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Case report

Successful use of camelid (alpaca) antivenom to treat a potentially lethal tiger snake (*Notechis scutatus*) envenomation in a dog

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ABSTRACT

This report describes a confirmed clinical case of tiger snake (*Notechis scutatus*) envenomation in a domestic dog that was successfully treated with a novel polyvalent camelid (alpaca; *Llama pacos*) antivenom. Samples collected from the dog were assayed for tiger snake venom (TSV) using a highly sensitive and specific ELISA. The TSV concentration in serum and urine at initial presentation was 365 ng/mL and 11,640 ng/mL respectively. At the time of initial presentation whole blood collected from the dog did not clot and the Prothrombin Time was abnormally increased (>300 s). Serum was also visibly hemolysed. The dog was administered antihistamine, dexamethasone and 4000 Units (sufficient to neutralise 40 mg of TSV) of a novel polyvalent alpaca antivenom diluted in 0.9% NaCl. At 4 h post-antivenom treatment the dog's clinical condition had improved markedly with serum TSV concentrations below the limit of detection (<0.015 ng/mL), consistent with complete binding of venom antigens by the alpaca antivenom. Coagulation parameters had begun to improve by 4 h and had fully normalised by 16 h post-antivenom. Venom concentrations in both serum and urine remained undetectable at 16 h post-antivenom. The dog made a complete recovery, without complications, suggesting that the alpaca-based antivenom is both clinically safe and effective.

1. Introduction

Australian veterinarians have been estimated to treat over 6000 cases of snakebite annually in dogs and cats (Mirtschin et al., 1998). However the true number of animals that are envenomed is not known and is likely to be higher than this estimate. The most common snake species involved in causing envenomation in animals in Australia are the eastern brown snake (*Pseudonaja textilis*), tiger snake (*Notechis scutatus*) and red bellied black snake (*Pseudechis porphyriacus*) (Heller et al., 2005; Mirtschin et al., 1998).

TSV contains neurotoxins, myotoxins and procoagulant toxins that lead to paralysis, muscle damage potentially resulting in secondary renal failure, and coagulopathy in envenomed animals. Due to the generally lower bodyweight of animals compared to humans, the clinical signs experienced by cats and dogs are likely to be expressed more rapidly and severely. Nevertheless, confirmation of the diagnosis of snakebite in animals can be challenging for veterinarians. In the absence of a definite history of snakebite, clinical signs and simple diagnostic tests such as clotting times and urinalysis are generally used for establishing the diagnosis. Treatment of tiger snake envenomation in dogs and cats is centred on prompt administration of effective monovalent or bivalent antivenom to neutralise circulating venom combined with ancillary supportive management (Indrawirawan et al., 2014). Unfortunately, few veterinary clinics in Australia have the facilities to provide artificial ventilation for prolonged time periods and the costs of this form of support can be beyond pet owner's financial reserves. Financial constraints are a key determinant of how an animal will be treated (Indrawirawan et al., 2014).

The procoagulant toxins contained in TSV rapidly lead to a reduction in plasma fibrinogen concentration and a significant prolongation of the Prothrombin Time in envenomed dogs (Best and Sutherland, 1991; Holloway and Parry, 1989). In a series of six confirmed tiger snake bite cases in dogs, five of these had markedly prolonged clotting times. One case had normal clotting times but also failed to develop any clinical signs of tiger snake envenomation even though venom was detected on a CSL venom test kit from the bite site. Two dogs died within 30 min of presentation and one dog

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was euthanased upon presentation without treatment. Pre-paralytic signs of tiger snake envenomation in dogs are vomiting, salivation, depression and dilated pupils (Hill, 1979). In another case series of tiger snake envenomation in dogs, six of the 125 described cases were reported to have collapsed within 20 min of being bitten, then appeared to recover and subsequently developed typical signs of tiger snake envenomation some hours later. Experimentally the time to onset of clinical signs in dogs is correlated with the number of lethal doses received (Lewis, 1994a,c). Dogs that survive the initial envenomation syndrome are reported to develop extensive myopathy, that may lead to renal complications (Lewis, 1994b).

Previous work by the authors has focused on developing antivenom using a camelid species – the alpaca. Alpaca are abundant in Australia with over 150,000 animals in the national herd. The antibody profile of this and other camelid species may be advantageous for the production of low immunogenicity, broadly neutralising and thermostable antivenom (Cook et al., 2010). Whilst camels have been the primary focus of previous camelid antivenom research, alpacas are more widely domesticated and so may be more practical than the former animal.

This report describes a clinical case of tiger snake envenomation in a dog that was successfully treated with a novel camelid-derived polyvalent antivenom.

2. Materials and methods

2.1. Animal ethics

The animal was treated under the framework of the Australian Pesticides and Veterinary Medicines Authority (APVMA) Small Scale Trial Permit (7250). Guidance on animal ethics was obtained from the Principal Veterinary Officer, Bureau of Animal Welfare, Department of Primary Industries, Victoria, Attwood. All procedures were performed by a registered veterinarian.

2.2. Tiger snake venom antigen ELISA

Frozen (−20 °C) serum and urine samples were retrospectively tested for the presence of whole tiger snake venom using ELISA. The concentration of TSV in urine and serum was measured using a highly sensitive and specific sandwich ELISA. A biotin labelled ELISA for TSV, similar to what has been described previously for TSV in humans (Isbister et al., 2012) was used. An alpaca anti-TSV capture antibody was produced by repeated immunisation of four alpaca with only tiger snake venom emulsified in Incomplete Freund's Adjuvant. A commercial affinity chromatography kit (AminoLink®, Pierce, USA) was used to prepare a column containing whole TSV bound to the matrix. Pooled alpaca serum was then passed through the column, unbound material discarded and the bound IgG eluted from the column using pH 2.7 elution buffer solution into 1M TBS buffer pH 7.4. To measure TSV concentrations in clinical samples, 96-well high binding microplates were coated with affinity purified alpaca anti-tiger Snake antibody at 5 μg/mL in carbonate coating buffer pH 9.6 and allowed to adsorb overnight at 4 °C. The following day the plate was washed 6 times in PBS-T20. A standard curve of TSV (5000 pg/mL) diluted in PBS-T20 + 0.5% BSA was applied to the plate in eight doubling dilutions. On each plate a 100 μL sample at 10% dilution of normal canine urine and normal canine serum was applied in doubling dilutions to measure non-specific binding and serve as a negative control. Clinical samples were initially diluted either 10% for serum or 1% for urine in PBS-T20 + 0.5% BSA buffer and eight doubling dilutions made on the plate. The plate was incubated for 60 min on a microplate shaker at 37 °C at 600 rpm. The plate was then washed 6 times in PBS-T20 to remove unbound venom and then 100 μL of the biotin labelled antibody (0.15 μg/mL) in PBS-T20 was incubated for 30 min at 37 °C with 600 rpm shaking. The plate was then washed 6 times to remove any unbound labelled antibody and 100 μL of streptavidin (1; 40,000 in PBS-T20) conjugated to horseradish peroxidase (Thermo Scientific, Australia) was added to each well and incubated as previously. The plate was washed again and a 100 μL volume of the chromogenic substrate TMB (TMB Ultra, Thermo Fisher, Australia) was applied to each well on the plate. The colour was allowed to develop for 4–10 min and the reaction stopped by addition of 50 μL of 10% sulphuric acid. The plate was read within 10 min at 450 nm in a microplate reader (Tecan Sunrise, Tecan, Australia). Raw optical densities of unknowns were interpolated from 5-point sigmoidal standard curve using a commercial software package (Magellan 7.1, Tecan, Australia). The first sample in each serial dilution series that resulted in the highest OD that was above the negative control OD was used for interpolation of the TSV concentration. The analytical limit of detection of whole tiger snake venom in this ELISA using PBS-T20 + 0.5% BSA as the venom diluent was 0.015 ng/mL. When samples of normal urine and normal serum were diluted 1:10 in 0.5% BSA PBS-T20 there was minimal non-specific binding (OD < 0.1). The standard curve based on serial dilutions of TSV assays showed excellent curve fit ($R^2 > 0.99$; Fig. 4).

2.3. Rapid snake venom detection kit

A commercial snake venom detection kit (SVDK) (Cox et al., 1992) produced by bioCSL (Parkville, Australia) was used to test for the presence of snake venom. The test is able to identify five families of snake venom from clinical samples (bite site, urine or blood). The test kit sensitivity for tiger and brown snake venoms is at least 10 ng/mL when the kit is used according to the manufacturer's directions, that is with a 10 min incubation time and visual reading (Cox et al., 1992; Moisidis et al., 1996). The control wells have been previously described (Cox et al., 1992). Briefly, the sixth well is a negative control with normal rabbit IgG as capture and an

Fig. 1. Photograph of the tiger snake (*Notechis scutatus*) brought in for identification by the owner of the dog.