Case report

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

Andrew M. Padula*, Kenneth D. Winkel

Australian Venom Research Unit, Department of Pharmacology and Therapeutics, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, 3010, Australia

**Article info**

**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.

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**1. Introduction**

The Australian Red-bellied Black snake (RBBS) (*Pseudechis porphyriacus*) is a large, distinctly 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). Its 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 pigmenturia, 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,
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 °C. 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) 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 standard curve using a 5-point fitted equation computed from commercial software (Magellan™ 7.2, Tecan, Austria).

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 thromboplastin 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

Alice, a 3-year-old female lactating English Springer Spaniel...
weighing 18 kg, was suspected by the owner to have been bitten on the nose by a RBBS at 2 p.m. on 3/4/2015. A RBBS had been seen in the vicinity of the dog earlier in the day. The dog was presented for veterinary treatment at 9.30 p.m. On initial examination the dog was bright and alert but had a large swelling over the dorsum of the nose. A urine sample collected at presentation was strongly positive for blood, negative for glucose and mildly positive for protein. The ACT was normal (70 s). The PT was 9.6 s and aPTT 18.8 s; both were considered normal. Serum obtained from centrifuged and clotted whole blood upon presentation was moderately haemolysed. Clinical biochemistry revealed no abnormalities with CK and AST within normal range. The dog was placed on intravenous fluids (0.9% NaCl) and administered one vial of tiger-brown snake anti-venom intravenously. The next day the dog appeared fully recovered and was sent home with the owner. The nose was still somewhat swollen. A serum sample collected 12-h after hospitalisation was still mildly haemolysed. RBBS venom was undetectable in serum and milk at both pre or post-antivenom administration. However, the urine sample collected prior to antivenom treatment contained RBBS venom at 2.2 ng/mL. A swab collected from bite site also yielded 8.2 ng/mL RBBS venom.

3.2. Case 2 – Banchee, male husky

Banchee, a 5-year-old desexed male Siberian Husky weighing 25 kg, was observed by the owner to have been bitten by a RBBS on 7/12/2014. The owner reported the dog had bite marks and a swelling developed on the dorsum of the nose shortly afterwards. The dog was presented for veterinary treatment within 1 h. On initial physical examination the dog was bright and alert, no vomiting or drooling and was able to walk normally. There was a small amount of dark bloody material on the dorsum of the nose. A urine sample collected at this time contained trace blood and negative for glucose. The dog was placed on intravenous fluids (0.9% NaCl) and administered one vial of tiger-brown snake anti-venom intravenously. Citrated plasma samples collected before antivenom and at 18 h post-antivenom and analysed revealed PT (14.4 s, 8.7 s), APTT (28.6 s, 16.4 s), and fibrinogen (1.6 g/L, 6.1 g/L). The next day the dog appeared normal and was sent home with the owner. Retrospective analysis of RBBS venom concentrations found extremely high venom concentration in the bite site swab (>5000 ng/mL). Serum RBBS venom concentration at presentation was 12 ng/mL, urine 1090 ng/mL and venom was undetectable in the serum sample collected at 15-min post-antivenom.

3.3. Case 3 – Lasky, 14-week old German Shepherd

Lasky, a 14 week old 15 kg entire female German Shepherd, was presented for veterinary treatment on 1.11.2014 at 8.30 p.m. due to vomiting, salivation and general lethargy. Earlier in the day the dog had appeared entirely normal. The owner had historically observed RBBS on the property. On initial examination the dog was mildly distressed and was making repeated attempts to swallow. Both front legs were covered with saliva because the dog had been drooling extensively. A soft non-painful swelling approximately 4 cm × 4 cm was present under the jaw on the ventral aspect of the neck. The dog was hospitalised and placed on intravenous fluids (0.9% NaCl). An ACT was performed using whole blood and was considered mildly prolonged at 100 s. Both the PT was 18.0 s and aPTT 84 s were mildly prolonged. The PCV at presentation was 43%. A urine sample was collected and this contained moderate amount of blood. A plasma sample collected at the same time was not visibly haemolysed. The dog was treated with one vial of tiger-brown snake antivenom intravenously. The administration of antivenom appeared to markedly reduce the dog’s anxiety. At 14 h after antivenom administration the dog appeared normal and was sent home. At 36 h post-presentation the dog returned because it was again lethargic. A full clinical haematology and biochemistry profile was performed that revealed the PCV had reduced to 19% (37–55) with haemoglobin 54 g/L (115–180) and evidence of regenerative anaemia with 6.9% reticulocytes. Examination of the blood smear revealed morphological abnormalities of the red blood cells including rouleaux formation +, polychromasia ± and a mild thrombocytopenia. Serum biochemistry revealed CK 1363 U (<400 U), ALT 329 U/L (<80) and Alkaline Phosphatase 164 U/L (<120 U/L). The dog was hospitalised and given a transfusion of 450 mL of freshly collected whole blood. The dog’s condition then improved markedly and it was discharged after a further 24 h of hospitalisation. Measurement of RBBS venom concentration demonstrated a high level of venom in serum at initial presentation (52 ng/mL) and urine (1300 ng/mL). No venom was detectable in serum after antivenom administration.

3.4. Case 4 – Lexi, 5 year old female Jack Russell Terrier

Lexi, a 5-year-old female Jack Russell Terrier of 6.2 kg bodyweight, was observed by the owner to have been bitten by a 30 cm long RBBS at 6 p.m. on 25/2/2014. The owner presented the dead snake to confirm the formal identification of the RBBS species. Upon examination at 8 p.m. the dog had an oedematous soft tissue swelling around 4 cm in diameter on the side of its face. The dog was bright and alert. A serum sample collected at initial presentation showed no signs of haemolysis and the PCV was 45%. Clinical serum biochemistry revealed normal CK and AST but markedly elevated amylase 2588 U/mL (400–1300) and lipase 555 U/mL (<70). However, a urine sample collected at this time point had a strong blood reaction and was negative for glucose. A citrated plasma sample collected, immediately frozen and subsequently was assayed for coagulation. The results were within expected normal ranges for a dog with PT 10.0 s, aPTT 21.4 s and fibrinogen 9.4 g/L. At 12 h post-antivenom the dog was negative for glucose and negative for protein. The ACT was performed and at 18 h post-antivenom and analysed revealed PT (0.9% NaCl) and administered one vial of tiger-brown antivenom intravenously. The next day the dog appeared clinically normal and was discharged from the veterinary hospital. RBBS venom concentration in serum measured by ELISA prior to antivenom treatment was 79 ng/mL and urine 69 ng/mL; venom was not detectable in serum after antivenom.

3.5. Case 5 – Macey, Staffordshire bull terrier cross

Macey, a seven-year-old cross bred dog weighing 28 kg, was presented on two occasions approximately 6 months apart for RBBS envenomation. On 24/9/2014 the dog was observed by the owner to have been bitten by a RBBS. The dog walked away after being bitten and then suddenly collapsed. The snake was identified as RBBS by a photograph of the snake taken by the owner. The dog was immediately transported to the veterinary clinic within 30 min of the envenomation. On initial examination the dog was anxious but was not drooling or vomiting but had an increased respiratory rate. An ACT was performed and no clot was detected after 10 min. The dog was placed on intravenous fluids (0.9% NaCl). A serum sample collected and centrifuged showed grossly visible haemolysis. Coagulation studies were performed retrospectively on frozen citrated plasma and revealed an initial PT of >300 s, aPTT >300 s and fibrinogen <0.43 g/L. One vial of tiger-brown snake antivenom was administered intravenously. As the antivenom was being infused the dog’s anxiety appeared to reduce and shortly afterwards the dog stood up and appeared normal. A urine sample collected 4 h post-antivenom showed large amounts of blood and was negative for glucose. At this time point PT had reduced to 78 s.
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but plasma was still grossly haemolysed. The dog was very bright and alert. The next day (24 h post-envenomation) the dog appeared clinically normal and the PT was now 9.2 s, aPTT 20 s and fibrinogen 3.4 g/L. The plasma was clear with no visible haemolysis and although the urine was also visibly clear, on dipstick testing the latter was moderately positive for blood. The dog was sent home with the owner. Retrospective analysis of serum and urine for RBBS venom concentrations showed the dog presented with serum RBBS venom 52 ng/mL and 15-min later this had risen to 65 ng/mL. The urine sample collected at 4 h contained 14 ng/mL RBBS venom. RBBS venom was not detected in any sample collected after antivenom.

Macey was presented for RBBS envenomation on a second occasion on 6/5/2015. The dog was again observed to be bitten on the face by a RBBS. The dog was presented within 1 h of envenomation and had a large soft swelling on the lower lip. A serum sample collected at initial presentation showed no visible haemolysis and the PCV was 46%. The PT was 8.8 s and aPTT 15 s and fibrinogen 1.9 g/L at presentation. The dog was placed on intravenous fluids (0.9% NaCl) and one vial of tiger-brown snake antivenom was administered intravenously. The next day the dog was clinically normal. RBBS venom concentration in the bite site swab was 21 ng/mL and in serum prior to antivenom administration was 7.9 ng/mL.

3.6. Case 6 – Susje, 5 year old desexed female jack russell terrier

Susje, a five-year-old desexed female 8 kg Jack Russell terrier, was presented on 16/12/2015 for lethargy and weakness. The owners had noticed 12 h earlier the dog had developed a large soft swelling on the side of the face but the dog otherwise appeared normal. A serum sample collected at initial presentation demonstrated marked haemolysis. A urine sample collected at this time point was very dark to black in colour with a strong positive reaction for blood and SG > 1.060. Coagulation testing revealed a normal PT of 8.8 s and mildly increased aPTT of 23.2 s. The dog was placed on intravenous fluids (0.9% NaCl) and one vial of tiger-brown snake antivenom administered intravenously. Serum biochemistry revealed elevations of CK 3587 U/mL (50–400), ALT 94 (5–80) and AST 424 (10–80). Haematological profiling demonstrated 3 + spherocytes and PCV 58%. The next day the dog was much brighter and alert but urine was still grossly haemoglobinuric although much less so than at initial presentation. The swelling had extended somewhat more ventrally down the neck but appeared to have reduced markedly at the suspected bite site. RBBS venom concentration in serum prior to antivenom administration was 7.4 ng/mL and urine at 4 h was 625 ng/mL.

3.7. Case 7 – Taylor, 6 year old male Boxer

Taylor, a six-year-old male Boxer dog weighing 38 kg, was presented on 29/10/2015 because the owner had found a dead RBBS in the dog’s yard. On initial examination the dog was bright and alert but had marked soft tissue swelling of the lip. A serum sample collected at this time was grossly haemolysed. The PT was 17.7 s and aPTT 20.3 s. The dog was placed on intravenous fluids (0.9% NaCl) and administered one vial of tiger-brown snake antivenom intravenously. The next day the dog was bright and alert and the swelling on the lip was no longer visible. The dog was discharged from the veterinary hospital having made a full recovery. RBBS venom concentration in serum prior to antivenom was 6.3 ng/mL.

3.8. Case 8 – unknown, Jack Russell terrier (died acutely)

A 3-year old male Jack Russell terrier was presented for veterinary treatment within 45 min of being observed by the owner to have been fighting with a large RBBS when it was observed to have been bitten on the foreleg. The dog was transported to the clinic and presented in extreme cardio-respiratory distress. However the dog died within minutes of presentation. The RBBS bite site was on the lateral aspect of the dog’s elbow and at the time of death was visibly swollen. The skin around the bite site also appeared to be covered with liquid venom. No samples were collected and no post-mortem was performed.

4. Discussion

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 (Heller et al., 2005, 2006) and presents some of the first data comparing bite swab, serum and urinary snake venom levels in naturally envenomed animals. It also presents pilot data on the use of a caprylic acid purified whole equine based bi-valent snake antivenom for black snake envenomation in veterinary practice and thereby complements human clinical studies of this envenomation (Churchman et al., 2010; Pearn et al., 2000).

Consistent with the limited canine literature (Gordon, 1958; Heller et al., 2005, 2006; Kellaway, 1930) this series describes a generally mild-moderate envenomation syndrome (cases 1–7) but with the potential for a fatal outcome (case 8). The consistent clinical findings were soft tissue swelling at the bite site (Figs. 1, 4 and 5), mild pro-coagulant type clotting disturbances, intravascular haemolysis (Fig. 2), mild myopathy and pigmenturia (Fig. 3). Soft tissue swelling was confined to within a 2–5 cm diameter of the bite site and swelling resolved within 2–3 days of onset.

Fig. 1. Photograph of Lexi showing facial swelling and oedema of soft tissues resulting from snakebite from Red-bellied Black snake (Pseudechis porphyriacus).
envenomation. Similar soft tissue swellings in RBBS envenomed humans has been described, occasionally resulting in necrosis requiring surgery if located on the extremities (Pearn et al., 2000). These cases also confirm the apparent clinical effectiveness of bivalent tiger and brown snake antivenom for the treatment of RBBS envenomation and the safety of this new whole equine IgG antivenom in dogs. The pilot data reported here follows a previous use of this product for a common tiger snake envenomation in a cat treated at the same hospital (Padula and Winkel, 2016). The ELISA data in that earlier case demonstrated a complete clearance of venom from serum within 15 min of treatment without detectable recurrence of venom antigenemia as measured out to 36 h. Given the positive experience of other equine based tiger/brown anti-venoms for the treatment of RBBS envenomation (Ong et al., 2015), it seemed highly likely that this whole IgG product would demonstrate comparable efficacy. Certainly the response to antivenom reported here was rapid as evident in the clinical recovery and absence of detectable venom components post-antivenom in

Fig. 2. Photograph of centrifuged, clotted whole blood sample from ‘Alice’ showing marked haemolysis of serum.

Fig. 3. Urine sample from Susje showing marked haemoglobinuria.

Fig. 4. a (top). Deceased dog witnessed by owner to have been bitten by Red-bellied Black snake and died within 45 min of bite. b (lower). Marked swelling of subcutaneous tissues over left elbow joint with venom grossly visible on skin.
cases where this was measured. It was also free of any obvious immediate or delayed adverse reactions, in contrast to the reported 36% rate of immediate hypersensitivity reactions noted in a recent human RBBS series treated with equine F(\(\text{ab}^\prime\))\(_2\) antivenoms (Churchman et al., 2010). The rapid clinical responsiveness reported here is consistent with earlier studies conducted in experimentally envenomed monkeys, injected with RBBS venom, and subsequently treated with equine antivenom (Sutherland et al., 1981). However a RBBS envenomation case report of a dog that was euthanased due to anuric renal failure, despite receiving bivalent CSL tiger/brown snake antivenom 15 h after envenomation, demonstrates the potential of un-neutralised circulating venom to cause secondary complications and death (Heller et al., 2006). The one envenomation in this series that clinically progressed after antivenom infusion, case 3, presented at an unknown time after the snakebite. Both of these cases illustrate the need for clinical vigilance concerning supportive snakebite management in that life-threatening envenomation complications can occur despite the administration of appropriate doses of antivenom. It also reiterates the value of early antivenin use once clinical indications appear.

Coagulation abnormalities associated with RBBS envenomation are generally mild, as observed in the majority of the cases presented here and reported for humans elsewhere (Churchman et al., 2010; Pearn et al., 2000; Sutherland and Tibballs, 2001). However case 5 did present with classic features of venom induced consumption coagulopathy with depleted fibrinogen and very prolonged \(\text{APTT}\). This combination, of prolonged clotting times accompanied by depleted fibrinogen and elevated fibrin degradation products (FDPs), has also been reported in a RBBS bite to a 1.5 year-old (Sutherland and Tibballs, 2001). One adult human case has also reported elevated D-dimer levels and FDPs after definite RBBS envenomation (Pearn et al., 2000). Hence, although uncommon, but consistent with the isolation of a prothrombin activator toxins in this venom (Chester and Crawford, 1982; St Pierre et al., 2005), a consumption-type, rather than anticoagulant-type, coagulopathy seems most clinically important for this venom in domestic animals and small children. This difference, from a recent human case series that did not report any evidence of RBBS venom-induced prothrombin activation (Churchman et al., 2010) might reflect species and developmental haematological differences. Indeed such age-related differences in human haematological responses to Australian snake venoms have been previously documented for coastal taipan (\textit{Oxyuranus scutellatus}) and common tiger snake (\textit{N. scutatus}) venoms (Kern et al., 2008).

RBBS venom contains a potent direct haemolysin that induces extensive haemolysis \textit{in vitro} (Doery and Pearson, 1961) and \textit{in vivo} (Martin, 1893). Consistent with these experimental findings and a previous RBBS bite case report (Heller et al., 2005, 2007), most animals reported here demonstrated some degree of haemolysis (cases 1, 3, 5, 6 and 7). Further, red blood cell abnormalities (spherocytosis) were observed in two cases in this series. RBBS venom has been show, \textit{in vitro}, to cause a range of red blood cell conformational abnormalities due to lipid membrane damage from phospholipase enzymes (Condrea, 1979). Indeed RBBS venom contains potent phospholipase enzymes that have been shown to be at least four times more active than those found in \textit{N. scutatus} (Ramasamy et al., 2004). The phenomena of red blood cell spherocyte formation following exposure to venom has also been reported in dogs suffering from tiger snake (\textit{N. scutatus}) envenomation (Ong et al., 2015). However in these tiger snake cases the effect was delayed several days rather than hours. \textit{In vitro} studies of other venoms have also shown that increasing doses of venom increase the degree of red cell membrane changes and spherocyte formation, particularly with viper venoms (Goddard et al., 2011; Walton et al., 1997). However, in this series, as with an earlier human study (Churchman et al., 2010), there was no clear correlation seen between the observed circulating venom levels and the severity of clinical effects such as the presence or absence of haemolysis. This could be explained by the limited pre-antivenom blood sampling undertaken in both studies resulting in highly truncated time series.

Venom concentrations in serum varied between non-detectable to less than 100 ng/mL, although some dogs had much higher urine concentrations — the first report of black snake venom antigen levels from the urine of naturally envenomed animals. In comparison, a series of human cases of RBBS envenomation serum venom concentrations were in the range 3—360 ng/mL (Churchman et al., 2010). Animal bodyweight varies widely and this likely explains why some animals experience more severe effects of RBBS venom than others. Notably in this case series the fatality was in a low bodyweight dog. Nevertheless the most severe coagulopathy was seen in a 28 kg dog (Case 5 — \(\text{APTT} > 300\) s) demonstrating that even larger animals should be considered vulnerable to this snake’s venom. The comparison here, of urine with other tissues, also shows that the latter is likely to have at least as high a venom concentration as that of serum. Hence, urine can be confidently sampled for immunoassays to aid the diagnosis and management of RBBS bite in dogs.

The venom of the RBBS is dominated by the presence of large quantities of the phospholipase A\(_2\) isoenzyme pseudexin that has neurotoxic effects on mouse diaphragm tissues (Vaughan et al., 1981) and chick biventer cervis nerve-muscle preparations (Ramasamy et al., 2004). Given the rapid clinical deterioration, it seems possible that the high levels of these neurotoxins, combined with small body weight, contributed to the rapid death of the dog in case 8. Alternatively, early cardiovascular collapse, strongly associated with the presence of prothrombin activators in other

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**Fig. 5.** RBBS bite site inside lower lip on Macey at second presentation for treatment.

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Austalian snake venoms (Allen et al., 2012), may be responsible for the rapid fatality. This is particularly plausible as no dog showed evidence of even mild neurotoxicity whilst the animal most affected by coagulopathy (case 5), also developed early post-bite ‘collapse’. Indeed, as case 5 and the earlier fatal case (Heller et al., 2006) demonstrate, both coagulopathy and myohaemoglobinuric-type acute renal failure are potentially lethal aspects of this envenomation in dogs.

The major limitation of this study was the small sample size resulting in significant variation in animal size (6–38 kg), time to presentation (30 min to at least 12 h post-bite), venom levels (urine levels from 2.2 to 1300 ng/ml). Despite this degree of variation, a normal aspect of veterinary practice, there was a remarkable consistency to the observed envenomation syndrome. Clearly it would be advantageous to enrol a larger series of animals and collect a larger number of bite site, serum and urine samples to more formally assess the relationship between venom antigenia across the various tissue compartments. A second limitation is the question as to how the tested antivenom compares to older tiger/brown bivalent antivenoms such as the CSL Limited (now Sequirus Limited) or Summerland Serums that are commonly used in clinical practice. The logical next step is a non-inferiority trial directed against various clinical end points for all three products. Given the apparent importance of RBBS envenomation in Australian veterinary practice (Heller et al., 2005), this seems a feasible and worthwhile goal.

In conclusion, although the envenomation syndrome in dogs caused by RBBS envenomation is usually mild in respect of clinical signs and coagulation disturbances, and is highly responsive to antivenom therapy, severe manifestations and complications can occur. Veterinary practitioners should be vigilant for such possibilities even after the administration of appropriate doses of antivenom.

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