# Developing a vaccine for tick paralysis

The Australian paralysis or 'scrub' tick, *Ixodes holocyclus*, is responsible for the deaths of many pets and livestock in the wetter areas of eastern coastal Australia.

This parasite may also attack man, causing a range of reactions from hypersensitivity — a heightened or accelerated response to salivary antigens — to paralysis and even death. It is the most consistently virulent paralysis tick in the world, secreting a toxin that causes muscular paralysis in victims. This leads to acute breathing difficulties — often complicated by cardiovascular problems and pneumonia.

At the CSIRO Division of Tropical Animal Science in Brisbane, Dr Bernard Stone and his colleagues have made progress towards the development of a protective vaccine that will give animals immunity to the toxin. They have produced an experimental vaccine based on detoxified tick secretion that has immunized test animals against tick paralysis. The idea came from early observations that some animals could acquire immunity to the more drastic effects of tick infestation.

### Hitchhiking bandicoots

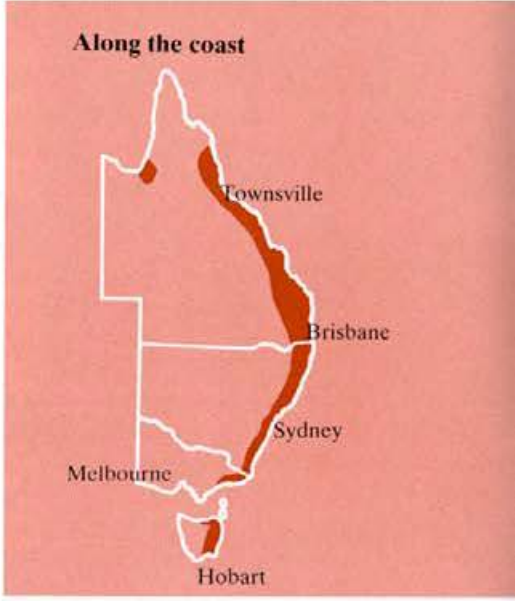
The paralysis tick's life cycle goes through three stages — larva, nymph, and adult — each requiring a new host animal, which may be the same or a different species. At each stage the tick attaches itself to the animal and feeds on blood until it becomes completely engorged and drops onto leaf litter.

Adult females cause most cases of paralysis, as they secrete much more toxin than larvae and nymphs; adult males don't suck blood at all but attach to and feed on female ticks. The highest incidence of tick paralysis coincides with a seasonal abundance of females in spring and early summer.

Many native animals — including dingoes, possums, wallabies, koalas, marsupial mice, echidnas, bush rats, and various birds — acquire immunity to the tick's potent poison. But paralysis ticks really favour bandicoots, especially the northern brown and long-nosed bandicoots, which have a very high immunity to the toxin.

Not only wildlife can naturally develop immunity to paralysis tick — cases have been observed among domestic animals and livestock. However, what may appear to be immunity to the toxin may be merely resistance of the host to tick feeding, which allows the animal to reject the feeding parasite.

The tick feeds on its host in alternating periods of blood-sucking and salivation. After concentrating the nutritional components of the blood, the tick returns surplus water to the host during salivation. Peak production of the toxin in the tick's salivary glands occurs about 5 days after the adult tick attaches to an animal.




**The distribution of paralysing ticks, including species other than *I. holocyclus*, along the eastern coast of Australia.**

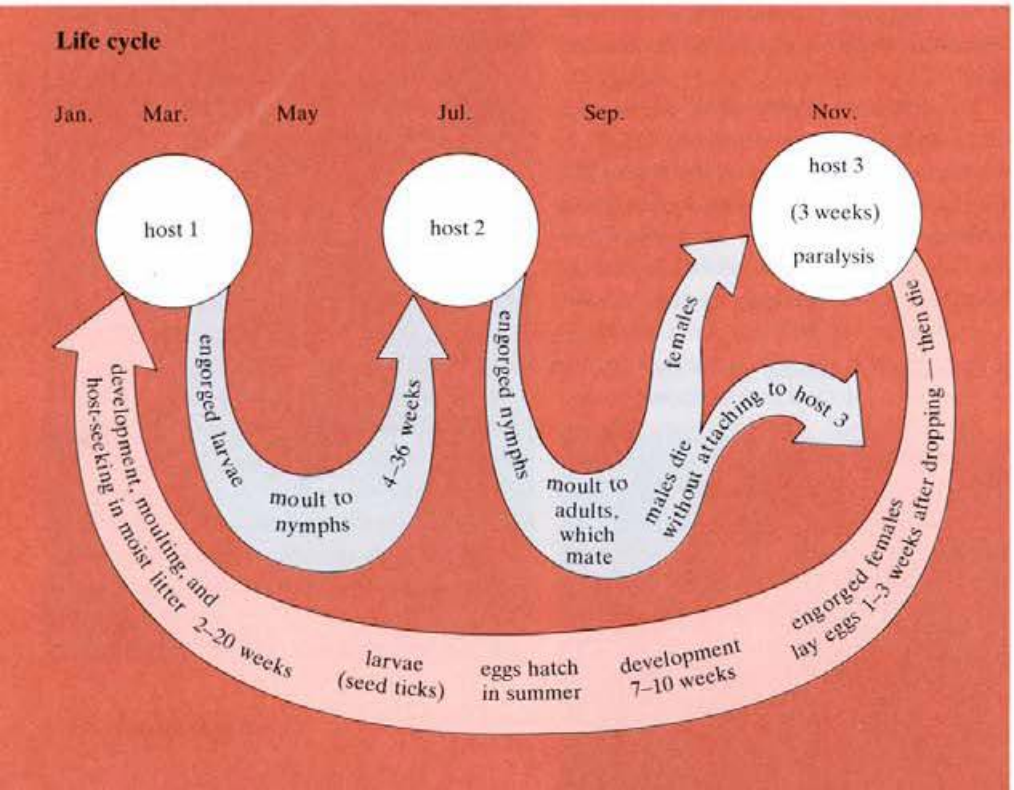
*It is the most consistently virulent paralysis tick in the world.*

In 1935, Sir Ian Clunies Ross of the then CSIR developed an antiserum from the blood of dogs that had developed immunity to tick paralysis. Dogs that become 'hyperimmunized' as a result of high levels of toxin in their blood-stream from repeated tick infestations have since been used in the production of commercial anti-paralysis serum. Injection of the parasite's salivary gland extract achieves similar 'hyperimmunity'.

Canine anti-paralysis serum is only used as treatment for paralysed animals. It is effective in the early stages of tick paralysis, but becomes increasingly unreliable as the disease progresses. Because it is produced in small amounts, the serum (with its associated veterinary care) is expensive enough to prohibit extensive use except for highly prized animals.

### Protective immunization

Its prevention of tick paralysis makes the protective vaccine being developed by CSIRO more effective than the serum. But how does the vaccine protect an animal? Acquired immunity is brought about by the




**The life cycle of the paralysis tick usually takes about a year to complete, depending on environmental conditions.**

action of the immune system, a network of lymph vessels and lymph nodes throughout the body. When 'foreign' particles such as bacteria or toxins invade the body, cells called lymphocytes produce antibodies — large globular proteins called immunoglobulins — which combine with the harmful antigenic molecules of the invader.

Most lymphocytes move continually around the lymphatic system, patrolling for antigens. When they encounter invaders the lymphocytes enlarge and divide, producing two types of cell. The first type — plasma cells — continue to divide and produce antibodies to the antigen.

The other cell type — memory cells — also manufacture antibodies, but continue to circulate long after the infection or the 'toxicosis' has been checked. Consequently, during a second invasion these cells can immediately initiate large-scale production of antibodies.

This rapid response by memory cells is the basis for vaccination. Vaccines are prepared in a number of ways — for example, using a pathogen closely related to the disease, as in smallpox virus. But in the case of tick paralysis, Dr Stone and his team prepared their vaccine by detoxifying the toxin with glutaraldehyde, producing an experimental vaccine that protected test animals from subsequent tick paralysis.

### Hyperimmunity

Although scientists knew how to produce antiserum, nobody had studied the way in which it worked. The development of a vaccine began when Dr Stone and Dr Ian Wright studied the pattern of artificially induced immune response in dogs.

*Normally, one tick feeding to repletion may kill a non-immune dog.*

Using ticks collected from infested bandicoots, they allowed the parasites to feed on beagles for a short time and then increased the number of ticks to 32 over a period of 42 weeks — the dogs withstood these numbers of ticks without producing toxicosis symptoms. Normally, one tick feeding to repletion may kill a non-immune dog.

**Salivary glands being removed from adult female ticks at the peak of their toxin-production cycle.**

The anti-toxin potency of the dogs' serum equalled that of commercial anti-paralysis serum after a maximum 32 ticks had become engorged on the dogs' blood. When the ticks were removed, the antiserum potency remained constant for about 8 weeks, declining to about one-third its maximum value in another 10 weeks. After the ticks were re-applied, antiserum potency returned to a value near the previous maximum within 12 weeks.

The tests indicated that only hyperimmune dogs, whose measured serum antitoxin levels had reached a plateau, could resist any further tick infestations. Fatalities were likely to occur among animals that had established lower levels of immunity.

In fact, hyperimmune dogs, kept tick-free for a year or more, did not succumb to the paralysing effect of 15 or more ticks, even though titres of serum antitoxin from them were at a minimum when the ticks were re-applied. Dr Stone emphasized that very few dogs could develop hyperimmunity as a result of natural exposure to ticks. One exception would be dingoes in paralysis tick areas.

Results of this study not only provided valuable information to anti-paralysis serum producers, but gave Dr Stone and his colleagues an insight into the feasibility of artificial induction of immunity. Dogs infested with ticks over a long period developed an immunological memory that could re-stimulate immunity upon further attacks.

The next step was inducing immunity in the dogs without the use of ticks by injecting salivary gland extract directly into them. The scientists used six injections over a period of 20 weeks. Neutralizing antibodies built up to levels similar to those produced in the tick feeding experiments. The dogs also appeared to attain a comparable hyperimmune state, and were able to withstand subsequent tick 'challenges'.

One of the problems with using a canine antiserum for human cases of tick paralysis is that humans can develop an allergic response to it. As an anti-paralysis serum is also needed for human use, the CSIRO team began investigating immunity in rabbits using tick salivary gland extract.

Like the dogs, the rabbits injected with this extract could withstand doses of toxin known to kill unimmunized animals.

*The CSIRO team prepared their vaccine by detoxifying the toxin.*

Further, the test rabbits retained their hyperimmunity for more than a year. The results confirmed the possibility of producing a purified tick-paralysis anti-toxin more suitable for human use than the anti-toxin derived from dog hyperimmune serum.

### Defusing the toxin

Dr Stone then focused his efforts on developing a modified toxin preparation that retained the effective antigenic properties of the natural tick secretion while causing little or no toxicity to the immunized animals.

By treating the toxin with glutaraldehyde, the scientists came up with a potent 'immunogenic toxoid' that they tested on rabbits.

**Oscillograph traces of an adult female tick's feeding pattern while attached to a mouse.**

**Tracing a tick's feeds**

**This female tick is feeding on human blood from a volunteer's hand, to which it has just attached.**

The toxoid proved successful — the rabbit serum samples registered very high antitoxin levels. Further, test animals required only half the number of injections and developed immunity in about one-third of the time needed by rabbits immunized with unmodified toxin.

No paralysis symptoms developed in immunized rabbits on which ticks were placed. Five months after immunization, the rabbits were unaffected by normally lethal doses of injected toxin.

Although the toxoid presents a promising basis for a vaccine, the scientists' next problem is to develop a method of producing the paralysis antigen (toxin) on a large scale. The CSIRO team explored the possibility of collecting toxic secretions from ticks feeding on liquid food medium under an artificial membrane. The scientists glued the ticks 'mouthparts' to the silicone rubber 'skin'. Although the parasites sucked and salivated in the normal manner, they produced a lower mean yield of soluble toxin per tick than did the naturally feeding ticks. Consequently, the artificial feeding research has been discontinued for the time being. Possible solutions are culture of the tick salivary gland cells or genetic engineering techniques, perhaps using cloned bacteria to synthesize the toxin.

*Many native mammals acquire immunity to the tick's potent poison.*

At the moment, priority is being given to isolation and purification of the toxin from salivary gland extracts. Dr Stone of CSIRO and Dr John Pearn of the Department of Child Health, University of Queensland, have received a grant from the CSIRO–University of Queensland Collaborative Research Fund to facilitate this research.

Ms Maryann Gauci has been appointed as a result of the grant to identify the protein components of the paralysis toxin using a process called isoelectric focusing. This involves separating individual proteins by subjecting the toxin to an electric field in a gel support in which a pH gradient has first been generated. (The technique can resolve the proteins of human blood plasma into 40 or more bands.)

Electrophoresis is normally an analytical tool, but Dr Stone and his team hope to be able to use it on a large scale to prepare quantities of the purified tick toxin. The purified toxin may then be applied in tests being developed to improve the bioassay for determining the potency of tick toxins and antitoxins. One spin-off of the research is that the purified toxin will become available for other purposes as well; for example, the genetic engineering processes mentioned above may use it in the production of commercial quantities of tick paralysis vaccine.

Mr John Morrison of the Department of Child Health has developed a simple laboratory test based on a biochemical technique called ELISA (Enzyme-Linked Immunosorbent Assay). The new assay uses coated plastic plates (containing 'wells'), rather than animals. Toxin and animal serum react in these wells in the presence of an enzyme and provide a measure of the antiserum's potency in terms of antitoxin units.

*Mary Lou Considine*

### More about the topic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**Female ticks engorging on a northern brown bandicoot.**

**Adult female ticks feed through an artificial (silicone rubber) membrane. An oscillograph monitors the feeding.**