Title

Rattlesnake envenomation in 2 Visayan warty pigs

Journal

Journal of Veterinary Diagnostic Investigation, 34(1)

ISSN

1040-6387

Authors

Henderson, Eileen E

Stadler, Cynthia K

Poppenga, Robert H

et al.

Publication Date

2022

DOI

10.1177/10406387211044555

Peer reviewed

Journal of Veterinary Diagnostic Investigation
2022, Vol. 34(1) 136–140

DOI: 10.1177/10406387211044555
jvdi.sagepub.com

Rattlesnake envenomation in 2 Visayan warty pigs

Eileen E. Henderson,[1] Cynthia K. Stadler, Robert H. Poppenga, Javier Asin Ros, Francisco A. Uzal

Abstract. Rattlesnake envenomation is an important cause of morbidity and mortality in humans and animals in the southwestern United States and elsewhere. Two Visayan warty pigs (*Sus cebifrons*) from a regional zoo were submitted for autopsy after being found dead close to a southern Pacific rattlesnake (*Crotalus helleri*) in their enclosure. Both pigs had severe regionally extensive cutaneous, subcutaneous, and muscle hemorrhage and edema with myonecrosis. Additionally, both pigs had lesions consistent with puncture wounds within the oral cavity, and one pig had a similar wound on a forelimb. The history, and gross and histologic findings, were consistent with envenomation by rattlesnake bite. There are few documented cases of snakebite envenomation in pigs, and it had been suggested that pigs may have some degree of resistance to envenomation. Our results indicate that warty pigs are susceptible to the action of rattlesnake venom.

Keywords: *Crotalus helleri*; envenomation; pigs; rattlesnakes; *Sus cebifrons*.

Venomous snakes are found throughout the continental United States.[2,3] The majority of venomous snakes in North America fall into the family *Viperidae* (subfamily *Crotalinae*), which includes rattlesnakes (*Crotalus* and *Sistrurus* spp.), copperheads, and water moccasins (*Agkistrodon* spp.). In California, there are several species of rattlesnakes, including western diamondback rattlesnake (*Crotalus atrox*), Mojave Desert sidewinder (*Crotalus cerastes cerastes*), Colorado Desert sidewinder (*Crotalus cerastes laterorepens*), southwestern speckled rattlesnake (*Crotalus pyrrhus*), southern Pacific rattlesnake (*Crotalus helleri*), Great Basin rattlesnake (*Crotalus lutosus*), northern Pacific rattlesnake (*Crotalus oreganus*), red diamond rattlesnake (*Crotalus ruber*), Panamint rattlesnake (*Crotalus stephensi*), and northern Mojave rattlesnake (*Crotalus scutulatus scutulatus*).

In the Southwest and other regions of the United States, snake bites are an important cause of morbidity and mortality in humans and other animals. An estimated 5,000–8,000 people and >150,000 pets are bitten by venomous snakes in the United States each year, and >50% of those reported envenomations are attributed to rattlesnakes.[2,7,9,14,17,19] In California, 5,365 snakebites were reported to the California Poison Control System (CPCS) between September 1, 1997 and September 20, 2017,[18] and annually >300 snakebites are reported to the CPCS. Morbidity and mortality associated with snakebites are somewhat variable and appear to be associated with several factors including volume of venom injected, relative toxicity of the venom, hospitalization and/or treatment, and the location of the bite. Deaths associated with snakebite envenomation are relatively low and, in one study, lethality in treated dogs was <5%.[14]

The vast majority of the literature regarding snakebite envenomation in veterinary species focuses on clinical management of dogs and cats. Very little has been published regarding the pathology of snakebite envenomation in other animal species. Given the difficulty in visualizing puncture wounds on most animals, rattlesnake envenomation is rarely suspected as a cause of morbidity or mortality unless the bite was witnessed, or a snake was present when the sick or dead animal was found. Visayan warty pigs (*Sus cebifrons*) are wild suids endemic to a few islands in the Philippines and are classified as critically endangered by the International Union for Conservation of Nature.[15] We report 2 cases of suspected rattlesnake envenomation in Visayan warty pigs in a southern California zoologic collection.

Between 2019 and 2020, 2 Visayan warty pigs housed in the same enclosure at a regional zoo were submitted to the San Bernardino branch of the California Animal Health and Food Safety Laboratory (CAHFS) for autopsy. In both cases, the pigs were found dead with a wild rattlesnake (identified as a southern Pacific rattlesnake) in the enclosure. In case 1, the rattlesnake was found dead; in case 2, the rattlesnake was still alive.

California Animal Health and Food Safety Laboratory System, University of California–Davis, San Bernardino (Henderson, Asin, Uzal) and Davis (Poppenga) Branches, CA, USA; Los Angeles Zoo and Botanical Gardens (Stadler), Los Angeles, CA, USA.

[1]Corresponding author: Eileen E. Henderson, California Animal Health and Food Safety Laboratory, University of California–Davis, 105 W Central Avenue, San Bernardino, CA 92408, USA. eehenderson@ucdavis.edu

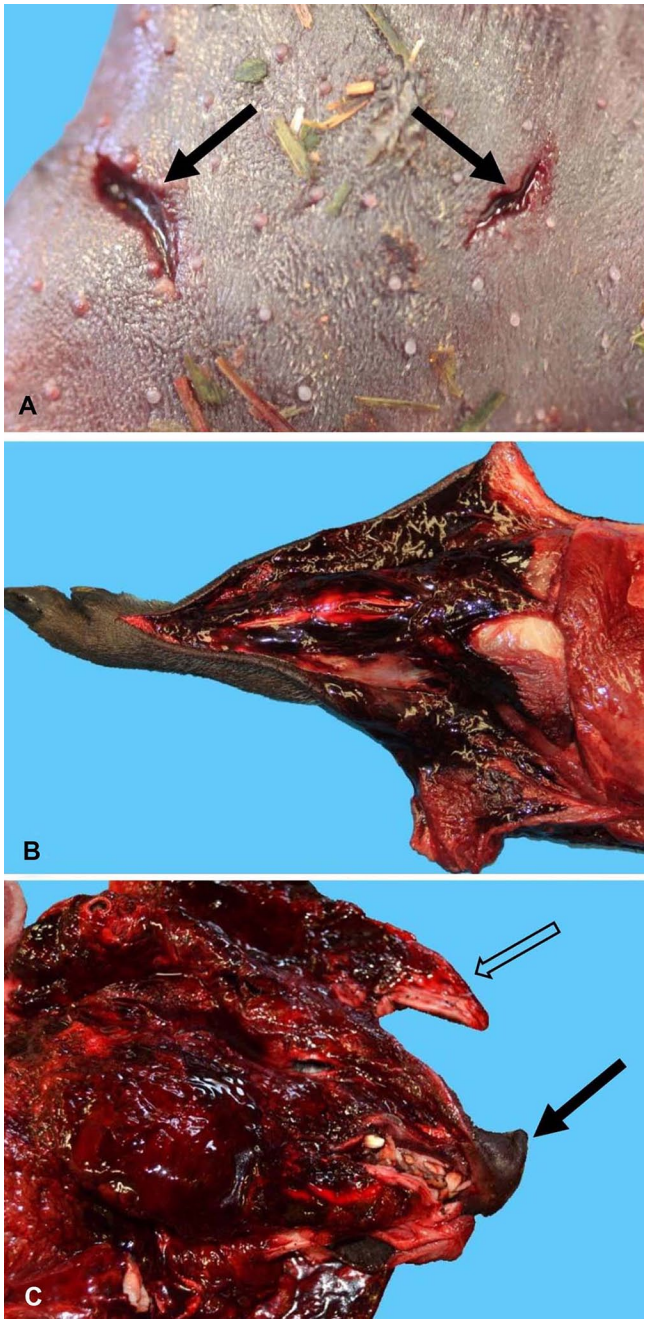

Figure 1. Gross lesions associated with rattlesnake envenomation in 2 Visayan warty pigs **A.** Two tract-like lesions within the tongue (arrows; presumed puncture wounds) in case 1. **B.** Cutaneous, subcutaneous, and muscle hemorrhage of the right forelimb of case 1. **C.** Cutaneous, subcutaneous, and muscle hemorrhage of the head and ventral neck of case 2. The solid arrow points to the snout. The open arrow indicates the skin reflected back from the right side of the mandible.

An autopsy was performed on both pigs. On gross examination, in case 1, 2 puncture wounds were noted on the dorsal and ventral aspect of the tongue (Fig. 1A), and 2 additional elongated puncture wounds were also present in the skin on the lateral aspect of the right forelimb fetlock area. Both animals had severe regionally extensive cutaneous, subcutaneous, and muscle hemorrhage and edema (Fig. 1). Lesions in the right forelimb extended from the coronary band to the shoulder (Fig. 1B), and in the neck, face, and mouth. In case 2, the cutaneous, subcutaneous, and muscle hemorrhage and edema was confined to the head and neck (Fig. 1C). Defects consistent with puncture wounds were noted on the buccal aspect of the oral mucosa.

Ancillary tests were performed following CAHFS standard operating procedures. Briefly, samples from brain, heart, skeletal muscle, haired skin, oral mucosa, kidney, spleen, liver, lung, adrenal gland, tongue, esophagus, stomach, small intestine, and large intestine were collected and fixed by immersion in 10% neutral-buffered formalin (pH 7.2) for a minimum of 24 h. These tissues were processed routinely to obtain 4-µm thick H&E-stained sections. Selected sections were also stained with phosphotungstic acid–hematoxylin (PTAH) and Masson trichrome. Immunohistochemistry was done on selected tissues with antibodies to smooth muscle actin (mouse anti-SMA, BioGenex MU128-UC, 1:300, HIER steamer [S2368], 30 min, PBS-Tween, NHS), desmin (1:400; Dako), and pancytokeratin (mouse anti-pancytokeratin, clone LU5, BioCare CM043C, 1:100, HIER steamer [S1699], 30 min, PBS-Tween, NHS). These special stains and immunohistochemical markers were performed to further investigate the vascular lesions noted microscopically in H&E sections.

Microscopic lesions were similar in both pigs. Sections of skin and muscle from the grossly affected regions of the forelimb or head and neck had cutaneous, subcutaneous, and muscle hemorrhage and edema with foci of acute myonecrosis (Fig. 2A, 2B). In sections of tongue from case 1, a linear tract was noted extending from the superficial epithelium into the deep subepithelial stroma (Fig. 2A). This tract was lined by few neutrophils and fibrin, and the arteries surrounding it exhibited changes similar to those described above. Special stains (PTAH, Masson trichrome, SMA, desmin, pancytokeratin) revealed vascular damage centered predominantly on the muscle fibers present within the tunica media (Fig. 2C–E).

Because gross lesions were compatible with clostridial myositis, fluorescent antibody testing (FAT) was performed to rule out clostridial infection. FAT for *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium septicum*, and *Clostridium sordellii* was performed on subcutaneous and muscle smears of both pigs; FAT was negative for all clostridia investigated in both cases.

Several species of dangerously venomous snakes are indigenous to California, and snake bite envenomation is an important cause of morbidity and mortality in several regions of the United States. Although incidents of snake bites are relatively common in dogs, cats, and other domestic animals, there are few documented cases of snakebite envenomation in pigs.[1] In a paper regarding the tolerance of the Virginia

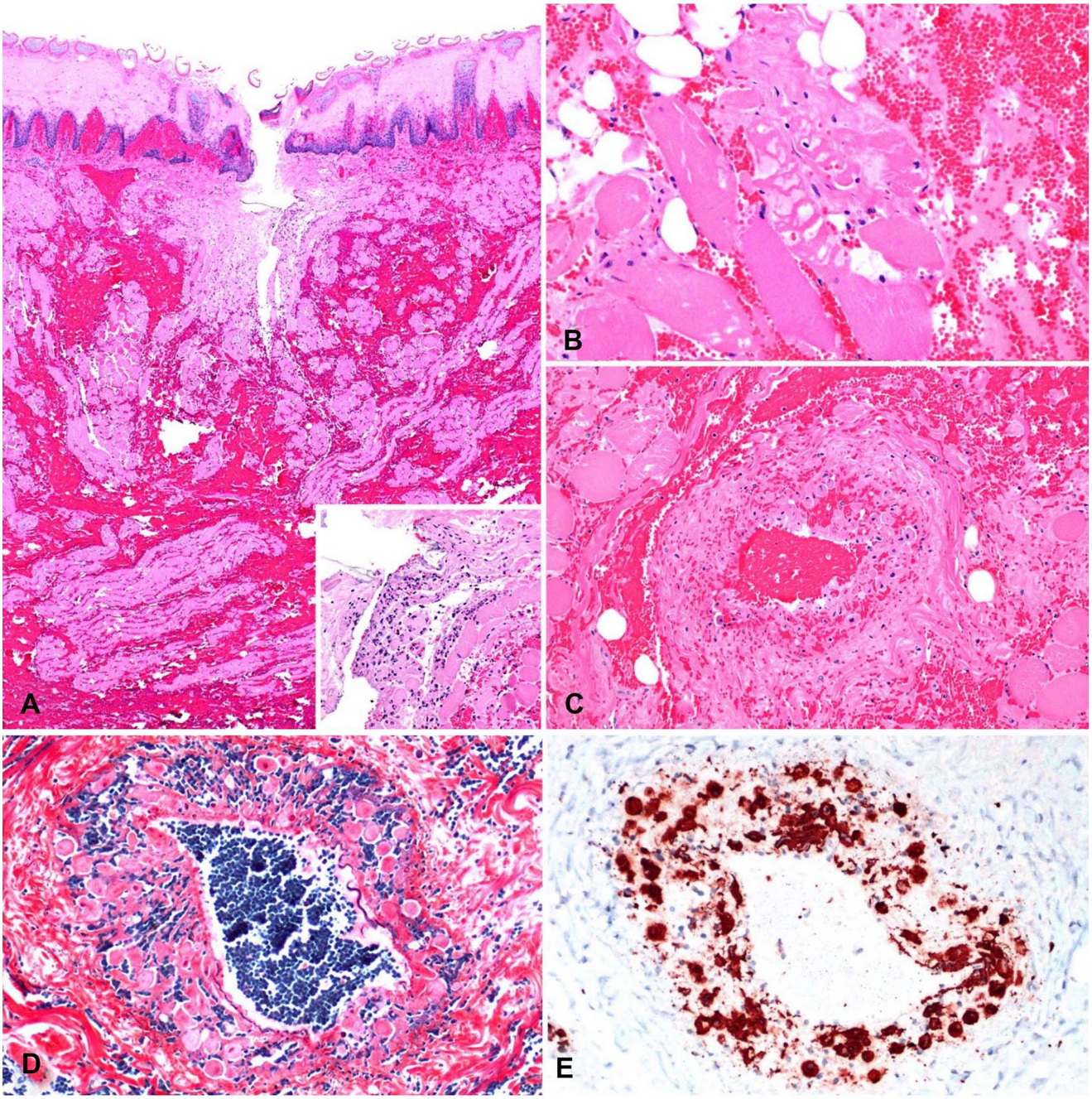

Figure 2. Microscopic lesions associated with rattlesnake envenomation in 2 Visayan warty pigs. **A.** Linear tract-like lesion noted within the tongue of case 2 (presumed puncture wound). Inset: tract was lined by few neutrophils and fibrin. H&E. **B.** Hemorrhage and acute myonecrosis noted in affected sections of skeletal muscle. H&E. **C.** Vascular changes noted within the affected skeletal muscle. Damage to the vessel was centered on the tunica media. H&E. **D.** Phosphotungstic acid–hematoxylin and **E.** smooth muscle actin highlight the muscular fragmentation within the vessel wall (affecting the tunica media predominantly).

opossum to snake venom,[8] brief mention is made of the alleged immunity of wild and domestic pigs to snakebite envenomation, which was attributed to "their tough skins and thick layers of subcutaneous fat which retards systemic absorption." Phylogenetic analysis indicates that pigs have functionally equivalent amino acid changes in their muscular nicotinic cholinergic receptors similar to other venom-resistant mammals (e.g., honey badgers, hedgehogs).[4] However, recent experimental studies suggest that pigs are not immune to the effects of snake venom.[10] Our results confirm that warty pigs are not immune to the effects of snake venom.

Mortality following snakebite envenomation is variable among species. Factors such as the volume of venom injected, toxicity of the venom, location of the bite, age

and species of the snake, and treatment appear to play a key role in clinical progression and prognosis.[14] In studies of rattlesnake envenomation in domestic animals, dogs were often struck on the head or neck, whereas cats were most often struck on a forelimb.[6,21] In both of our cases, the warty pigs had lesions consistent with puncture marks within the oral cavity and one of the pigs also had puncture wounds on the right forelimb. The location of these lesions suggests that these animals were likely investigating or attacking the snakes rather than avoiding them. A study in dogs demonstrated that when presented with newspapers scented with odors from several different animals including different types of snakes, mice, and snails, the dogs chose to sniff the newspaper with odor(s) from rattlesnakes for a significantly greater time than most other provided scents.[16] The study suggests that dogs display no innate fear of rattlesnakes, and their interest in investigating this odor may play a role in why dogs are bitten. No such information is available about pigs.

Rattlesnake venom is composed of a variety of enzymes, peptides, small organic molecules, and inorganic components, and the composition of the venom often has direct correlation with the lesions associated with envenomation.[20] In particular, metalloproteinases are a major component of most snake venoms and contribute significantly to the toxicity of pit viper venom.[13] Several studies have demonstrated that there is intra-species–specific variation in venom profiles with differences between juveniles and adults, males and females, and individuals from different geographic locations.[20] Rattlesnake venoms are often broadly characterized as hemotoxic and/or cytotoxic or neurotoxic, and produce both localized and systemic effects. Although the venom of southern Pacific rattlesnakes (*C. helleri*) is often thought of as being predominantly hemotoxic and/or cytotoxic, a subset of southern Pacific rattlesnakes produces venom with predominantly neurotoxic effects. Hemotoxic effects of pit viper venom typically target the coagulation cascade, and affected individuals often have hypofibrinogenemia, elevated prothrombin time, and thrombocytopenia.[3] In our cases, the venom was most likely hemotoxic and/or cytotoxic; however, given that both pigs were found dead, we cannot definitively rule out a neurotoxic effect.

Thromboelastographic evaluation of dogs bitten by rattlesnakes revealed that most envenomated animals had a clot strength below the 25th percentile (compared to control dogs), suggestive of a hypocoagulable state.[11] Rattlesnake venom is also reported to damage the endothelium and basement membrane of blood vessels and results in localized rhabdomyolysis. The gross and histologic findings in our warty pig cases are consistent with those described for rattlesnake envenomation in other species. Although renal changes were not evident in our cases, renal degeneration, tubular necrosis, and hyaline and granular intratubular casts have been reported in cases of lancehead pit viper (*Bothrops* spp.) envenomation.[12] In a case report of a dog with ascending paralysis following envenomation by a southern Pacific rattlesnake, postmortem changes were limited to the lungs, and the constellation of histologic findings was suggestive of acute respiratory distress syndrome.[5] The lack of renal lesions in the pigs of our study may have been related to the rapid clinical course of the disease after the bite.

Gross and histologic findings were limited largely to the skin, subcutis, and skeletal muscle adjacent to the puncture wounds, and were characterized by regionally extensive hemorrhage, edema, and myonecrosis associated with damage of the surrounding blood vessels. The findings in these cases were consistent with those reported for pit viper envenomation in other species.

Acknowledgments

We thank the animal care staff for their help in maintaining these animals, and the personnel at the histotechnology laboratories at the San Bernardino and Davis branches of CAHFS for production of the histologic slides.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Eileen E. Henderson https://orcid.org/0000-0002-5043-9308

Javier Asin Ros https://orcid.org/0000-0002-6178-4801

Francisco A. Uzal https://orcid.org/0000-0003-0681-1878

References

1. Bolon E, et al. Snakebite in domestic animals: first global scoping review. Prev Vet Med 2019;170:104729.
2. Buchanan JT, Thurman J. Crotalidae Envenomation. StatPearls, 2021.
3. Corbett B, Clark RF. North American snake envenomation. Emerg Med Clin North Am 2017;35:339–354.
4. Drabeck DH, et al. Why the honey badger don't care: convergent evolution of venom-targeted nicotinic acetylcholine receptors in mammals that survive venomous snake bites. Toxicon 2015;99:68–72.
5. Hoggan SR, et al. Mojave toxin-type ascending flaccid paralysis after an envenomation by a Southern Pacific rattlesnake in a dog. J Vet Emerg Crit Care 2011;21:558–564.
6. Hoose JA, Carr A. Retrospective analysis of clinical findings and outcome of cats with suspected rattlesnake envenomation in Southern California: 18 cases (2007–2010). J Vet Emerg Crit Care 2013;23:314–320.
7. Kanaan NC, et al. Wilderness Medical Society practice guidelines for the treatment of pitviper envenomations in the United States and Canada. Wilderness Environ Med 2015;26:472–487.

8. Kilmon JA Sr. High tolerance to snake venom by the Virginia opossum, *Didelphis virginiana*. Toxicon 1976;14:337–340.
9. Lavonas EJ, et al. Medically significant late bleeding after treated crotaline envenomation: a systematic review. Ann Emerg Med 2014;63:71–78.
10. Lewin MR, et al. Delayed LY333013 (oral) and LY315920 (intravenous) reverse severe neurotoxicity and rescue juvenile pigs from lethal doses of *Micrurus fulvius* (Eastern coral snake) venom. Toxins 2018;10:479.
11. Lieblick BA, et al. Thromboelastographic evaluation of dogs bitten by rattlesnakes native to southern California. Am J Vet Res 2018;79:532–537.
12. Machado M, et al. Fatal lancehead pit viper (*Bothrops* spp.) envenomation in horses. Toxicon 2019;170:41–50.
13. Markland FS Jr, Swenson S. Snake venom metalloproteinases. Toxicon 2013;62:3–18.
14. McCown JL, et al. Effect of antivenin dose on outcome from crotalid envenomation: 218 dogs (1988–2006). J Vet Emerg Crit Care 2009;19:603–610.
15. Meijaard E, et al. *Sus cebifrons*. The IUCN Red List of Threatened Species, 2017. [cited 2016 June 11]. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T21175A44139575.en
16. Mulholland MM, et al. The nose may not know: dogs' reactions to rattlesnake odours. App Anim Behav Sci 2018;204:108–112.
17. Peterson ME. Snake bite: pit vipers. Clin Tech Small Anim Pract 2006;21:174–182.
18. Phillips C, et al. Snakebites and climate change in California, 1997–2017. Clin Toxicol 2019;57:168–174.
19. Seifert SA, et al. AAPCC database characterization of native U.S. venomous snake exposures, 2001–2005. Clin Toxicol 2009;47:327–335.
20. Sunagar K, et al. Intraspecific venom variation in the medically significant Southern Pacific Rattlesnake (*Crotalus oreganus helleri*): biodiscovery, clinical and evolutionary implications. J Proteomics 2014;99:68–83.
21. Witsil AJ, et al. 272 cases of rattlesnake envenomation in dogs: demographics and treatment including safety of F(ab′)2 antivenom use in 236 patients. Toxicon 2015;105:19–26.