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THE CLINICAL PATHOLOGY OF HEARTWATER. I. HAEMATOLOGY AND BLOOD CHEMISTRY

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ABSTRACT

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Clinical pathological studies were undertaken in 5 calves with experimentally-induced heartwater. The most important findings include a progressive anaemia which may be associated with bone marrow depression and fluctuations in the total and differential white cell count, of which an eosinopenia and a lymphocytosis were the most marked. A severe drop in serum protein, especially in the albumin levels, was observed in all 5 cases.

This disease is probably associated with an increased capillary permeability, as the protein content of the pericardial fluid in 1 case that died, approximated that of the serum. The osmolality of the effused fluid was also higher than that of the blood. No significant changes in the serum electrolyte levels occurred, except for total calcium levels which tended to decrease to below normal during the acute stage of the disease.

Marked increases in total bilirubin were recorded. This, however, was not associated with liver pathology or haemolysis and may possibly be ascribed to a fasting hyperbilirubinaemia. Darkening of plasma colour was associated with peak rises in total biluribin.

Increases in both blood urea and creatinine levels indicate interference with renal glomerular filtration during the acute stage of the disease.

INTRODUCTION

Studies on the clinical pathology of heartwater have been carried out by Graf (1933), Clark (1962), Owen, Littlejohn, Kruger & Erasmus (1973), Abdel Rahim & Shommein (1977), Ilemobade & Blotkamp (1978) and Metelerkamp, Litthauer, Naude, Oelofsen & Gruss (1986). The subject was also reviewed by Camus & Barré (1982), whereas Van Amstel, Guthrie, Reyers, Bertschinger, Oberem, Kileen & Matthee (1987) specifically reviewed the clinical pathology and pathophysiology of the disease.

From these studies it appears that an increased capillary permeability is the main underlying cause for the subsequent pathophysiological changes which may lead to the death of the animal (Clark, 1962; and Owen *et al.* 1973). The inciting cause for this increased capillary permeability has not been elucidated, but Bezuidenhout (1982), Jackson & Neitz (1932) and Prozesky (1982), as cited by Camus & Barré (1982), suggest that a toxin may play a role. The effects of the decrease in blood volume caused by this increase in capillary permeability may be further compounded by a sympatholytic decrease in blood pressure resulting in the death of the animal through vascular collapse (Clark, 1962).

The current study describes the haematological and blood chemical findings associated with experimentally-induced heartwater in calves.

MATERIALS AND METHODS

Experimental animals

Heartwater was induced in each of 5 healthy 6–10 month-old Friesland calves (identified as 1066, 1067, 1099, 0549 and 0605) by the intravenous inoculation of the Ball 3 strain of *Cowdria ruminantium* contained in 5 mℓ of blood from an infected sheep. This strain of the organism present in the blood of sheep suffering from heartwater is routinely issued on a commercial basis by the Veterinary Research Institute, Onderstepoort as a "vaccine". The rectal temperature of each animal was recorded twice daily. Clinical disease was considered to have commenced when this reached 40 °C. Rectal temperatures above 40 °C were recorded in all 5 calves from 14–24 days post-infection. Blood was collected from all the calves for haematology and blood chemistry on Days 7 and 11 post-infection and then daily from Day 14 until death or recovery. The latter was considered to have occurred on Day 25 in surviving animals when they were regarded as having recovered. No treatment was instituted.

Effused fluid was collected for chemical analysis from the pericardial sac of Calf 1066 shortly after death. This included total serum protein (TSP) and protein electrophoresis, sodium (Na), potassium (K), calcium (Ca) and measurement of the osmolality using the appropriate methods outlined below.

Haematology and blood chemistry

Complete blood counts (CBC) were conducted, using a Coulter (Model Fn) electronic cell counter[1] which measured the red cell count (RCC), mean corpuscular volume (MCV) and white cell count (WCC). For the differential WCC, thin blood films were stained by the Diff-Quick[2] method. Haemoglobin (Hb) was determined by the cyanmethaemoglobin method on a haemoglobinometer[1]. Haematocrit (Ht) determinations were carried out using microhaematocrit capillaries (75 mm/$\mu\ell$) in a microhaematocrit centrifuge. The mean corpuscular haemoglobin concentration (MCHC) was calculated from measured Hb and Ht values.

Blood chemistry studies were directed towards the evaluation of protein and electrolyte homeostasis and liver and kidney function. The possible presence and severity of stress was determined by measuring blood glucose and cortisol levels.

The following methods were used:

1. Total serum protein (TSP) and protein electrophoresis.
   - 1.1 TSP. Technicon RA 1000[3] method (Cat. No. T01-1301-02) using the biuret reaction.
   - 1.2 Albumin. A Technicon RA1000 method (Cat. No. T01-1377-02) using bromocresyl green dye binding.

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1.3 Protein electrophoresis was carried out using the microzone electrophoresis system[4] on cellulose acetate membranes, with ponceau-S staining.

2. Serum electrolytes.

2.1 Sodium (Na) and potassium (K).

Na and K determinations were carried out using an ion sensitive electrode on a Baker Analyte Model +1[4].

2.2 Calcium (Ca).

Ca levels were determined on an atomic absorption spectrophotometer (Varian AA-275)[5], using a strontium chloride (1,5 %) diluent.

3. Blood and fluid osmolality.

Osmolalities were determined by freezing-point depression, using a Knauer Osmometer[6].

4. Blood urea (UN) and creatinine (Crt) for the evaluation of kidney function.

4.1 UN. Technicon RA 1000 method (Cat. No. T01-1821-56).

4.2 Crt. Technicon RA 100 method (Cat. No. T01-1304-53).

5. Gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and total and direct bilirubin for evaluation of liver function and excretion.

5.1 GGT. (EC 2.3.2.2) Technicon RA 1000 method (Cat. No. T01-1916-01), using nitrosalinide substrate.

5.2 ALP. (EC 3.1.3.1) Technicon RA 1000 method (Cat. No. T01-1811-01), using PNPP substrate in diethanolamine buffer.

5.3 Total bilirubin. Technicon RA 1000 method (Cat. No. T01-1508-01).

5.4 Direct bilirubin. Determinations done on a LP6 spectrophotometer (Dr Lange), using a Boehringer Mannheim test kit (Cat. No. 123919).

TABLE 1 Duration of temperature reactions of 40 °C or above

Animal identification	Duration of temperature reaction Days post-infection
0549	15–20
0605	14–19
1066	16–18*
1067	18–24
1099	15–19

* This calf died on Day 18

TABLE 2 Haemoglobin values (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	104	104	110	ND	73	84	80	75	66	72	83	78		
0605	97	100	93	83	94	81	80	83	78	81	81			
1066	102	105	101	87	85	80	84							
1067	102	110	111	97	88	93	83	88	79	70	71	69	68	67
1099	107	111	79	68	78	68	65	68	69	66	74	73		

Normal value = 80–140 g/ℓ: Schalm *et al.* (1975)

ND = Not done

* = First fever reaction of 40 °C or above

TABLE 3 Haematocrit values (ℓ/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	0,31	0,31	0,32	ND	0,24	0,24	0,22	0,22	0,21	0,22	0,24	0,23		
0605	0,28	0,29	0,27	0,23	0,25	0,23	0,23	0,24	0,22	0,24	0,23			
1066	0,30	0,31	0,30	0,26	0,24	0,23	0,25							
1067	0,29	0,32	0,31	0,28	0,27	0,26	0,24	0,25	0,22	0,20	0,21	0,20	0,19	
1099	0,30	0,32	0,23	0,19	0,20	0,19	0,18	0,19	0,20	0,19	0,22	0,21		

Normal value = 0,24–0,46 ℓ/ℓ: Schalm *et al.* (1975)

ND = Not done

* = First fever reaction of 40 °C or above

[4] Baker Instrument Corporation, Allentown, Pennsylvania, USA

[5] Varian Techtron (Pty) Ltd, Victoria, Australia

[6] Knauer, Berlin

TABLE 4 White cell count ($\times 10^9/\ell$)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	13,4	9,8	6,9	ND	7,2	7,1	9,5	12,4	7,6	8,3	16,1	14,8		
0605	8,3	5,5	4,2	5,4	5,3	6,9	7,9	7,8	5,9	6,5	15,4			
1066	8,9	5,8	4,1	5,0	5,0	6,0	12,6							
1067	12,7	8,8	8,6	8,7	7,4	4,7	5,0	5,8	5,8	6,7	8,6	9,3	9,2	9,6
1099	11,4	7,1	4,4	5,9	6,8	7,8	7,9	10,2	10,2	6,2	14,9	13,2		

Normal value = $4-12 \times 10^9/\ell$: Schalm *et al.* (1975)
ND = Not done
* = First fever reaction of 40 °C or above

TABLE 5 Neutrophil count (mature cells: absolute numbers) ($\times 10^9/\ell$)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	6028	4606	966	ND	1440	1349	1995	1364	456	498	1288	3700		
0605	4648	2200	882	594	1431	1311	1501	2106	1239	1755	2156			
1066	4272	2146	1681	850	950	1080	3024							
1067	4572	3432	3182	2436	1480	611	1500	870	986	734	1376	651	1104	1056
1099	5351	2059	1364	472	680	624	711	1696	1122	796	1788	528		

Normal value = $600-4\,000 \times 10^9/\ell$: Schalm *et al.* (1975)
ND = Not done
* = First fever reaction of 40 °C or above

TABLE 6 Lymphocyte count (absolute number) ($\times 10^9/\ell$)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	6713	4606	5589	ND	5472	5112	7315	10188	6536	7470	13202	10064		
0605	3154	2005	3150	4158	3710	5106	5767	5148	3953	4615	12012			
1066	3293	3306	2296	3850	3950	4680	8694							
1067	7360	4576	5074	5742	5846	3854	3450	4814	4060	5195	6822	8091	7728	7872
1099	5814	4686	2728	5133	5848	6084	6715	8056	8364	5518	12218	12144		

Normal value = $2\,500-7\,500 \times 10^9/\ell$: Schalm *et al.* (1975)
ND = Not done
* = First fever reaction of 40 °C or above

6. Blood glucose and cortisol levels.

6.1 Glucose. Technicon RA 1000 method (Cat. No. T01-1825).

6.2 Plasma cortisol levels were measured with a radioimmunoassay (RIA) technique, employing Coat-a-Count ^{125}I.

RESULTS

Rectal temperatures

The duration of temperature reactions above 40 °C for each calf is shown in Table 1. From Table 1 it can be seen that Calf 1067 became ill at a later stage than the other 4 calves.

TABLE 7 Eosinophil count (absolute numbers) ($\times 10^9/\ell$)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	137	196	0	ND	0	0	0	124	0	83	0	148		
0605	249	275	42	0	0	138	0	0	0	0	154			
1066	801	116	82	0	0	0	126							
1067	381	88	86	87	0	0	0	0	0	0	0	0	0	0
1099	0	0	44	0	0	0	0	0	0	0	0	0		

Normal value = 0–2 400 $\times 10^9/\ell$: Schalm *et al.* (1975)
ND = Not done
* = First fever reaction of 40 °C or above

TABLE 8 Blood glucose (mmol/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	3,9	3,6	3,7	3,8	4,0	3,9	3,7	3,4	4,7	3,6	3,7	4,2		
0605	3,7	3,3	3,2	3,6	3,8	3,3	3,4	3,3	4,0	4,1	3,8			
1066	3,6	3,4	3,5	3,5	3,7	3,3	3,8							
1067	3,6	3,4	3,5	3,6	3,8	3,3	3,7	3,4	3,6	3,5	3,5	3,6	3,6	3,6
1099	4,2	3,8	3,7	4,0	4,1	3,7	3,8	3,9	4,2	4,3	4,6	4,5		

Normal value = 3,1–4,7 mmol/ℓ: Schmidt (1979)
* = First fever reaction of 40 °C or above

TABLE 9 Blood cortisol (μmol/ℓ)

Days post infection	5	6	7	11	12	13	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification																		
0549	31,7	9,2	9,6	20,2	8,4	19,2	15,5	ND										
0605	29,0	13,8	19,3	53,1	37,6	29,4	26,9	19,4	30,8	33,1	16,8	24,0	13,1	20,8	6,6	7,5	10,7	
1066	2,8	28,4	4,8	50,8	7,0	28,4	23,5	46,6	21,5	19,6	80,3	83,0						
1067	12,5	46,1	12,9	27,8	16,3	20,1	27,7	140,7	36,7	17,9	8,6	17,4	12,5	34,2	14,2	35,4	34,5	10,0

Normal value = 0–30 μmol/ℓ: Coubrough, Bertschinger & Kühne (1980)
ND = Not done
* = First fever reaction of 40 °C or above

Haematology

Haematological findings associated with these cases of experimentally-induced heartwater include a proportional decrease in both the Hb and Ht values (Tables 2 & 3). The initial reduction in these parameters coincided with or started shortly after the fever reaction and continued throughout the course of the disease but never reached critically low levels. Both the Hb and Ht were still at below normal levels by Day 25, when the surviving calves were judged to be clinically normal.

No changes were obtained in the MCHC or MCV values during the course of the disease. These results indicate the presence of a normocytic, normochromic type anaemia which could be associated with bone marrow depression (Schalm, Jain & Carroll, 1975). This concept was further strengthened by the absence of circulating immature red cells (Schalm *et al.*, 1975).

All 5 calves showed a transient reduction in their total white cell count. This relative leukopenia, mainly due to a drop in the neutrophil count (Table 5) was present as

early as Day 11 and persisted throughout the early phase of the temperature reaction (Table 4).

A progressive lymphocytosis developed in all 5 calves during the course of the disease, being most marked at the end of the disease (Table 6). Its cause is not known, but Abdel Rahim & Shommein (1977) speculated that it might be due to an immunological response.

A total absence of eosinophils was recorded in all 5 calves during the acute stage of the disease (Table 7). The presence of the agranulocytosis (neutropenia and eosinopenia), although apparently transient, may be further evidence of the presence of bone marrow depression (Schalm *et al.*, 1975). Thrombocyte levels, however, were within normal limits.

An eosinopenia may be caused by elevated levels of endogenous cortisol, in which case a lymphopenia and a hyperglycaemia should also be present (Schalm *et al.*, 1975). However, in these cases a lymphocytosis was present and no marked increases in blood glucose levels could be demonstrated (Table 8). Likewise, blood cortisol levels remained mostly within normal limits with the exception of those of Calf 1066 which died after having shown a distinct terminal increase (Table 9). One other

TABLE 10 Total serum protein levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	57,5	61,0	61,5	54,9	55,0	54,1	50,5	50,5	49,6	51,9	54,9	55,2		
0605	54,9	61,4	59,1	54,1	55,2	56,3	55,6	57,4	54,9	56,9	60,7			
1066	58,4	60,6	59,3	54,1	53,4	51,6	47,8							
1067	66,8	70,0	68,6	65,9	67,6	68,6	65,0	65,2	59,0	57,5	56,9	56,9	57,7	59,0
1099	59,5	61,0	55,6	51,0	54,6	53,0	51,2	52,6	65,1	55,0	59,0	57,6		

Normal value = 67,4–74,6 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

TABLE 11 Albumin levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	31,8	32,8	32,7	28,6	29,0	28,4	26,6	26,4	25,9	27,0	27,5	27,8		
0605	31,8	33,2	32,1	29,1	29,0	29,5	29,4	29,9	27,9	28,7	30,4			
1066	34,2	34,6	32,9	29,7	29,3	28,0	25,7							
1067	34,5	34,9	34,6	32,0	33,7	33,7	31,1	31,7	29,4	28,5	28,1	27,9	28,3	29,0
1099	34,2	34,2	30,2	27,8	28,9	28,0	26,7	27,5	28,4	28,1	29,1	28,9		

Normal value = 30,3–35,5 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

TABLE 12 Globulin levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	25,7	28,2	28,8	26,3	26,0	28,4	23,9	24,1	20,7	24,9	27,4	27,4		
0605	23,1	28,2	27,0	25,0	26,2	29,5	26,2	27,5	27,0	28,2	30,3			
1066	24,2	26,0	26,4	24,4	24,1	28,0	22,1							
1067	32,3	35,1	34,0	33,9	33,9	33,7	33,9	33,5	29,6	29,0	28,8	29,0	29,4	30,0
1099	25,3	26,8	25,4	23,2	25,4	28,0	24,5	25,1	25,7	26,9	29,9	28,7		

Normal value = 30–34,8 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

TABLE 13 Alpha-globulin levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	9,2	10,7	10,0	9,1	9,0	8,6	8,5	8,8	8,4	9,1	9,7	9,8		
0605	8,9	11,1	10,6	9,1	8,9	10,2	9,6	9,6	10,2	10,4	11,1			
1066	8,3	9,8	9,2	8,3	7,6	8,0	7,4							
1067	10,1	11,6	10,1	10,7	10,9	10,7	10,1	10,9	9,7	9,3	9,1	9,8	9,5	10,7
1099	9,7	10,9	9,6	8,6	9,4	9,4	9,3	9,4	9,7	9,4	10,9	10,4		

Normal value = 7,5–8,3 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

TABLE 14 Beta-globulin levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	5,0	5,9	5,2	5,0	5,4	5,5	4,9	5,4	5,4	5,4	5,9	5,5		
0605	4,7	6,1	5,3	5,1	4,8	5,9	5,6	5,8	5,6	6,2	7,2			
1066	4,6	5,4	4,9	5,0	4,5	5,5	4,2							
1067	5,7	6,6	5,5	6,2	6,6	6,6	5,5	5,7	5,4	5,5	5,5	6,2	6,3	5,9
1099	5,8	5,9	5,5	5,5	5,7	5,7	5,2	6,3	6,1	6,3	6,6	6,6		

Normal value = 8,0–11,2 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

TABLE 15 Gamma-globulin levels (g/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	14,1	14,4	15,8	13,3	13,6	13,4	12,6	11,9	11,4	12,7	14,0	15,2		
0605	12,1	14,7	14,4	11,7	11,6	14,0	13,6	12,7	12,2	14,5	14,3			
1066	13,7	15,0	14,5	12,5	11,6	12,9	11,9							
1067	18,9	19,7	19,6	18,0	18,6	18,9	18,1	17,6	15,2	15,3	15,5	15,1	15,7	15,8
1099	14,1	14,6	14,1	10,4	12,4	13,1	12,3	11,8	11,8	14,6	15,0	14,7		

Normal value = 16,9–22,5 g/ℓ: Hoffmann (1981)
* = First recorded fever reaction of 40 °C or above

calf (1067) showed a plasma cortisol level of 140,7 μmol/ℓ on Day 15. The reason for the increase is not immediately apparent, as this calf developed a temperature rise of over 40 °C only on Day 18 and reached the peak of clinical disease on Day 21. It may, however, have been associated with handling.

In this study, total serum protein and all its fractions showed a reduction which coincided with the initial temperature reaction and persisted throughout the acute stage of the disease (Tables 10, 11, 12, 13, 14 & 15). This reduction in portein was due mostly to a drop in albumin (Table 11), probably since albumin is a smaller protein than globulin (Ganong, 1975) and passes through endothelium more easily. Albumin levels remained depressed even after the apparent clinical recovery of the animal (Table 11). When the protein content of the blood was compared with that effused in the pericardium of the fatal case, Calf 1066, it was found that the concentrations of both albumin and globulin were high in this fluid (Table 16). The most severe drop in serum protein levels occurred in this calf that died on Day 18.

No marked fluctuations occurred in the blood Na and K concentrations and osmolality in any of the calves. In the calf that died, both Na and particularly the K concen-

trations were higher in the effused fluid than in the blood (Table 16). This resulted in a higher osmolality of the effused fluid as compared to that of the blood. Blood Ca levels did show a reduction during the acute stage of the disease (Table 17). In the calf that died (1066), the level of the calcium in the effused fluid was almost as high as that of the blood (Table 16).

TABLE 16 Chemical analysis of the blood and pericardial fluid of Calf 1066

Day 18 post-infection		
Parameter	Blood	Pericardial fluid
TSP	47,8 g/ℓ	41,7 g/ℓ
Albumin	25,7 g/ℓ	20,2 g/ℓ
Globulin	22,1 g/ℓ	21,5 g/ℓ
α-globulin	7,4 g/ℓ	6,0 g/ℓ
β-globulin	4,2 g/ℓ	4,1 g/ℓ
γ-globulin	11,9 g/ℓ	11,4 g/ℓ
Na	142,9 mmol/ℓ	151,6 mmol/ℓ
K	4,71 mmol/ℓ	21,63 mmol/ℓ
Ca (total)	1,82 mmol/ℓ	1,4 mmol/ℓ
Osmolality	293 mOsm/kg	328 mOsm/kg

Both UN and Crt level elevations occurred during the febrile stage of the disease but never exceeded the accepted top normal levels (Tables 18 & 19).

Increases in total bilirubin occurred in 4 of the 5 calves. These increases coincided with the febrile stage of the disease (Table 20). Darkening of the plasma colour coincided with peaks in bilirubin levels (Table 20). Measurements of direct bilirubin, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALT) and haptoglobin remained within normal limits (Results not shown).

DISCUSSION

Changes in haematological and blood chemistry parameters coincided with the onset of the febrile reaction. Of the haematological changes, a proportional drop in both haemoglobin and the haematocrit was the most marked. The resultant anaemia was of a normocytic, normochromic type, as no changes occurred in the MCV or MCHC, and could have been the result of a bone marrow depression (Shalm *et al.* 1975). This concept is further strengthened by the presence of a developing agranulocytosis (neutropenia and eosinopenia). The eosinopenia did not appear to be linked to changes in the blood cortisol levels. Of the remaining leukocytes, a lymphocytosis was observed, which also corresponded with the febrile stage. Abdel Rahim & Shommein (1977) postulated that this lymphocytosis was associated with

TABLE 17 Blood calcium levels (mmol/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	2,12	2,49	2,47	2,26	1,93	2,1	2,1	1,86	2,14	2,22	2,26	2,22		
0605	2,15	2,52	2,40	2,26	2,03	2,15	2,24	1,94	2,33	2,40	2,37			
1066	2,12	2,54	2,29	2,20	1,93	1,95	1,82							
1067	2,31	2,42	2,43	2,28	2,09	2,30	2,23	2,01	2,03	2,04	1,97	1,92	2,10	2,25
1099	2,53	2,67	2,29	2,28	2,22	2,23	2,19	2,0	2,47	2,40	2,33	2,33		

Normal value = 2–3 mmol/ℓ: Doxey (1979)
* = First recorded fever reaction of 40 °C or above

TABLE 18 Blood urea levels (mmol/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	0,6	1,1	1,2	1,4	2,8	5,8	7,2	4,0	2,7	2,7	2,7	2,5		
0605	1,3	1,4	1,6	2,1	3,6	3,2	2,1	4,1	2,0	1,8	2,3			
1066	0,9	1,7	1,5	1,6	2,7	8,5	10,1							
1067	0,4	1,6	0,7	0,9	1,9	3,5	2,1	6,0	6,2	6,0	5,4	4,9	3,4	1,9
1099	1,2	1,8	1,8	2,3	4,2	8,4	8,8	4,7	3,8	3,8	3,3	2,0		

Normal value = 3,6–10,7 mmol/ℓ: Schmidt (1979)
* = First recorded fever reaction of 40 °C or above

TABLE 19 Blood creatinine levels (μmol/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	84,4	73,7	93,4	90,0	97,8	104,6	117,0	95,9	88,8	76,8	80,6	82,5		
0605	67,8	59,7	83,7	81,3	82,8	77,6	72,6	69,7	63,6	60,8	64,4			
1066	81,2	72,6	88,6	95,7	99,4	120,3	119,1							
1067	85,3	81,6	86,9	82,7	87,1	87,7	94,2	77,4	90,5	101,7	104,5	107,2	89,0	76,1
1099	82,2	75,3	93,1	93,2	115,8	109,0	104,6	92,7	79,4	72,1	74,3	74,6		

Normal value = 133 μmol/ℓ: Schmidt (1979)
* = First recorded fever reaction of 40 °C or above

TABLE 20 Total bilirubin levels (μmol/ℓ)

Days post-infection	7	11	14*	15	16	17	18	19	20	21	22	23	24	25
Animal identification														
0549	5,3	6,3	6,4	5,4	7,3^{+}	11,5^{++}	10,3^{+}	7,9	9,0	4,6	6,6	5,7		
0605	4,3	6,7	6,9	4,8	6,3^{+}	8,7	7,2	7,8	7,2	5,4	4,5			
1066	3,7	5,0	6,6	5,3	9,5	19,3^{+++}	17,6^{++}							
1067	4,2	5,4	5,6	4,8	5,1^{+}	10,0^{+}	8,7	12,4	13,8^{+}	10,7	12,6	15,6^{++}	8,3	5,6
1099	4,7	5,5	8,8	6,1	8,2	14,9^{+}	11,7	9,5	7,9	4,2	5,1	4,6		

Normal value = 8,6 μmol/ℓ
* = First fever reation of 40 °C or above
$^{+}$ = Colour change of plasma
$^{+}$ = slight orange yellow
$^{++}$ = moderately orange yellow
$^{+++}$ = dark orange yellow

an immunological response. This, however, was not reflected in the protein electrophoresis as no increases in the gamma-globulin levels occurred at the time of clinical recovery.

This study suggests that endogenous cortisol does not have a major influence on the pathogenesis of the disease because of both the absence of elevated measured levels and of some of its metabolic effects which include a hyperglycaemia and lymphopenia (Shalm *et al.*, 1975). The use of exogenous cortisone in the symptomatic treatment of heartwater may thus be well indicated because of its membrane stabilizing effect (Jenkins, 1984) which may limit the oedema present in clinical cases of heartwater (Van Amstel, 1987).

The drop in blood protein levels, especially albumin, and the high protein content of the effused fluid suggests an increase in capillary permeability which occurs during the febrile stage. Protein levels tend to return to pre-febrile levels at the time of apparent clinical recovery, although albumin levels tend to remain somewhat depressed. This leaking of protein and electrolytes results in a higher osmolality in the effused fluid, as compared with plasma which will further act to withdraw fluid from the intravascular compartment.

No marked fluctuations occurred in blood electrolytes except in total blood Ca levels, which declined during the acute stage of the disease. In the calf that died, both Na and particularly the K concentration was higher in the effused fluid than in the blood. The significance of the lowered Ca levels is not known, as only total Ca and not ionized Ca was measured, the latter being the pharmacologically active fraction (Schalm *et al.*, 1975).

Increases in total bilirubin occurred in all the calves. Peaks in bilirubin seem to correspond with the severity of the disease. This bilirubinaemia does not appear to be associated with liver disease or haemolysis but may be associated with anorexia which the calves showed during the acute stage of the disease. The coincidence of darkening of plasma with peaks in bilirubin confirms the finding of Metelerkamp *et al.*, (1986) that bilirubin is responsible for this phenomenon.

Small increases in UN and Crt during the acute stage of the disease suggests some interference with glomerular filtration (Schalm *et al.*, 1975). This may be due to a reduction in blood volume, as described by Clark (1962).

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