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SWEATING SICKNESS: RELATIVE CURATIVE EFFECT OF HYPERIMMUNE SERUM AND A PRECIPITATED IMMUNOGLOBULIN SUSPENSION AND IMMUNOBLOT IDENTIFICATION OF PROPOSED IMMUNODOMINANT TICK SALIVARY GLAND PROTEINS

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ABSTRACT

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Although of low morbidity, sweating sickness is readily induced in calves by infestation with positive *Hyalomma truncatum* adult ticks. This epitheliotrophic disease has no specific cure except by the administration of hyperimmune serum obtained from animals which have recovered and are subsequently immune to the disease. Treatment with hyperimmune serum, however, has associated problems of donor availability, possible serum contamination and i.v. administration of a relatively large volume. This paper compares the treatment and cure of sweating sickness using unrefined hyperimmune serum and that of an experimental suspension. The latter proved relatively inefficient probably due to a low concentration of effective immunoglobulins. Immunoblot analyses of the sera of affected animals, using tick salivary glands as antigen during the course of the trial revealed 4 tick salivary gland proteins with molecular masses of between 27–33 kDa. These are proposed as being associated with sweating sickness immunodominance.

INTRODUCTION

Sweating sickness (SS) is a tick-borne toxicosis transmitted by some strains of *Hyalomma truncatum* and affects particularly young calves (Neitz, 1959; Bezuidenhout & Malherbe, 1981). Treatment of SS cases in the past relied mainly on fluid replenishment and the administration of antibiotic against secondary bacterial infection (Van Amstel, 1984).

Despite low prevalence of the disease within the distributional area of the vector, the severity of the disease and the lack of a specific treatment prompted investigation into the production of an effective hyperimmune serum (Oberem, Van Amstel, Matthee & Bezuidenhout, 1985), similar to the antiserum developed against *Ixodes holocyclus* paralysis in Australia (Oxer & Ricardo, 1942).

However, the production of SS hyperimmune serum in cattle is costly, requires infrastructure to enable tick challenge of donor animals and freezing facilities for long term storage. Also, administration of the relatively large volume (100 mℓ) as an effective dose is by intravenous drip (Oberem *et al.*, 1985). This prompted investigation into the production and efficacy of an experimental precipitated immunoglobulin suspension as a cure for SS.

SS is similar to other South African tick-borne toxicoses of major economic importance but, unlike Karoo paralysis (Spickett & Heyne, 1988), it is easily induced under laboratory conditions with positive ticks, which can be routinely bred in large numbers (Bezuidenhout & Malherbe, 1981). The availability of large numbers of ticks for salivary gland extraction, sera from affected animals during the trial and hyperimmune serum presented the opportunity to attempt the biochemical identification of the proteins responsible for immunity to SS by means of immunoblot analysis.

MATERIALS AND METHODS

Experimental animals

Ten, 10-month-old Friesian calves, obtained after quarantined internal parasite experimentation and presumed to be tick naive, were randomly assigned to 4 treatment groups (Table 1). A further 2 cattle from the same source were later assigned to the experiment (Group 5, Table 1).

TABLE 1 Assignation of experimental animals and treatment groups

Group	Treatment	Number of animals
1	SS-positive ticks Positive control group Treatment in critical phase to save animals	2
2	SS-positive ticks Treated with hyperimmune serum in advanced phase	3
3	SS-positive ticks Treated with experimental precipitated suspension in advanced phase	3
4	SS-negative ticks Negative control group Untreated	2
5	SS-negative ticks Immunoblot control group Treated with hyperimmune serum	2

Each calf in Group 1, 2 and 3 was infested with 40 ♂ and 40 ♀, laboratory-bred, SS-positive *H. truncatum* (Zululand) ticks. Group 4 and 5 calves were each infested with 40 ♂ and 40 ♀, laboratory-bred, SS-negative *H. truncatum* (Mkuzi) ticks. The day of tick infestation is regarded as Day 0. Rectal temperatures of all calves were taken from Day 1 until the end of the experiment. About 20 mℓ of blood was collected from each calf 1 day before and on each day after infestation up to Day 12. The blood was allowed to clot at room temperature for 4 h, kept overnight at 4 °C, centrifuged at 1 000 r.p.m. for 10 min and serum collected for biochemical analysis.

Serum was also collected before and up to 8 days after subsequent tick challenge which took place on Day 27. All animals were monitored daily for the typical clinical signs of SS and classes according to severity as in Table 2.

Treatment and challenge

Serum used to treat SS cases was taken from a hyperimmune donor ox which had received its last SS-positive tick challenge, *H. truncatum* (Zululand),

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TABLE 2 Clinical signs of sweating sickness classified according to severity

Severity		Clinical signs
Initial phase	+	Variable temperature reaction Hyperaemia of the oral mucous membranes
Middle phase	++	Variable temperature reaction pronounced hyperaemia and swelling of the lips Hyperaemia of the groin area Serous oculo-nasal and oral discharge
Advanced phase	+++	Variable temperature reaction Pronounced hyperaemia of the skin Excess ocula-nasal and oral salivation Listlessness, ataxia
Critical phase	++++	Extreme temperature variability Extreme hyperaemia and sloughing of certain skin areas Pronounced excess oculo-nasal and oral salivation Pronounced malaise, recumbent Anorexic

14 months prior to the experiment. Efficacy and hyperimmunity of the serum obtained from this animal and kept frozen was proven by the successful treatment of induced and natural cases of SS in sheep and cattle, at intervals, up to the time of the experiment.

An experimental precipitated immunoglobulin suspension (500 mℓ) was prepared from 5 000 mℓ blood obtained from the donor ox by a pepsin digestion method modified from Pope (1939). The method is used routinely by the Onderstepoort Vaccine Factory for the production of tetanus antitoxin.

Instead of allowing valuable experimental animals to die, 1 calf of the original control group (Group 1) was treated by i.v. drip administration of 100 mℓ hyperimmune serum on Day 8 at the critical phase when death seemed imminent. The other calf in this group received 20 mℓ of the experimental precipitated immunoglobulin suspension i.v. in the critical phase on Day 8, supportive treatment[1] from Day 8–12 and a further 100 mℓ unrefined hyperimmune serum i.v. on Day 11 when no response to treatment was evident. Group 2 calves were treated with 100 mℓ hyperimmune serum i.v. and Group 3 calves with 20 mℓ precipitated suspension i.v. on Day 7, while progressing through the advanced phase of the condition. Group 4 calves remained normal and were not treated. A further 2 cattle (Group 5), later infested with SS-negative ticks, were treated with 100 mℓ unrefined hyperimmune serum i.v. on Day 7 post infestation to obtain negative sera from treated animals as a control for the immunoblot analyses.

All calves were challenged on Day 27 with the same number and strain of ticks as used in the original infestation.

Immunoblots

Salivary glands were dissected from semi-engorged SS-positive and SS-negative female ticks of 20–50 mg body mass. Crude extracts were made by sonification in phosphate buffered saline (PBS) and subsequent centrifugation in a micro-centrifuge at 8 000 *g* for 5 min.

[1] Duplocillin* (Procain penicillin, benzathine penicillin)
* Registered trademark of Coopers Animal Health
20 mℓ i.v. daily.
Phenylarthrite* (Phenylbutazone)
* Registered trademark of Panvet
20 mℓ i.v. daily

Samples containing 30–50 µg of protein were dissociated in a boiling waterbath for 5 min in the presence of 1,6 % (w/v) dithiotreitol, 2,05 (w/v) sodium dodecyl sulphate and 0,02 M Tris-HCl, pH 6,8. Electrophoreses of the samples was done on a 12 % poliacrylamide gel according to the mehtod described by Laemmli (1970). After electrophoresis, the separated proteins were transblotted onto a polyvinylidine difluoride membrane (PVDF), using 10 mM 3-(Cyclohexylamino)-1-propanesulphonicacid(CAPS), pH 9,0 at 0,17 A for 1 h and 45 min (Moose, Nguyen & Lui, 1988). Staining of the transblotted molecular mass markers was done in 0,25 % (w/v) Coomassie brilliant blue R250 in 5:1:5 methanol:acetic acid: water for 2 h. Destaining was done overnight in 5:1:5 methanol:acetic acid:water.

The immunological detection of the antigens was performed by incubation of the separate membranes in 3:2 dilutions of the appropriate sera with incubation buffer consisting of 1 % (w/v) skimmed milk powder, 0,05 % (v/v) Tween 20 in Tris buffered saline (TBS), pH 7,4 (2,4 g Tris base + 9 g NaCl in 1 000 mℓ water).

SS-negative antigens were incubated with sera from Group 4 and 5 calves, while SS-positive antigens were incubated with sera from Group 2 calves. An additional incubation of SS^+ antigens with the experimental precipitated suspension was also performed. Incubation was carried out at 25 °C for 1,5 h and the membranes subsequently washed 3 times with washing buffer [0,1 % (w/v) skimmed milk powder in TBS]. A further incubation for 1,5 h at 25 °C in 1:500 peroxidase conjugated rabbit anti-bovine IgG (H + L):incubation buffer followed. Washing was repeated. Visualisation of the antigens was done by incubating the membranes at 25 °C with substrate (0,06 g 4-chloro-1-naphthol, 20 mℓ cold methanol, 60 µl 30 % H_2O_2 and 100 mℓ TBS) for 10–20 min. Substrate development was stopped by rinsing the membranes with distilled water. The membranes were stored at 4 °C, protected from light.

RESULTS AND DISCUSSION

Reactions and treatment

All calves infested with SS-positive ticks showed initial clinical signs of SS on Day 5 post infestation. The disease progressed rapidly to the advanced phase on Day 7 and calves in Group 1 reached the critical phase on Day 8 (Fig. 1 & 2). The death of Group 1 calves seemed unavoidable and it was decided to attempt their treatment even at this late stage. The calf in Group 1 (calf 1, Fig. 1), treated

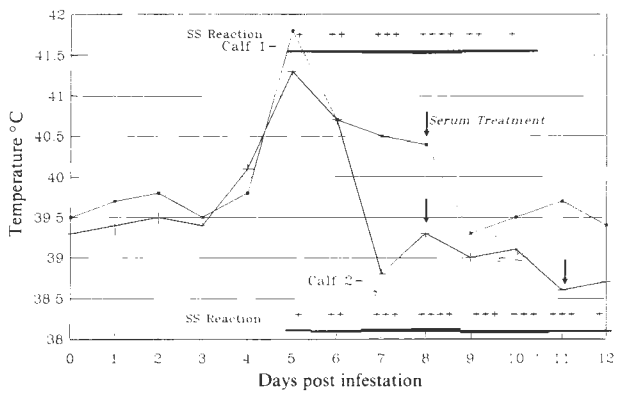

FIG. 1 Temperature reaction, sweating sickness reaction and treatment of Group 1 calves, infested with SS-positive *Hyalomma truncatum* and treated at the critical phase of the condition

with hyperimmune serum on Day 8, in the critical phase, responded remarkably well, did not require supportive treatment and had recovered by Day 11. The other calf in Group 1 (calf 2, Fig. 1), treated in the critical phase with the experimental precipitated suspension on Day 8, responded poorly, required supportive treatment and recovered only after the further administration of unrefined hyperimmune serum on Day 11.

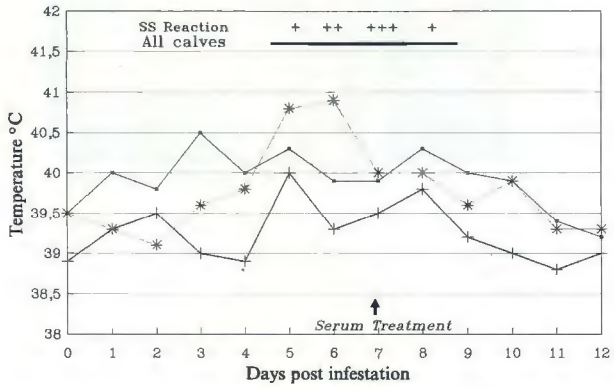

FIG. 2 Temperature reaction, sweating sickness reaction and treatment of Group 2 calves infested with SS-positive *Hyalomma truncatum* and treated with hyperimmune serum at the advanced phase of the condition

All 3 calves in Group 2, treated with hyperimmune serum on Day 7, in the advanced phase of the condition, recovered without incident within 2 days of serum administration (Fig. 2). Group 3 calves responded slowly to treatment with the experimental precipitated suspension but did recover 3 days later (Fig. 3). It is unlikely that these calves would have recovered spontaneously as rapid progression of the disease is indicated by clinical symptoms.

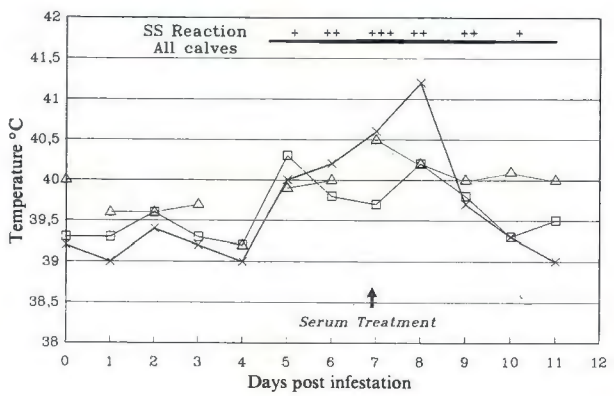

FIG. 3 Temperature reaction, sweating sickness reaction and treatment of Group 3 calves infested with SS-positive *Hyalomma truncatum* and treated with an experimental precipitated immunoglobulin suspension at the advanced phase of the condition

On subsequent challenge with SS-positive ticks on Day 27 all calves in Group 1, 2 and 3, showed no reactions, expressing immunity to the disease. Group 4 calves, infested and challenged with SS-negative ticks, appeared normal throughout the experiment (Fig. 4) as did the Group 5 animals which showed almost identical temperature reactions to those of Group 4.

From the reaction to treatment it appears that unrefined hyperimmune serum, is effective in curing sweating sickness, even if administered at the critical phase of the condition. Although the animals treated

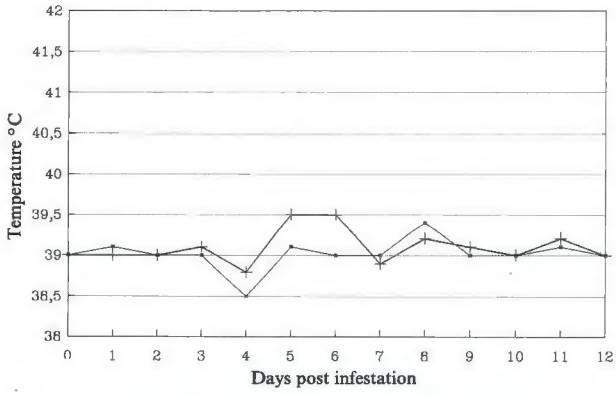

FIG. 4 Temperature reaction of Group 4 calves infested with SS-negative *Hyalomma truncatum* and treated on Day 7 with hyperimmune serum

with the experimental precipitated supsension in the advanced phase recovered, their recovery was slower than those treated with unrefined hyperimmune serum. The 1 animal treated in the critical phase with experimental precipitated suspension, could not recover without coincidental supportive and subsequent unrefined hyperimmune serum treatment. Although only a small number of animals were used and response to treatment was variable, it appears that the experimental precipitated suspension is less effective than unrefined hyperimmune serum once severity has progressed beyond the advanced phase of the disease.

Immunoblots

Immunoblot analyses of sera from Group 2 and 4 calves during the course and treatment of the disease yielded 4 antigen bands with molecular masses of 27–33 kDa which are proposedly associated with sweating sickness immunodominance.

The immune response during the course of Group 2 infestation with SS-positive ticks, is shown in Fig. 5 by way of immunoblots of sera taken at Days 0 (naive); 2; 4; 6; 7; 8; 10; 27 and 35. Four prominent bands of 27–33 kDa molecular mass became visible from Day 27 onwards. The immune response during the course of Group 4 investation with SS-negative ticks is similarly shown in Fig. 6, for sera taken at Days 0 (naive); 5; 6; 7; 8; 27 and 35. The 4 antigen

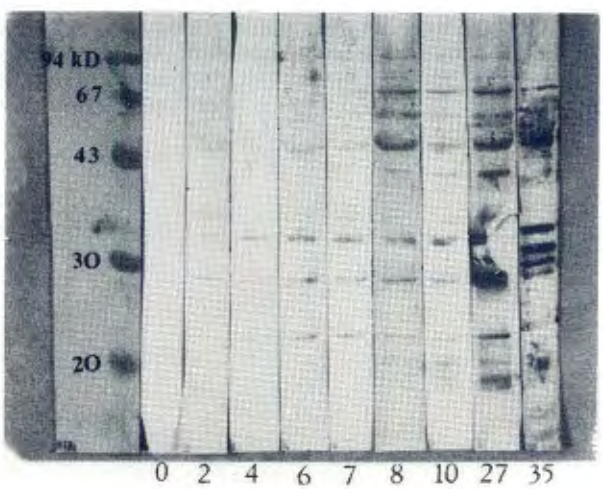

FIG. 5 Immunoblot of sera from Group 2 calves infested with SS-positive ticks and treated with unrefined hyperimmune serum on Day 7 post infestation. Sera from Days 0 (naive); 2; 4; 6; 7; 8; 10; 27 and 35 and incubated with SS-positive antigen

bands prominent in Fig. 5 are absent in the Group 4 response. The immunoblot with SS-positive antigens, screened against the experimental precipitated immunoglobulin suspension also yielded no recognition of the 4 antigen bands as seen in the Group 2 response. A further immunoblot (Fig. 7) of sera taken from cattle subjected to SS-negative tick infestation and treated with unrefined hyperimmune serum (Group 5, Table 1), showing no antigen bands in the 27–33 kDa range, confirms that the antigen bands were associated with SS-positive salivary secretion and not with the unrefined hyperimmune serum.

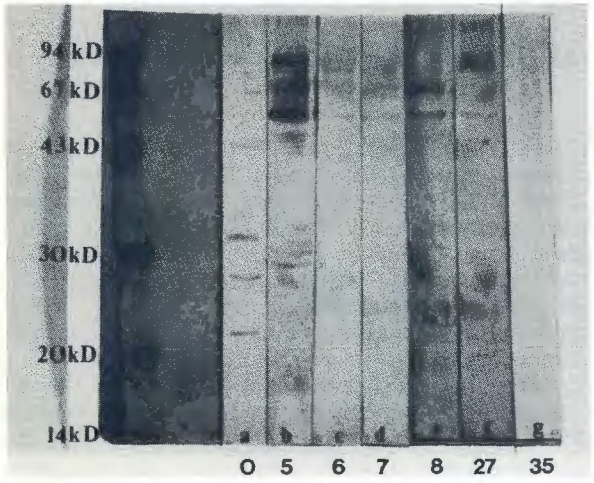

FIG. 6 Immunoblot of sera from Group 4 calves infested with SS-negative ticks and not treated with unrefined hyperimmune serum. Sera from Days 0 (naive); 5; 6; 7; 8; 27 and 35 and incubated with SS-negative antigen

Cattle that are subjected to SS-positive tick infestation thus become immune to the disease upon treatment due to the apparent eliciting of an immune response against certain salivary antigens secreted by SS-positive ticks. These antigens are absent in SS-negative ticks.

Immunoglobulins in the experimental precipitated suspension could not visually recognise the antigens responsible for the immune response. Group 3 animals, however, did survive the SS-positive infestation after treatment with the suspension, indicating that immunoglobulins are present which recognise the antigens, but that their concentration is very low. Alternate or modified preparation techniques to refine hyperimmune serum for practical application as a cure for SS should thus be investigated. Salivary

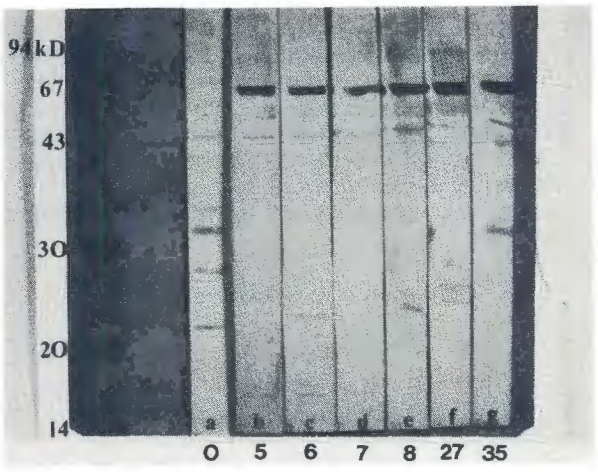

FIG. 7 Immunoblot of sera from Group 5 cattle infested with SS-negative ticks and treated with unrefined hyperimmune serum on day 7 post infestation. Sera from Days 0 (naive); 5; 6; 7; 8; 27 and 35 and incubated with SS-negative antigen

gland proteins identified to be proposedly associated with immunodominance would have potential in the biochemical preparation of a practical treatment.

REFERENCES

BEZUIDENHOUT, J. D. & MALHERBE, A., 1981. Sweating sickness: A comparative study of virulent and avirulent strains of *Hyalomma truncatum*. *Proceedings of an International conference on tick biology and control.* Rhodes Univesity, Grahamstown, Republic of South Africa, 7–12.

LAEMMLI, U. K., 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature,* 277, 680–685.

MOOSE, M., NGUYEN, N. Y. & LUI, T., 1988. Reproducible hight yield sequencing of proteins electrophoretically separated and transferred to an inert support. *The Journal of Biological Chemistry,* 263, 6 005–6 008.

NEITZ, W. O., 1959. Sweating sickness: the present state of our knowledge. *Onderstepoort Journal of Veterinary Research,* 23, 3–38.

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.

OXER, D. T. & RICARDO, C. L., 1942. Notes on the biology, toxicity and breeding of *Ixodes holocyclus* (Neumann). *Australian Veterinary Journal,* 18, 194–199.

POPE, C. G., 1939. The action of proteolytic enzymes on the antitoxins and proteins in immune serums. I. True digestion of the proteins. *British Journal of Experimental Pathology,* 20, 132–149.

SPICKETT, A. M. & HEYNE HELOISE, 1988. A survey of Karoo tick paralysis in South Africa. *Onderstepoort Journal of Veterinary Research,* 55, 89–92.

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