REVIEW

Tick paralysis in Australia caused by *Ixodes holocyclus* Neumann

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Ticks are obligate haematophagous ectoparasites of various animals, including humans, and are abundant in temperate and tropical zones around the world. They are the most important vectors for the pathogens causing disease in livestock and second only to mosquitoes as vectors of pathogens causing human disease. Ticks are formidable arachnids, capable of not only transmitting the pathogens involved in some infectious diseases but also of inducing allergies and causing toxicoses and paralysis, with possible fatal outcomes for the host. This review focuses on tick paralysis, the role of the Australian paralysis tick *Ixodes holocyclus*, and the role of toxin molecules from this species in causing paralysis in the host.

Many forms of tick toxicosis affect humans and other animals (Gothé and Neitz, 1991; Mans *et al.*, 2004). According to Gothé (1984), a tick toxicosis is defined as a 'generalized, experimentally standardizable, reproducible disease syndrome induced by one or a few potent ticks, even on first infestation of a physiological susceptible vertebrate species without participation of an immunopathological reaction during or following the tick feeding'. Such toxicoses are mostly caused by ixodid or hard ticks (Stone and Wright, 1981) and, in their most severe form, result in paralysis

of the infested host. About 69 species of ticks from around the world are capable of inducing paralysis (Gothé and Neitz, 1991), the most important being *Ixodes holocyclus* in Australia, *Dermacentor andersoni*, *De. variabilis* and *Argas (Persicargas) radiatus* in North America, *Ix. rubicundus* in South Africa, *Rhipicephalus evertsi evertsi* and *Ar. (Pers.) walkerae* in Ethiopia, and *Ar. (Pers.) radiatus* in the Nearctic region of North America (Stone, 1986). Some important paralysing ticks and their distributions are summarized in Table 1. In Australia, *Ix. holocyclus* can cause paralysis in humans, dogs, cats, sheep, cattle, goats, pigs and horses but predominantly infests dogs, cats and humans (Stone, 1986). It appears to be the most potentially toxic tick species, with a single tick capable of killing a large dog (Stone and Wright,

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1981) or sheep (Sloan, 1968). Although the Tasmanian paralysis tick *Ix. cornuatus* has been reported to cause bulbar paresis and respiratory failure in humans (Tibballs and Cooper, 1986) and dogs (Beveridge *et al.*, 2004), its habitat is more restricted than that of *Ix. holocyclus* and very few cases of paralysis have been associated with this tick. In Australia, tick paralysis has mainly been seen as a problem in veterinary medicine, affecting approximately 10,000 companion animals/year (Stone and Aylward, 1987). Occasionally, however, the climatic conditions become

particularly favorable to tick survival, tick densities reach very high levels, and the number of humans being bitten by *Ix. holocyclus* increases. Although rarely severe, tick paralysis caused by *Ix. holocyclus* can be fatal.

Ixodes holocyclus is found along the eastern coastal strip of Australia and is most abundant from September to about March. Paralysis is induced by a toxin that is transmitted to the host in the saliva of a female *Ix. holocyclus* when the tick takes a bloodmeal. During feeding, toxicity in the salivary glands increases, peaking after 4–5 days of engorgement

TABLE 1. Distribution of the main tick species that cause paralysis (Gregson, 1973; Murnaghan and O'Rourke, 1978; Gothe *et al.*, 1979)

Tick species	Country/region	Host(s)
IXODID		
<i>Amblyomma maculatum</i>	Uruguay	Dogs
	U.S.A.	Humans
<i>Dermacentor andersoni</i>	Northern U.S.A. and Canada	Livestock, humans
<i>Dermacentor auratus</i>	India	Domestic animals
<i>Dermacentor occidentalis</i>	U.S.A.	Cattle, horses, deer
<i>Dermacentor</i> sp.	Italy	Cattle, goats, sheep
<i>Dermacentor variabilis</i>	Eastern U.S.A.	Dogs, humans
<i>Haemaphysalis cinnabarina</i>	U.S.A.	Humans (one fatal case)
<i>Haemaphysalis kutchensis</i>	India	Rabbits
<i>Haemaphysalis punctata</i>	Crete	Sheep, dogs, cats
	Macedonia, Bulgaria	Sheep, goats, calves, chickens
<i>Hyalomma truncatum</i> *	South Africa	Humans (rarely), sheep, goats, pigs, dogs
<i>Ixodes brunneus</i>	U.S.A.	Birds
<i>Ixodes crenulatus</i>	Ukraine	Sheep
<i>Ixodes hexagonus</i>	England and France	Humans
<i>Ixodes holocyclus</i>	Australia	Cats, pigs, cattle, horses, sheep, goats, humans
<i>Ixodes ricinus</i>	Crete	Livestock, dogs, cats
	Former Yugoslavia	Cattle, sheep, goats
	Turkey	Sheep, goats, calves
	Azerbaijan	Foals
<i>Ixodes rubicundus</i>	Africa	Humans (rarely), sheep, dogs, cattle, jackals
<i>Ixodes scapularis</i>	U.S.A.	Dogs
<i>Rhipicephalus nuttalli</i>	South Africa	Dogs
<i>Rhipicephalus evertsi</i> †	Africa	Lambs, calves, dogs
<i>Rhipicephalus sanguineus</i>	Venezuela	Dogs
<i>Rhipicephalus simus</i>	Africa	Humans (rarely)
ARGASID		
<i>Argas persicus</i>	Africa	Chickens
<i>Argas walkerae</i>	Africa	Chickens
<i>Ornithodoros lahorensis</i>	Kazakhstan	Sheep
<i>Ornithodoros savignyi</i>	Nigeria	Cattle
<i>Otobius megnini</i>	Africa	Cattle

*Cause of 'sweating sickness'.

†Cause of 'spring lamb paralysis'.

(Goodrich and Murray, 1978). The toxin produced by *Ix. holocyclus* inhibits acetylcholine release at the neuromuscular junction and tends to cause more severe neurological impairment than that associated with *De. andersoni* or *De. variabilis* in North America (Grattan-Smith *et al.*, 1997).

Historically, the Australian explorers Hamilton Hume and William Hovell described ticks biting humans as early as 1824, during their journey between Lake George (Canberra) and Port Phillip (Victoria) (Gregson, 1973). Bancroft (1884) was the first to report on tick toxicosis in humans in Australia, describing two cases with weakness and blurred vision. A human fatality caused by the bite of an (apparently uninfected) tick was first described by Cleland (1912). Between 1914 and 1942, 20 human fatalities in Australia were attributed to tick bite, all but three of the victims being children (Murray and Koch, 1969).

CLINICAL PRESENTATION

Although tick paralysis in humans is most commonly reported in children aged 1–5 years, the problem does also occur in older children and adults (Grattan-Smith *et al.*, 1997; Inokuma *et al.*, 2003). The clinical presentation usually involves a combination of lethargy, weakness and unsteadiness of gait, often with ataxia, loss of appetite, dilated pupils, ascending symmetrical paralysis, slurred speech and depressed deep-tendon and gag reflexes (Grattan-Smith *et al.*, 1997; Edlow and McGillicuddy, 2008). Laboured breathing, bradycardia, decreased oxygen saturation and asystole have also been reported (Grattan-Smith *et al.*, 1997). In addition to neuromuscular paralysis, the toxin from *Ix. holocyclus* can produce myocarditis in children (Pearn, 1966). In older children or adults, double or blurred vision and photophobia may develop (Grattan-Smith *et al.*, 1997; Edlow and McGillicuddy, 2008). Facial weakness or paralysis may be present and ascending flaccid paralysis can also occur if the tick is not removed quickly (Pearn, 1977). It is also worth

noting that the removal of *Ix. holocyclus* from a patient can worsen the patient's condition. In one case described by Miller (2002), for example, facial-nerve paralysis occurred hours after the removal of *Ix. holocyclus* ticks from around the patient's face. Similarly, tick removal apparently led to deterioration in four of the six cases described by Grattan-Smith *et al.* (1997). Stone *et al.* (1989) hypothesised that such delayed toxicity was caused by the disruption of the feeding lesion during the removal of the tick, which caused the release of toxin that had been bound to cells or tissues. Surprisingly, despite the continued deterioration and then slow recovery seen in many Australian patients following the removal of *Ix. holocyclus*, removal of *De. andersoni* and *De. variabilis* from North American patients results in recovery within a day (Garrettson, 1984).

DIFFERENTIAL DIAGNOSIS

Edlow and McGillicuddy (2008) suggested that '*the first and most important factor in diagnosing tick paralysis is thinking of it*' and that Guillain-Barré syndrome (GBS) may be commonly confused with tick paralysis. In addition to GBS, viral encephalomyelitis, botulism, myasthenia gravis, spinal-cord compression, transverse myelitis, organophosphate poisoning, porphyria, heavy-metal poisoning and diphtheria may also need to be considered in a differential diagnosis. Edlow and McGillicuddy (2008) presented an informative guide to some clinical markers that may be of use in differential diagnosis (see Table 2).

TREATMENT

In humans, untreated tick paralysis can be fatal. Despite the risk of a short-term worsening in the patient's symptoms, locating and removing all ticks from the patient's body is an important part of therapy (Edlow and McGillicuddy, 2008). Ticks are most commonly found on the scalp behind an ear

but, as multiple ticks may be attached, a thorough investigation of the patient’s entire body may be warranted. Although tick anti-toxin is available, the heterologous nature of the antiserum can induce serum sickness and anaphylactic shock in humans (Stone *et al.*, 1982). Progression of paralysis may require mechanical respiratory ventilation in an intensive-care unit (Grattan-Smith *et al.*, 1997). The administration of antibiotics, to protect against tick-borne pathogens such as *Rickettsia* and *Orientia*, may also be justified (Inokuma *et al.*, 2003).

NATURAL HOSTS OF *Ixodes holocyclus*

The most common native hosts of *Ix. holocyclus* are the long-nosed bandicoot (*Perameles*

nasuta), the short-nosed or northern brown bandicoot (*Isodon macrourus*) and the southern brown bandicoot (*Is. obesulus*) (Doubé, 1975) but this tick has been found on a wide variety of native animals (Table 3). *Ixodes holocyclus* also parasitises livestock (sheep, goats, horses, pigs, chickens and ducks) and companion animals (cats and dogs) and is frequently associated with humans.

PRODUCTION OF *Ix. holocyclus* TOXIN IN THE TICK’S SALIVARY GLANDS

The toxin from *Ix. holocyclus* is produced in the salivary glands (SG) of feeding female ticks and transferred to the host via the tick’s saliva (Stone *et al.*, 1983*a*). The SG of ticks consist of numerous alveoli, called acini,

TABLE 2. Factors to be considered in the differential diagnosis of tick paralysis [an adaptation of the factors described by Edlow and McGillicuddy (2008)]*

Sign or symptom	Condition or disease					
	Tick paralysis	Guillain–Barré syndrome	Transverse myelitis	Spinal-cord compression	Botulism	Viral encephalomyelitis or poliomyelitis
Fever	Absent	Rare	Variable (depending on aetiology)	Absent (except in some cases of epidural abscess)	Absent	Present
Pain	Rare	Rare	Frequent	Frequent	Absent	Variable
Sensory abnormalities	Generally absent	Frequent prodromally	Frequent	Frequent	Absent	Not prominent
Dilated pupils	Can be present (Australia) but rare in North America	Rare	Absent	Absent	Present	Absent
Protein in cerebrospinal fluid	Normal	Elevated	Often elevated	Variable (depending on aetiology)	Normal	Elevated
Leucocytes in cerebrospinal fluid	Normal	Normal	Elevated	Variable (depending on aetiology)	Normal	Elevated
Results of MRI scan	Normal	Usually normal	Abnormal	Abnormal	Normal	Abnormal

*This is a partial list that is not meant to be encyclopaedic. MRI, Magnetic-resonance imaging.

classified into four types known as I, II, III and IV. Both genders have acini of types I, II and III but only males have type IV. The acini produce and release biologically active compounds. Each acinus consists of one or two layers of cells around a lumen. Agranular cells characterise the type-I acinus whereas the cells in type-II and type-III acini are granular and are categorised, according to their histology, into cell types A–F, with cell types A–C and D–F occurring in type-II and type-III acini, respectively (Kaufman, 1983). Although

Binnington and Stone (1981) interpreted the results of histological investigations as indicating that cells of type B (from the type-II acini of *Ix. holocyclus*) might contain the toxin responsible for tick paralysis, Stone *et al.* (1989) subsequently identified cells of type E (from type-III acini) as the probable source.

A tick's saliva contains a plethora of biologically active molecules that modulate the host's immune response to tick infestation, facilitating the formation of a feeding pool in the host's tissue (Ribeiro, 1995;

TABLE 3. *The many native hosts of Ixodes holocyclus in Australia (Roberts, 1960; Domrow and Derrick, 1965; Marks and Cribb, 1966; Doube, 1975; Jackson et al, 2000; Campbell et al., 2003)*

Latin name of host	Common English name of host
<i>Aepyprymnus rufescens</i>	Australian marsupial bettong (rat-kangaroo)
<i>Ailuroedus crassirostris</i>	Green cat bird
<i>Antechinus flavipes</i>	Marsupial mouse
<i>Corvus coronoides</i>	Australian raven
<i>Corvus orru</i>	Australian crow
<i>Cracticus nigrogularis</i>	Pied butcher bird
<i>Dasyurus maculatus</i>	Tiger quoll
<i>Dendrolagus lumholtzii</i>	Tree kangaroo
<i>Gymnorhina tibicen</i>	Australian magpie
<i>Isodon obesulus</i>	Southern brown bandicoot
<i>Isodon macrourus</i>	Short-nosed bandicoot
<i>Hydromys chrysogaster</i>	Eastern water rat
<i>Macropus</i> sp.	Kangaroos
<i>Manorina melanoccephala</i>	Noisy miner
<i>Melomys cervinipes</i>	Mosaic-tailed rat
<i>Melomys lutillus littoralis</i>	Brown rat
<i>Perameles nasuta</i>	Long-nosed bandicoot
<i>Phascogale tapoatafa</i>	Red-tailed phascogale
<i>Phaseolartos cinereus</i>	Koala
<i>Pitta</i> sp.	Pitta bird
<i>Platycercus elegans</i>	Crimson rosella
<i>Pteropus conspicillatus</i>	Spectacled flying fox
<i>Ptilonorhynchus violaceus</i>	Satin bower bird
<i>Rattus conatus</i>	Native rat
<i>Ra. culmorum</i>	Pale field rat
<i>Ra. fuscipes</i>	Australian bush rat
<i>Ra. rattus</i>	Black rat
<i>Ra. sordidus</i>	Cane field rat
<i>Strepera graculina</i>	Pied currawong
<i>Tachyglossus aculeatus</i>	Echidna
<i>Thylogale stigmatica</i>	Red-legged pademelon
<i>Trichosurus caninus</i>	Mountain brush-tail possum
<i>Tr. vulpecula</i>	Coppery brush-tail possum
<i>Uromys caudimaculatus</i>	White-tailed native rat
<i>Vombatus ursinus</i>	Wombat
<i>Wallabia dorsalis</i>	Black-striped wallaby
<i>W. rufogrisens</i>	Red-necked wallaby

Wikel, 1996, 1999; Schoeler and Wikel, 2001). To allow successful feeding over several days, ticks need to overcome the host's haemostatic, inflammatory and immune mechanisms (Ribeiro and Francischetti, 2003), and they produce many compounds, including anti-coagulants (such as gelatinases), fibrinolytic enzymes and anti-platelet and vasodilator substances (Kemp *et al.*, 1983), to achieve this goal. Anti-coagulant has been reported in the saliva of *Ar. persicus* (Hoepli and Feng, 1933), *De. sinicus* (Hoepli and Feng, 1933), *Ornithodoros moubata* (Hawkins and Hellmann, 1966), *Ix. ricinus* (Ross, 1926) and *Ix. holocyclus* (Kaire, 1966). These substances generally interfere with coagulation via activated factor X (fXa) or thrombin. The anti-platelet substance apyrase has been detected in the saliva of *Ix. dammini* (Ribeiro *et al.*, 1985) and *Orn. moubata* (Ribeiro *et al.*, 1991) and various forms of prostaglandins are also produced in tick saliva to hinder platelet formation (Bowman *et al.*, 1995). Prostaglandins act as vasodilators as well as having anti-inflammatory, immunosuppressive and antihemostatic activities (Bowman *et al.*, 1996). Usually, tick saliva contains at least one anti-clotting agent, one anti-platelet agent and a vasodilator, which together prevent coagulation (Ribeiro and Francischetti, 2003). Although the saliva of some tick species (which have hypostomes that only penetrate a small way into the host dermis) also contains cement to anchor the tick to the host, the hypostome of *Ix. holocyclus* reaches up to 980 μm into a host's skin (Allen *et al.*, 1977) and does not need the support of any such cement.

The diversity of the compounds used by hematophagous arthropods is astounding (Ribeiro, 1995) and the biological functions of many of the salivary proteins secreted by such arthropods remain unknown.

ISOLATION AND CHARACTERISATION OF THE *Ix.* *holocyclus* TOXIN

Kaire (1966) was the first to isolate the toxin from *Ix. holocyclus* and found it was resistant

to digestion with pepsin, trypsin and papain. In a series of elegant experiments, Stone *et al.* (1983a) then proved that the toxin was injected into the tick's hosts with the tick's saliva. In these investigations, *Ix. holocyclus* were allowed to feed on mice for 4 days prior to their exposure to an in-vitro feeding system in which they fed on tissue-culture medium through an artificial membrane. The medium that contained saliva from the pre-fed ticks was then concentrated and injected into mice, where it produced classic tick paralysis, similar to that induced by inoculation of *Ix. holocyclus* salivary-gland extracts (SGE). The toxin found in *Ix. holocyclus* was called holocyclotoxin. Separation of *Ix. holocyclus* SGE by gel filtration and high-performance liquid chromatography (HPLC) subsequently yielded proteins of 50–80 kDa (Stone *et al.*, 1979, 1983b; Stone and Aylward 1987; Stone, 1988). Holocyclotoxin was found to be a proline-rich glycoprotein that was resistant to digestion with pronase (B. F. Stone, unpubl. obs.). While it was initially thought that apparent inconsistencies seen in the molecular sizes of fractions isolated from *Ix. holocyclus* SGE were the result of variable resolution in the electrophoresis protocols that had been used, Malik (1991) suggested that several different toxic components were present in the SGE, including the holocyclotoxin (Stone and Wright, 1981) that causes paralysis and another toxin that was lethal but did not induce flaccid paralysis.

In an attempt to produce an immunogenic but less toxic antigen from the SGE of *Ix. holocyclus*, as a first step in the production of a vaccine against tick paralysis in Australia, Stone *et al.* (1986) treated SGE with glutaraldehyde. Although this led to a better antitoxin response, it was decided that the number of engorged ticks (and hence the volume of SGE) that could be produced was too low to meet the demands for the production of a vaccine based on glutaraldehyde-treated SGE.

Thurn *et al.* (1992) isolated a neurotoxin from *Ix. holocyclus* by binding SGE to

pinched-off nerve terminals in rat-brain synaptosomes. Three polypeptides, known as holocyclotoxin 1, 2 and 3 (HT-1, HT-2 and HT-3) were isolated. Each had a molecular mass of about 5 kDa. Subsequently, HT-1 was isolated and defined by N-terminal amino-acid sequencing (Thurn, 1994). The corresponding gene, which was isolated, sequenced and characterised by Masina and Broady (1999), showed a high homology with the gene coding scorpion neurotoxin.

Antibodies directed against an immunogenic recombinant form of HT-1 have been found to be protective against holocyclotoxin (Thurn *et al.*, 1992) and the recombinant antigen shows great promise as a vaccine candidate. HT-1, HT-2 and HT-3 seem to be part of the same gene complex (Nicholson *et al.*, 2006).

The 68-kDa toxin in *Rhipiceph. evertsi* SGE and the 60-kDa toxin in *Ar. walkerae* SGE also cause ascending flaccid paralysis but perhaps via a different mechanism to that occurring with *Ix. holocyclus*, since these toxins impair the conduction of impulses along peripheral nerve fibres (Gothe *et al.*, 1979; Gothe and Laemmler, 1982; Viljoen *et al.*, 1986, 1990; Crause *et al.*, 1994).

TOXICITY OF TICKS

An interesting clinical phenomenon is the fact that *Ix. holocyclus* need several days of engorgement on their host before signs of paralysis manifest. Ross (1935) and Goodrich and Murray (1978) observed that the SG from *Ix. holocyclus* only produced marked toxicity, when injected into mice, if they came from ticks that had previously fed on dogs for 5 days. The SG collected from ticks that had fed for only 3 or 4 days on dogs produced much less toxicity when injected into mice (Kaire, 1966; Goodrich and Murray, 1978). Binnington and Stone (1981) subsequently confirmed that the toxicity of homogenates of *Ix. holocyclus* SG depended on the duration of tick feeding. Thus, although SG collected 1 or

2 days after the onset of feeding showed very little toxicity, the toxicity of SG increased with progressive feeding, peaking 4–5 days post-infestation and then decreasing until complete repletion. In both *Ix. holocyclus* (Binnington and Stone, 1981) and *Rhipiceph. evertsi* (Neitz and Gothe, 1986), the timing of the peak production of toxin in the SG was found to coincide with the rapid engorgement phase of feeding (on days 4–5 post-infestation). The paralysis caused by *Ix. rubicundus* or *De. andersoni* is easily reversed, at least in its early stages, by removing the attached tick(s) (Stone, 1988). Paralysis in chickens induced by larvae of the genus *Argas* disappears when the replete larvae drop off the host (Mans *et al.*, 2004). Paralysis caused by *Ix. holocyclus*, however, cannot be easily reversed and affected animals may still die despite removal of the ticks.

Potency levels within one tick species, such as *De. andersoni* (Gregson, 1973), can vary considerably; it is suspected that *De. andersoni* has geographical variants with different levels of toxicity. Although *Ix. holocyclus* ticks generally appear to be consistently toxic, with one tick potent enough to kill a dog, there is also some evidence of variation in toxin potency in this species (Alexander, 1986; Curtin, 1986; Jones, 1986; Pursell, 1986; Atwell and Fitzgerald, 1994).

Toxicity of the Immature Stages of Ticks

Riek (1957) homogenized the eggs from each of 22 species of tick [17 ixodids (including *Ix. holocyclus*) and five argasids] and injected the homogenates into laboratory animals to test for toxic reactions. Toxaemia in the animals injected with ixodid-egg homogenates, leading to death without paralysis, was observed. Similar egg toxicity (attributed to an 'ixovotoxin') has since been reported for additional ixodid species (Gregson, 1973). The relationship between ixovotoxin and the toxins found in SGE is not known (Sonenshine, 1993).

When injected into guinea pigs, extracts from larval *Ix. holocyclus* caused paralysis (Oxer and Ricardo, 1942) whereas extracts from the larvae of *Rhipiceph. (B.) microplus*, *Ix. ricinus*, *Hyalomma dromedarii* and *Haemaphysalis bispinosa* proved to be lethal — with clinical signs similar to those induced by ixovotoxin — but produced no paralysis (Riek, 1957).

Extracts from the nymphal stages of *Ix. holocyclus* and the Karoo paralysis tick, *Ix. rubicundis*, also induced paralysis, and were fatal, when injected into guinea pigs (Oxer and Ricardo, 1942) and rabbits (Spickett *et al.*, 1989), respectively. In contrast, extracts of the nymphs of *Rhipiceph. (B.) microplus* or *Ha. bispinosa* were non-fatal when injected into guinea pigs (Riek, 1957).

PHYSIOLOGICAL REACTION OF THE HOST TO TICK TOXIN

Toxin from *Ix. holocyclus* is now believed to induce motor paralysis as the result of action at the host's neuromuscular junctions (Cooper and Spence, 1976). In dogs paralysed by *Ix. holocyclus*, Cooper *et al.* (1976) found nerve action potentials and maximum nerve conduction velocities to be normal but muscle action potentials to be reduced. In humans and other animals affected by holocyclotoxin, the cortex of the central nervous system (CNS) seems generally unaffected, since the cases remain alert and conscious and sensations are retained (Stone, 1986).

Although Ross (1926) concluded that the toxin of *Ix. holocyclus* acts on motor neurones in the CNS (because humans paralysed by the toxin showed loss or modification of reflexes and normal contractions in their paralysed muscles), this conclusion was not supported by the results of subsequent research. Using phrenic-nerve-hemidiaphragm preparations, Cooper and Spence (1976), for example, demonstrated a temperature-dependent inhibition of transmitter release by holocyclotoxin

at neuromuscular junctions; at relatively low temperatures, muscle contraction following nerve stimulation occurred more readily than at higher temperatures. Cooper and Spence (1976) concluded that holocyclotoxin inhibits transmitter release at neuromuscular junctions via an intermediate step between the terminal membrane and the release of acetylcholine.

When Campbell and Atwell (2002) investigated cardiovascular functions in dogs with tick paralysis, they found that the dogs were suffering from acute left-sided congestive heart failure. Prolonged QT intervals (which represent the time needed for ventricular depolarization and repolarization) were seen in electrocardiographic investigations of dogs (Campbell and Atwell, 2002), bats (Campbell *et al.*, 2003) and rats (Campbell *et al.*, 2004) affected by tick paralysis caused by *Ix. holocyclus*. The salivary toxin of *Ix. holocyclus* directly affects vascular and cardiac potassium channels by blockade, and this action differs from the respiratory distress caused by progressive muscle paralysis (Atwell *et al.*, 2001).

It is not known if the limb paralysis and cardiovascular changes seen in mammals bitten by *Ix. holocyclus* are elicited by one or two separate toxins. In animals paralysed by the toxin produced by *De. andersoni*, paralysis was found to be the result of presynaptic blockage of conduction at neuromuscular junctions (Murnaghan and O'Rourke, 1978; Gothe *et al.*, 1979; Stone, 1988). When acetylcholine was injected into paralysed muscle preparations, the muscles responded normally, indicating that acetylcholine is either not produced or not released in the paralysed muscle (Murnaghan and O'Rourke, 1978).

VACCINE

Over the last three decades there has been some research into a vaccine that will protect against tick paralysis. As already mentioned, Stone *et al.* (1986) treated a

partially purified toxin from *Ix. holocyclus* SG with glutaraldehyde to produce an inactivated and stabilized antigen (toxoid). In rabbits, compared with untreated SGE, this preparation showed enhanced immunogenicity, with relatively high toxin neutralization titres that were achieved in a relatively short time (Stone and Neish, 1984). In dogs, the toxoid showed promising results (Stone *et al.*, 1986). Based on the results of limited terminal amino-acid sequencing of toxic proteins isolated from *Ix. holocyclus* SG, a putative toxin-coding gene was cloned and expressed recombinantly in bacteria, to produce large quantities of toxin for vaccine research and development (Beckmann, 1989). Unfortunately, the monoclonal antibodies that were generated for toxin isolation were later shown to be unsuccessful in neutralizing the *Ix. holocyclus* paralysis toxin (B. F. Stone, unpubl. obs.). In another study (Crause *et al.*, 1994), neutralizing monoclonal antibodies were produced to the paralyzing toxin of *Rhipiceph. evertsi* and these antibodies were shown to cross-react with the paralysis toxin from *Ar. walkerae*.

SUMMARY AND CONCLUSIONS

In Australia, tick paralysis is a potentially fatal illness of importance to both human and veterinary medicine, largely attributable to holocyclotoxin from *Ix. holocyclus*. This toxin is produced in the salivary glands of feeding ticks and transferred to the host via the ticks' saliva. In the host, the toxin results in a presynaptic decrease in acetylcholine release at the neuromuscular junction. Clinical presentation usually involves a combination of lethargy, weakness, unsteadiness of gait, dilated pupils, ascending symmetrical paralysis, slurred speech and depressed deep-tendon and gag reflexes. Laboured breathing, bradycardia, decreased oxygen saturation and asystole may also be observed. Treatment involves removing the tick, although the removal of the tick may

trigger further deterioration in the patient's clinical condition, and respiratory support may be required in an intensive-care unit. Tick anti-serum is available but may cause acute allergic reactions.

Monoclonal antibodies to *Ix. holocyclus* toxin are required for the identification and characterisation of tick-toxin components and purification of toxin-specific antigens. The production of potent neutralizing antibodies may provide the basis for a more standardized and specific immunotherapy against tick paralysis.

An immuno-assay to detect and quantify antibodies to tick toxin would be extremely useful, not only for evaluating the immune status of patients but also for monitoring sera following immunization for monoclonal-antibody production, and for screening the resultant hybridomas.

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