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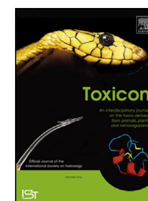

Case report

Fatal presumed tiger snake (*Notechis scutatus*) envenomation in a cat with measurement of venom and antivenom concentration

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ABSTRACT

A fatal outcome of a presumed tiger snake (*Notechis scutatus*) envenomation in a cat is described. Detectable venom components and antivenom concentrations in serum from clotted and centrifuged whole blood and urine were measured using a sensitive and specific ELISA. The cat presented in a paralysed state with a markedly elevated serum CK but with normal clotting times. The cat was treated with intravenous fluids and received two vials of equine whole IgG bivalent (tiger and brown snake) antivenom. Despite treatment the cat's condition did not improve and it died 36 h post-presentation. Serum concentration of detectable tiger snake venom components at initial presentation was 311 ng/mL and urine 832 ng/mL, this declined to non-detectable levels in serum 15-min after intravenous antivenom. Urine concentration of detectable tiger snake venom components declined to 22 ng/mL at post-mortem. Measurement of equine anti-tiger snake venom specific antibody demonstrated a concentration of 7.2 Units/mL in serum at post-mortem which had declined from an initial high of 13 Units/mL at 15-min post-antivenom. The ELISA data demonstrated the complete clearance of detectable venom components from serum with no recurrence in the post-mortem samples. Antivenom concentrations in serum at initial presentation were at least 100-fold higher than theoretically required to neutralise the circulating concentrations of venom. Despite the fatal outcome in this case it was concluded that this was unlikely that is was due to insufficient antivenom.

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1. Introduction

Snakebite is an infrequent reason for presentation of animals to veterinarians in Australia (Mirtschin et al., 1998). A mailout survey of Australian veterinarians concluded that approximately 6,000 cases of snakebite are treated annually (Mirtschin et al., 1998). Envenomed cats and dogs were reportedly seen by veterinarians in similar proportions. A survey of veterinary practices in NSW found that red bellied black (44%), brown snake (40%), tiger snake (7%) accounted for the majority of snakebite cases presented to veterinarians (Heller et al., 2005). By contrast published veterinary case data from around Melbourne found that definite or suspected snakebites cases in both canine and feline were almost exclusively tiger snake (Indrawirawan et al., 2014). The current taxonomy of tiger snakes defines only a single species within the *Notechis* genus across Australia (Cogger, 2000).

The epidemiology of a series of 41 cases of confirmed tiger snake envenomations in cats was described in the Werribee area of Victoria (Hill and Campbell, 1978). A subsequent study in the same geographical area of 115 cats and 125 dogs provided more epidemiological data and further clinical findings (Barr, 1984). The predominant clinical signs in cats were dilated pupils, flaccid paralysis, weakness and hindlimb ataxia (Hill and Campbell, 1978). In another case series from the same geographical region most tiger snake bite cases in cats were presented on average 21 h post-bite compared to 2 h for dogs (Holloway and Parry, 1989). This late presentation of cats reportedly made diagnosis based on coagulation findings alone difficult as most cats presented with normal Prothrombin Times (PT) compared to dogs in which the PT was very prolonged (Holloway and Parry, 1989). By contrast, another study reported that 34–50% of cats with definite and suspected tiger snakebite presented with some degree of coagulopathy (Indrawirawan et al., 2014).

This case report of a fatal outcome in a cat from a tiger snake bite is the first report describing Notechis-specific venom component concentrations pre- and post-antivenom together with antivenom concentrations in serum and urine. It is also the first report to

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describe the use of camelid antibodies as a tool for snakebite immuno-diagnosis and venom quantitation.

2. Methods

2.1. Coagulation parameters

A semi-automated analyser (CoaData 2000, USA) using 50 μL citrated plasma and 100 μL thromboplastic reagent (Helena Laboratories, Australia) was used to measure the PT.

2.2. Clinical biochemistry

A clinical biochemistry profile was performed upon a serum sample collected prior to antivenom administration at a commercial veterinary diagnostic laboratory (Gribbles Veterinary Pathology, Clayton, Victoria).

2.3. ELISA for tiger snake venom

The concentration of tiger snake venom (TSV) components in urine and serum was measured using a highly sensitive and specific sandwich ELISA. The biotin labelled ELISA for TSV was similar to what has been described previously for TSV in humans (Isbister et al., 2012). An alpaca anti-TSV antibody was produced by repeated immunisation of four alpaca with tiger snake venom emulsified in incomplete freund's adjuvant. An affinity chromatography kit (AminoLink®, Pierce, USA) was used to prepare the column containing whole TSV bound to the matrix. Pooled alpaca serum was passed through the column, unbound material discarded and the bound IgG eluted from the column using pH 2.7 buffer solution into 1M TBS buffer pH 7.4. To measure TSV concentrations in clinical samples 96-well high binding microplates coated with affinity purified alpaca anti-tiger Snake antibody at 5 μg/mL in carbonate coating buffer and allowed to adsorb overnight at 4 °C. Next 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 doubling dilutions. On each plate a 100 μL sample at 10% dilution of normal feline urine and normal feline 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 0.5% BSA PBS-T20 buffer and eight doubling dilutions made on the plate. The plate was incubated for 60 min at 37 °C with 600 rpm shaking. The plate was washed 6 times in PBS-T20. Next, 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. Next, 100 μL of streptavidin conjugated to horse radish 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 10% sulphuric acid. The plate was immediately read at 450 nm in a microplate reader (Tecan Sunrise, Australia). Raw optical densities were interpolated from the standard curve using a commercial software package (Magellan 7.1, Tecan, Australia). The first sample in each serial dilution that resulted in the highest OD that was above the negative control OD was used for interpolation of the venom 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).

2.4. ELISA for equine IgG

The concentration of equine IgG in clinical samples was measured using a highly sensitive and specific ELISA. Briefly, 96-well high binding microplates were coated with goat anti-horse IgG-Fc (Jackson Immuno, USA) at 2.5 μg/mL. The capture antibody used was highly specific for the Fc portion of the equine IgG molecule. There is non-detectable cross reactivity with feline IgG and equine IgG (Esteves, 1972). The capture antibody was allowed to adsorb overnight at 4 °C then plates washed next day. A standard curve of purified equine IgG (ChromPure Equine IgG, Jackson Immuno, USA) in PBS-T20 was applied to the plate as serial dilutions (117 ng/mL–0.91 ng/mL). An internal positive control sample was run on each plate. Normal feline urine and normal feline serum were applied at an initial dilution of 10% to assess non-specific binding. Clinical samples were applied at an initial dilution of 1% for urine or 0.01% for serum and eight doubling serial dilutions made on the plate. Plates were incubated for 1 h at 37 °C with shaking at 600 rpm. Plates were then washed 6 times in PBS-T20. A goat anti-horse IgG (heavy and light chains) secondary antibody conjugated to horseradish peroxidase was applied at a dilution of 1:20,000 (Jackson Immuno, USA). Plates were washed again and the chromogen TMB (TMB Ultra, Thermo Fisher, Australia) applied to each well. Colour was allowed to develop for 4–10 min and the reaction stopped using 10% sulphuric acid. The intensity of the colour reaction was read at 450 nm in a microplate reader (Tecan Sunrise). Unknown values were interpolated from the standard curve using a commercial software package (Magellan 7.1, Tecan).

2.5. ELISA for equine anti-tiger snake venom antibodies

An indirect ELISA was used to measure the concentration of equine anti-tiger Snake venom neutralising antibody. The biological potency of a calibration serum was determined in mice and used to construct a standard curve. Previous work on the Thai Cobra has demonstrated a very strong correlation between ELISA titres and mouse bioassay results (Pratanaphon et al., 1997; Rungsiwongse and Ratanabanangkoon, 1991). Due to the close relationship between the venoms of Tiger and Cobra an assumption was made that the ELISA for equine anti-Tiger Snake venom antibody would also correlate closely with the *in vivo* neutralising potency. The plate coating antigen used for ELISA for equine anti-Tiger Snake venom antibody was a purified fraction of the neurotoxins less than 30kD molecular weight present in tiger snake venom (Harris et al., 1973). The ELISA was similar otherwise to that used for total equine IgG. Urine samples were assayed at an initial dilution of 10% and serum at 1%.

2.6. Sample collection

All clinical samples were collected as part of the treatment course. Serum was collected from whole blood samples that had been left at room temperature for at least 1 h to clot and separated by centrifugation and frozen at −20 °C until assayed. Some previous work has demonstrated a marked decline in serum venom concentrations in samples not frozen soon after collection (Kulawickrama et al., 2010).

2.7. Case report

An 8-year old non-desexed male cat was presented to the attending veterinarian having been found by its owner in a collapsed state inside the house. The owner reported the cat had appeared normal 20 h previously and had been confined inside the

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