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Case report

Suspected anaphylaxis and lack of clinical protection associated with envenomation in two dogs previously vaccinated with Crotalus atrox toxoid

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ABSTRACT

Objective: to describe the clinical presentation of two canines present in anaphylactic shock secondary to rattlesnake envenomation. In both cases, there was no previous documented previous envenomation event and the initial sensitization required for anaphylactic response is believed to be secondary to Crotalus atrox toxoid vaccine.

Case description: In the first case, a 12-year-old golden retriever present for collapse, severe hematochezia, and vomiting after first time envenomation from a suspected western diamondback rattlesnake. The patient presented in severe hypovolemic shock and required aggressive fluid therapy, antivenom, anti-emetics, and pain management. The patient made a full recovery within 24 hours. In the second case, an 8-year old English setter presented for acute collapse, vomiting, and facial swelling after suspected first time envenomation from a suspected Prairie rattlesnake. The patient presented in severe hypovolemic shock with cardiac arrhythmias and required aggressive fluid therapy, antivenom, pain control, anti-emetics, and antibiotics. The patient made a full recovery after three days of hospitalization. Both patients had been previously vaccinated with the *C. atrox* vaccine.

Conclusion: This case report documents suspected anaphylaxis in two canine patients after first time envenomation by a rattlesnake. Both patients were previously vaccinated by the *Crotalus atrox* toxoid, which is hypothesized to be the initial inciting trigger.

1. Introduction

There are approximately 8000 venomous snakebites occurring in the human population in the United States each year (Gold, 2004) and are a significant clinical problem for the small animal veterinarian. The majority of venomous bites sustained in the United States are from the family *Viperidae,* subfamily *Crotalinae,* which includes rattlesnakes, copperheads, and water moccasins. It is thought that the eastern and western diamondback rattlesnakes (*Crotalus adamanteus, Crotalus atrox,* respectively) are responsible for the most morbidity and mortality due to their venom potency and widespread geographic distribution (Juckett and Hancox, 2002).

Venom from a rattlesnake contains multiple substances, including small peptides and enzymes that contribute to multisystemic disease, with hematologic/coagulopathic abnormalities, local tissue necrosis/inflammation, and in some cases, neurologic impairment (Armentano and Schaer, 2011). The mainstays of therapy for rattlesnake envenomation include fluid resuscitation, antivenom administration, and pain management.

In rare instances, patients can present in anaphylactic shock with venom playing the role as the trigger leading to immunologic reaction and mast cell degranulation (Hogan and Dire, 1990). In most instances, this is noted in patients with a previously known envenomation event. In this case series, two patients presented for rattlesnake envenomation in concurrent anaphylactic shock with no previous sensitization event. It is hypothesized in both patients

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that the previous sensitization event was from repeat vaccinations with *C. atrox* toxoid vaccine.

2. Case report

2.1. Case 1

A previously healthy 12-year old female spayed golden retriever presented to the emergency service in Maricopa County, Arizona for rattlesnake envenomation. The owner witnessed a rattlesnake bite to the left rostrolateral muzzle immediately prior to collapse and approximately 30 minutes prior to emergency room presentation. The dog suffered acute collapse, vomiting, and hematochezia within minutes of the bite. The patient suffered no previous rattlesnake envenomation. Three prior injection of the *C. atrox* toxoid vaccine were administered one year apart, with the last vaccination given one year prior to this envenomation (March 2012, March 2013, April 2014).

Physical examination showed marked obtundation, lateral recumbency, and inability to stand, tachycardia (200 bpm), weak femoral pulses, muddy mucous membranes with a prolonged capillary refill time (CRT) > 3 seconds, tachypnea (60bpm) and a rectal temperature of 101.8F (39.7C). Doppler blood pressure was measured at 60 mmHg and electrocardiogram identified a narrow complex tachycardia. Two small puncture wounds with mild hemorrhage and minimal swelling were noted on the left rostolateral muzzle. Retching with rhythmic abdominal contractions and multiple episodes of large volume hematochezia occurred during initial assessment.

The dog was treated with oxygen delivered via loose fitting facemask, shock fluid therapy in titrated aliquots that consisted of the following during the emergency phase of resuscitation: 60mL/kg of Normosol-R,[1] 3mL/kg of 7.1% NaCL,[2] and 5ml/kg of Vetstarch.[3] Fluid therapy was continued following optimized endpoints of resuscitation at 4.8mL/kg/hr of Normosol-R[1] and 1.6mL/kg/hr of Vetstarch.[3] Hydromorphone[4] (0.1mg/kg intravenous) was administered for analgesia. The dog was admitted to the intensive care unit for ongoing care and two vials of F(ab′)2 Antivenom, Crotalidae polyvalent, equine origin[5] (Venom Vet) were administered over two hours. A single dose of maropitant citrate[6] (1mg/kg) and pantoprazole[7] (1mg/kg) were administered. Fentanyl[8] was provided as a constant rate infusion of 3 μg/kg/hr.

While resuscitation efforts were underway, the following diagnostics were obtained: venous blood gas and electrolytes, complete blood count, biochemistry profile, prothrombin time, activated partial thromboplastin time, and blood smear analysis. Initial venous blood gas showed a lactic acidosis (pH 7.33, reference interval [7.34–7.42], Lactate 7.5mmol/L, reference interval [0.3–3.4]). Packed cell volume measured 56%, reference interval (37–55%) and total plasma protein of 42g/L, reference interval (52–82g/dL). The serum biochemistry panel showed a hypoalbuinemia (1.6g/dL, reference range [2.2–3.9]) and hypoglobulinemia (1.9g/dL, reference range [2.5–4.5]). Automated machine complete blood count was within normal limits. Citrate whole blood prothrombin time (PT) was mildly prolonged at 19s [11–17 seconds] and activated partial thromboplastin time (aPTT) was markedly prolonged at >300 seconds [72–102 seconds]. A blood smear analysis showed moderate thrombocytopenia (129 K/μL, reference interval [200–500 K/μL]) with estimated 75% echinocytes and occasional spherocytes. An abdominal focused assessment with sonography for trauma (AFAST) was performed by the author during stabilization (Lisciandro, 2011), showing a double walled gallbladder with no peritoneal free fluid (Quantz, 2009). No pleural or pericardial effusions were appreciated on thoracic focused assessment with sonography for trauma.

A repeat aPTT two hours following completion of antivenom administration showed a mildly prolonged aPTT (110 seconds, reference interval [72–102 seconds]). A blood smear analysis showed no echinocytes or other red blood cell abnormalities. PCV was 42% and total plasma protein was 30g/dL. Venous blood gas and electrolytes were measured 18 hours post presentation, with no abnormalities. A blood smear again evaluated, showing no echinocytes and adequate platelets. PCV was 42% and total plasma protein was 42g/dL with clear serum.

The tachycardia and hypotension resolved at approximately 90 minutes following emergency presentation, with Doppler systolic blood pressure remaining ≥100 mmHg for the duration of hospitalization. Large volume hematochezia persisted for approximately 6 hours following admission, tapering off to smaller volumes throughout remainder of hospitalization. Occasional runs of wide complex pre-mature beats at an accelerated rate of 140 bpm were noted on the electrocardiogram approximately 12 hours following presentation. Specific treatments for this arrhythmia were not pursued and the arrhythmia was much less frequent at the time of discharge to home. The dog was walking and eating with no vomiting, retching, or regurgitation approximately 12 hours following presentation. The dog was discharged to home at 24 hours post presentation on the following medications with instructions to follow up: Gabapentin[9] (6mg/kg every 12 hours), Tramadol[10] (3-4-5mg/kg every 8 hours), and Proviable[11] once daily. The owner was contacted three weeks post discharge and noted that the patient was doing well.

2.2. Case 2

An eight year old, male intact English setter presented to the emergency service in Fort Collins, CO for acute collapse following suspect rattlesnake envenomation. The rattlesnake envenomation was not witnessed, however, two black puncture wounds were noted on the oral mucosa during the physical examination and the dog was out hiking in an area with known rattlesnake encounters. The dog vomited a large amount and then collapsed following the presumptive envenomation. The dog had a previous documented episode of facial swelling after being in the yard approximately two years prior, which required no medical intervention at this time. It is suspected that this prior event was mild facial edema secondary to an insect sting, as rattlesnakes had not been witnessed in the yard of this home and the dog did not develop any other signs consistent with a rattlesnake envenomation at that time. Two prior injections of the *C. atrox* toxoid vaccine were administered one year part, with the last vaccination given 3 months prior to this suspected envenomation (April 2014, April 2015).

The presenting physical examination showed a sinus tachycardia, lateral recumbency, pale mucous membranes with a CRT > seconds, obtunded mentation, moderate unilateral swelling of the rostral muzzle with a well-demarcated 5 cm × 3 cm region of

[1] Normosol-R, Hospira, Inc. Lake Forest, IL.
[2] 7.2% NaCL, MWI. Boise, ID.
[3] Vetstarch, Abbott Laboratories. North Chicago, IL.
[4] Hydromorphone, West-ward. Eatontown, NJ.
[5] Venom Vet, Instituto Biologico. Argentino S.A.I.C.
[6] Maropitant citate injectable, Zoetis. Parsippany, NJ.
[7] Pantoprazole, Zycomed GmBH. Konstanz, Germany.
[8] Fentanyl, West-ward. Eatontown, NJ.
[9] Gabapentin, Amneal Pharamceuticals. Paterson, NJ.
[10] Tramadol, Amneal Pharmaceuticals. Paterson, NJ.
[11] Proviable. Nutramax Laboratories. Lancaster, SC.

black, suspected necrotic oral mucosa associated with two small puncture wounds consistent with a rattlesnake bite. Doppler systolic blood pressure measured during emergency fluid resuscitation showed mild hypotension (92 mmHg systolic).

Diagnostics measured from blood collected on entry included a venous blood gas and electrolytes panel, complete blood count, serum biochemistry profile, prothrombin time, activated partial thromboplastin time, and blood smear analysis. Polycythemia (HCT 57%, reference range [40–55%]), thrombocytopenia (70K/μL, reference range [200–500]) with type 3 echinocytes and gross hemolysis noted on entry. Chemistry panel showed a hypoglycemia (54mg/dL [70–115]), likely associated with delay in sample analysis, elevated creatinine (1.8 mg/dL, reference interval [0.6–1.6]), hyperbilirubinemia (0.8mg/dL, reference range [0.0–0.2]), and severely elevated ALT (4614 U/L, reference range [10–90]), severely elevated AST (4573 U/L, reference range [15–45]), severely elevated GGT (86 U/L, reference range [0–9]), and mildly elevated ALP (168 U/L, reference range [15–140]). Venous blood gas on entry showed hyperlactatemia (1.6mmol/L [0.2–1.44]) with normoglycemia (108 mg/dL, reference range [70–115]) and coagulation profile showed mildly prolonged PT (9.2s, reference range [7.1–9.1]) and aPTT (16.7s, reference range [9.4–15]).

The initial dose of parenteral fluids consisted of a single 15ml/kg bolus of an isotonic crystalloid solution, followed by a constant rate infusion of 2.5–3mL/kg/hr. The dog was admitted to the ICU for ongoing treatments. Analgesia was provided with Fentanyl[8] (3 μg/kg IV bolus, followed by constant rate infection of 3 μg/kg/hr). A single vial of F(ab′)2 antivenom[12] (Veteria Laboratories) was administered over 30 minutes, additional analgesia was provided with a single dose of metadone[13] (0.2mg/kg IV once). Gastrointestinal support was initiated with maropitant citrate[6] (1mg/kg IV q24h) and Pantoprazole[7] (1mg/kg I q24h). Metronidazole[14] was initiated (10mg/kg IV q12h) as well as N-acetylcysteine[15] (140mg/kg IV once, followed by 70mg/kg IV q6h for 6 total doses). Ventricular arrhythmias was noted and monitored via continuous ECG during hospitalization. Specific anti-arrhythmia medications were not initiated at the time and the arrhythmia did improve over time. PT (9.0s), aPTT (14.6s), and manual platelet count (234 K/μL) were repeated twelve hours after infusion of the first vial of antivenom and no abnormalities noted.

The following medications were added after 12 hours of hospitalization; sucralfate[16] (50mg/kg PO q8h in slurry), ondansetron[17] (1mg/kg IV q8h), ketamine[18] (2 μg/kg/min), and metoclopramide[19] (2mg/kg/day). A second vial of antivenom[12] was administered at 36 hours prior to presentation to treat suspect re-envenomation, evidenced by relapsed thrombocytopenia (52K/μL), recurrent idioventricular rhythm, moderate lethargy, increased regurgitation, and anorexia. Ampicillin sulbactam[20] (50mg/kg IV q8h) was also added at this time. All clinical signs improved after the additional vial of antivenom. The dog was discharged to home four days following presentation and appeared to be doing well. He was sent home on tramadol[10] (5mg/kg PO q8h for pain), metronidazole[21] (12mg/kg PO q12h for three additional doses), omeprazole[22] (1mg/kg PO q24h for three days), maropitant[23] (1.6mg/kg PO q24h for two days), sucralfate[16] (50mg/kg PO q8h in slurry), and amoxicillin/clavulanic acid[24] (18mg/kg PO q12h for three additional doses).

3. Discussion

Anaphylaxis is defined as a severe life-threatening systemic hypersensitivity reaction with acute onset of respiratory distress, gastrointestinal upset, and/or hypotension. Most commonly, anaphylaxis is a type I hypersensitivity reaction, requiring prior antigen exposure and subsequent antibody production. Upon antigen re-exposure, crosslinking of Ig-E antibodies and complement occurs resulting in mast cell or basophil degranulation. Mediators released during anaphylaxis include tryptase, histamine, heparin, and platelet-activating factor, which are capable of trigger vasodilation, bronchoconstriction, increased vascular permeability, and coagulopathies. As both patients in this case study had not been known to have previous exposure to natural rattlesnake envenomation but did experience clinical signs consistent with anaphylaxis, it is proposed that the sensitizing antigen was provided by the *C. atrox* vaccine.

Anaphylaxis secondary to initial rattlesnake envenomation is rare in both the human and veterinary patient, likely due to limited exposure to venomous snakes in the environment. In the few case reports in human medicine, many patients suffering from anaphylactic reaction occurred from a second envenomation event, with a previous rattlesnake bite noted (Hogan and Dire, 1990). The most common cause of anaphylaxis associated with rattlesnake envenomation is thought to be secondary to the administration of xenogeneic antivenom (De Silva, 2016) instead of direct exposure to the venom components themselves. Recommendations for administration of antivenom include slow initial administration, monitoring for changes in heart rate, respiratory rate, blood pressure, or temperature. There is no method of preventing systemic reactions to antivenom that has been proven effective, including prophylactic epinephrine (Warrel, 2010).

Anaphylaxis in the dog is most often characterized by severe gastrointestinal signs. This is most likely due to a relatively high concentration of mast cells in the liver and gastrointestinal tract. When these mast cells degranulate, it results in vasodilation and increased blood flow in the hepatic artery while the hepatic vein undergoes vasoconstriction (Miyaji, 2012; Thomas et al., 2013). This combination leads to hepatic congestion, portal hypertension, and the clinical signs of vomiting, diarrhea, and gastrointestinal hemorrhage.

Rattlesnake venom contains numerous enzymes and peptides including hyaluronidase, metalloproteinases, collagenase, and phospholipase, resulting in a venom-induced coagulopathy, endothelial cell damage, and local necrosis. Common physical exam findings following rattlesnake envenomation include tachycardia, mild to severe local tissue swelling, hemorrhage, and ecchymosis at the site of envenomation. Reported hematologic abnormalities include hemoconcentration, echinocytosis, thrombocytopenia, hyperlactatemia, and prolonged coagulation parameters (Brown, 1994). In some patients, rattlesnake envenomation alone without concurrent anaphylaxis can cause systemic collapse, with severe shock secondary to coagulopathy, third spacing of vascular fluid secondary to vasculitis, and subsequent hypovolemia.

As hypothesized above, because both patients had received no previous exposure to rattlesnake venom, the toxoid vaccine is thought to be the initial sensitization in these two cases. A *C. atrox*

[12] F(ab′)2 antivenom, Veteria Laboratories. Ciudad de Mexico, Mexico.
[13] Methadone, Mylan Instititutional LLC. Rockford, IL.
[14] Metronidazole injectable. Baxter Healthcare Corp. Deerfield, IL.
[15] N-Acetylcystine, APP Pharmaceutical, LLC. Schaumburg, IL.
[16] Sucralfate, TEVA Pharmaceuticals USA Inc. North Wales, PA.
[17] Ondansetron, West-ward. Eatontown, NJ.
[18] Ketamine, Akorn. Lake Forest, IL.
[19] Metoclopramide, TEVA Pharmaceutical USA Inc. North Wales, PA.
[20] Ampicillin Sulbactam, Myalan Institutional LLC. Rockford, IL.
[21] Metronidazole tablets, Unichem Pharmacy (USA) Inc. Hasbrock Heights, NK.
[22] Omprazole, Kremers Urban Pharmacy, Inc. Seymore, IN.
[23] Maropitant citrate tablets, Zoetis. Kalamazoo, MI.
[24] Amoxicillin/Clavulanic Acid, Zoetis. Kalamazoo, MI.

toxoid vaccine developed by Hygieia Biological Laboratories[25] is approved for use in canines. The rationale for using this vaccine as snakebite prophylaxis includes promoting antibody formation to venom with the hope of minimized the severity of clinical signs secondary to natural envenomation. In patients previously vaccinated, standard treatment including fluids, pain medications, and antivenom are still recommended. There have no peer-reviewed publications providing evidence of clinical efficacy in snakebitten dogs, and there is no clear vaccination schedule available. A recent study comparing the protective effect of the *C. atrox* rattlesnake toxoid vaccine in mice showed statistically significant increase in survival time in vaccinated mice as compared to unvaccinated (Cates, 2015). However, there was a subset of vaccinated mice that died or required euthanasia due to cardiovascular collapse following exposure to venom earlier than their unvaccinated counterparts. Sensitization to a component(s) of the venom, with a Type I hypersensitivity reaction following subsequent exposure, is thought to play a role in this development. A prospective study in equines comparing the antibody response to natural rattlesnake envenomation compared to a series of the *C. atrox* vaccine found that 28% of horses demonstrated no measurable response to the vaccine series (Gilliam, 2013). The study found that the vaccine did not predictably result in measureable antibodies in all horses, and in those with measurable antibodies, it was not a sustained response. Protective titers have not been established in animals, making a measurable antibody response of dubious significance in regards to mitigating clinical signs following natural venom exposure. A retrospective study evaluating 272 cases of natural envenomation in dogs documented no statistical difference in survival or measures of severity for vaccinated versus unvaccinated dogs (Witsil, 2015). With no proven increase in survivability, changes in treatment recommendations, and the potential for adverse reactions, further peer reviewed, prospective studies should be considered for the *C. atrox* toxoid vaccine.

This case series documents atypical clinical presentations secondary to rattlesnake envenomation. No previous envenomation was noted in the first case highlighted. The second case is complicated by a possible prior natural exposure, based upon focal facial edema and echinocytes observed on a blood smear. This prior event required no medical intervention and there was no pain or classic rattlesnake bite lesion noted. It is in the authors' opinion that this prior event was likely a reaction to insect envenomation, however, we cannot definitively rule out a very low dose of rattlesnake venom.

Cardiovascular collapse, severe gastrointestinal signs (vomiting, hematochezia), and respiratory distress can be associated with severe hypovolemic shock secondary to capillary leakage and third spacing, which can occur following severe rattlesnake envenomation. It is the authors' experience that these clinical signs are exceptionally rare for dogs experiencing rattlesnake envenomation in the Sonoran Desert, and particularly unexpected for victims of the Prairie Rattlesnake (*C. viridis viridis*). An alternative mechanism we propose for the acute cardiovascular collapse in these cases is anaphylaxis to component(s) of the venom. Both of these dogs had previously been vaccinated with the *C. atrox* toxoid vaccine on more than one occasion, which may have served as the initial sensitization required for the development of anaphylaxis.

The treatment for anaphylaxis is focused on intravenous fluid resuscitation and early utilization of intravenous or intramuscular epinephrine (Mink, 2004). These dogs were not treated with epinephrine because the suspicion of anaphylaxis did not occur during the emergency phase of treatment. Anaphylaxis is not a typical result of rattlesnake envenomation and has been observed anecdotally by veterinarians after the rattlesnake toxoid vaccination started being administered to dogs. These two cases are presented in an effort to provide evidence for the suspicion of this unintended consequence of vaccinations. Treatment for rattlesnake envenomation includes intravenous fluids, adequate pain management, and antivenom. Quick identification and treatment for both of these conditions is imperative in a successful outcome and improved prognosis.

Conflict of interests

The authors declare no conflict of interests.

Transparency document

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[25] Hygieia Biological Laboratories. Woodland, CA.