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INVESTIGATIONS INTO THE TOXIN FROM THE AUSTRALIAN PARALYSIS TICK, *IXODES HOLOCYCLUS*

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Dogs are most commonly and severely affected by tick paralysis caused by *Ixodes holocyclus* in Australia. The toxin acts by reducing the release of acetylcholine. However, little is known about the site of binding of the toxin and the active polypeptide component of the toxin. Commercially produced anti-tick serum is available for treatment. However, this product shows great variability in efficacy to neutralise tick toxin and some dogs with paralysis will die even with this treatment. The only assay available to test this anti-tick serum is an expensive and relatively inaccurate *in vivo* system.

The aims of this project are to develop a novel *in vitro* assay to measure toxin-neutralising antibody levels in commercial anti-toxin serum. In addition, investigations into the immunochemical nature of the toxin are carried out by producing monoclonal antibodies to the toxin and identifying polypeptides associated with toxin activity that may serve as highly specific antigens in these assays.

We have developed a first generation enzyme-linked immunoassay (ELISA), using a partially purified toxin from the tick salivary gland, to specifically detect anti-toxin antibody in rat and dog sera. However, dog sera show a significant level of background due to impurities in the antigen. To address this we produced monoclonal antibodies (mAbs) by immunising rats with controlled tick infestation and inoculations with partially pure toxin. Monoclonal antibodies that effectively neutralise tick toxin *in vivo* have been selected for the identification of the active toxin components and to purify specific antigen for the ELISA.

Currently, a second generation ELISA using mAbs is assessed for its specificity to detect antibodies to tick toxin in dog sera. Also, toxin is probed with mAbs and used to identify specific bands in Western blots that can be purified and sequenced.