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# Changes in the blood-gas status of sheep with experimentally induced heartwater

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**ABSTRACT**

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The blood-gas status of seven sheep with experimentally induced heartwater during the acute and terminal stages was investigated. Changes in blood gas included a decline in arterial oxygen tension ($pO_2$) combined with a respiratory alkalosis. Although the sheep became hypoxaemic, blood-gas changes associated with respiratory failure were not observed.

## INTRODUCTION

Studies on the blood-gas status in heartwater have been carried out by Ilemobade & Blotkamp (1978), Clark (1962), Owen, Littlejohn, Kruger & Erasmus (1973) and Van Amstel, Guthrie, Oberem, Killeen & Matthee (1988). In these studies no clear consensus was reached as to the exact nature of the blood-gas changes associated with experimentally induced disease nor the role they played in the cause of death. Differences in results could possibly be due to differences in the collection, handling and assay of samples as well as the time of collection during the course of the disease.

In their previous study Van Amstel *et al.* (1988) found a tendency towards the development of a respiratory alkalosis during the early, acute febrile stage of the disease. This they associated with hyperventilation resulting from fever and handling. Neither lowering of arterial carbon dioxide ($pCO_2$) nor oxygen tension ($pO_2$) were marked. Based on this they questioned the importance of lung oedema as the major cause of death in acute cases of the disease. These authors did not, however, record any blood-gas changes during the terminal stages (last 12 h) of the disease. As alveolar flooding appears to be a terminal event, it would appear important that such studies be included. In the present study, blood-gas determinations were carried out during 12 d pre-infection, 12 d post-infection and also during the last 12 h before death.

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## MATERIALS AND METHODS

### Experimental animals

Seven healthy adult Merino sheep were used (identified as 3535, 3499, 3428, 3437, 3459, 3559 and 3438). To facilitate the collection of arterial blood samples a portion of the left common carotid artery was subcutaneously relocated in the jugular groove, by use of the technique described by Butler (1962). Complete healing of the surgical wounds occurred during a 2-week period, after which pre-infection samples were drawn over a period of 12 d. Thereafter, heartwater was induced in the sheep by inoculating each of them intravenously with a single dose

(5 mℓ of infected blood) of the "Welgevonden" stock of *Cowdria ruminantium* (Du Plessis 1985).

### Clinical parameters

The rectal temperature of each animal was recorded twice daily. Only the morning temperature was entered into the records, the afternoon reading serving as a double-check. On no occasion was there a significant discrepancy between the two readings. The acute (febrile) phase of the disease was considered to have commenced when the recorded rectal temperature reached 40 °C.

Respiratory rate was recorded daily, in the morning, by counting the number of full respiratory movements over 1 min. In the event of an animal panting, no value was entered in the records, but the panting was noted.

Heart rate was recorded daily, in the morning, by auscultation of the heart for a full minute.

Habitus and appetite were also recorded daily, in the morning, using a subjective, semi-quantitative scoring system of 0–5+.

### Confirmation of heartwater

The presence of heartwater was confirmed by microscopic demonstration of *Cowdria ruminantium* organisms in Romanowski-stained (Cam's Diff Quick, CA Milch, Krugersdorp, RSA) smears prepared from the hippocampus at autopsy after euthanasia or disease-induced death (days 12–15 post-infection).

### Experimental procedures

*Blood-gas parameters*

Arterial blood was collected via a 23 G hypodermic needle introduced percutaneously into the relocated portion of the carotid artery. Blood samples were collected anaerobically in 5-mℓ heparinized, disposable syringes, which were sealed immediately after collection. Blood-gas analysis was performed within an hour of sample collection using a Nova Stat Profile 7 blood-gas analyser (Nova Biomedical, Waltham, USA).

Blood samples were collected on days –12, –6, –5, –1, on the day of infection (day 1) and then once daily from days 6–13 post-infection. On day 13 samples were collected within 12 h of death or euthanasia. In three sheep that died on day 13 (3459, 3437, 3499) and one sheep that survived to day 15 (3559) two samples were taken on day 13 during the 12-h period preceding the death of the former three. The results of three samples, one on day –6 (sheep 3459) and one each on days 8 and 9 (sheep 3499) were not included as doubt existed whether the blood was of arterial origin.

*Pericardial effusions and thoracic effusions*

After removal of the abdominal organs at autopsy, the animal was placed in dorsal recumbency. An incision was made through the abdominal attachment of the diaphragm to expose the chest. The incision was carefully enlarged until the thoracic effusion could be visualized. By means of a 6-cm teat canula, the thoracic fluid was aspirated into a 50-mℓ disposable syringe and the volume recorded.

During this procedure care was taken not to accidentally cut into the pericardial sac during the opening of the chest. A 18 G needle attached to a 50-mℓ disposable syringe was used to aspirate the pericardial effusion after penetration of the pericardial sac. Following this, the pericardial sac was carefully incised in order to reach any remaining fluid, which was then also aspirated and recorded.

*Statistical analysis*

Data for each measured variable were analysed by means of the computer-based statistical analysis programme "Statgraphics" (Statistical Graphics Corporation, USA, 1990). The data were subjected to the Least Significant Difference (LSD) multiple-range test (Snedecor & Cochran 1980) to compare sampling-day mean values. A difference was considered significant at the $P < 0,05$ level. Means from days –12, –6, –5, –1 and 0 were grouped as "pre-infection" levels.

## RESULTS

### Fever

Rectal temperatures daily mean values remained at the pre-infection level of 38,6–39,1 °C up to and including day 6 (Fig. 1). On day 7 six of the seven sheep recorded a temperature of 40,0 °C and thereafter rectal temperatures rose progressively to exceed 41,5 °C by day 12. All temperature means from

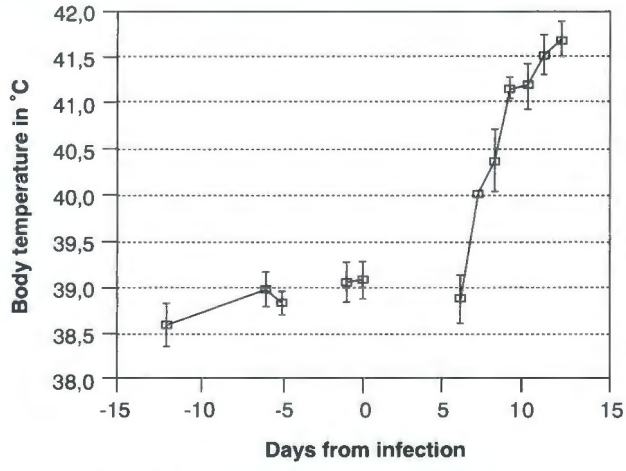


FIG. 1 Results of mean rectal body temperature in °C ± 2 SE

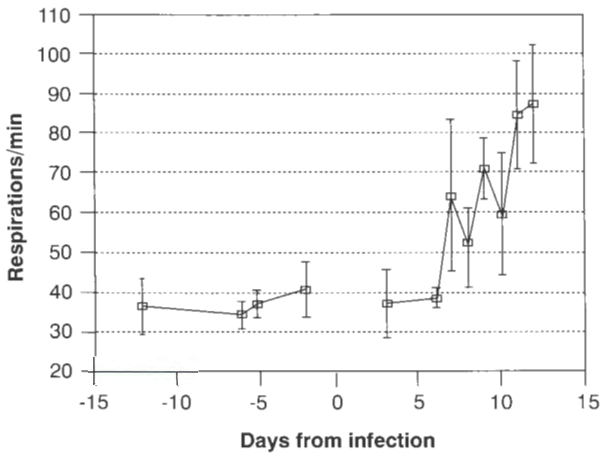


FIG. 2 Results of mean respiratory rates (per min) ± 2 SE

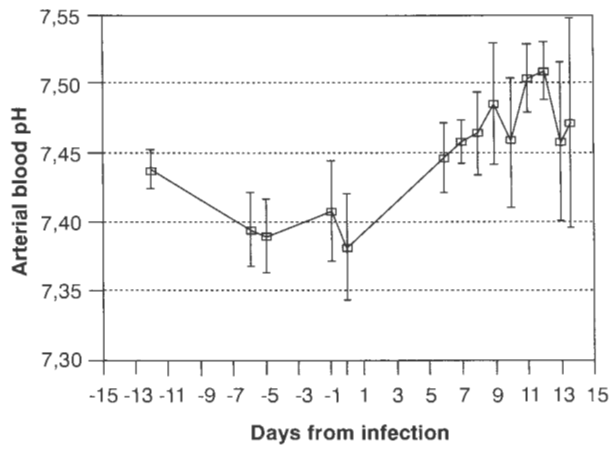


FIG. 3 Results of mean arterial blood pH ± 2 SE

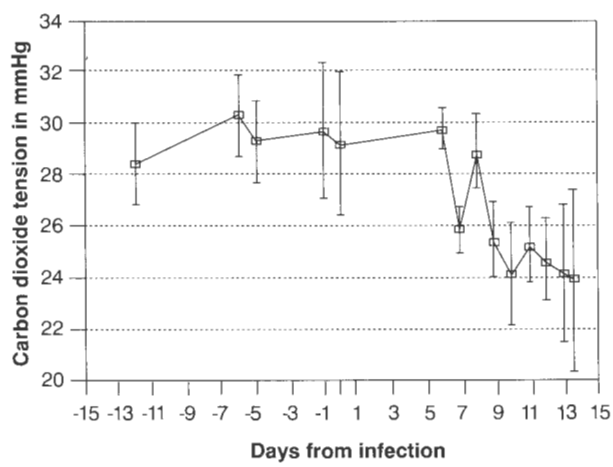


FIG. 4 Results of mean arterial blood partial carbon dioxide tension in mm mercury ± 2 SE

day 7 were significantly higher than pre-infection levels as well as that recorded on day 6. Based on the above, the acute (or febrile) phase of the disease was identified as day 7 to death or euthanasia.

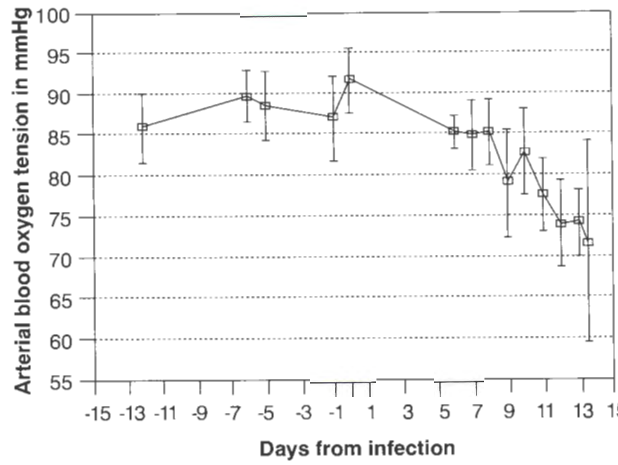


FIG. 5 Results of mean arterial blood partial oxygen tension in mm mercury ± 2 SE

## Outcome

Sheep 3428 died on day 12. Three sheep (3499, 3437 and 3459) died on day 13. Two sheep (3535 and 3438) were euthanased on day 13 owing to the severity of clinical signs. Sheep 3539 survived day 13 but had to be euthanased on day 15.

## Respiratory rate

Fig. 2 shows that, like rectal temperature, respiratory rate mean values remained in the pre-infection range of 34–40/min until day 6. From day 7 all respiratory rate means were significantly higher than pre-infection rates as well as the mean value obtained on day 6. Respiratory rates continued to rise during the acute (febrile) phase with the means recorded on days 11 and 12 being significantly higher than those on days 7 and 8.

## Blood-gas/acid-base status

Fig. 3 demonstrates that all pH mean values during the acute febrile phase exceeded 7,45, with values in excess of 7,5 on days 11 and 12. The mean pH values during this phase were significantly higher than all pre-infection means values except those on day –12. Thus it can be said that an alkalosis existed during the entire acute (febrile) phase.

Arterial partial pressure of carbon dioxide (Fig. 4) was significantly lower (with the exception of day 8) during the acute (febrile) phase than during the pre-infection period as well as on day 6.

Arterial $pO_2$ (Fig. 5), although decreasing after day 8, showed mean values which were significantly lower than pre-infection levels only on days 11, 12 and 13.

TABLE 1 Results of the arterial partial oxygen tension during the last 12 h

| Sheep identification | $pO_2$ mmHg | |
|---|---|---|
| | First reading | Second reading |
| 3499 | 71,6 | 54,9 |
| 3437 | 82,0 | 75,7 |
| 3459 | 70,3 | 84,3 |

TABLE 2 Results of effusion measured at autopsy and the last recorded arterial oxygen tension and respiratory rates

| Sheep | Pericardial effusion (mℓ) | Thoracic effusion (mℓ) | $pO_2$ (mmHg) | Respiratory rate ($min.^{-1}$) |
|---|---|---|---|---|
| 3535 | 70 | 257 | 77 | 60 |
| 3499 | 59 | 300 | 55 | 100 |
| 3428 | 79 [a] | 135 | 79 | 80 |
| 3437 | 15 | 218 | 76 | 110 |
| 3459 | 5 | 33 | 84 | 104 |
| 3438 | 50 | 446 | 75 | 60 |
| 3559 | 12 [b] | ND [c] | 72 | 94 |

[a] = Day 12 [b] = Day 15 [c] = Not done

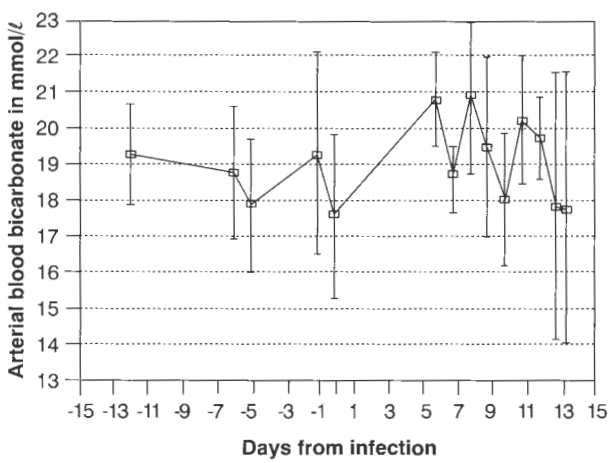


FIG. 6 Results of mean arterial blood bicarbonate in mmol/ℓ

Only one of the three sheep which died on day 13 showed a significant terminal hypoxaemia (Table 1).

From Fig. 6 it is clear that blood-bicarbonate concentrations, although showing great variability, did not exhibit any changes during the trial period which correlated with any of the other observed patterns.

No significant correlations could be demonstrated between effusion volumes and $pO_2$ or respiratory rate. Table 2 clearly demonstrates the lack of any close correlations.

## DISCUSSION

These results confirm an earlier report of an alkalosis being present during the acute (febrile) phase of the disease (Van Amstel *et al.* 1988). This alkalosis was shown to persist into the terminal stages. The changes exhibited by the respiratory rate and $pCO_2$ clearly demonstrate that the alkalosis is respiratory in origin.

Such a respiratory alkalosis could be brought about by two possible mechanisms:

- Loss of $CO_2$ through hyperpnoea triggered by a hypoxaemia. Lung oedema and/or alveolar flooding would be expected to cause the observed hypoxaemia. This would cause a compensatory hyperpnoeic response. Carbon dioxide, being more easily solubilized, would pass through a fluid barrier with greater facility than oxygen, leading to a respiratory alkalosis as a result of excessive carbon-dioxide loss. The data from this trial do not lend much support for this mechanism as the hypoxaemia was not very severe (except in sheep 3499) and trailed the alkalosis chronologically.
- Loss of $CO_2$ through hyperpnoea triggered by fever. In an attempt to restore normal body temperature by increasing evaporative heat loss through hyperventilation, excessive $CO_2$ would be "blown off" leading to a respiratory alkalosis. The data from this trial appear to confirm that this mechanism was probably responsible. Respiratory-rate increase closely followed the increase in temperature (sheep 3437 even panting on day 8), mirrored by a decrease in $pCO_2$.

There was a gradual reduction in the arterial $pO_2$ levels during the acute phase of the disease. Except for sheep 3459, the $pO_2$ of all the other animals (Table 2) were clearly below the mean pre-infection $pO_2$ levels of 85–90 mmHg (Fig. 5). However, in an attempt to determine whether respiratory failure could be identified as the cause of death, these data do not, in general, support such a conclusion. Only sheep 3499, with a terminal $pO_2$ of 54 mmHg, approaches the degree of hypoxaemia which would be consistent with such a mechanism (Huber 1984).

The hypoxaemia, although not generally very severe, and occurring late in the acute (febrile) phase, appears to be only loosely (although not significantly) correlated with the observed volume of pericardial and/or thoracic effusions. This relationship was, however, particularly noticeable in sheep 3499 and 3459, representing two extremes.

## ACKNOWLEDGEMENTS

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