COMPARATIVE STUDY IN SERUM BIOCHEMICAL CHANGES IN SHEEP AND GOATS WITH HEARTWATER

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SUMMARY

A comparative serum biochemical study was done. The AST levels rose significantly in both test sheep and goats. The BUN did not show significant changes in sheep but showed a significant rise in test goats. The TSP was significantly lower in test sheep and significantly higher in test goats. The albumins were significantly lower in both test sheep and goats while the globulins were significantly higher in both test sheep and goats compared to the controls. This is the first comparative study on sheep and goats reported. Further research is necessary before biochemical tests which can be used as an indicator of this disease are identified.

INTRODUCTION

Serum biochemical changes in sheep and goats infected with *Cowdria ruminantium* are equivocal, with some workers suggestinginconsistentalterations(Graf, 1933), others non-significant shifts (Clark, 1962) and still others reporting changes in acid-base balance (Owen *et al*, 1976), low total serum protein with increases in alpha globulins, serum pyruvate and serum lactate (Ilemobade and Blotkamp, 1978). Because of this dearth of information on such an important subject it was decided to carry out a comparative study of possible changes in a selected serum biochemical profile in sheep and goats experimentally infected with heartwater.

MATERIALS AND METHODS

Animals

Ten sheep (Dopper) and ten goats (Red Masai) of mixed sex and ages varying from 1-2 years from a heartwater free area of Kenya were used. They were clinically examined, including blood smear evaluation for haemoparasites and rickettsia and found to be healthy. Five sheep and five goats were used as test animals while the remainder served as controls.

Preparation of Stabilate

The stabilate of *Cowdria ruminantium* used in these experiments was obtained from a field case of bovine heartwater from Mavueni location of Kilifi District in Kenya. It was maintained through passage at the Veterinary Research Laboratories, Kabete. Ten milliliters of blood was collected from the animals at the point when they had peak fever. It was then cryoprotected with 10% Dimethyl sulphoxide (DMSO) and stored in liquid nitrogen (-196°C) until required. For infection studies three 10ml vials of cryopreserved *C.ruminantium* stabilate was thawed for half a minute in warm water (37°C) and inoculated into the first three sheep.

After infection had set in, and at the peak of fever 10ml of blood was harvested from the first set of experimental animals. This was inoculated into other subsequent experimental animals. To facilitate this transmission donor and recipient animals were placed in close proximity side by side. After inoculation

the animals were clinically examined daily and 7ml of blood obtained from the jugular vein for assessment of Aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Total Proteins (TSP), Albumins (A) and Globulins (G).

Table 1: The Mean Daily Serum Biochemical Values for the Ten Sheep Experimentally Infected with Heartwater

Days Post-Infection	AST (SF units/ml)	BUN (mg/100ml)	TSP (g/100ml)	Albumin (mg/100ml)	Globulin (g/100ml)
0	67.5	12.3	6.4	3.0	3.4
1	73.7	12.1	6.4	3.0	3.4
2	70.0	12.1	6.4	3.0	3.4
3	68.4	12.7	6.4	2.9	3.5
4	71.0	12.5	6.3	2.9	3.4
5	74.5	23.5	6.3	2.9	3.4
6	84.0	24.0	6.8	3.2	3.6
7	84.9	25.5	6.4	3.0	3.4
8	87.7	34.7	6.6	3.1	3.5
9	86.2	31.1	6.2	3.2	3.0
10	86.5	30.9	6.3	3.1	3.2
11	85.2	32.0	6.3	3.1	3.2
12	73.8	30.5	6.3	3.1	3.2
13	75.5	30.3	6.2	2.9	3.3
14	75.5	30.3	6.2	2.9	3.4
15	71.6	16.6	6.3	2.9	3.3
16	71.4	16.3	6.1	3.0	3.1
17	77.9	18.1	6.3	3.0	3.3
18	68.6	17.5	6.2	2.7	3.5
19	64.1	14.3	6.2	2.7	3.5
20	65.3	13.6	6.3	2.7	3.6
21	69.0	14.3	6.3	2.7	3.6
22	70.4	14.1	6.3	2.9	3.4
23	70.5	13.0	6.5	2.8	3.7
24	62.0	15.5	6.4	2.7	3.7
25	70.0	12.7	6.4	2.6	3.8
	SE=4.7	SE=2.7	SE=0.1	SE=0.1	SE=0.1

SE= Standard Error
Day 0 = Average value for 5 days before infection
AST = Aspartate amino-transferase
SF = Sigma - Frankel Units.
BUN = Blood Urea Nitrogen.
TSP = Total Serum Proteins.

The AST was determined using the method of Reitman and Frankel (1957) using the Boehringer AST kits (Boehringer Mannehin GMHB, West Germany), The values were read from an Eppendorf photometer using a mercury filter at 526nm and values

expressed in Sigma-Frankel (S-F) units per ml of blood. The BUN was determined using the Urastrat dip strips (General Diag. Div. Warner and Lambert Co., N.J., U.S.A.) and values obtained expressed in milligrams per 100 ml of blood. The TSP was determined using the Biuret method as described by Reinhold (1953). The difference of the TSP and Albumin gave the globulin levels. The TSP, Albumins and globulins were recorded in grams per 100ml of blood. The globulins were not differentiated into alpha, beta and gamma.

Table II: Mean Daily Serum Biochemical Values for the Ten Control Sheep

Days Post-Infection	AST (SF units/ml)	BUN (mg/100ml)	TSP (g/100ml)	Albumin (mg/100ml)	Globulin (g/100ml)
0	73.3	16.1	6.5	3.1	3.4
1	76.7	17.8	6.5	3.0	3.5
2	73.1	17.1	6.3	3.0	3.4
3	65.6	18.5	6.4	2.9	3.5
4	76.8	17.9	6.4	2.9	3.4
5	73.4	17.6	6.6	3.0	3.6
6	70.1	18.5	6.7	3.0	3.4
7	70.8	17.7	6.8	3.3	3.5
8	76.7	15.5	6.5	3.3	3.4
9	71.2	17.4	6.6	3.1	3.3
10	71.8	17.7	6.8	3.3	3.6
11	71.3	17.3	6.8	3.2	3.7
12	67.9	17.8	6.8	3.1	3.7
13	71.8	16.9	6.6	3.1	3.5
14	66.8	17.2	6.5	3.1	3.4
15	74.1	17.3	6.4	3.1	3.3
16	73.0	17.1	6.6	3.1	3.5
17	71.6	17.1	6.6	3.1	3.5
18	70.7	16.9	6.5	3.1	3.4
19	68.0	17.0	6.5	3.1	3.4
20	66.0	17.6	6.5	3.1	3.4
21	68.2	16.9	6.2	3.1	3.1
22	73.4	16.7	6.5	3.1	3.4
23	65.9	16.4	6.5	3.1	3.4
24	61.5	16.2	6.7	3.3	3.4
25	67.0	17.4	6.2	2.9	3.3
	SE=4.0	SE=1	SE=0.1	SE=0.1	SE=0.1

Day 0= Average for 5 days before test animals were infected
SE= Standard Error
AST = Aspartate amino-transferase
BUN = Blood Urea Nitrogen
TSP= Total Serum Proteins.
SF Units = Sigma - Frankel.

Statistical analysis of the results was done using the paired Student t-test as described by Steel and Torrie (1980).

The sheep died or recovered fully by day twenty five thus t^{25} while all goats died by day sixteen thus t^{16}.

RESULTS

The serum biochemical changes observed are summarised in Tables I, II, III and IV for test sheep, control sheep, test goats and control goats, respectively.

In test sheep the AST values rose slightly from day six after infection and remained so, up to day eleven. It was found that the test sheep had significantly higher AST values compared to the controls ($t^{25} = 2.49$). The test goats showed a dramatic rise in AST values from day eight after infection and this dropped from day twelve but never went as low as was the pre-inoculation levels. The test goats showed significantly higher AST values than the controls ($t^{16} = 3.82$).

The BUN in test sheep did not show

Table III: The Mean Daily Serum Biochemical Values for the Ten Goats Experimentally Infected with Heartwater

Days Post-Infection	AST (SF units/ml)	BUN (mg/100ml)	TSP (g/100ml)	Albumin (mg/100ml)	Globulin (g/100ml)
0	33.9	13.8	8.1	2.9	5.2
1	33.3	15.5	8.4	3.2	5.2
2	32.4	15.7	8.2	3.2	5.0
3	33.4	15.8	8.3	3.1	5.2
4	33.4	15.5	8.1	3.1	5.0
5	36.2	16.2	8.1	3.1	5.0
6	41.7	15.8	8.0	3.1	4.9
7	41.0	18.3	7.7	3.1	4.6
8	46.5	18.5	7.9	3.2	4.7
9	58.4	19.4	7.7	3.2	4.5
10	74.9	22.4	7.6	3.1	4.5
11	85.1	23.4	7.6	3.1	4.5
12	95.2	24.9	7.8	3.2	4.6
13	60.5	28.6	7.5	3.0	4.5
14	54.0	39.0	7.8	3.0	4.8
15	54.0	39.0	7.8	3.0	4.8
16	53.0	37.0	7.6	3.0	4.6
	SE=2.7	SE=2.0	SE=0.2	SE=0.10	SE=0.2

Day 0 = Average of 5 days before infection
SE = Standard Error for 5 days before infection
AST = Aspartate amino-transferase
SF = Sigma - Frankel Units.
BUN = Blood Urea Nitrogen.
TSP = Total Serum Protein

significant difference with the controls except for a mild rise ($t^{25} = 1.60$). In test goats however the BUN rose steadily from day seven reaching a peak on day fourteen. The test goats showed significantly higher BUN values compared to the controls ($t^{26} = 2.26$).

The TSP in both test sheep and controls remained fairly constant but was found significantly lower in the test group than the controls ($t^{25} = 5.54$).

The albumins in the test sheep were significantly lower than in the controls ($t^{25} = 5.37$) while the globulins were significantly higher in the test group than the controls ($t^{25} = 5.22$).

A mild rise in globulins was noted in the test sheep during the last few days with corresponding drop in the albumins.

In goats the TSP was found significantly higher in the test group compared to controls ($t^{16} = 2.36$).

There was a mild drop in TSP values especially from day nine after infection and this coincided with a drop in both the albumins and globulins but more so with globulins. The albumins were found significantly lower in the test goats ($t^{16} = 5.21$) while the globulins were significantly higher ($t^{16} = 4.88$). The trend of the albumin and globulins in both

Table IV: The Mean Daily Serum Biochemical Values for the Ten Control Goats

Days Post-Infection	AST (SF units/ml)	BUN (mg/100ml)	TSP (g/100ml)	Albumin (mg/100ml)	Globulin (g/100ml)
0	30.1	15.3	7.8	3.4	4.4
1	30.5	15.6	7.5	3.3	4.2
2	35.7	24.1	7.2	3.5	3.7
3	35.3	24.1	7.2	3.5	3.7
4	35.3	16.4	7.3	3.4	3.9
5	34.7	16.4	7.2	3.2	4.0
6	33.1	17.1	7.4	3.3	4.1
7	33.9	17.1	7.4	3.3	4.1
8	30.8	17.1	7.4	3.3	4.1
9	31.2	17.1	7.3	3.3	4.0
10	32.5	17.0	7.3	3.3	4.0
11	30.3	17.0	7.2	3.3	3.9
12	30.5	17.7	7.3	3.4	3.9
13	29.2	18.3	7.3	3.2	4.1
14	31.0	15.3	7.4	3.3	4.1
15	31.0	15.2	7.4	3.2	4.2
16	36.0	15.6	7.4	3.2	4.2
	SE=0.8	SE=0.8	SE=0.2	SE=0.1	SE=0.2

Day 0 = Average values for 5 days before test animals were inoculated
SE = Standard Error for 5 days before infection
AST = Aspartate amino-transferase. SF = Sigma - Frankel Units.
BUN = Blood Urea Nitrogen. TSP = Total Serum Protein

groups appeared similar except for the statistical differences

DISCUSSION

Ilemobade and Blotkmap (1978) observed a drop in TSP, a rise in alpha-globulins accompanied by a fall in gammaglobulins. In this study a mild drop in TSP was noted in both test sheep and test goats especially after the onset of fever. Both the albumins and globulins however contributed to the drop. This partly agrees with the findings of Ilemobade and Blotkamp (1978). Further investigation is necessary to ascertain the cause of the drop in these factors with rise in body temperature.

Previous reports on the biochemical changes during heartwater infections have not dealt with AST and BUN. However, AST activity has been known to rise with tissue necrosis (Henson *et al.*, 1965; Cardinet, 1971; Nagode *et al* 1966; Kiptoon *et al.*, 1984). Cornelius and Kaneko (1960) noted that serum enzymes which are usually intracellular only increased in large amounts in circulation if the cells were damaged or the cell membranes lost their integrity causing an increase in their permeability.

Pathological findings in tissues of the animals that succumbed to heartwater did not indicate necrosis of the tissues. It was thus assumed that factors produced by *C. ruminantium* during its replication caused alteration of the membrane permeability of the host cells causing a rise in AST values. Detailed ultra-microscopic studies of the tissues with high concentration of AST i.e. the heart, liver, intestines and kidney may help to point out the nature of membrane alteration, since little damage was noted on these tissues on light microscopy.

The BUN was found significantly higher in goats than in sheep. According to Coles (1967) the BUN levels rise due to pre-renal, renal and post-renal causes. Nephritis of acute, subacute or chronic forms may cause abnormal rise in BUN levels, either by interfering with glomerular or tubular functions. In the present study the kidneys especially in goats showed leukocytic cellular infiltration in the glomerulus, congestion of blood vessels and mild degeneration of the tubules. This possibly was responsible for the increased BUN. Increased protein breakdown in the liver could also have led to increased BUN but this needs further investigation to ascertain.

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