Onderstepoort J. Vet. Res., 54, 45–48 (1987)

FURTHER STUDIES ON THE CLINICAL PATHOLOGY OF SWEATING SICKNESS IN CATTLE

S. R. VAN AMSTEL[1], F. REYERS[1], P. T. OBEREM[2] and O. MATTHEE[2]

ABSTRACT

VAN AMSTEL, S. R., REYERS, F., OBEREM, P. T. & MATTHEE, O., 1987. Further studies on the clinical pathology of sweating sickness in cattle. *Onderstepoort Journal of Veterinary Research*, 54, 45–48 (1987)

Experimentally-induced cases of sweating sickness in calves were used in an effort to correlate the blood chemistry with some of the known pathological changes. Results showed that the "sweating" associated with necrotic dermatitis did not alter blood electrolyte levels. Laboratory evidence of a disseminated intravascular coagulopathy was found which correlated with the microthrombi described in cases of sweating sickness. A high blood cortisol level was found in one of the animals that died from the disease and could possibly be used as a prognostic indicator in clinical cases. Recommendations are made with regard to the supportive treatment based on the clinical pathological findings.

INTRODUCTION

Sweating sickness toxin has a selective affinity for stratified squamous epithelium of the skin, resulting in a necrotic dermatitis. Other structures lined with this epithelium may undergo a diphtheric or a necrotic pseudomembranous inflammation (Kriek, 1977). The clinical pathological changes associated with these lesions have been investigated (Van Amstel, 1984) and pathological changes of parenchymatous organs in sweating sickness have been described. One of the underlying pathogenetic mechanisms involved in sweating sickness appears to be an extensive microangiopathy which results in a disseminate intravascular coagulopathy (DIC) with the microthrombi appearing as hyaline globules (Kriek, 1977). The objectives of this study were to determine whether the histological evidence of a consumption coagulopathy could be verified by chemical means, and whether the severe cutaneous "sweating" has any influence on blood electrolyte levels.

MATERIALS AND METHODS

Experimental animals

Five experimental cases of sweating sickness were produced by infesting healthy 5-6-month-old calves with strains of *Hyalomma truncatum* ticks (Tick Unit, Veterinary Research Institute, Onderstepoort) known to consistently provoke the disease. Twenty male and 40 female ticks were placed into each of 5 tick bags which were glued onto the animals' backs in the thoraco-lumbar area. The animals were then observed daily for typical signs of sweating sickness. Six days after attachment of the ticks all the calves had developed relatively severe clinical signs of the disease which were characterized by pyrexia, markedly congested mucous membranes and severe hyperaemia of the skin with exudation (sweating) and exfoliation. The ticks were allowed to remain attached throughout the experimental period. A sixth uninfested calf, maintained under the same conditions, served as control. Blood was collected for clinical pathological tests from all the animals, except Calf 1204, 6, 8, 10 and 13 days after attachment of the ticks. Calf 1204 was only bled twice on the 6th and 8th days, respectively, as it died on the 10th day after infestation.

All the affected animals, except one (1204), were treated with hyperimmune serum, each receiving 200 mℓ intravenously on Days 6, 8 and 10. Sweating sickness hyperimmune serum was prepared as described by Oberem, Van Amstel, Matthee & Bezuidenhout (1985). In addition, they were each given procaine penicillin intramuscularly at a dose rate of 30 000 i.u./kg on Day 10, except for Calf 1204, which died on that day. The other 4 experimental cases made a slow recovery.

[1] Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110

[2] Veterinary Research Institute, Onderstepoort 0110

Received 21 October 1986—Editor

Blood chemistry

Parameters evaluated to examine possible derangements in blood coagulation included the following:

1. The prothrombin time (PT), using the "Hepato-Quick", Boehringer Mannheim's test kit (Cat. No. 126527), according to the manufacturer's directions.
2. The partial thromboplastin time (PPT), using Boehringer Mannheim's test kit (Cat. No. 126551), according to the manufacturer's directions.
3. Fibrin degradation products (FDP), using the Trombo-Wellco test (Rapid latex) Test HA 13, Wellcome Laboratories.

A blood sample obtained from the control calf was used to monitor the PT and PTT test results.

As both liver failure and endotoxaemia can play a role in the pathogenesis of DIC (Morris & Beech, 1983), serum activities of the following enzymes were determined:

1. Gamma glutamyl transpeptidase (GGT) (EC 2.3.2.2). A Technicon RA 1000 method (Cat. No. T01-1528) with L-γ-glutamyl p-nitroanilide substrate was used.
2. Glutamate dehydrogenase (GLDH) (EC 1.4.1.3). A Boehringer Mannheim reagent (Cat. No. 124320) was used on a Lange LP 6 U.V. spectrophotometer, according to the manufacturer's directions.
3. Aspartate transaminase (AST) (EC 2.6.1.1). A Technicon RA 1000 method (Cat. No. T01-1528) was used.

All enzyme activity results are expressed at 25 °C.

The LAL chromogenic endotoxin assay was perfomed with the Whittaker MA Bioproducts test kit (Cat. No. 50-6404), according to the manufacturer's directions.

Blood electrolyte levels were determined with a Varian Techtron single-beam, atomic, absorptiometer Model 275. Sera were diluted 1:25 in 0,92 % Lanthanum chloride and aspirated into an air-acetylene flame.

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

RESULTS

The results of the PT, PTT and FDP determinations on the experimental sweating sickness cases and the control are shown in Tables 1, 2 & 3.

All the experimental sweating sickness cases showed increased protrombin times for variable periods from the 6th day after attachment of the ticks (Table 1). This indicated the presence of an abnormality in the extrinsic pathway of their blood coagulation mechanism. Only one calf (1205) showed a marked increase in the partial thromboplastin time, an indication of an abnormality of

* Diagnostic products Corporation, Los Angeles, California

TABLE 1 Prothrombin times

Days after infection	6	8	10	13
	Sec.	Sec.	Sec.	Sec.
Control	37	47	45	40
1202	59	52	68	45
1204	60	28	Death	
1205	46	90	66	45
1203	53	ND	46	45
1201	56	20	44	40

ND = Not done

TABLE 2 Partial thromboplastin times

Days after infection	6	8	10	13
	Sec.	Sec.	Sec.	Sec.
Control	28	34	27	37
1202	31	27	40	42
1204	31	34	Death	
1205	41	55	42	59
1203	32	ND	43	38
1201	40	38	38	27

ND = Not done

TABLE 3 Fibrin degradation products

Days after infection	6	8	10	13
	µg/mℓ	µg/mℓ	µg/mℓ	µg/mℓ
Control	3	3	3	2
1202	3	3	3	2
1204	3	3	Death	
1205	3	40	40	2
1203	3	3	3	2
1201	3	3	3	2

TABLE 4 Aspartate transaminase (AST) activity

Days after infection	6	10	13
	u/ℓ @ 25°C*	u/ℓ @ 25°C*	u/ℓ @ 25°C*
Control	22	22	23
1202	119	222	90
1204	102	Death	
1205	118	208	133
1203	ND	37	37
1201	66	51	33

ND = Not done

* Normal value = < 80 u/ℓ Schmidt (1979)

TABLE 5 Glutamate dehydrogenase (GLDH) activity

Days after infection	8	10	13
	u/ℓ @ 25°C*	u/ℓ @ 25°C*	u/ℓ @ 25°C*
Control	2,2	2,6	1,1
1202	7,3	59,5	10,2
1204	4,0	Death	
1205	76,7	38,0	1,8
1203	ND	3,3	5,1
1201	4,4	3,3	1,8

ND = Not done

* Normal value = <8 u/ℓ Schmidt (1979)

the intrinsic pathway of blood coagulation (Table 2). This calf also had elevated levels of fibrin degradation products on Days 8 and 10 after tick attachment, which suggests the presence of DIC.

The results of the serum enzyme activities are shown in Tables 4, 5 & 6.

Two calves (1202 and 1205) showed increases in AST, GLDH and GGT activities indicative of hepatic/ hepato-biliary pathology.

TABLE 6 Serum gamma glutamyl transpeptidase activity

Days after infection	8	10	13
	u/ℓ @ 25°C*	u/ℓ @ 25°C*	u/ℓ @ 25°C*
Control	10	10	4
1202	8	29	16
1204	9	Death	
1205	24	22	26
1203	ND	8	7
1201	11	10	8

ND = Not done

* Normal value = <25 u/ℓ Schmidt (1979)

TABLE 7 Plasma cortisol levels

Days after infection	8	10	13
	µmol/ℓ*	µmol/ℓ*	µmol/ℓ*
Control	35,0	6,7	5,0
1202	82,0	32,4	1,1
1204	139,9	Death	
1205	23,5	100,9	6,9
1203	ND	0	39,4
1201	10,7	10,6	21,3

ND = Not done

* Normal value = 0-30 Emol/ℓ Coubrough, Bertschinger & Kühne (1980)

TABLE 8 Serum sodium levels

Days after infection	6	8	10	13
	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*
Control	136,3	155,9	137,4	ND
1202	164,3	148,7	154,4	142,4
1204	146,3	ND	Death	
1205	140,7	152,3	137,5	139,9
1203	ND	142,3	ND	150,3
1201	150,4	155,7	136,9	148,7

ND = Not done

* Normal value = 136–145 mmol/ℓ Doxey (1977)

TABLE 9 Serum potassium levels

Days after infection	6	8	10	13
	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*
Control	4,91	4,77	4,12	ND
1202	4,10	3,1	5,55	5,73
1204	4,07	ND	Death	
1205	3,34	2,7	4,7	4,44
1203	ND	4,9	ND	5,03
1201	2,93	4,73	5,0	5,38

ND = Not done

* Normal value = 3,6–5,6 mmol/ℓ Doxey (1977)

No detectable levels of endotoxins were measured in any of the animals.

The results of the plasma cortisol levels are shown in Table 7.

Markedly elevated cortisol levels were found in 3 calves (1202, 1204 and 1205), the highest level being in the calf that died 10 days after tick attachment.

Serum electrolyte levels in the calves are shown in Tables 8, 9, 10 and 11.

All the calves showed quite a marked fluctuation in sodium levels, but without any specific pattern. Affected animals, but not the control, showed increasing levels of potassium as the condition progressed. There were no marked alterations in the magnesium levels in any of

them. The calcium level of Calf 1204 that died was markedly depressed, whereas the levels in the other calves were within normal limits.

TABLE 10 Serum calcium levels

Days after infection	6	8	10	13
	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*
Control	2,07	2,96	2,77	ND
1202	2,86	2,33	2,91	2,46
1204	1,45	ND	Death	
1205	2,19	2,66	2,31	2,41
1203	ND	2,41	ND	2,74
1201	2,20	2,47	2,42	2,67

ND = Not done

* Normal value = 2–3 mmol/ Doxy (1977)

TABLE 11 Serum magnesium levels

Days after infection	6	8	10	13
	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*	mmol/ℓ*
Control	0,76	1,096	0,96	ND
1202	0,86	0,67	0,68	0,81
1204	0,75	ND	Death	
1205	0,80	0,94	0,64	0,83
1203	ND	0,65	ND	0,90
1201	0,83	0,96	0,76	0,93

ND = Not done

* Normal value = 0,65–1,23 mmol/ℓ Doxey (1977)

Discussion

An increased prothrombin time can be caused by hepatitis, consumption coagulopathy, vitamin K deficiency or the presence of vitamin K analogs (Hall, 1972). In sweating sickness, the first 2 causes are the most likely. In this study, all the sweating sickness cases showed prolonged PT times during some stage of the condition (Table 1). Two of the calves (1202 and 1205) showed laboratory evidence of liver disease (Tables 4, 5 & 6). In DIC, which can be caused by a wide variety of disease processes, simultaneous activation of the coagulation and fibrinolytic system results in microvascular thrombosis or a bleeding tendency as clotting factors and platelets are depleted (Green, 1983). This will result in a prolonged PTT. The latter can also be caused by prolonged liver disease, as the factors in the intrinsic coagulation pathway have a half life of 2–4½ days compared with 4–6 h for Factor VII (Hall, 1972). The single calf (1205) that had a prolonged PTT also had elevated FDP levels, indicating that consumption of clotting factors was probably responsible for the prolonged PTT. A diagnosis of DIC in Calf 1205, based on the laboratory findings, was considered justified.

This diagnosis may indicate that the histopathological changes of DIC, as recorded by Kriek (1977), were those of a comsumption coagulopathy. It is interesting to note that Calf 1205, considered to be suffering from DIC, recovered without any treatment directed at this coagulation disorder. This calf, however, did receive specific treatment in the form of hyperimmune serum.

Plasma cortisol levels were markedly raised in 3 of the calves (Table 7). The calf with the highest cortisol level, which died on Day 10 after tick attachment, did not receive hyperimmune serum. Of the other 2 calves with raised cortisol levels, one (1202) had laboratory evidence of liver disease, while the other (1205) had both DIC and liver disease.

From the foregoing it appears that there may be a correlation between the severity of the condition and blood cortisol levels. As can be seen from Table 7, the cortisol levels did not remain constantly raised during the course of the condition, and this may limit its use as a prognostic indicator.

The results suggest that endotoxins do not play a significant role in the pathogenesis of the disease.

Despite severe "sweating" in most of the calves, there were no marked abnormalities in the serum levels of sodium, potassium or magnesium (Tables 8–11). The calf that died, however, did have a markedly depressed serum calcium level. The significance of this is not immediately apparent.

The specific treatment of sweating sickness has been discussed by Oberem, *et al.* (1985). Recommendations, based on previous clinical pathological studies (Van Amstel, 1984) and the results of this study, can now be made with regard to the supportive treatment.

The presence of a severe inflammatory process in the stratified squamous epithelium has been shown on both histopathology (Kriek, 1977) and clinical pathology (Van Amstel, 1984). For this reason the use of anti-inflammatory agents is indicated.

The use of corticosteriods as anti-inflammatory agents, however, seems contra-indicated, as high levels of endogenous cortisol may already exist, as demonstrated in this study. The adverse effects of corticosteroids on the immune system is well known and may be compounded by the presence of the leucopaenia demonstrated by Van Amstel (1984). The non-steroidal, anti-inflammatory drugs are probably preferable but, as they may be potentially nephrotoxic (Trillo, Sots & Gunson, 1984), care should be taken with their use in sweating sickness, as a concurrent nephrosis very often exists (Van Amstel, 1984).

Despite the absence of an endotoxaemia, the use of antibiotics in sweating sickness is indicated because of the likely immunosuppression in severe cases resulting from the elevated cortisol levels, as well as from the leucopaenia which may develop (Van Amstel, 1984).

The use of specific treatment to prevent intravascular coagulation appears to be indicated. It has been shown that heparin, in doses of 10–20 i.u. per kg, significantly reduces the incidence of experimentally-induced laminitis in horses in which it has been shown that DIC plays a significant role (Hood & Stephens, 1985).

Finally, from the results of this study, the use of liver supportive treatment appears to be indicated and drugs which can aid in the synthesis of cellular membranes may be of benefit. These include cyanocobalamine, choline, methionine and essential phospholipids.

Acknowledgement

The authors wish to thank Prof. H. J. Bertschinger for his assistance.

References

COUBROUGH, R. I., BERTSCHINGER, H. J. & KÜHNE, K. J., 1980. The practical implications of genital histopathological changes, enzyme and hormonal levels in the cow caused by malnutrition. *Proceedings of the 11th World Congress on Diseases of Cattle, Tel Aviv, Israel,* 1, 867–874.

DOXEY, D. L., 1977. SI units: The new method of recording laboratory results for diagnostic purposes. *Veterinary Record,* 100, 555–556.

GREEN, R. A., 1983. Bleeding disorders. *In:* ETTINGER S. J. (ed.). Textbook of veterinary internal medicine. Diseases of the dog and cat, Volume 2, 2087–2088. Phildelphia: W. B. Saunders.

HALL, D. E., 1972. The haemostatic mechanism and its components in the dog. *In:* HALL, D. E. (ed.). Blood coagulation and its disorders in the dog, 1–33. London: Bailliére Tindall.

HOOD, D. M. & STEPHENS, K. A., 1985. Pathophysiology of equine laminitis. *In:* WHITLOCK, R. H. (ed.). The compendium collection. Equine medicine and surgery in practice. 5th anniversary series, 136–141. Veterinary Learning Systems, Lawrenceville, New Yersey.

KRIEK, N. P. J., 1977. The pathology of sweating sickness. M. Med. Vet. thesis, University of Pretoria.

MORRIS, D. D. & BEECH, J., 1983. Disseminated intravascular coagulation in six horses. *Journal of the American Veterinary Medical Association*, 183, 1068–1072.

OBEREM, P. T., VAN AMSTEL, S. R., MATTHEE, O. & BEZUIDENHOUT, J. D., 1985. The efficacy of hyperimmune serum in the treatment of sweating sickness. *Onderstepoort Journal of Veterinary Research*, 52, 283–287.

SCHMIDT, M., 1979. Laboratory testing in veterinary medicine. *In:* SCHMIDT, M. (ed.). Laboratory testing in veterinary medicine. Diagnosis and clinical monitoring, 48–64. Mannheim: Boehringer Mannheim GmbH Diagnostica.

TRILLO, M. A., SOTO, G. & GUNSON, D. E., 1984. Flunixin toxicity in a pony. *Equine Practice*, 6, 21–30.

VAN AMSTEL, S. R., 1984. Aspects of the clinical pathology of sweating sickness in cattle. *Proceedings of the 13th World Congress on Diseases of Cattle, Durban, Republic of South Africa*, 1, 520–525.

Printed by the Government Printer, Private Bag X85, Pretoria, 0001.