ELSEVIER

International Journal for Parasitology 29 (1999) 535–541

INTERNATIONAL Journal for PARASITOLOGY

Invited review

Tick paralysis: development of a vaccine

S. Masina, K.W. Broady *

Department of Cell and Molecular Biology, University of Technology, Sydney, Westbourne St., Gore Hill, NSW 2065, Australia

Received 19 June 1998; received in revised form 18 December 1998; accepted 18 December 1998

Abstract

The paralysis tick of Australia, *Ixodes holocyclus*, causes a severe toxicosis in domestic animals such as dogs and cats, livestock, and in some cases, humans. It is characterised by a rapidly ascending flaccid paralysis. The causative agent of the toxicosis is a neurotoxin(s) produced in the tick salivary glands. The current treatment for tick paralysis is in the form of a polyclonal dog antiserum. This antiserum treatment is expensive and effective only in the early stages of paralysis. The aim of current research is to develop a recombinant veterinary vaccine based on the tick neurotoxin peptide sequence. A successful vaccine would provide cost-effective, long-term protective immunity against tick-induced paralysis.

Keywords: *Ixodes holocyclus*; Neurotoxins; Tick paralysis; Toxicosis; Vaccine

1. Introduction

Ticks are parasitic arthropods of vertebrates that belong to the class Arachnida, which includes spiders and scorpions, and to the subclass Acari, which includes mites. The suborder Ixodida comprises three families of ticks: the Argasidae, Ixodidae and Natalliellidae [1]. Paralysis induced by ticks has been determined to be the result of salivary neurotoxins transferred to the host during feeding. The functional significance of a toxin to a parasite is not clear. The reduction of host mobility, exertion of local anaesthesia and the prevention of blood coagulation during feeding are some suggestions for toxin generation. There has also been a suggestion that paralysis may be a vestigial function conserved when the tick evolved a parasitic lifestyle [2].

Tick-induced paralysis occurs in different regions of the world. Over 60 of the 820 tick species (belonging to 10 different genera) have been implicated as capable of causing paralysis [3]. Most of these are Ixodid species (hard ticks), but at least nine species of Argasid ticks (soft ticks) have been found to cause paralysis. Those species which are of major veterinary and human importance are *Dermacentor andersoni*, *Dermacentor variabilis* of North America; *Ixodes rubicundus*, *Rhipicephalus evertsi evertsi* and *Argas (Pericargas) walkerae* of Africa; and *Ixodes holocyclus* of Australia.

The objective of this review is to direct attention to current research ideas and concepts

* Corresponding author: Tel: 61-2-9514-4101; Fax: 61-2-9514-4026; e-mail: Kevin.Broady@uts.edu.au

0020-7519/99/$20.00
PII: S0020-7519(99)00006-5

regarding the biology, biochemical characterisation of the tick neurotoxin and treatment of tick paralysis in Australia.

2. The Australian paralysis tick, *I. holocyclus*

The Australian paralysis tick, *I. holocyclus*, occurs along the eastern coastal strip of Australia from North Queensland down to Lakes Entrance in Victoria [4] (Fig. 1). The very limited distribution of *I. holocyclus* appears to depend on its great susceptibility to slight variations in environmental conditions, particularly humidity. The highest incidence of tick paralysis coincides with a seasonal abundance of female ticks in spring and early summer [5]. Another factor governing the incidence of the tick is the presence of its natural host, the bandicoot; either the long-nosed bandicoot, *Perameles nasuta* or the Northern brown bandicoot, *Isodon macrourus* [6]. It has been suggested that these natural hosts survive heavy infestations as a result of acquired immunity rather than having an intrinsic resistance to the toxin. This is supported by the observations that, when a bandicoot carrying high numbers of *I. holocyclus* is captured and held in tick-free conditions, its ability to resist

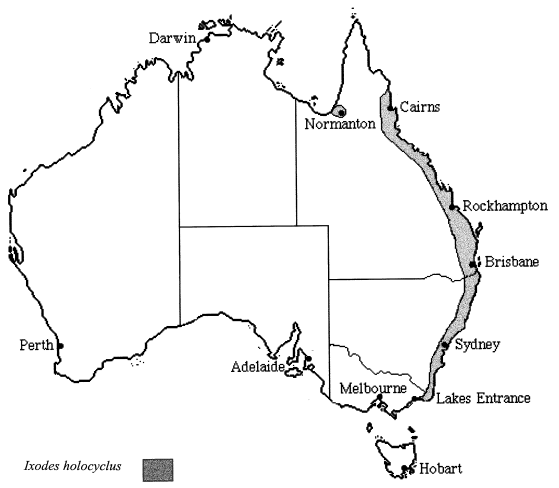

Fig. 1. Distribution of the Australian paralysis tick *Ixodes holocyclus*. (Adapted from [12]).

tick paralysis diminishes over several months [http://www.peg.apc.org/ ~ ullavet/tick.html] and immune animals (including hyperimmune dogs) are seen to have decreased levels of immunity when no longer exposed to tick infestation.

The principle accidental host of *I. holocyclus* is the dog, but it regularly attaches to other domestic pets and livestock including cats, cattle and horses. It occasionally affects humans, with 20 fatal cases due to paralysis having been reported [7]. Humans are more commonly affected by allergic reactions to tick bites, and several allergens to tick extracts and tick saliva have been described [8, 9]. Allergic reactions range from mild skin reactions (urticaria and pruritis) to anaphylactic reactions, which are life-threatening.

As a measure of the severity of tick toxicosis in non-immune animals, a single adult female *I. holocyclus* tick may be responsible for many cases of tick paralysis and a single tick can cause death of the host [10]. Infestations by tick nymphs have been reported [11] to produce paralysis, but only rarely, while the larvae have not been associated with paralysis, only local irritation [12]. This contrasts with the paralysis caused by the tick *A. (P.) walkerae* (the common poultry tick in South Africa), where only the larvae cause paralysis following at least 4 days infestation [13]. Larval ticks of this species feed over several days, whereas the other stages are fully engorged within 1–2 h, during which time insufficient toxin is produced to cause paralysis [13].

2.1. Tick attachment and feeding

All ticks are blood-sucking ectoparasites of terrestrial vertebrates [14]. The active stages of larva, nymph and adult require blood as a nutritive source and, in the case of adults, for sperm or egg production. *Ixodes holocyclus* is a three-host tick. During the three developmental stages, the tick attaches to and feeds on a new warm-blooded host (which may be of the same or a different species) until it becomes completely engorged, then drops off and undergoes further development on the ground [5]. On abandoning

the host, the fully engorged adult female lays 2000–3000 eggs and dies. The male tick does not attach to the host or suck blood, but may be found wandering on the host seeking a mate [15].

Because of the mechanical processes and salivary secretions associated with blood feeding, the tick–host parasitic interaction is complex. Tick salivary secretions play a major role in animal disease because of the introduction of toxic compounds that may affect general metabolism or flaccid paralysis. Female *I. holocyclus* ticks have relatively long mouth-parts that can be thrust deeply into the skin, so that the hypostome armed with backward-projecting spines anchor the feeding tick causing injury to the surrounding tissues [1] (Fig. 2). The male is smaller than the female, with shorter mouth parts that are incapable of piercing the skin of mammals; it feeds off the female and not directly from the host [16].

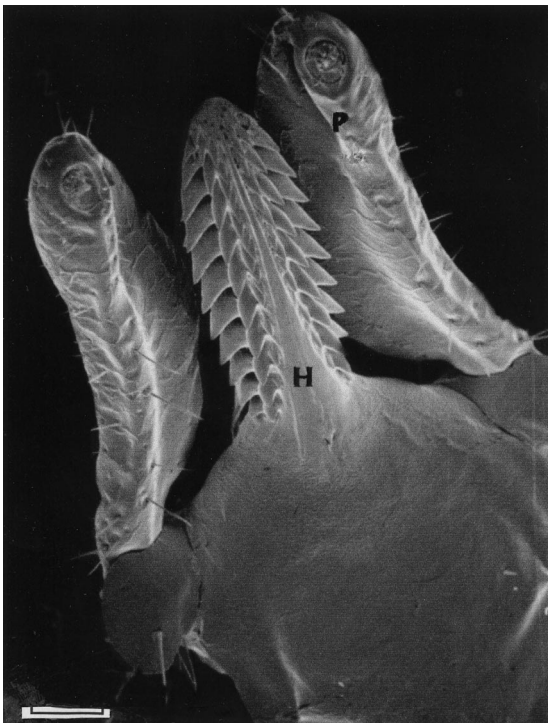

Fig. 2. An electron micrograph showing the mouthparts of *Ixodes holocyclus*. The hypostome (H) penetrates and is anchored by the teeth to the host's skin during attachment. The palps (P) protect the hypostome from being damaged before attachment. Scale-bar = 100 μm.

Unlike other species, *I. holocyclus* does not produce a cement which fixes the mouth-parts into the skin. The mouth-parts are instead inserted directly into the tissues. In contrast, the North American wood tick, *D. andersoni*, which affects domestic pets and cattle, feeds more superficially and secretes an external cement with only shallow penetration of the hypostome. This method of feeding results in a much less severe paralysis than that of *I. holocyclus* and a quicker recovery period once the tick has been removed due to the absence of circulating or bound toxins [2].

Feeding of the adult tick occurs in two phases. The slow phase taking 1–4 days, during which the body parts of the tick develop to allow for the rapid increase in size of the tick in the final stage of rapid engorgement. This second stage usually lasts approximately 24 h and is characterised by a 10-fold increase in weight of the tick. This figure, however, underestimates the total volume of blood taken during feeding by Ixodid ticks. The meal is concentrated by removal of fluid, which is then taken up by the salivary glands and secreted back into the hosts circulation, thus concentrating the nutrient components of the meal [1, 16]. Components of tick saliva suppress the immune and inflammatory response of the host, permitting the ticks to remain on the host for an extended period of time [17]. This trauma to the host, coupled with the introduction of various salivary products, may evoke a wide range of responses including local inflammation, oedema and haemorrhage. The destruction of host tissues that develops as a result of tick feeding is due to the presence of active compounds in the salivary secretions. These components have been partially characterised, but their functions still require further investigation [17].

Toxicosis caused by *I. holocyclus* is characterised initially by a weakness of the hind limbs, followed by a rapidly ascending flaccid paralysis. The paresis then increases and extends to the forelimbs and respiratory muscles [12]. Other symptoms include loss of appetite and voice, inco-ordination, excessive vomiting, respiratory distress and often, in the absence of timely antitoxin treatment, death [2]. The adult female tick

does not secrete detectable amounts of toxin until the third day of attachment to the host. This contrasts to the tick *D. andersoni*, where there is a latent period of about 5 days before sufficient toxin can be produced to cause paralysis. Paralysis by *D. andersoni* can be avoided if the tick is removed during this latent period [18]. The onset of paralysis symptoms is usually not seen before the fourth or the beginning of the fifth day for *I. holocyclus*. This period corresponds to the onset of a final rapid stage of engorgement of the tick and has been shown to be accompanied by a marked increase in the size and activity of the salivary glands of the parasite [19].

2.2. Prevention and treatment of tick paralysis

Tick paralysis can be prevented by avoidance of tick-infested areas, daily searches for attached ticks, and application of acaricidal chemicals. Attached ticks need to be removed carefully, and there is much debate about the best method of removal and whether the tick should be killed before removal. The problem revolves around the observation that paralysis and allergic reactions can progress following removal of a tick, presumably due to the secretion of more toxin during the removal process [2].

Acaricidal preparations can be administered to animals either orally or directly onto the skin (sprays and collars). Cythioate (Proban, Boehringer-Ingelheim) is an orally administered organo-phosphate that is absorbed from the gut into the bloodstream, where it is ingested by the ticks during engorgement causing death of the tick. Fipronil (Frontline, Rhone-Merieux), used as a spray and rinse, is reported to kill ticks in situ within 24 h [20]. Other organo-phosphates (coumaphos) and synthetic pyrethrins (permethrin) have been used topically and the chemicals carbamate propoxur and amitraz have been employed in impregnated collars [1].

These acaricides have a number of deficiencies. First, there is the need for regular application and administration of the acaricides. Second, the ticks are able to attach to areas (such as the lips, ear canal and anus) that are not covered by topical applications. The oral treatment has the added disadvantage of not being recommended for use in cats, and some veterinarians report it as not being completely effective [www.ozemail.-com.au/ ~ norbertf/]. Finally, there is concern about using acaricides in young or pregnant animals, and also about the presence of acaricidal residues in the flesh of food-producing animals [12]. Antitoxin immunity induced by vaccination is currently under development and will provide significant advantages over the other methods of prevention.

Treatment of tick paralysis involves removal or killing of the tick(s) followed by anti-toxin antiserum administration and veterinary support, such as the administration of epinephrine to treat anaphylaxis and vasodilators such as acepromazine to aid respiratory distress [21]. Removal of the offending tick(s) may be sufficient during early infestation (1–2 days) and after mild paralysis has occurred, with no further treatment being required [12]. The ability of dogs to become immune to *I. holocyclus* toxin following gradual or controlled exposure to tick infestation was recognised early [6, 22], and hyperimmune dog serum produced for the treatment of tick paralysis [11] has been marketed ever since. There are several disadvantages to the current antiserum treatment. It is effective only in the early stages of the disease [12] while the toxin is still either circulating or in the tick lesion, that is, before it has bound to its site of action at the neuromuscular junction, and is unreliable when a number of ticks are involved [5]. The cost of treatment is high and, in many cases, the cost of antiserum exceeds the commercial value of farm animals. The efficacy of hyperimmune serum varies between manufacturers and between batches, so precise doses are difficult to recommend. Finally, a large number of animals develop adverse reactions, such as anaphylaxis or serum sickness, associated with the use of large doses of foreign serum proteins [2]. The veterinary aspects of tick paralysis are discussed in detail on two Internet sites: www.ozemail.com.au/ ~ norbertf/

treatment-dogs.htm and www.peg apc.org/~ullavet/tick.html.

Veterinary and agricultural authorities acknowledge that the Australian paralysis tick is a significant commercial problem and is one of the more important causes of morbidity and mortality in companion and domestic animals in Eastern Australia. There is general agreement that the development of an anti-tick vaccine would be the most effective alternative to current anti-serum therapy and chemical control, because of the advantages of target-species specificity, environmental safety, lack of human health risks, ease of administration and cost. The observation that the *I. holocyclus* toxin secreted during infestation induces a protective antibody response suggests that a vaccination approach is practical. However, despite the efforts of a number of research units over the past 30 years, the tick neurotoxin has, until recently, defied complete isolation and characterisation.

3. Biochemical characterisation of tick paralysis

Ross [6, 22] demonstrated that mice and dogs injected with extracts of salivary glands from *I. holocyclus* produced characteristic symptoms of tick paralysis. Since then, salivary glands have been used almost exclusively as the source of starting material whenever the toxin has been investigated. Stone and coworkers [8] verified that the neurotoxin was secreted into the host with the saliva in a study where *I. holocyclus* females, which had been partially engorged (4 days) on mice, were attached to an artificial membrane and allowed to feed on a culture medium supplemented with foetal bovine serum. The feeding medium was collected, concentrated and shown to produce paralysis in mice identical to that observed with salivary-gland extracts.

A toxic fraction of *I. holocyclus* was first isolated by Kaire [23]. This fraction produced paralysis in dogs, consistent with tick toxicosis. A marked immunological similarity to native neurotoxin was demonstrated as both mice and dogs passively immunised with hyperimmune anti-tick serum were protected against the toxic fraction and dogs acquired immunity to the toxic fraction upon immunisation. The toxic component of this fraction was also shown to be resistant to digestion with proteases (pepsin, trypsin and papain), heating at 75°C for 15 min, and moderate pH (3–9) changes.

Numerous attempts have been made to isolate the neurotoxin from *I. holocyclus* [3, 8, 17, 18, 23–28]. These studies concluded that the neurotoxic activity was associated with a high molecular mass (40–80 kDa) protein fraction isolated from whole-engorged-tick extracts or salivary-gland extracts. Similar results were reported in studies of other ticks, such as *R. e. evertsi* and *A. (P.) walkerae*, which also induce an ascending flaccid paralysis, although the toxins may function by different mechanisms. The neurotoxins from *R. e. evertsi* and *A. (P.) walkerae* impair the conduction of nerve impulses along peripheral nerve fibres [29, 30], while preliminary evidence suggests that paralysis induced by *I. holocyclus* results from the inhibition of acetylcholine release from the neuromuscular junction [31].

The neurotoxin isolated from the salivary glands of the female spring lamb paralysis tick *R. e. evertsi* has a high molecular mass of 68 kDa with an isoelectric point of 6 [32, 33]. A non-homogeneous protein toxin inducing paralysis has been isolated from the tick *A. (P.) walkerae* [12]. The toxin has a molecular mass of approximately 60 kDa. The protein sequences for both these tick toxins are yet to be determined. More recently, Crause and coworkers [32] have reported the production of a mAb that is able to recognise and neutralise the paralysing toxin of *R. e. evertsi* and cross-reacts with the paralysis toxin present in *A. (P.) walkerae*.

Partially purified toxic preparations from *I. holocyclus*, when treated with glutaraldehyde, were shown to produce a superior antitoxin response [34] and, while demonstrating that a vaccine could be developed from the native toxin, also indicated the impracticability of using a native source of immunogen, due to the limited availability of engorged ticks and low levels of toxin.

Recent studies [35] describe the isolation of neurotoxins from *I. holocyclus* that bind to rat brain synaptosomes (pinched off nerve terminals) in a temperature-dependent manner. The neurotoxins (holocyclotoxins; HT-1, HT-2 and HT-3) are polypeptides with an apparent molecular mass of 5 kDa. The molecular mass is comparable with that of other arachnid neurotoxins previously isolated from spiders, in the range of 3–11 kDa, [36] and scorpions of 5–8 kDa [37]. The observed temperature dependence of the neurotoxins binding to synaptosomes coincide with the in vivo observation of Cooper and Spence [31] that paralysis is inhibited by low temperatures.

The gene for the neurotoxin HT-1 has been isolated using PCR technology from partial protein sequence data. The gene sequence contains characteristic features of a leader peptide of 18 residues, stop and start codons, polyadenylation signal peptide and polyA tail. The derived protein sequence of the mature toxin had a calculated molecular mass of 5.9 kDa and a calculated basic p*I* of 8.86. The HT-1 gene sequence has high homology to the scorpion neurotoxin genes and the protein sequences have a similar number and arrangement of cysteine residues, indicating a strong structural homology (paper in preparation). The size and composition of residues in the leader sequence and the basic nature of the protein are also similar to those of scorpion neurotoxins. This relatedness of HT-1 with scorpion neurotoxins is encouraging, as it suggests that *I. holocyclus* neurotoxins may have homologies with toxins from other tick species.

A recombinant form of the toxin HT-1 has been produced and shown to be immunogenic. The antibodies produced against the recombinant fusion protein show a significant level of protection against the native toxins, indicating that the development of a recombinant vaccine is possible. The native neurotoxins HT-2 and HT-3 have not been characterised nor their genes isolated, and thus it is not known if they represent distinct but structurally related neurotoxins as commonly occur in scorpions [38] or post-translational modifications of HT-1.

4. Conclusion

Further investigation into the identification and molecular characterisation of the neurotoxins responsible for paralysis from other offending tick species is necessary to help unravel the mystery of the structure of these potent toxins, which in turn will assist in the developmental research for producing the necessary vaccines against tick paralysis.

References

[1] Cupp EW. Biology of ticks. Vet Clin North Am (Small Anim Pract) 1991;21:1–26.
[2] Stone BF, Binnington KC, Gauci M, Aylward JH. Tick/host interactions for *Ixodes holocyclus*: role, effects, biosynthesis and nature of its toxic and allergenic oral secretions. Exp Appl Acarol 1989;7:59–69.
[3] Stone BF. Toxicosis induced by ticks and reptiles in domestic animals. In: Harris JB, editor. Natural toxins. Oxford: Oxford University Press, 1987;56–71.
[4] Roberts FHS. Australian ticks. Melbourne: CSIRO, 1970;56–63.
[5] Concidine ML. Developing a vaccine for tick paralysis. Ecos 1985;43:12–14.
[6] Ross IC. Tick paralysis: a fatal disease of dogs and other animals in eastern Australia. J Counc Sci Indust Res Aust 1935;8:8–13.
[7] Kaufman WR. Tick–host interaction: a synthesis of current concepts. Parasitol Today 1989;5:47–56.
[8] Stone BF, Neish AL, Wright IG. Tick (*Ixodes holocyclus*) paralysis in the dog—quantitative studies on immunity following artificial infestation with the tick. Aust Vet J 1983;60:65–8.
[9] Dorey CN, Broady KW. Characterisation of the major 35 kDa allergen of the Australian paralysis tick. Allergy 1995;50 Suppl: 389 (Abstr P-0922).
[10] Ilkiw JE, Turner DM, Howlett CR. Infestation in the dog by the paralysis tick *Ixodes holocyclus*. Aust Vet J 1987;64:137–44.
[11] Oxer DT, Ricardo CL. Notes on the biology, toxicity and breeding of *Ixodes holocyclus* (Neumann). Aust Vet J 1942;18:194–9.
[12] Albiston HE. Arthropod infestation—ticks and mites. In: Diseases of domestic animals in Australia. Part 3. Canberra: Australian Government Department of Health, 1968.
[13] Viljoen GJ, Wyngaardt SV, Gothe R et al. The detection and isolation of a paralysis toxin present in *Argas (Persicargas) walkerae*. Onderstepoort J Vet Res 1990;57:163–8.

[14] Klompen JSH, Black WC, Keirens JE, Oliver JH. Evolution of ticks. Annu Rev Entomol 1996;41:141–61.
[15] Cooper BJ, Cooper HL, Ilkew J, Kelly JD. Tick paralysis. In: Hungerford T, editor. Proceedings No. 30 from the University of Sydney Postgraduate Committee in Veterinary Science. 1976;57.
[16] Beckman R. When a tick takes hold. Ecos 1989;59:13–17.
[17] Bowman AS, Coons LB, Needham GR, Sauer JR. Tick saliva—recent advances and implications for vector competence. Med Vet Entmomol 1997;11(3):277–85.
[18] Stone BF, Double, BM, Binnington KC, Goodger BV. Toxins of the Australian paralysis tick *Ixodes holocyclus*. In: Rodriguez JG, editor. Recent advances in acarology, vol. 1. New York: Academic Press, 1979;347–56.
[19] Stone BF, Binnington KC. The paralysing toxin and other immunogens of the tick *Ixodes holocyclus* and the role of the salivary gland in their biosynthesis. In: Sauer JR, Hair JA, editors. Morphology, physiology and behavioural biology of ticks. Chichester: Ellis Horwood, 1986;75–99.
[20] Searle A, Jensen CJ, Atwell RB. Results of a trial of fipronil as an adulticide on ticks (*Ixodes holocyclus*) naturally attached to animals in the Brisbane area. Aust Vet Pract 1995;25(3):157–8.
[21] Ilkiw JE, Turner DM. Infestation in the dog by the paralysis tick *Ixodes holocyclus*: 5. Treatment. Aust Vet J 1988;65:236.
[22] Ross IC. An experimental study of tick paralysis in Australia. Parasitology 1926;18:410–29.
[23] Kaire GH. Isolation of tick paralysis toxin from *Ixodes holocyclus*. Toxicon 1966;4:91–7.
[24] Stone BF. Chemical characterisation studies on salivary gland toxins of the paralysis tick *Ixodes holocyclus*. In: Chubb IW, Geffen LB, editors. Neurotoxins: fundamental and clinical advances. Adelaide: Adelaide University Press, 1979;273.
[25] Stone BF. Tick paralysis, particularly involving *Ixodes holocyclus* and other *Ixodes* species. In: Harris KF, editor. Advances in disease vector research, vol. 5. New York: Springer, 1988;61–85.
[26] Stone BF, Aylward JH. Tick toxicosis and the causal toxins: tick paralysis. In: Gopalakhrishnakone P, Tan CK, editors. Progress in venom and toxin research. Singapore: National University of Singapore Press, 1987;594–682.
[27] Davey MW, Ratcliffe R, Titchen DA. Aspects of the symptomology and intoxication produced by *Ixodes holocyclus*. In: Baldo BA, Harle DG, editors. Proceedings of the Sydney Allergen Group, vol. 6. Adverse reactions to arthropods in Australia, 1989.
[28] Thurn MJ, Broady KW. A tick toxin. In: Watters D, Lavin M, Maguire D, Pearn J, editors. Toxins and targets. New York: Harwood Academic, 1992;75.
[29] Gothe R, Lammler M. Secretional classification of the toxicity of the paralysis-inducing female *Rhipicephalus evertsi evertsi* Neumann 1897, during repletion. Zbl Vet B 1982;29:37–50.
[30] Gothe R, Buvheim C, Hoogstraal H. The mechanisms of pathogenicity in the tick paralyses. J Med Entomol 1979;16:357–69.
[31] Cooper BJ, Spence I. Temperature-dependent inhibition of evoked acetylcholine release in tick paralysis. Nature 1976;263:693–5.
[32] Crause JC, van Wyngaardt S, Gothe R, Neitz AWH. A shared epitope found in the major paralysis inducing tick species of Africa. Exp Appl Acarol 1994;18:51–9.
[33] Viljoen GJ, Bezuidenhout JD, Oberem PT et al. Isolation of a neurotoxin from the salivary glands of female *Rhipicephalus evertsi evertsi*. J Parasitol 1986;72:865–74.
[34] Stone BF, Neish AL, Morrison JJ, Uren MF. Toxoid stimulation in dogs of high titres of neutralising antibodies against holocyclotoxin, the paralysing toxin of the Australian paralysis tick *Ixodes holocyclus*. Aust Vet J 1986;63(4):125–6.
[35] Thurn MJ, Gooley A, Broady KW.Identification of the neurotoxin from the Australian paralysis tick, *Ixodes holocyclus*. In: Gopalakrishnakone P, Tan CK, editors. Recent advances in toxicology research, vol. 2. Singapore: Venom and Toxin Research Group, National University of Singapore, 1992;250–1.
[36] Kawai N. Neuroactive toxins of spider venoms. J Toxicol Rev 1991;10:131–67.
[37] Rochat H, Bernard P, Couraud F. Scorpion toxins: chemistry and mode of action. Adv Cytopharmacol 1979;3:325–34.
[38] Leberton F, Delepierre M. Primary and NMR three-dimensional structure determination of a novel crustacean toxin from the venom of the scorpion *Centruroides limpidus limpidus* Karsch. Biochemistry 1994;33:11135–49.