# THE CLINICAL PATHOLOGY AND PATHOPHYSIOLOGY OF HEARTWATER: A REVIEW

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## ABSTRACT

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This paper reviews the available literature on the clinical pathology and pathophysiology of heartwater and makes comparisons with unpublished results obtained from a recent study in experimentally-induced heartwater in calves. The pathophysiological changes seem to center on an increased capillary permeability the result of which is reflected most noticeably in cardiac and lung function. There is a marked drop in cardiac output in severe cases and some workers have recorded a severe drop in diastolic blood pressure in the advanced stage of the disease. Changes in lung function are variable, depending on the stage of the disease, and may change from a respiratory alkalosis in the early febrile stage to a respiratory acidosis in more advanced cases. The basic cause for the increased capillary permeability is not known. The main clinical pathological changes measured include a progressive anaemia, fluctuations in total and differential white cell count, of which an eosinopenia and a lymphocytosis are the most marked, increases in total bilirubin which coincide with darkening of plasma colour, and a drop in total serum proteins mostly shown in the albumin levels.

## CLINICAL PATHOLOGICAL CHANGES

### Haematology

Haematological parameters investigated included haemoglobin concentration (Hb); haematocrit (Ht, or packed cell volume—PCV), mean corpuscular volume (MCV), mean corpuscular haemoglubin concentration (MCHC), red cell count (RCC) and total and differential white cell counts.

### Haemoglobin

Graf (1933), in his examination of 12 experimental cases of heartwater in sheep, came to the conclusion that the disease was not associated with any specific consistent change in the blood Hb content. Illemobade & Blotkamp (1978) found significantly lowered Hb levels in six experimentally infected goats when compared with three normal control goats. Owen, Littlejohn, Kruger & Erasmus (1973) also found a gradual lowering of Hb levels in venous blood samples in their study. Abdel Rahim & Shommein (1977) in their haematologic studies on experimentally induced heartwater in 20 male goats also demonstrated a progressive drop in haemoglobin. This has been confirmed in a recent study by Van Amstel, Guthrie, Reyers, Bertschinger, Oberem, Killeen & Matthee (unpublished data, 1986) in 5 cases of experimentally-induced heartwater in calves. They found that this decrease in Hb concentration started occurring shortly after the onset of the febrile reaction.

### Haematocrit (Ht), red cell count (RCC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)

Clark (1962) found that there was a general tendency for the Ht to fall during the course of the disease and to rise again terminally. Illemobade & Blotkamp (1978) also observed a drop in the Ht in the experimental group during the disease, but this did not differ significantly from the control group. Owen *et al.* (1973) observed a similar drop in venous Ht. Abdel Rahim & Shommein (1977), on the other hand, found a statistically significant drop in the Ht during the course of the disease. This was confirmed by Van Amstel *et al.* (unpublished data, 1986).

Clark (1962), Owen *et al.* (1973), Abdel Rahim & Shommein (1977) and Van Amstel *et al.* (unpublished data, 1986) found the drop in the haemoglobin to be proportional to that of the Ht. A concurrent drop in RCC, although not statistically significant, was demonstrated by Abdel Rahim & Shommein (1977) and Van Amstel *et al.* (unpublished data, 1986). Furthermore, Abdel Rahim & Shommein (1977) demonstrated statistically significant lowerings of both the MCHC and MCV values from which they concluded that a microcytic hypochromic anaemia develops during the course of the disease. This haematologic change is characteristic of the iron deficiency anaemias or a failure to utilize iron (Schalm, Jain & Carroll, 1975). As increased phagocytic activity leads to macrophage trapping of iron (Feldman & Kaneko, 1980), this possibility may exist in heartwater.

Van Amstel *et al.* (unpublished data, 1986) could not demonstrate any changes in the MCHC or MCV during the course of the disease and concluded that the anaemia seen in heartwater is more of a normocytic, normochromic type. Such an anaemia could result from acute blood loss, acute haemolysis, sequestration of red blood cells in the spleen, haemodilution or bone marrow depression.

Anaemia resulting from acute blood loss can be ruled out as no significant haemorrhage is generally associated with the disease (Prozesky, 1987). There may be some evidence for haemolysis as an increase in total bilirubin levels does occur concurrently with the decrease in both Hb and Ht (Van Amstel *et al.*, unpublished data, 1986). These researchers have shown that the increases in bilirubin were not associated with a decrease in haptoglobin, which is the carrier protein for free haemoglobin associated with haemolysis. Sequestration of red cells in the spleen could partially account for the drop in Hb, Ht and RCC as a tumor splenis generally exist (Prozesky, 1987). Haemodilution could not have played a role in the pathogenesis of the proposed normocytic normochromic anaemia as changes in plasma osmolality did not occur (Van Amstel *et al.*, unpublished data, 1986). Bone marrow depression can be associated with a normocytic normochromic anaemia (Schalm *et al.*, 1975). Other evidence supporting the possible existence of a bone marrow depression in heartwater includes absence of immature circulating red cells and an agranulocytosis (neutropenia and eosinopenia), as demonstrated by Van Amstel *et al.* (unpublished data, 1986).

### Total and differential white cell count

#### Total white cell count

Ilemobade & Blotkamp (1978) reported a leucopenia. The leucopenia developed 3 days after infection. This

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was also found by Van Amstel *et al.* (unpublished data, 1986). This leucopenia occurred prior to the onset of the febrile reaction. Abdel Rahim & Shommein (1977) reported a leucocytosis associated with the clinical disease.

### Neutrophils

A neutropenia was reported by Illemobade & Blotkamp (1978), Abdel Rahim & Shommein (1977) and confirmed by Van Amstel *et al.* (unpublished data, 1986). Camus & Barré (1982), quoting Perreau (1981), reported on the presence of a neutrophilia.

### Lymphocytes

A lymphopenia was reported by Illemobade & Blotkamp (1978) but both Abdel Rahim & Shommein (1977) and Van Amstel *et al.* (unpublished data, 1986) found a marked lymphocytosis developing shortly after onset of the febrile reaction.

### Monocytes

Illemobade & Blotkamp, Abdel Rahim & Shommein (1977) and Van Amstel *et al.* (unpublished data, 1986), observed no changes in the monocyte count. The presence of activated monocytes characterized by the presence of a large "foamy" or vacuolated cytoplasm has been regularly observed in field cases of heartwater (F. Reyers & A. van Heerden, Department of Medicine, Onderstepoort, unpublished data).

### Eosinophils

Clark (1962) observed the disappearance of circulating eosinophils before the onset of the temperature reaction. This was supported by Owen & Blotkamp (1973) except that the eosinopenia was preceded by a rise in eosinophil count. Both Abdel Rahim & Shommein (1977) and Van Amstel *et al.* (unpublished data, 1986) recorded significant decreases in eosinophil levels which corresponded with the febrile reaction.

From the above it seems that a neutropenia, an eosinopenia and a lymphocytosis are the most marked and consistent changes seen in the haemogram associated with heartwater. Both neutrophils and eosinophils are granulolytes formed in the bone marrow (Schalm *et al.*, 1975) and this finding could serve as evidence for the existence of a bone marrow depression, as already indicated. An eosinopenia could also be caused by elevated levels of endogenous cortisone (Schalm *et al.*, 1975). However it is well recognized that elevated cortisol levels, apart from causing an eosinopenia, also cause both a neutrophilia and a lymphopenia. As there is both a neutropenia as well as a lymphocytosis present in heartwater it seems unlikely that the eosinopenia found is caused by a cortisol response. Furthermore, Van Amstel *et al.* (unpublished data, 1986) in some preliminary studies on cortisol, were unable to demonstrate any significant increases in cortisol levels during the course of the disease.

## Chemical pathology

### Blood glucose

A rise in blood glucose was reported by Clark (1962), Graf (1933) and Illemobade & Blotkamp (1978). This rise seems to appear terminally and can result in a glucosuria (Clark, 1962). Results obtained by Van Amstel *et al.* (unpublished data, 1986), did not support this finding.

### Blood proteins

The blood proteins were investigated by Clark (1962), Illemobade & Blotkamp (1978) and Van Amstel *et al.* (unpublished data, 1986). Clark (1962) did not find significant changes in the blood proteins whereas Illemobade & Blotkamp (1978) reported a drop in the gamma globulins during the course of the disease and a rise in

alpha globulins. Clark ascribed the first change as being due to the lymphocyte depletion, which was observed in the follicles of the spleen and lymph nodes. This explanation seems unlikely in view of the fact that gamma globulins have a half-life of 17–22 days (Tizard, 1982). A rise in alpha globulins is a non-specific response to tissue damage and may be seen in many conditions (Schalm *et al.*, 1975).

Van Amstel *et al.* (unpublished data, 1986) found reductions in all protein fractions in their study of experimentally-induced heartwater in calves. This reduction in protein was reflected mostly in the albumin fraction, which seems probable as albumin is a smaller protein than globulin (Ganong, 1975) and would pass through the endothelium with greater ease. However, when the protein content of the blood was compared with the protein content of the effused fluid in the same animal, Van Amstel *et al.* (unpublished data, 1986) found that the concentrations of both albumin and globulin were high in the effused fluid (albumin 25,7 g/$\ell$ in blood as against 20,2 g/$\ell$ in the effused fluid and globulin 22,1 g/$\ell$ in the blood and 21,5 g/$\ell$ in the fluid).

Clark (1962), commenting on his findings, postulated that the plasma protein concentrations remain unchanged, despite the loss resulting from capillary leakage, because of a concurrent reduction in blood volume, which he determined using the Evans blue method. The results of Van Amstel *et al.* (unpublished data, 1986) are not contrary to those of Clark (1962), but suggest that a disproportionate loss of fluid and protein from the vascular compartment occurs; in other words, relatively larger losses of protein in contradistinction to fluid may take place.

### Blood urea nitrogen (UN) and creatinine (CRT)

Both Clark (1962) and Graf (1933) found increases in the UN whereas the CRT remained within normal limits. This tends to indicate that the rise in UN was caused by pre-renal factors and not by kidney damage as such. Pre-renal increases in UN can be caused by several factors including high nutritional levels of protein, dehydration, decreased glomerular filtration resulting from blood pressure changes, or high levels of cortisol resulting in an increase in protein catabolism (Ganong, 1975). As blood pressure changes are associated with heartwater, as shown by Clark (1962) and Owen *et al.* (1973), this could be responsible for the increase in UN levels.

Van Amstel *et al.* (unpublished data, 1986) when following daily levels of UN and CRT in calves with experimentally-induced heartwater, found elevations above baseline values starting shortly after the onset of the febrile reaction. These elevations were not severe and did not reach values in excess of what is regarded as being at the upper limit of what is normal for the species.

As creatinine is excreted through the kidney by means of glomerular filtration only, and not reabsorbed to any extent (Ganong, 1975), measurement of CRT levels can act as an indicator of glomerular filtration rate. The findings by Van Amstel *et al.* (unpublished data, 1986) would therefore suggest that some interference in glomerular function occurs in cases of heartwater.

### Plasma colour

Clark (1962) and Gruss (1981) reported that blood plasma becomes progressively more dark and may attain a dark orange colour. Van Amstel *et al.* (unpublished data, 1986) confirmed this and, in addition, showed that the darkening of plasma is linked with elevations in total bilirubin levels. Metelerkamp, Litthauer, Naudé, Oelofsen & Gruss (1986) in chromatographic studies on serum obtained from experimentally-induced heartwater in angora goats, came to the conclusion that the darkening

of the serum was associated with bilirubin and/or its conjugates.

## Serum electrolytes

Clark (1962), Owen et al. (1973) and Van Amstel et al. (unpublished data, 1986) determined electrolyte levels during experimental heartwater including sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), inorganic phosphate (SIP) and calcium (Ca).

### Sodium (Na) and chloride (Cl)

Owen et al. 1973 reported a slight rise in Na and Cl during the febrile phase. Clark (1962) and Van Amstel et al. (unpublished data, 1986) did not find this change. However, the latter authors found Na levels in the effused fluid to be in excess of those in the serum.

As both Na and Cl are distributed mainly extracellularly, increases of these ions may occur with primary water loss (Ganong, 1975), as can occur during febrile state with inadequate water intake.

### Potassium (K)

Owen et al. (1973) reported a terminal drop in blood K levels. Other investigators, to whom reference has been made, found no marked changes in K levels, with the exception of Van Amstel et al. (unpublished data, 1986), who found potassium levels in the effused fluid to be far in excess of those in the serum. This, together with the Na, resulted in the osmolality of the effused fluid being higher than that of the plasma.

### Calcium

Camus & Barré (1982) quoting Malan reported on fluctuations in blood calcium levels resulting in a terminal hypocalcaemia. Clark (1962), did not confirm this finding.

Van Amstel et al. (unpublished data, 1986), measuring total calcium, found decreases occurring during the course of the disease to below the lowest normally accepted level of 2 mmol/ℓ for calves. This drop in total calcium could be expected in cases of heartwater as some of the carrier protein will effuse through resulting in a lowering of total blood calcium levels. Measuring total calcium in effused fluid, Van Amstel et al. (unpublished data, 1986) found a level of 1,4 mmol/ℓ compared with 1,82 mmol/ℓ in the blood of the same animal.

It should be considered, however, that ionized calcium is the pharmacologically active fraction in the blood and that levels of ionized calcium do not necessarily follow those of total calcium (Ganong, 1975). This should be taken into account when calcium therapy is considered for clinical cases of heartwater.

### Magnesium (Mg) and inorganic phosphate (SIP)

Clark (1962) and Van Amstel et al. (unpublished data, 1986) did not find any variations in the Mg or SIP levels.

## Blood acid/base and respiratory findings

Aspects of the blood acid-base status and respiratory function were examined by Clark (1962), Ilemobade & Blotkamp (1978), Owen et al. (1973) and Van Amstel et al. (unpublished data, 1986).

### Blood acid-base

*Arterial and venous pH, arterial $PO_2$ and $PCO_2$, standard bicarbonate ($HCO_3$), blood pyruvate and lactate.* Ilemobade & Blotkamp (1978) found terminal increases in blood pyruvate and lactate levels and a simultaneous drop in venous blood pH. They speculated that these changes indicate the presence of a metabolic acidosis. This may be supported by a terminal drop in bicarbonate ($HCO_3$) as demonstrated by Clark (1962). The existence of a metabolic acidosis in terminal cases of heartwater is likely, as Clark (1962) also demonstrated a drop in blood volume, prior to death, which would result in anaerobic muscle metabolism giving rise to the above changes (Ganong, 1975).

Owen et al. (1973) demonstrated a tendency towards $CO_2$ retention and respiratory acidosis on the 12th day post infection. Standard bicarbonate values in their study did not change significantly, thus indicating little or no metabolic component of the acidosis. At the same time $O_2$ diffusion was affected, resulting in a significantly lowered arterial oxygen tension ($PO_2$) which persisted until the day of death.

Van Amstel et al. (unpublished data, 1986) found a tendency towards the development of a respiratory alkalosis during the early stages of the disease. This resulted from hyperventilation at this stage which was probably caused in part by fever, although handling of the calves must also have played a role. Both retention of $CO_2$ and lowering of arterial oxygen tension ($PO_2$) were never marked. A developing metabolic acidosis was never reflected in the blood gas analyses.

### Respiratory function

Respiratory function was evaluated by Owen et al. (1973) by measuring oxygen consumption, respiratory rate, tidal and minute volumes and the ventilatory equivalent ($VEO_2$), which is defined as the volume of inspired gas necessary to give an oxygen uptake of 100 mℓ/min. They found that the most pronounced changes in these parameters took place when the animals showed the most severe clinical signs of respiratory distress which was 12 days post infection. Increases in both the respiratory rate and the tidal volume resulted in an increased minute volume. There was a simultaneous increase in $VEO_2$ showing that an increased volume of gas was necessary for oxygen extraction at a rate of 100 mℓ/min to occur. Owen et al. (1973) concluded that from the three findings of respiratory acidosis, hypoxia and an increased ventilatory equivalent for $O_2$, confirm the marked pulmonary dysfunction suggested by clinical and post mortem findings.

The pathophysiological mechanisms of pulmonary dysfunction in heartwater have not been fully elucidated. Van Amstel et al. (unpublished data, 1986) hypothesized that changes in ventilation and perfusion occur during the severe stages of the disease and for this reason studies of physiological and alveolar dead space and venous admixture were carried out.

### Plasma and blood volumes

Clark (1962) demonstrated a marked drop in blood volume in 7 of 10 sheep just prior to death. He postulated that this drop in blood volume could be caused by an increase in capillary permeability as no change in blood protein concentration could be demonstrated associated with this drop in volume. He said that this hypothesis is further strengthened by the fact that transudates in heartwater frequently show coagulation, indicating that large protein-like fibrinogen can pass through the wall.

The cause for this increase in capillary permeability remains an open question. Bezuidenhout (1982), Jackson & Neitz (1932) and Prozesky (1982), as cited by Camus & Barré (1982), suggest that a toxin may play a role in the pathogenesis of the disease. It is well known that endotoxin from gram negative organisms can increase capillary permeability through a direct effect or by their role in the synthesis of vasoactive inflammatory products such as serotonins, leucotrienes and prostaglandins (Jenkins, 1984). This possibility should still be investigated.

### Cardiovascular function

Cardiovascular funtion was examined by Clark (1962), Owen et al. (1973) and Van Amstel et al. (unpublished data, 1986) and included eletrocardiographic

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recordings (EKG), systolic and diastolic blood pressure, right ventricular pressure, cardiac output and stroke volume.

### Electrocardiographic recordings (EKG)

Owen *et al.* (1973) and Van Amstel *et al.* (unpublished data, 1986) reported that no significant EKG changes occurred during the course of the disease suggesting little if any primary cardiac damage. The absence of any arrhythmias indicates that conduction remains normal.

### Systolic and diastolic blood pressure, cardiac output and stroke volume

Clark (1962) reported a precipitous drop in diastolic blood pressure just prior to death and postulated that this was caused by a sympatholysis resulting in a peripheral vasodilatation. This finding was confirmed by Owen *et al.* (1973) who, in addition, also recorded a progressive drop in systolic blood pressure during the course of the disease. The latter finding was caused by a progressive decrease in stroke volume probably associated with pericardial effusion (Owen *et al.* 1973). They found however that cardiac output was maintained until death, despite the decreasing stroke volume, and was induced by an increase in heartrate.

Van Amstel *et al.* (unpublished data, 1986) found that decreases in cardiac output may play an important role in the advanced stage of the disease in calves.

### SUMMARY

Most clinical pathological and pathophysiological changes coincide with the onset of the febrile reaction. The most marked haematological changes include a proportional drop in both haemoglobin and haematocrit. There seems to be some controversy as the exact type of anaemia which develops. Abdel Rahim & Shommein (1977) postulate that it is a microcytic hypochromic anaemia as seen with the iron deficiency anaemias. Van Amstel *et al.* (unpublished data, 1986) found no changes in the mean corpuscular volume and the mean corpuscular haemoglobin concentration and so concluded that it is a normocytic normochromic anaemia as may be seen with bone marrow depression. Other support for this concept is the development of a agranulocytosis (neutropenia and a very marked eosinopenia) and the absence of circulating immature red blood cells.

Of the chemical pathological changes found the following are the most important: Elevations in bilirubin levels occurred shortly after the onset of the febrile reaction. This finding does not seem to be associated with haemolysis (Van Amstel *et al.*, unpublished data, 1986). Darkening of plasma colour as described by Clark (1962) and Gruss (1981) is associated with this increase in bilirubin.

Leaking of protein fractions into fluid effusions occur as was shown by protein determinations on the effused fluid (Van Amstel *et al.*, unpublished data, 1986). These researchers found that plasma protein levels dropped accordingly.

There were no noteworthy changes in serum electrolytes except total calcium, the levels of which decreased to below that regarded as normal.

Both sodium, and especially potassium levels, were higher in the effused fluid compared with the serum values. The result was that the effused fluid had a higher total osmolality when compared with the serum (Van Amstel *et al.*, unpublished data, 1986).

Mild increases occurred in both the urea nitrogen and creatinine levels. The former could be associated with the decrease in blood volume whereas elevations of both can be involved in reduced glomerular function.

Changes in the acid base seemed to be closely associated with respiratory function.

Changes in respiratory function include hyperventilation, increased minute volume and ventilatory equivalent, respiratory acidosis and hypoxia.

Changes in cardiovascular function include a decrease in stroke volume and cardiac output and a drop in both systolic and diastolic blood pressure. Changes in heart function are probably associated with pericardial effusion as no changes in the EKG were seen. The drop in systolic blood pressure is probably linked with the decrease in stroke volume whereas the drop in diastolic pressure may be associated with a CNS-induced sympatholysis as postulated by Clark (1962).

### CONCLUSION

Future research in the field of the clinical pathology and pathophysiology of heartwater should be directed towards finding the exact mechanism responsible for the increase in capillary permeability. Further study is also necessary into the pathophysiological mechanisms of caridac and respiratory function. This will enable us to institute rational supportive therapy which could be life saving in a high percentage of field cases of this disease.

It seems unlikely that a specific diagnostic test will be found for clinical cases of heartwater based on clinical pathological changes.

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