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272 cases of rattlesnake envenomation in dogs: Demographics and treatment including safety of F(ab′)2 antivenom use in 236 patients

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ABSTRACT

Medical records of 272 rattlesnake envenomations of canines from 5 veterinary emergency centers in Maricopa County, Arizona between 2010 and 2012 were investigated. The objectives were to examine the patient demographics, severity of clinical signs, and treatment modalities employed, in order to discuss the outcomes of certain therapies including glucocorticoid use, antibiotic use, rattlesnake vaccination, and safety of antivenom administration in dogs. Evaluation was performed to model each response (survival, proposed canine snakebite severity score (cSSS), and length of stay) as a function of multiple variables. Of the 272 bite incidences, 8 dogs had a fatal outcome. In dogs older than 10 years, there was a greater likelihood of fatal outcome associated with a longer delay between the bite and presentation. 236 of the envenomated patients were treated with a F(ab′)2 antivenom, 24 with a whole immunoglobulin antivenom, and 12 with both products. Overall incidence of acute hypersensitivity reaction was 0.7% with one incident observed in each antivenom group and F(ab′)2 antivenom administration having the lowest rate of acute hypersensitivity reactions; no reactions were life-threatening. Antivenom administration was found to be generally safe in treatment of canine rattlesnake envenomation. In view of the results of this study, in dogs with rattlesnake envenomation, there is no evidence that use of glucocorticoids, diphenhydramine, prophylactic antibiotics, or vaccination lessen morbidity or mortality.

1. Introduction

Snake envenomation is a clinically significant cause of presentation to advanced care facilities for both humans and animals. Approximately 162 snake taxa are native to the United States, about 27 of which are front-fanged venomous taxa, with the majority of these belonging to the family Viperidae, Subfamily Crotalinae. Pit vipers (Crotalidae), including rattlesnakes (*Crotalus* spp), copperheads and water moccasins (*Agkistrodon* spp), and pygmy rattlesnakes and massasaugas (*Sistrurus* spp), are responsible for approximately 99% of the venomous bites sustained in the US (Peterson, 2006). This study examined snake envenomations in Maricopa County, Arizona. Maricopa County is situated in the Sonoran desert, a region reaching over 100,000 square miles with more species of rattlesnakes than any other region in the world (Phillips and Comus, 2000). Maricopa County is home to approximately 30 different species of snake of which 8 are venomous, and 7 of these venomous snakes crotalines (Brennan and Holycross, 2006).

It is common practice to label pit viper venom as tissue toxic and hemotoxic with elapid venom considered neurotoxic; such classification is an oversimplification and misleading (Lavonas, 2012; Russell et al., 1975). Marked variation in venom activity occurs within a species of snake and variation in clinical signs occurs within individual patients. Venom toxins are known to have both local and systemic effects ranging from local tissue necrosis, pain, and vascular endothelial damage, to induction of coagulopathies and other hematological changes, effects on the kidneys secondary to hypoperfusion, primary renal toxins, and rhabdomyolysis, as well as cardiac effects and direct assault on the nervous system (Gopalakrishnakone et al., 1980; Powell et al., 2004).

The therapeutic approach to snake envenomation in dogs is multimodal. The only means of prevention is avoidance, but attempts at envenomation prophylaxis include aversion training,

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environmental control, and use of a rattlesnake vaccine available for dogs, *Crotalus atrox* Toxoid manufactured by Hygieia Biological Laboratories and distributed by sister-company Red Rock Biologics. This rattlesnake vaccine claims efficacy against *C. atrox* venom, and the manufacturer also notes possible protection against venoms of *Crotalus viridis* (including *C. viridis viridis*, *Crotalus oreganus lutosus*, *C. oreganus oreganus*, and *C. oreganus helleri*), *Crotalus cerastes*, *Crotalus horridus*, *Sistrurus catenatus*, *Agkistrodon contortrix*, as well as *Crotalus adamanteus* (Red Rock Biologics). Although the vaccine manufacturer cites evidence during the product's licensing process of formation of protective antibodies against rattlesnake venom in dogs, no canine challenge studies or peer-reviewed studies have been released documenting the vaccine's clinical efficacy (Armentano and Schaer, 2011; Red Rock Biologics). Moreover, boostering recommendations ranging from once yearly to three times yearly following the initial vaccine sequence contribute to difficulty in assessing appropriate use and effectiveness of the product (Red Rock Biologics). The authors have found no peer-reviewed documented studies supporting prophylaxis of snakebite by using avoidance training, behavioral modification, or prophylactic vaccines.

The mainstay of treatment in rattlesnake envenomation is prompt administration of antivenom. In veterinary medicine in North America one antivenom product has predominated for many years: Antivenin (Crotalidae) Polyvalent (ACP), an equine origin pit viper antivenom based on whole immunoglobulin G (IgG) molecules. Newer options include digesting the whole IgG to cleave off the antigen (venom) binding region, termed fragment antigen binding (Fab) region, from the fragment crystallizable (Fc) portion (Fig. 1a, b, and c). The creation of a smaller product lacking the Fc portion of the molecule is believed to not only increase the volume of distribution, but also possibly result in a less antigenic product (Gutierrez et al., 2003; Lavonas, 2012; Morais and Massaldi, 2009; Seifert and Boyer, 2001). These Fab-based antivenoms include Crotalinae Polyvalent Immune Fab (Crofab™), an ovine origin single Fab-based molecule antivenom, and Fab dimer (F(ab′)2) equine origin antibody-derived antivenoms. Crofab™ is often judged to be cost-prohibitive for use in veterinary medicine while use of more cost effective lyophilized F(ab′)2 antivenom was found to be more common among clinics in this study. In addition to use of antivenom, treatment for canine envenomation includes supportive measures such as fluid therapy for intravascular volume support and diuresis, pain management, treatment for blood loss if indicated, respiratory support as needed, with surgical debridement and amputation rarely necessary. In general, glucocorticoid administration is not recommended unless a reaction to antivenom administration is noted (Armentano and Schaer, 2011; Peterson, 2006). In the case of antibiotic prophylaxis, the consensus in human medicine is that antibiotics are not indicated unless evidence of an infection develops; however use of antibiotics in cases of snake envenomation in veterinary medicine remains widespread (Clark et al., 1993; LoVecchio et al., 2002).

The aim of this study was to describe a large population of dogs that were treated for rattlesnake envenomation examining their demographics, severity of clinical signs, and modality of treatments employed. We sought to provide a starting point for further discussion and investigation into the efficacy of different antivenoms and use of rattlesnake vaccination. Little evidence has been produced comparing antivenom effectiveness or describing prophylactic rattlesnake vaccination in veterinary medicine. Moreover, despite lack of evidence in support of such practices, many clinicians continue to recommend the rattlesnake vaccine and will administer glucocorticoids and antibiotics to envenomated patients. By evaluating the impact of such practices on outcome we hope to provide more objective data regarding treatment of rattlesnake envenomated canines.

2. Methods

Two hundred seventy-two (272) dog cases were used in analysis. Inclusion criteria were dogs with a witnessed rattlesnake envenomation or clinical signs consistent with envenomation (rapid swelling, pain, puncture wounds) plus access at the time of onset to rattlesnake(s). Attempts to record or formally verify the envenoming crotaline species were not made. We proposed an adapted canine snakebite severity scoring system based on the already validated human snakebite severity score (hSSS) (Dart et al., 1996). The human SSS (hSSS) has been used as a tool for evaluating the severity and progression of North American pit viper envenomations in adults. It is based on scoring of severity in 6 categories including: the pulmonary, cardiovascular, and gastrointestinal systems as well as character of the local wound and development of hematological abnormalities (Fig. 2). While the scale has a utility in management of envenomation cases, individual patient variables not accounted for, such as prior exposure to snake venom or antivenom products, mean such scales should not be narrowly interpreted. Due to the challenge of grading animals (i.e., lack of verbal descriptive feedback), costs associated with routine measurement of all components of the hSSS that must be paid by the pet owner, and the retrospective nature of the data at hand, an adapted scale, the canine snakebite severity score (cSSS), was created for use in dogs. Financial limitations in veterinary medicine often prohibit diagnostic testing, such as that of PT, aPTT, or fibrinogen for instance, that are necessary for use of an unadapted hSSS. Alternative tests including the 20 min whole blood clotting test (WBCT 20) have been used in resource poor areas in human medicine to detect coagulopathy in snake envenoming and guide treatment with antivenom; this testing is easy to perform requiring only a blood sample and clean glass tubes, however is variably sensitive for detecting coagulopathy and would remain a limitation in using an unadapted hSSS (Lee and White, 1913; Warrell et al., 1977; Sano-Martins et al., 1994). As a result, many veterinarians are forced to prioritize treatment of patients with neutralizing antivenom and supportive care over acquisition of laboratory measurements when client finances are limited. The overall canine snakebite severity score (cSSS) in this study is a summation of the grading of Bite Factor scores and Clinical Sign scores (Fig. 3). In this system each Bite Factor (swelling, ecchymosis, pain, and drainage) is given a score of either 0 (if absent) or 1 (if noted). Clinical signs, similar to the system-based grading in the human SSS, were noted and each clinical sign assigned a score of 1. The sum total of Bite Factor and Clinical Sign scoring resulted in each patient's overall canine snakebite severity score, a baseline score created on initial presentation with higher scores correlating to a generally more severe condition. It should be noted that these records were from hospitals that are within a network and share common case report forms and other data capture methods that enhance standardization of the analysis.

Antivenom administered included Antivenin *Crotalus durissus* and *Bothrops asper* ("F(ab′)2 AV"), a F(ab′)2 product manufactured by Instituto Bioclon, S.A. de C.V, and Antivenin (Crotalidae) Polyvalent (ACP) ("IgG AV"), a whole IgG product manufactured by Boehringer Ingelheim Vetmedica, Inc. F(ab′)2 AV was supplied in a 20 mL labeled injection vial containing a lyophilized white porous solid. Protocol for administration of F(ab′)2 AV at the 5 emergency veterinary hospitals participating included reconstitution of the lyophilized product using 10 mL of sterile 0.9% saline, bacteriostatic water, Lactated Ringers Solution, or Plasmalyte fluid. The reconstituted antivenom was administered on a syringe pump intravenously typically within 30 min depending on severity of clinical signs, patient tolerance of infusion, and clinician preference.

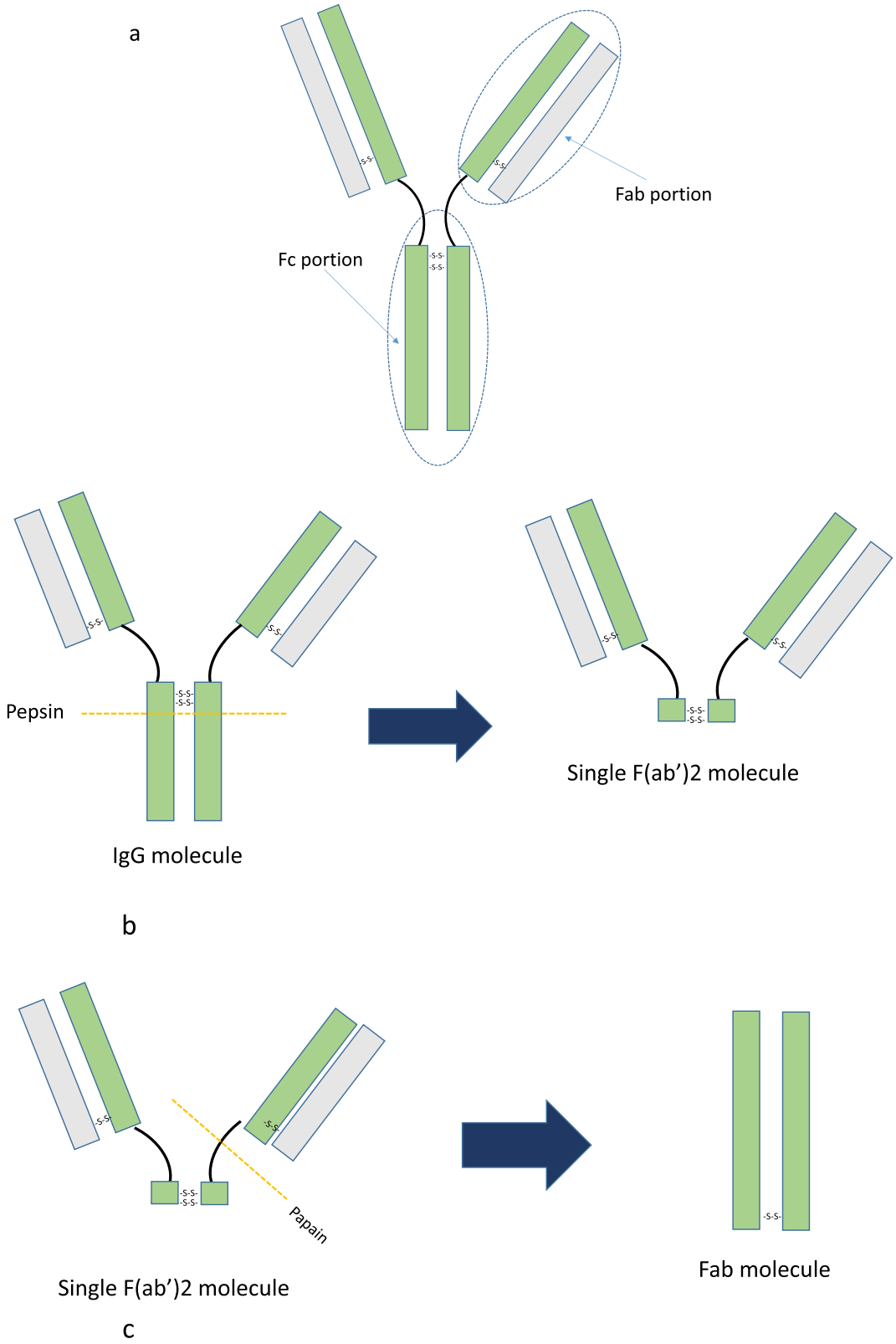

Fig. 1. a Adapted from Lavonas (2012). Schematic representation of an immunoglobulin G (IgG) molecule composed of a single fragment crystallizable (Fc) portion and two identical fragment antigen binding (Fab) regions. b Adapted from Lavonas (2012). Digestion of the whole IgG molecule using the enzyme pepsin to cleave off the fragment crystallizable (Fc) portion creating a single F(ab′)2 molecule. c Adapted from Lavonas (2012). Digestion of the F(ab′)2 molecule using the enzyme papain to create a single F(ab′) molecule.

The Human Snakebite Severity Scoring System [1]

Criterion	Points
Pulmonary System	
No symptoms/signs	0
Dyspnea, minimal chest tightness, mild or vague discomfort, or respirations of 20 to 25	1
Moderate respiratory distress (tachypnea, 26 to 40 breaths/minute; accessory muscle use)	2
Cyanosis, air hunger, extreme tachypnea, or respiratory insufficiency/failure	3
Cardiovascular System	
No symptoms/signs	0
Tachycardia (100 to 125 beats/minute), palpitations, generalized weakness, benign dysrhythmia, or hypertension	1
Tachycardia (126 to 175 beats/minute) or hypotension, with systolic blood pressure greater than 100 mm Hg	2
Extreme tachycardia (>175 beats/minute}, hypotension with systolic blood pressure <100 mm Hg, malignant dysrhythmia, or cardiac arrest	3
Local Wound	
No symptoms/signs	0
Pain, swelling, or ecchymosis within 5 to 7.5 cm of bite site	1
Pain, swelling, or ecchymosis involving less than half the extremity (7.5 to 50 cm from bite site)	2
Pain, swelling, or ecchymosis involving half to all of extremity (50 to 100 cm from bite site)	3
Pain, swelling, or ecchymosis extending beyond affected extremity (more than 100 cm from bite site)	4
Gastrointestinal System	
No symptoms/signs	0
Pain, tenesmus, or nausea	1
Vomiting or diarrhea	2
Repeated vomiting, diarrhea, hematemesis, or hematochezia	3
Hematologic	
No symptoms/signs	0
Coagulation parameters slightly abnormal: PT, <20 seconds; PTT, <50 seconds; platelets, 100,000 to 150,000/mL; or fibrinogen, 100 to 150 ug/mL	1
Coagulation parameters abnormal: PT, <20 to 50 seconds; PTT, <50 to 75 seconds; platelets, 50,000 to 100,000/mL; or fibrinogen, 50 to 100 ug/mL	2
Coagulation parameters abnormal: PT, <50 to 100 seconds; PTT, <75 to 100 seconds; platelets, 20,000 to 50,000/mL; or fibrinogen, <50 ug/mL	3
Coagulation parameters markedly abnormal, with serious bleeding or the threat of spontaneous bleeding: unmeasurable PT or PTT; platelets, <20,000/mL; or undetectable fibrinogen; severe abnormalities of other laboratory values also fall into this category	4
Central Nervous System	
No symptoms/signs	0
Minimal apprehension, headache, weakness, dizziness, chills, or paresthesia	1
Moderate apprehension, headache, weakness, dizziness, chills, paresthesia, confusion, or fasciculation in area of bite site	2
Severe confusion, lethargy, seizures, coma, psychosis, or generalized fasciculation	3

PT, prothrombin time; **PTT,** partial thromboplastin time

Points are assessed on the basis of manifestations caused by the venom itself (antivenom reactions not included). Ranges given are for adults; appropriate compensation should be made for age.

[1] Dart, R. C., Hurlbut, K. M., Garcia, R., and Boren, J., 1996. Validation of a severity score for the assessment of Crotalid snakebite. Ann. of Emerg. Med. 27(3), 321-326.

Fig. 2. The human snakebite severity scoring system[1].

Infusion was initiated slowly at 0.5 mL/kg/hr for the first 5 min to monitor for immediate complications before increasing the rate of infusion. Administration of IgG AV in this study was primarily by referring clinics prior to transport to the participating emergency hospitals. The IgG AV is supplied as a lyophilized reddish-white opaque porous solids in a 10 mL vial for injection. Reconstitution for administration is generally similar to that described for F(ab′)2 AV, however precise methods for administration of the IgG AV product in each patient were not uniformly recorded by referring clinics and cannot be described in this paper.

Of the two hundred seventy-two data sets employed, one dog presented twice within a 3 month period for separate envenomation incidents; these data were analyzed as repeated measure. Outcome in the dataset was evaluated initially by the canine snakebite severity score and overall by survival. Evaluation was performed to model each response (survival, canine Snakebite Severity Score, and length of stay in hospital) as a function of variables including age, prior rattlesnake vaccination, location of bite, antibiotic use, type of antivenom administered, number of vials of antivenom used, use of glucocorticoids, use of diphenhydramine, and time to presentation. Further analysis was performed evaluating the relationship between body weight and vials of antivenom and location of bite, antibiotic type employed, and month of envenomation.

The data were converted into ranks and parametric statistical analyses were performed. Linear regression was used to evaluate differences of continuous outcomes among categorical variables. Medians were used to describe the score data. Some variables such as age and time to presentation were categorized into meaningful categories for analysis. A correlation was used when both the variables of interest were continuous. If there are 2 categorical variables, a Fisher's exact test was used to determine the limit of statistical significance. A p-value of 0.05 was used to determine statistical significance.

The Canine Snakebite Severity Scoring System (cSSS)	
Criterion	Points
Bite Factors	(0=absent, 1=present)
Swelling	0/1
Ecchymosis	0/1
Pain	0/1
Necrosis	0/1
Drainage	0/1
Bite Factor Sum	**Sum**
Clinical Sign Score	
One point assigned to each clinical sign noted whether cardiovascular, pulmonary, gastrointestinal, neurological, thermal, or other	
Clinical Sign Score Sum	**Sum**
Canine Snakebite Severity Score = Bite Factor Sum + Clinical Sign Score Sum	

Fig. 3. The canine snakebite severity scoring system (cSSS).

3. Results and discussion

Two hundred eighty-seven records (287) involving rattlesnake envenomation of canines were identified amongst five emergency veterinary hospitals in Maricopa County Arizona between 2010 and 2012. Of these two hundred eighty-seven incidents, fifteen were excluded from analysis due to a lack of vital data, principally the type of antivenom administered and number of vials used. In total, 272 bite incidents were included for analysis.

3.1. Patient characteristics and epidemiological features of envenoming

Fig. 4 depicts characteristics of the envenomated patients. Ages ranged from 5 months to 14½ years with males and females nearly equally represented. Fifty-eight different breeds of dog were represented with the largest proportion being Labrador Retrievers or mixes thereof (36 of 271 dogs). The envenomations occurred throughout the year with the earliest bite occurring January 5th, and the latest bite occurring December 16th; most bites, however were clustered in the late spring to early fall.

Patient Characteristics
Age Range: 5 mo - 14.5 yrs Median: 2.75 yrs
Sex Male: 129 (113 neutered, 16 intact) Female: 143 (117 spayed, 26 intact)
Weight: Range: 0.9 kg - 66.1 kg Median: 19.8 kg
Breeds Most Encountered (number cases) Labrador Retriever (36); Chihuahua (15); Jack Russell Terrier (13), Dachshund (12); Yorkshire Terrier (10)

Fig. 4. Patient characteristics.

3.2. Outcome: the data were analyzed for outcome based on survival and snakebite severity scoring (cSSS)

3.2.1. Survival

Of the two hundred seventy-two bite cases, 4 dogs died and 4 were euthanized associated with the envenomation. The low number of fatalities prohibited statistical comparison of the data on survival. Subjective evaluation of the data revealed that none of the four dogs to die presented to a hospital within 2 h of the envenomation. This is consistent with previous studies noting that the timing of antivenom administration in relation to bites likely plays a role in outcome (Hackett et al., 2002) and that specifically the neutralizing ability of antivenom against hemorrhagic, edema-forming, and myotoxic activities possibly decreases as the time-lapse between envenomation and antivenom administration increases (Leon et al., 1997). It is reasonable to infer that the sooner antivenom is administered after envenomation, the more effectively it can blunt the complex cascade of venom-induced pathophysiological effects.

Further evaluation of fatal cases revealed that all 8 dogs received F(ab′)2 AV prior to death, each dog receiving an average of 2.12 vials. For reference, surviving dogs received an average of 1.46 vials. It is suspected that the higher dose of antivenom received by dogs with a fatal outcome is an indication of the severity of the patient's condition and the clinician's subsequent decision to employ a higher dose of antivenom, not that higher doses of antivenom result in nonsurvival. Five of the 8 fatal cases received glucocorticoids (1 of which also received diphenhydramine), with only 8% of surviving patients having received glucocorticoids (9% having received diphenhydramine). While the potential for benefit related to glucocorticoid administration has been discussed in terms of decreased inflammation and pain, the potential for glucocorticoids

to accentuate the effects of venom has also been cited (Armentano and Schaer, 2011; Hackett et al., 2002). The results of this study provide no support for the administration of glucocorticoids. It is suspected that glucocorticoids are administered, often ineffectively, to patients that are most severely affected and not responding to conventional therapy.

Other factors found to be related to survival include location of bite and patient age. The majority of patients in this study (87%) were bitten on the head, including 6 of the 8 non-survivors. This may reflect the increased risk of venom more rapidly entering the vascular system in facial bites due to lymphatic drainage, microvascular network, or other anatomical or physical differences between the face and extremities, or the potential for swelling that may interfere with respiration. Moreover, of the 49 presentations of patients under 2 years of age, none died or were euthanized. 2%, 4% and 7% of individuals between 2 and 6 years of age, 6 and 10 years of age, and over 10 years of age, respectively, died or were euthanized. The higher percentage of patients to die or be euthanized over 10 years of age may be related to complicating comorbid conditions and/or reduced intrinsic mechanisms to manage toxin elimination. Specific clinical changes or comorbidities leading up to cardiopulmonary arrest or factoring into decisions to euthanize were not recorded.

Additional data evaluating prior medical history and acute clinical changes such as presence of arrhythmias, renal impairment, or neurological deterioration just prior to death, for instance, may have better allowed for assessment of the role of concurrent conditions and age in outcome. Moreover, formal verification of the species of envenoming Crotaline is not routine in veterinary medicine with the absence of pre-hospital personnel to assist in identification and recovery of the snake. Seven species of rattlesnake are present in the region studied, with populations of *Crotalus scutulatus* and *tigris* both having venom containing the neurotoxic component Mojave toxin which may significantly influence clinical signs and outcome (Brennan and Holycross, 2006; Powell et al., 2004; Wilkinson et al., 1991). Species identification may have proved useful in assessing outcome in terms of survival, as well as length of stay and snakebite severity score. Investigation into the role finances play in owner decisions should also be evaluated in the future, as owner cost invariably impacts care and survival in veterinary medicine.

3.2.1.1. Rattlesnake vaccination. A total of 15 of the 271 individuals included in this study were known to be vaccinated with the rattlesnake vaccine. Of the 8 fatal cases, only 1 dog had a history of being vaccinated with the rattlesnake vaccine. 96.5% of unvaccinated individuals survived envenomation with 93.3% of vaccinated individuals surviving envenomation. No significant correlation was found between the canine snakebite severity scores and a history of having had the rattlesnake vaccine. In this study, no measurable benefit could be identified associated with rattlesnake vaccination.

One of the challenges present in this dataset was the lack of detailed background information such as vaccine history for each patient. Rattlesnake vaccination was recorded based on owner's confirmation at the time of presentation. Further information such as date of last vaccination and number of boosters given was not available. Antivenom antibody titer measurements were not measured in these patients. Such information would be helpful in interpretation of results, especially in light of the vaccine manufacturer's suggestion that some dogs may require up to 3 doses of the vaccine in the initial sequence of vaccination to develop sufficient antibody levels (Red Rock Biologics). Scarce information has been published regarding antivenom antibodies titers produced in animals following rattlesnake vaccination. The limited studies available are largely involving horses used in the production of antivenom. Previous reports underline the variability in duration of antibody titers in a variety of species including humans, goats, and horses with protocols for horses used in antivenom production including vaccination (immunization with venom) up to every 2–14 days (Freitas et al., 1991; Gilliam et al., 2013; Glenn et al., 1970; Guidlolin et al., 2010). One study involving 36 horses evaluated venom antibody titers in response to vaccination with the same rattlesnake vaccine marketed for use in canine patients. 28% of the horses demonstrated no response to the rattlesnake vaccine series, with 2 horses developing peak titers 30 days after the first vaccination, 9 following the second vaccination, and 15 after the third vaccination in the series (Gilliam et al., 2013). These data reflect an unpredictable effect of the vaccine on immunity, albeit in a different species than evaluated in this study (Gilliam et al., 2013). Taking this information into account and the fact that no measurable benefit to rattlesnake vaccination could be identified in our study, vaccination for protection of the general canine population from rattlesnake envenomation cannot be recommended by these authors.

3.2.2. Snakebite severity scoring

Two hundred and seventy-two snakebite incidents were graded using the aforementioned canine snakebite severity scoring system (cSSS) (Fig. 3) upon clinical presentation. The overall canine snakebite severity scores ranged from 1 to 11 with most bites scoring between 2 and 5. Prior rattlesnake vaccination was evaluated for association with snakebite severity scores. 203 envenomations involved non-vaccinated animals, 15 involved vaccinated animals, and vaccine status was unknown in 53 cases. No significant association was present between cSSS and vaccination status. Age groups were created for ease of evaluation. Groupings included <2 years of age, 2–6, 6–10, and greater than 10 years of age. Age groups between 6 and 10 ($p = 0.01$, cSSS: 3.73) and greater than 10 ($p = 0.02$, cSSS: 3.93) years of age showed significantly higher snakebite severity scores when compared to those less than 2 (cSSS: 3.14) and 2–6 (cSSS: 3.25) years of age. Bite location was described as head, specifically intraocular, neck, torso, or extremity; with most cases involving bites to the head (221). Bites to the extremities, however showed a significantly ($p = 0.03$) higher cSSS (3.93) when compared to bites of the head (cSSS: 3.28). It is possible that this is secondary to increased pain from focal swelling and compartment syndrome resulting in decreased perfusion to the limb. Two patients were bitten in both the head and extremity during the same incident; these patients showed significantly higher ($p < 0.001$) cSSS (5.0) when compared to bites to the head alone (cSSS: 3.28). Dogs with significantly higher cSSS were more likely to receive glucocorticoids ($p = 0.0005$) as well as antibiotics ($p < 0.0001$). Those that received IgG AV (24 bite incidents) had significantly lower snakebite severity scores (mean score 2.88) compared with those that received F(ab′)2 AV (238 bite incidents, mean score 3.48) and those that received both IgG AV and F(ab′)2 AV (12 bite incidents, mean score 3.58). Since the cSSS in this study was measured at baseline and not a reflection on change in condition, the use of IgG AV in patients with lower baseline cSSS does not have clinical relevance. No significant association was observed between snakebite severity scores and subsequent diphenhydramine administration nor time to initial presentation.

3.2.2.1. Length of stay. Length of stay was significantly ($p < 0.0001$) longer with snakebites affecting both the head and extremity as well as extremity alone as compared to bites solely on the head. Significantly longer durations of hospital stays were found in dogs that received antibiotics ($p = 0.0004$) and dogs that received glucocorticoids ($p = 0.05$). A non-significant positive correlation between hospital stay and number of vials of antivenom was

appreciated, with longer stays being associated with more vials, perhaps due to severity of clinical picture. Similarly, the administration of antibiotics and glucocorticoids may be secondary to the clinician's perception that antibiotics and glucocorticoids are of value in patients' envenomated by pit vipers, although this perception is unfounded. Age, time to presentation, and administration of diphenhydramine were not noted to be significantly associated with length of stay in hospital.

3.2.2.2. Body weight. The number of vials of antivenom administered was evaluated against patient weight. A significant ($p = 0.03$) inverse correlation was noted such that with decreasing body weight higher numbers of vials of antivenom were administered. Although this is logically reasonable in terms of volume of distribution of the toxic components of venom, and likely based on clinical response, it is possible that the number of vials administered to patients was skewed by clinician expectation of this correlation. Other factors including financial means of dog owners in administration of antivenom must also be considered in interpreting these results.

3.2.2.3. Antivenom side effects. In this study, 236 rattlesnake envenomations were recorded in which F(ab′)2 AV was administered, 24 in which IgG AV was administered, and 12 in which both antivenom products were administered. One of the main goals in this retrospective analysis was to describe the safety of antivenom administration in a large population of rattlesnake bitten canines. 2 of the 272 patients in the study experienced apparent acute hypersensitivity reactions to the antivenom. One patient developed urticaria during administration of IgG AV. The other patient was noted to develop a rapid increase in body temperature during administration of F(ab′)2 AV. This corresponds to an acute hypersensitivity reaction rate of 0.7% of all cases in which antivenom was administered (0.4% of cases in which F(ab′)2 AV was administered alone, 2.7% of cases in which IgG AV was administered either alone or in conjunction with the F(ab′)2 AV). Neither patient that reacted had ever been treated for a rattlesnake envenomation previously or received prior vials of antivenom. The patient experiencing a reaction to IgG AV was later treated with a vial of F(ab′)2 AV without reaction noted.

Our findings not only underline the general safety of the aforementioned antivenom products in canine patients, but also suggest that administration of F(ab′)2 AV may be associated with a lower rate of acute hypersensitivity reaction. Removal of the Fc portion that can bind to receptors of basophils and mast cells involved in acute hypersensitivity reactions may explain this association (Lavonas, 2012; Morais and Massaldi, 2009). This finding is consistent with prior results documenting acute hypersensitivity reaction rates in dogs associated with IgG AV administration ranging from 4 to 7% (Berdoulay et al., 2005; McCown et al., 2009). Moreover, when F(ab′)2 AV was examined in healthy dogs, these dogs showed no adverse effects when administered 3 vials of a F(ab′)2 antivenom within 1 h, and only self-limiting edema of the head or neck, vomiting, and subclinical total hypocalcemia in 13% of patients receiving 6 vials within a 1 h period (Woods and Young, 2011). In terms of delayed reactions, in humans the incidence of serum sickness associated with IgG AV administration is reported to affect 50–75% or more of the patients; this risk has also been noted to be proportional to the number of vials of antivenom administered (Corrigan et al., 1978; Jurkovich et al., 1988). Follow-up of patients beyond discharge was not performed in this group of dogs, and it is possible that complications including delayed hypersensitivity reactions or serum sickness were not identified. Alternatively, the relatively low number of vials administered to canine patients on average may diminish the risk of these complications.

3.2.2.4. Antibiotics. Of the 272 bite incidents, only 64 cases (23%) received antibiotic administration. 207 cases received no antibiotics, and antibiotic history was unknown in one case. Three of the 8 cases with a fatal outcome were treated with antibiotics. Of the patients that did not receive antibiotics ($n = 207$), 5 (2.4%) died or were euthanized. The relatively low incidence of antibiotic use in this study is particularly important as previous large retrospective studies reporting rattlesnake envenomations in dogs have documented antibiotic usage rates of 87–90% (Hackett et al., 2002; McCown et al., 2009). In the human literature, prophylactic antibiotics are not considered indicated as the incidence of rattlesnake bite wound infection is low (Clark et al., 1993; LoVecchio et al., 2002). This is suspected to be due to the relatively sterile nature of venom compared to snake saliva as well as the bactericidal nature of crotaline venom against many aerobic pathogens (Clark et al., 1993; Talan et al., 1991). Follow-up data for this canine population were not available, therefore patients with subsequent wound infection could be unrepresented. The most commonly isolated organisms from crotaline species' saliva included *Pseudomonas aeruginosa*, Proteus species, coagulase-negative Staphylococcus, Clostridium species, and *Bacteroides fragilis* (Goldstein et al., 1979). Antibiotics most frequently administered in this study were betalactams (48 cases) providing appropriate coverage for the expected oral flora of the snake saliva or secondary infection from commensal skin organisms. Future studies with follow-up data regarding the rate of bite wound infection in canine rattlesnake envenomations would be interesting; similarly further evaluation of the influence of bite location on the rate of wound infection may prove informative. Most of the patients that were treated with antibiotics in our dataset suffered wounds to the head. We suspect that extremity bites would be more susceptible to infection due to compromised lymphatic drainage and circulation to the limbs as compared to the head and torso, as well as increased risk for nosocomial infections as these patients walk through the hospital during treatment.

4. Conclusion

In conclusion, antivenom is not only the treatment of choice, but also a safe treatment of canine rattlesnake envenomation with a low risk of acute hypersensitivity reaction. Moreover, administration of a single vial of the antivenom proved sufficient in treatment for most of the dogs, but an inverse correlation exists between body weight and vials used indicating that smaller dogs may require more vials of antivenom. This is particularly relevant in veterinary medicine, where cost is invariably a crucial factor in driving pet owner decisions to finance treatment. Despite the fact that many veterinarians continue to administer glucocorticoids, diphenhydramine, and antibiotics to rattlesnake envenomated patients, the routine use of these drugs in rattlesnake envenomated canines is not supported by our results. Furthermore, no measurable benefit could be identified associated with use of the rattlesnake vaccine and its administration cannot be recommended based on the results of this study. Additional prospective studies evaluating response in terms of cSSS to therapy as well as follow-up for delayed complications would be beneficial to our understanding of canine rattlesnake envenomation.

Ethical statement

All authors have read the manuscript and agree to its publication in Toxicon. The rules of ethics presented in the Elsevier's Ethical Guidelines for Journal Publication have been adhered to and affirmed by all authors.

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