowest concentration at week 10 (< 7.0 g/dL). While in control group the mean Hb concentration remained as high as (7.6- 8.9g/dL) throughout the study period.

3.1.2. PCV

The mean values of PCV in the infected group were found to be lower than that of the control group ranging between (22-28%) and (26-29%), respectively (Table 1). However, the differences were statistically significant ($P<0.05$) at weeks 8,9 and10 representing the lowest value (<22%).

3.1.3. White Blood Cells (WBCs)

The mean WBCs counts were (8.1±2.12and 8.3±2.24) in the control and infected group, respectively. As shown in Table (1) there was a gradual decrease in WBCs counts in the infected group and at week 5 and week 6 the counts dropped to significant levels ($P<0.05$). However, at week 7 to week10 the counts increased to the starting level (8-11) in both groups.

Table 1. Haematological values (mean± SD) in *Theileria lestoquardi*-infected Sudanese desert ewes

Weeks post tick application	Hb	PCV	WBCs
1	8.27±1.20[a] 8.10± 1.35[a]	25.11±4.7[a] 24.11±5.62[a]	8.12±2.12[a] 8.36±2.24[a]
2	7.65±1.12[a] 6.91±1.49[a]	28.72±8.3[a] 25.56±6.44[a]	7.96±3.23[a] 7.52±3.25[a]
3	8.40±0.95[a] 7.44±1.04[a]	27.34±5.16[a] 23.00±5.17[a]	7.02±1.82[a] 6.69±1.66[a]
4	8.00±0.61[a] 7.88±0.79 [a]	29.47±4.71[a] 27.88±4.58[a]	6.20±2.56[a] 6.10±1.93[a]
5	7.97±0.78[a] 7.91±1.04[a]	28.79±4.9 [a] 28.11±5.25 [a]	6.20±2.84[a] 4.96±1.41[b]
6	8.38±0 .64[a] 8.26±0.64[a]	25.87±4.73[a] 23.00±4.44[b]	7.20±2.25[a] 6.31±4.65[b]
7	7.67±1.13[a] 6.78±1.10[b]	26.00±5.04[a] 25.43±4.65[a]	6.92±2.11[a] 6.78±2.23[a]
8	8.35±1.82[a] 4.20±0.01[b]	27.93±5.16[a] 21.89±6.39[b]	9.13±2.16[a] 9.33±2.29[a]
9	8.55±0.33[a] 6.92 ±1.7[b]	26.82±5.56[a] 22.15±6.41[b]	11.0±3.24[a] 8.92±1.09[a]
10	8.90±1.13[a] 6.98±1.27[b]	27.64±5.39[a] 22.30±4.00[b]	8.45±1.75[a] 8.92±1.09[a]

Values with different small superscripts within the same column were significantly different at $P<0.05$

3.2. Biochemical Parameters

3.2.1. Serum Total Proteins

Decreases in the weekly serum total protein concentrations was observed for up to 10 weeks post tick application in the infected animals compared to the controls (Table 2). However, the difference was significant (P < 0.05) at weeks 3 –week7, whereas, in week 8- 10, the mean values of serum total proteins showed insignificant difference ($P>0.05$) between the two groups.

3.2.2. Serum albumin

Although the mean values of serum albumin during the sampling period of 10weeks, were slightly lower in the infected group compared to the control group, yet the differences were insignificant ($P>0.05$).

3.2.3. Serum globulins

A gradual decrease in the mean concentration of serum globulin was noticed in the infected group from (> 4.0g/dl) at week 3 to (<3.0g/dl) at week 7 post infection (Table 2),however, at week 8 the values began to increase to the control level and by week 10 there were no significant difference in globulin concentration between both group.

3.2.4. Serum creatinine

The mean serum creatinine concentrations (mg/dl) were significantly higher in the infected group of sheep compared to the control one's (Table 2). These differences were observed during the first 7 weeks reaching the highest value (22.05±14.4 mg/dl) at week 5. Then it started to drop again to the baseline level. Later, at week 10 the

creatinine levels reached its lowest values in both groups (4- 4.3 mg/dl).

3.2.5. Serum urea

The weekly serum urea concentrations varied between (30 - 34mg/dl) in the infected group which is significantly higher (P<0.05) than in the control group (28-31mg/dl) during the first eight weeks, whereas, during weeks 9-10, there were no significant differences in urea concentration of both groups where they showed relatively constant value (32.4-32.9mg/dl) as presented in Table 2.

Table 2. Serobiochemical values (mean± SD) in *Theileria lestoquardi*-infected Sudanese desert ewes

Weeks post tick application	Total Proteins (g/dl)	Albumins (g/dl)	Globulins (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)
1	7.31± 0.32 [a]	3.32 ±0.33[a]	3.98± 0.54 [a]	5.32± 5.86 [a]	34.76± 5.58 [a]
	7.34± 2.05[a]	3.31± 0.82 [a]	4.03± 1.37 [a]	7.59± 5.03 [b]	33.86± 3.19 [b]
2	7.45 ± 0.49 [a]	3.38± 0.32 [a]	4.07± 0.76 [a]	5.46± 0.87 [a]	32.84± 3.26 [a]
	7.73± 2.62[a]	3.41± 0.57 [a]	4.32± 2.01 [a]	10.14± 3.85 [b]	34.24± 2.45 [b]
3	7.35 ± 0.78 [a]	3.00± 0.27 [a]	4.34± 0.88 [a]	4.26± 3.74 [a]	31.38± 2.89 [a]
	5.56± 1.11[b]	2.84± 0.97 [a]	2.72± 1.03 [b]	8.99± 5.99 [b]	32.89± 7.19 [b]
4	7.27 ± 0.37 [a]	3.12± 0.32 [a]	4.15± 0.51 [a]	6.83± 3.59 [a]	28.51± 4.10 [a]
	6.11± 0.15[b]	2.99 ±0.61[a]	2.87± 0.78 [b]	11.34± 2.55 [b]	30.02± 3.19 [b]
5	7.19 ± 0.51 [a]	3.26± 0.58 [a]	3.93± 0.81[a]	7.00± 3.07 [a]	30.71± 3.62 [a]
	5.73±. 69 [b]	3.14 ±0.27[a]	2.83± 0.68[b]	22.05± 14.7 [b]	33.71± 6.63 [b]
6	7.24 ± 0.40 a	3.24± 0.24 a	3.97± 0.51a	6.16± 5.51 a	31.52± 4.92 a
	5.98± 1.36b	3.09± 0.43 a	2.84± 1.12b	10.27± 4.17 b	32.88± 6.24 b
7	7.37 ± 0.44 a	3.21± 0.17 a	4.16± 0.54 a	4.29± 2.20 a	31.39± 2.39 a
	5.83± 1.25b	3.07 ±0.22a	2.98± 1.21 b	9.21± 3.59 b	34.78± 3.90 b
8	7.19 ± 0.38 a	3.31± 0.33 a	3.89± 0.60 a	10.26± 1.21 a	31.70± 4.06 a
	7.15± 1.96a	3.12 ±0.35a	3.79± 1.59 a	12.74± 4.12a	33.36± 3.32 b
8	7.17 ± 0.36 a	3.13± 0.29 a	4.04± 0.49 a	7.38± 3.79 a	32.40± 3.24 a
	7.63± 1.69a	3.12± 0.52 a	4.25± 0.91 a	7.12± 4.38a	32.95± 11.34a
10	7.19 ± 0.59 a	3.15 ±0.31a	4.15± 0.52 a	4.30± 2.81 a	32.31± 4.25 a
	7.54± 0.82a	3.11 ±0.42a	4.16± 0.29 a	4.13± 3.25 a	32.59± 5.68 a

Values with different small superscripts within the same column were significantly different at *P*<0.05

4. Discussion

Attempts to explain the haematological changes on pathophysiological basis, revealed that in infection with *T. lestoquardi,* there was a marked decrease in haemoglobin, packed cell volume and white cell counts. This decrease fluctuated in weeks following tick application, after which, the values returned back to normal. Our findings were similar to the findings of other worker (Ahmed, 2004; Mehta *et al*., 1988; Rayules and Hafeez, 1995; Sandhu *et al*., 1998; Singh *et al*., 2001). Nazifi *et al*. (2010) also reported that, as the parasitaemia increased, a significant decrease was observed in RBCs, PCV and Hb. In contrast, with an increase in the parasitaemia rate, a significant increase in the mean corpuscular volume, haptoglobin (Hp), serum amyloid A (SAA), ceruloplasmin and fibrinogen was evident. The decline of the above mentioned values might probably be attributed to the destruction of erythrocytes by macrophages in the lymph nodes, spleen and other organs of the reticuloendothelial system as previously suggested. Singh *et al.* (2001) and Omer *et. al.* (2002) reported that these changes in blood parameters pictured by the decrease in haemoglobin, packed cell volume and white blood cell counts may finally lead to the occurrence of severe anemia.

With respect to the biochemical changes as a result of *T. lestoquardi* infection, the study demonstrated that there was an apparent decrease in the concentrations of serum total protein, serum albumin and serum globulins. This decrease fluctuated in weeks following the tick application, after which, these values returned back to normal. This finding is in line with that of Ahmed (2004), Singh *et al*. (2001), Ramazan and Uguruslu (2007), and Yadav and Sharma (1986). However, Sandhu *et al.* (1998) reported an insignificant decrease in these parameters. Stockham *et al.* (2000) attributed the decrease in the concentrations of the serum protein and the serum albumin to the extra vascular accumulation of proteinaceous fluids resulting from affected lymph nodes. On the other hand, Singh *et. al.* (2001) and Omer *et al.* (2003) attributed the decrease in serum proteins to hypoalbuminaemia and hypoglobulinaemia arising from liver failure.

Our findings indicated that ewes infected with *Theileria lestoquardi* had higher concentrations of serum creatinine and serum urea. This increase fluctuated in week3-7 following tick application, after which, they returned to normal levels. These findings were in agreement with the findings of Ramazan and Uguruslu (2007), Ahmed (2004), and Yerham *et al*. (1998) who attributed the increase in creatinine to damage observed in the liver and kidney in babesiosis in sheep. The increase in urea level was similar to that reported by Singh *et al*. (2001) and Sandhu *et al*. (1998). However, our observation contradicts that of Omer *et al.* (2003) who showed significant decrease in urea and creatinine in cattle naturally infected with *Theileria annulata.*

5. Conclusions and Recommendations

In conclusion it could be stated that infection of desert sheep with *Theileria lestoquardi* had significantly and adversely affected the haematological and biochemical parameters under this study.

Further investigations were needed to trace the causes of such fluctuations in serum and blood parameters.

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