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Case report

Red-bellied black snake (*Pseudechis porphyriacus*) envenomation in the dog: Diagnosis and treatment of nine cases

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ABSTRACT

The clinical signs, biochemical changes and serum and urine venom concentrations for a series of nine cases of Red bellied black snake [RBBS] (*Pseudechis porphyriacus*) envenomation in eight dogs seen in a regional Australian veterinary hospital are described. Although the resulting envenomation syndrome was, in most cases, relatively mild and responded rapidly to intravenous administration of a novel bivalent caprylic acid purified whole IgG equine antivenom for tiger (*Notechis scutatus*) and brown snake (*Pseudonaja textilis*), one fatality prior to antivenom treatment was recorded. The latter case occurred within 1 h of envenomation prior to receiving antivenom treatment. Intravascular haemolysis, pigmenturia, bite site swelling, lethargy, and generally mild coagulopathy were present in most cases. Detectable RBBS venom specific components were found in serum, bite site swab or urine using a standard sandwich ELISA approach. Serum levels fell within the range previously reported for human RBBS envenomation cases (6–79 ng/ml) whilst bite site and urine samples varied more markedly (8.2 to >5000 ng/ml and 2.2–1300 ng/ml respectively). No venom was detected from serum after antivenom treatment. The envenomation syndrome in dogs is similar to what is described for humans, with the exception of the presence of potentially severe venom induced consumption coagulopathy in one case (aPTT > 300 s and fibrinogen < 0.43 g/L) and potential for fatal outcomes. This series represents the largest and most detailed examination of RBBS envenomation in animals yet reported. It reinforces the emerging view that the potential severity of this envenomation has been underappreciated by veterinary practitioners and highlights the possibility of severe venom induced consumption coagulopathy in canine cases.

1. Introduction

The Australian Red-bellied Black snake (RBBS) (*Pseudechis porphyriacus*) is a large, distinctively coloured and moderately venomous snake distributed widely throughout south-eastern Australia. The RBBS is diurnal and typically found near waterways, swamps and lagoons where it feeds predominantly on frogs (Cogger, 2000). It's venom was first investigated by Charles Martin in the 1890's as a prototype for Australian elapids (Hawgood, 1997) and then, more comprehensively, by Charles Kellaway in the 1930's (Kellaway, 1930). The venom is notably less toxic than that of other Australian elapids with an LD_{50} of 2.52 mg/kg in 18–21 g mice (Broad et al., 1979) and contains neurotoxins, procoagulants and myotoxins (Pearn et al., 2000). The venom of the RBBS is effectively neutralised by antivenom directed against tiger snake (*Notechis scutatus*) venom (Best and Sutherland, 1991).

Despite its wide distribution there are very few published reports of envenomation in animals (Gordon, 1958; Heller et al., 2006). Surveys of veterinarians in Australia have revealed that RBBS envenomation is relatively common in certain geographical areas. For example, in one study 44.6% of animal snakebite cases treated in 253 veterinary clinics in New South Wales were for presumed RBBS envenomation (Heller et al., 2005). The most common clinical findings in such presumed, but not formally confirmed cases, were pigmenturina, weakness, ataxia and salivation (Heller et al., 2005). To address the paucity of veterinary clinical data on this envenomation type, and to complement recent human findings, we examined RBBS envenomation in dogs treated at a regional Victorian veterinary hospital. Hence this report describes the clinical features, serum biochemistry, haematological,

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coagulation status, venom concentrations and treatment outcomes, in nine cases of RBBS envenomation in eight dogs.

2. Materials and methods

2.1. RBBS venom specific ELISA

The concentration of RBBS venom was measured in clinical samples (serum, urine, bite site swab) using a sandwich ELISA. The ELISA was similar to that previously utilised for measuring taipan venom (Churchman et al., 2010; Kulawickrama et al., 2010). RBBS venom specific antibodies were purchased from a commercial supplier (Harry Perkins Institute, Perth, Western Australia). The rabbit anti-RBBS IgG was purified by passing the crude rabbit serum through a Protein G column and eluting the bound IgG. A biotin label was applied to the rabbit anti-RBBS IgG preparation using a commercial kit (EZ-Link, Pierce, USA). The RBBS ELISA was performed by coating 96-well polystyrene high binding microplates (Maxisorp™, Nunc, USA) with 100 μL/well of rabbit anti-RBBS IgG at 10 μg/mL in carbonate coating buffer pH 9.6. Plates were incubated at room temperature for 3 h and then placed in a refrigerator overnight at 4°. Next day the plates were washed three times with PBS-T20 and unbound sites blocked with 300 μL/well of ELISA blocking buffer consisting of PBS-T20 + 0.5% BSA (Bovostar, Bovogen, Australia). Controls, standards and test samples were pipetted into each well in a volume of 100 μL and incubated for 30 min on a plate shaker at 600 rpm for 30 min. Unbound venom was removed by washing plates three times as described above. Next, 100 μL of the secondary biotin labelled rabbit anti-RBBS (0.15 μg/mL) in blocking buffer was pipetted into each well and incubated as described above. Following incubation, unbound biotin antibody was then removed by washing the plate three times. Streptavidin-HRP (Thermo Fisher, Australia) was then added at a 1:40,000 dilution in blocking buffer and incubated the same as for the secondary antibody. Plates were washed for a final time and 50 μL of TMB (Ultra TMB, Thermo Fisher, Australia) was added to each well and colour allowed to develop for 4–10 min. The enzyme reaction was then stopped by addition of 50 μL of 10% sulphuric acid. Plates were read within 10 min in a Tecan Sunrise microplate reader at 450 nm. Unknown samples were interpolated against the standard curve using a 5-point fitted equation computed from commercial software (Magellan™ 7.2, Tecan, Austria).

Serum, urine and bite site swabs were initially diluted to 10% in ELISA blocking buffer and applied to Row 1 on the microplate. Doubling dilutions were then prepared down the plate to provide a range of sample concentrations from 1:10 to 1:1280. A standard curve was run in duplicate on each plate consisting of RBBS venom (Venom Supplies Pty Ltd, Tanunda, South Australia) dissolved in ELISA blocking buffer to a final concentration of 5 ng/mL.

Negative control samples were run in each assay run consisting of pooled normal dog urine and serum collected from ten non-envenomed dogs. Raw optical density (OD) values of unknown samples that were below the OD of the negative control for each matrix were not used for calculations. The mean OD of eight blank wells was subtracted from the OD reading of each standard, control or test sample well. The sensitivity of the RBBS venom ELISA was 0.30 ng/mL. The RBBS ELISA was evaluated for cross reactivity with Tiger snake (*Notechis scutatus*) and Eastern brown snake (*Pseudonaja textilis*) venoms by assaying doubling dilutions of these venoms starting at 1000 ng/mL. The RBBS venom ELISA was found to be highly specific for RBBS venom with only 0.7% cross-reactivity with Tiger and <0.01% with Brown snake venom that are the other significant venomous snakes in this geographical area.

2.2. Clinical biochemistry, haematology, urine biochemistry

Clinical serum biochemistry and haematology was performed at commercial veterinary pathology laboratory (Gribbles Veterinary Pathology, Clayton, Victoria, Australia). Urine biochemistry was performed using commercial indicator test strips (Multistix® 7, Siemens, Poland). Urine specific gravity (SG) was measured using a manual refractometer.

2.3. Coagulation studies

A semi-automated coagulation analyser (CoaData 2000, USA) was used to measure the Prothrombin Time (PT) using 50 μL citrated plasma and 100 μL thromboplastic reagent (Helena Laboratories, Australia). The activated-PT was measured using the same analyser and commercial reagents (Helena Laboratories, Australia). Citrated plasma fibrinogen was similarly assayed using a commercial reagent (Helena Laboratories, Australia) utilising the Clauss method. Whole blood activated clotting time (ACT) was performed by collecting 2 mL of whole blood and rapidly transferring to a commercial ACT tube (Actalyke, Helena Laboratories, Australia). The tube was gently mixed in the tube in a water bath at 37 °C for 30 s and then inverted every 5–10 s to monitor for clot formation (See et al., 2009). A stopwatch was then used to manually time the interval until solid clot formation.

2.4. Antivenom

The antivenom used in all cases was a whole IgG formulation produced by progressively immunising horses with venoms from tiger snake (*N. scutatus*) and eastern brown snake (*Pseudonaja textilis*). The immunoglobulin fraction was concentrated using caprylic acid method as previously described (Rojas et al., 1994) and dialysed against 0.9% NaCl. The potency of the antivenom was determined in a mouse bioassay where 1 Unit is the amount of antivenom required to neutralise 0.01 mg of whole venom. The product was formulated to contain no less than 4000 Units of tiger snake and 4000 Units of brown snake antivenom per vial. The experimental antivenom was used under the conditions of a small scale trial permit (PER 7250) from the Australian Pesticides and Veterinary Medicines Authority. Guidance on animal ethics was obtained from the Principal Veterinary Officer, Bureau of Animal Welfare, Department of Primary Industries, Victoria, Attwood. All veterinary medical procedures were performed by a registered veterinarian.

2.5. Clinical sampling handling

Whole blood samples were collected into commercial clinical sample collection tubes and centrifuged; plasma or serum was then separated, tubes labelled, and samples stored frozen at −20 °C within 2 h of collection. Bite site swabs were collected using a moistened 7.5 cm cotton tip applicator (Propax®, BSN Medical, Germany). The bite site was swabbed by rolling the applicator across the skin and then placing the tip into a 1.5 mL flip-top tube containing 0.5 mL of PBS-T20 solution for 5 min to release venom. The cotton tipped applicator was then discarded and the remaining solution frozen at −20 °C within 2 h of collection. Immediately prior to assay the samples were thawed at 37 °C.

3. Case reports

3.1. Case 1 – Alice, 3 year old Springer Spaniel

Alice, a 3-year-old female lactating English Springer Spaniel