# From "Snakebites in the New Millennium: A State-of-the-Art Symposium"

## University of Nebraska Medical Center, October 21–23, 2005, Omaha, Nebraska

### RECURRENCE OF COAGULATION ABNORMALITIES IN AN ELDERLY PATIENT AFTER ENVENOMATION BY AN UNSEEN CROTALINE SNAKE IN WESTERN NEBRASKA

*Audi J, Seifert, SA*

*Nebraska Regional Poison Center, Omaha, NE. Email: jaudi@unmc.edu*

**Background:** The venom of North American crotalines contains anticoagulants, procoagulants, fibrinolysins and hemorrhagins, as well as components that alter endothelial and platelet function. Effects include thrombocytopenia, prolonged PT and PTT, fibrinolysis, hypofibrinogenemia, and hypoprothrombinemia. Recurrence of these effects has been described after cessation of Fab antivenom treatment. This case illustrates an obscured presentation of envenomation and a common decision-making problem with recurrent hematologic effects.

**Case Report:** An 80-year-old female, with a history of hypertension, presented after having fallen in her kitchen, sustaining a laceration to the back of her scalp. On presentation, she was confused, diaphoretic, and hypotensive, with a systolic BP ~65 mmHg. She was given an IV NS bolus and started on dopamine. Two punctures at the base of her right thumb were noted, with swelling and ecchymosis of the hand. History was obtained from her of gardening a short time before her fall and possibly being bitten on the hand by something unseen. Initial lab showed platelets 15,000/mm$^3$, serum fibrinogen 115 mg/dL, and INR 1.8. A presumptive diagnosis of rattlesnake bite was made. She was treated with 6 vials of ovine Fab antivenom (CroFab®) over an hour, and then 2 vials every six hours for three additional doses. A CT scan of the head showed no intracranial bleeding. Blood pressure stabilized to 92/48, and continued to improve following antivenom. Progression of swelling stopped at the level of the elbow and did not recur. Platelets rebounded within the hour to 230,000/mm$^3$, but fibrinogen continued to fall, with a nadir of 65 mg/dL and the INR increased to a maximum of 2.3. Dopamine was discontinued on hospital day 2. At discharge on hospital day 5, fibrinogen was 338 mg/dL, INR 1.1 and platelets 140,000/mm$^3$. Two days post-discharge, the patient had a recurrent hypofibrinogenemia of 50 mg/dL. INR was 1.2 and platelets 177,000/mm$^3$. She had hematuria on urine dipstick, was given two vials of antivenom IV, and followed daily. Fibrinogen was 110 mg/dL the following day and remained in that range for several more days. She had no recurrence of thrombocytopenia or elevation of her INR, hematuria did not recur, and she did not have clinically significant bleeding at any time in her course.

**Discussion:** Recognition of signs and symptoms of a rattlesnake envenomation in an endemic area, and rapid response to crotaline antivenom, confirms the diagnosis. Although early, severe hematologic effects are risk factors for late, severe recurrence of the same components, this patient had a recurrence only of one of her hematologic abnormalities, demonstrating the unpredictable nature of this phenomenon. Neutralization, exhaustion or persistence of particular venom components will determine the specific clinical effects. This patient improved following additional antivenom given in response to recurrence of severe hypofibrinogenemia and evidence of bleeding.

**Conclusion:** Recognition of crotaline envenomation is critical in endemic areas. Potentially severe recurrence of coagulation abnormalities after initial control should warrant close monitoring and consideration of additional antivenom for severe coagulation defects or bleeding.

## TREATMENT OF ENVENOMATION BY THE SOUTHEASTERN CORAL SNAKE (*MICRURUS FULVIUS*)

*Gennaro JF, Skimming JW, Van Mierop LHS, Kitchens CS*

*University of Florida-Medical Center Gainesville, FL. Email: jdjanuary@cox.net*

**Objective:** To present an envenomation by a Southeastern coral snake (*Micrurus fulvius*) in a 16-year-old male.

**Case Report:** The patient, a North Florida resident, was bitten on his left index finger by a Southeastern coral snake. He arrived at the local hospital 170 minutes after the bite. Initially there was only a mild swelling of his hand and fingers. Vital signs were wnl. The wound resembled a scratch mark, without an obvious puncture. He was given 1 gm Cefazolin and 8 vials of antivenom (Wyeth Antivenin, *Micrurus fulvius*) intravenously (iv) in saline at the rate of 250 ml/hr. Before administration of antivenom he was given methylprednisolone and diphenhydramine iv. Within one hour he experienced respiratory difficulties and mild bilateral ptosis with fully reactive pupils. By 1.75 hours, the ptosis had increased; there was local pain and numbness proximal to the bite, and a feeling of fullness in his throat. Three hours after the start of the antivenom infusion he became combative, dysarthragic, and had increasing respiratory distress. He was sedated with midazolam 2 mg iv and paralyzed with pancuronium 4 mg iv in preparation for elective endotracheal intubation. At 3 hours post intubation (~10 hrs post bite), he exhibited progressive diffuse muscle weakness, complete ptosis, and paralysis of the extraocular muscles. The bite site swelling had extended into his forearm. He was given 7 more vials of antivenom. Vital signs remained wnl. Copious intrapulmonary secretions necessitated frequent suctioning. Initial laboratory evaluation showed normal hematocrit, white blood cell count 24,000/mm$^3$, PT 11.6 sec (nl) and PTT 23.0 sec (nl). His serum CPK rose to a maximum of 358U/L in the first 24 hours and then began decreasing. Extremity weakness persisted and he continued to be unable to breathe without mechanical assistance. He appeared fully conscious, as determined by purposeful movements of his eyebrows and toes. The fifth day after the bite, he could open his eyelids fully and communicate by moving his eyes up and down or side to side. CXR showed consolidation and tracheal aspirates grew *Klebsiella pneumoniae* and coagulase-positive *Staphylococcus*. He was started on ceftriaxone and oxacillin. Copious intrapulmonary secretions continued and he made no spontaneous respiratory effort for the remainder of the first week. Eight days after the bite, he was able to move his hands, feet, head, and gradually, his extremities. Recovery was faster in the contralateral extremities, with flexor muscles recovering more rapidly than the extensors and the distals more quickly than the proximal ones in all extremities. Ventilatory support was discontinued unsuccessfully 15 days post-bite and successfully 19 days post-bite. He was discharged home 24 days post-bite.

**Conclusion:** This case illustrates (a) the pathophysiology and typical clinical course of coral snake envenomation; (b) the apparent inefficacy of the commercially available antivenom in preventing paralysis; and (c) an apparently favorable effect of the antivenom in ameliorating tissue damage.

## JUST A NAJA ENVENOMATION

*Seifert SA*

*Nebraska Regional Poison Center, Omaha, NE. Email: sseifert@nebraskamed.com*

**Objective:** To describe a privately-collected cobra envenomation in the U.S.

**Case Report:** A 23-year-old female was bitten by her privately collected *Naja Kaouthia*. At presentation, two puncture wounds were seen. There was bruising and some swelling at the bite site, but no persistent bleeding. The prothrombin time (PT) was prolonged at 33 seconds. South African Polyvalent Antivenom (SAIMR) was obtained from the nearest zoo, a process that took many hours. The patient remained otherwise asymptomatic for approximately 8 hours post-bite, when she developed diplopia, dysphagia, and progressive respiratory muscle weakness, ultimately requiring endotracheal intubation. The patient was given one vial of antivenom by slow IV push and an additional 9 vials IV over one hour. She remained intubated overnight and also given propofol and fentanyl, but remained awake and responding appropriately. Vital signs remained stable, with HR ~115, BP 100/80. Following antivenom, the PT decreased to 22 seconds, but the platelet count decreased, with a nadir of 54,000, and fibrin degradation products (FDP) were detected without an abnormal fibrinogen. The patient did not develop any spontaneous bleeding and the Hgb/Hct remained stable. The patient's respiratory motor function improved over 24 hours and she was extubated without difficulty. A small area of necrosis developed at the bite site.

**Discussion:** The patient developed mild local tissue injury, abnormal coagulation parameters without bleeding, and respiratory muscle paralysis requiring intubation. Local injury from cobra envenomations is variable, ranging from minimal effects to massive necrosis. Coagulation abnormalities are also variable, but usually remain mild, without spontaneous bleeding. The major toxicity is neurologic, with death from respiratory muscle paralysis. She was treated with SAIMR antivenom, followed by relatively rapid recovery of respiratory muscle function. This Antivenom is not listed by the manufacturer as indicated for *N. kaouthia* and there are no

clinical reports in the literature regarding its use with this species. Although the post-synaptic motor blockade of cobra venom is considered irreversible and recovery may take days to weeks, there is evidence that patients treated with antivenom recover motor function more quickly. This patient's recovery over 24 hours following antivenom, suggests its efficacy against venom neurologic toxicity. The abnormal PT improved following antivenom, but the thrombocytopenia did not, suggesting either partial activity against venom coagulation factors or an insufficient dose for this effect.

**Conclusion:** Non-native envenomations pose a challenge to U.S. clinicians. Definitive determination of the genus and species of snake may not be possible. Locating and obtaining an appropriate antivenom may be difficult and may result in significant treatment delays. SAIMR Polyvalent Antivenom may offer partial efficacy against *N. kaouthia* envenomation.

## MALES BITING MALES: DOES TESTOSTERONE SHAPE BOTH SIDES OF THE SNAKEBITE EQUATION?

*Cardwell, MD, Bush SP, Clark RT, Dugan EA*

*Loma Linda University, Loma Linda, CA. Email: mikecardwell@comcast.net*

**Objective:** To examine the potential correlation between snakebite epidemiology recorded at a major southern California trauma center and behavior observed in surrounding wild rattlesnake populations.

**Methods:** The seasonality of snakebites was compared to the behavior of wild populations of the rattlesnake species responsible for bites. When possible, biting snakes were identified to species and sex.

**Results:** Of the 78 rattlesnake bites presenting in 2003 and 2004, 65 (83%) patients were male. Most bites occurred in May ($n = 17$), June ($n = 13$), August ($n = 11$) and September ( $n = 11$). A nearby 4-year field study of Mohave rattlesnakes (*Crotalus scutulatus*) established a bimodal mating period (March / April / May and August / September / October). During these months in 2003 and 2004, mean daily movement of male Mohave rattlesnakes (47 meters/day, ±6.93 SE) was more than 3 times that of females (14 meters/day, ±2.36 SE) ($P = 0.001$). Similarly, the mean home range utilized by males (20.4 hectares, ±2.59 SE) was nearly 10 times greater than that utilized by females (2.2 hectares, ±0.43 SE) ($P = 0.001$). Available data for other rattlesnake species responsible for most of the trauma center's bites (*C. helleri, C. ruber, C. cerastes*) also suggest that males are more motile than females with greatest movement occurring during their mating seasons. Of the 12 rattlesnakes responsible for bites and for which sex could be determined, excluding neonates, 10 (83%) were determined to be males ($P = 0.039$, assuming a background population of 50:50 males to females).

**Discussion:** Although limited by small sample sizes in some aspects, our study suggests that male rattlesnakes are more likely than females to bite humans, at least partly because mature male rattlesnakes are significantly more motile than females during their mating seasons and, therefore, encounter humans more frequently. Consistent with previous investigators, our data suggest that male humans are more likely than females to be bitten by rattlesnakes, probably because they more often choose to interact with the snakes.

**Conclusion:** The severity of a snakebite season may be predictable as the factors that stimulate sexual behavior in rattlesnakes become better understood.

## CLINICAL CONTROVERSIES IN NORTH AMERICAN CROTALINE SNAKEBITE

*Dart RC*

*Rocky Mountain Poison and Drug Center, Denver, CO. Email: Richard.Dart@rmpdc.org*

The fundamentals of North American crotaline snakebite treatment have been established for many years. As they have become established, the rate of antivenom use seems to have increased and the use of fasciotomy less common (although it is likely still overused). Nevertheless, many areas of controversy remain. Perhaps the largest topic of disagreement involves the significance and treatment of recurrence. Recurrence has been reported following use of a variety of antivenoms involving snakebites from a range of countries. Most cases of recurrence involve platelet or coagulation abnormalities. The appropriate treatments for these forms of recurrence remain unclear. Equally as controversial is the effect of antibody form on recurrence. It has been presumed that the size of the molecule and the elimination half-life (Fab vs. Fab2 vs IgG) determines the rate of recurrence. The data available to date are insufficient to prove this point. Further, the issue of clinical significance has not been addressed. Another concern involves death from snakebite, which continues to occur despite the availability of antivenoms. There are 3 categories of these deaths. The simplest is death from lack of antivenom administration (i.e. medical error). Two other types of death are equally as troubling. The first is death before antivenom can be administered. Typically these patients suffer sudden cardiovascular collapse or airway compromise within

minutes of their envenomation. To address these deaths, a predictive approach is needed. Finally, there are cases in which the patient dies despite the seemingly adequate administration of antivenom. There at least two potential causes of these problems: time and complexity. An antivenom may contain the correct antibodies, but these antibodies are not present at the right place and at the right time. For example, recurrence of coagulation abnormalities may be due to excretion of the antivenom before the venom has been completely absorbed or distributed. Alternatively, it is possible that a neutralizing antibody was never present in the antivenom. Venoms are notable for their variety and complexity. While some are simple, others are very complex and not well understood. It is unlikely that antibodies to every active component of a venom can be produced in an economically feasible product. It is also unlikely that a single administration schedule will be appropriate for any drug. A new paradigm is needed to understand and investigate the clinical effects of snake venoms as well as the limitations and alternatives to the use of antivenom.

## UNUSUAL PRESENTATION OF ELAPID ENVENOMATION IN THE U.S.

*Rose SR, Poulson BS, Waring ER, Whitlow KS*

*Virginia Poison Center, Richmond, VA. Email: kwhitlow@mcvh-vcu.edu*

**Background:** Envenomations from non-indigenous snakes rarely occur in the U.S. The appropriate antivenom may be difficult to identify, locate, or obtain. Poison centers are uniquely positioned to locate and facilitate delivery of the appropriate antivenom. We present a case of elapid envenomation in the U.S. in which zoos were utilized to obtain antivenom.

**Case Report:** A 42-year-old amateur snake collector was bitten once on his right hand while feeding his black Pakistani cobra (*Naja Naja*). On arrival to the tertiary care center, two hours post envenomation, he began to develop bilateral ptosis and weakness to all extremities. One hour later he was intubated for airway protection and impending respiratory collapse. Tetanus toxoid was administered. Solumedrol and diphenhydramine were administered for urticaria. The nearest metropolitan zoo referred the poison center to the Bronx Zoo in New York and the Florida Antivenin Bank at Miami-Dade Fire Rescue Service. Arrangements were made through the regional poison center for emergent transport of two antivenoms—one via commercial airline from Miami, and the other via police helicopter from New York City. Fifteen vials of non-FDA approved Indian polyvalent (Haffkine) antivenom were administered 8 hours after envenomation. The patient was treated with steroids, antihistamines, and epinephrine for allergic reaction prophylaxis. His platelet, fibrinogen, and coagulation levels remained normal. Within 12 hours of antivenom administration, he was able to move all extremities and was extubated. His right arm, which had a constriction bandage dressing applied prior to antivenom administration, remained swollen and painful for several days. He was discharged the next day with a prednisone taper and levofloxacin for prophylaxis against *Salmonella*, which is endemic in cobras. He was hospitalized ten days later for treatment of an abscess and cellulitis to his right hand and arm, the culture of which grew out *Bacteroides* and *Enterococcus*.

**Conclusion:** Exotic snake envenomations are capable of producing local and systemic symptoms that can culminate in life-threatening toxicity. Having a mechanism in place for the rapid location, acquisition, and administration of antivenom is important for optimal patient care.

## BACTERIAL FLORA IN ORAL CAVITIES OF VENOMOUS REPTILES

*Wiley KL, Harrison JR*

*Kentucky Reptile Zoo. Email: kyreptil@pop.mis.net*

**Objective:** To provide a broad survey of bacterial flora that can occur in snakes' mouths.

**Methods:** A total of 47 wild and captive-bred snakes were tested by mouth swabs that were cultures for bacterial growth. Four groups of animals represented all main venomous reptile families. The first group were cobras (*Naja kaouthia*) captive born at Kentucky Reptile Zoo that were at least two years old. The second group consisted of wild-caught western diamondbacks (*Crotalus atrox*) that had been captured less than one month previous to the culture being taken. The third group were African bush vipers (*Atheris chlorechis*) that were captive born at a different facility and had been at Kentucky Reptile Zoo for only two days. The fourth group contained beaded lizards (*Heloderma horridum*) that were received from a different facility seven days prior to the culture, but the origin of the animals was unknown.

**Results:** 9% of tested animals (N = 4) had pathogenic bacteria in their mouths. In all of these, the bacteria found were *Pseudomonas aeruginosa*. A surprising result of this study was that these cultures did not find any pathogenic bacteria in either the recently captured *C. atrox,* or in the *N. kaouthia* that were captive-born at KRZ. However, both groups of animals that came from a different facility contained animals that harbored these potentially infectious bacteria.

## PROSPECTIVE MEASUREMENT OF INTRA-COMPARTMENTAL PRESSURE FOLLOWING CROTALINE SNAKEBITES AS A DETERMINANT FOR COMPARTMENT SYNDROME DEVELOPMENT

*Shum S[a], Jaramillo JE[b], Franklin R[c], Fernandez M[c]*

[a] *Texas Panhandle Poison Center*

[b] *Department of Surgery, Texas Tech School of Medicine, Amarillo, TX*

[c] *South Texas Poison Center, San Antonio, TX. Email: shumshu@yahoo.com*

**Objective:** To prospectively monitor intra-compartment pressure by direct measurement with an indwelling catheter of a Stryker Monitor on the involved extremity of victims of envenomation and to determine if conservative treatment with CroFab and mannitol has an effect on intra-compartmental pressure.

**Methods:** After obtaining informed consent, patients are being enrolled in one of two participating poison centers in Texas. A Stryker Monitor intra-compartmental pressure-indwelling catheter is inserted to monitor the envenomated extremity's intracompartmental pressure. Once envenomation and progression are documented, pressure measurements are recorded prior to CroFab administration, one hour post-infusion after initial control, and after each subsequent dose of CroFab. If an increased intracompartmental pressure is determined (>30 mmHg), the investigator administers an additional 6 vials of CroFab by iv infusion and 1 gm/kg iv of mannitol, given over 30 minutes. If the intra-compartmental pressure of the involved extremity remains >30 mmHg one hour after completion of the infusion of CroFab and mannitol, evaluation by a surgeon is recommended. If the insertion site becomes infected, the catheter is removed and the patient treated for the infection. The indwelling catheter is removed at the conclusion of the study period or prior to any surgical intervention.

**Results:** This study is currently in-progress. Preliminary results from one patient showed no increase in intra-compartmental pressure of the envenomated extremity.

**Discussion:** Experimental data from rabbits indicated that superficial crotaline envenomation did not raise the intracompartmental pressure of the envenomated extremity. However, deep intramuscular injection of crotaline venom did result in elevation of the intra-compartmental pressure. Envenomated patients present with pain and swelling of the extremities mimic compartment syndrome. Surgeons may perform fasciotomies early, without any supporting data concerning the intra-compartmental pressure of the involved extremity, or prior to adequate antivenom and other pressure-reducing measures.

**Conclusion:** The hypothesis is that prospectively collected data will support conservative, non-surgical management of Crotaline envenomation. Data collection is in-progress.

## PRAIRIE RATTLESNAKE (*CROTALUS VIRIDIS VIRIDIS*) ENVENOMATION: RECURRENT COAGULOPATHY IN A CHILD TREATED WITH IMMUNE FAB

*Lintner CP, Keyler DE, Bilden EF*

*Hennepin Regional Poison Center, Minneapolis, Minnesota. Email: chris.lintner@co.hennepin.mn.us*

**Background:** Local and systemic recurrence phenomena following North American crotaline snake envenomation have been described in adults treated with antivenoms. However, this phenomenon has not been reported previously in cases of prairie rattlesnake (*Crotalus viridis viridis*) envenomation. Information in the pediatric population is limited, specifically with regard to recurrent coagulopathy due to prairie rattlesnake envenomation following Fab treatment.

**Case Report:** We report a case of a 2-year-old boy who developed recurrent coagulopathy after being bitten on the leg by a prairie rattlesnake and treated with Crotalidae Polyvalent Immune Fab® (CroFab). He presented to a rural hospital where initial labs included an INR of 1.16 and platelets 118,000/mm$^3$. He was transported to a second hospital 2 hours after envenomation. Labs at the second hospital included hgb 6.9 g/dl, fibrinogen 117 mg/dl, INR 1.6, and platelets 8,000. Four hours after envenomation, swelling and ecchymosis had spread to the groin and 6 vials of antivenom were infused; an additional 6 vials were given 2.5 hours later. Eight hours after envenomation, the hgb dropped to 5.3, INR was 1.4, and platelets were 280,000. Local swelling had not progressed. The patient received packed RBC's. At 21 hours after envenomation, hgb was 8.1, fibrinogen was 92, INR was 1.2, and platelets were 220,000. The patient's labs remained stable until 68 hours after envenomation, when the platelets dropped to 31,000. Two more vials of antivenom were given. Labs normalized, and pt was discharged 110 hrs after envenomation. He returned 7 days after envenomation, and his platelets had dropped to 42,000. Two more vials of antivenom were given and platelets increased to 88,000. No further complications were noted.

**Conclusion:** This case documents delayed recurrent coagulopathic effects of prairie rattlesnake venom in a Fab-treated pediatric patient. Late coagulopathy can be a serious concern in pediatric patients, warrants close monitoring, but does respond to Fab therapy.

## EPIDEMIOLOGY OF SNAKEBITES IN CHITWAN AND NAWALPARASI DISTRICTS, NEPAL

*Pandey DP*

*Birendra Multiple Campus, Trubhuwan University, Bharatpur, Chitwan, Nepal. Email: vultdeb@yahoo.com*

**Objective:** To characterize the epidemiology of snakebite in Chitwan and Nawalparasi Districts, Nepal.

**Methods:** From each district, about 20% of snakebite reports were sampled at random and abstracted by enumerators who used pre-designed and pretested data collection sheets. Data collection activities were regularly reviewed for consistency and accuracy.

**Results:** 2,186 snakebite cases were studied in two districts of southern part of Western and Central Nepal from April to September 2005, of which 1,438 (66%) were from Nawalparasi and 748 (34%) from Chitwan. Of all snakebites, 1,265 were nonvenomous and 921 were venomous. Of the venomous snakebites, 253 were found dead (a 27% pre-hospital case fatality rate of venomous snakebite). Of the total, 54% were males and 46% were females. 27% of patients were between 10 and 20 years of age and 63% were between 10 and 40 years of age. The most snakebites occurred in Brahmin (46%) and fewest in Dalit and Chhetri caste (7%). The largest percentage of snakebites occurred in the evening (26%) followed by nighttime (25%), daytime (24%), and morning (24%) respectively. The most common victims were farmer, students, teachers, housewives, drivers, army and police, and businessman. 61% of bites occurred outdoors and 59% in those performing agriculture-related activities, with 31% occurring specifically while working in the field. More cases were seen in the southern part of Western and Central Nepal than in Eastern Nepal. Snakebite during sleep occurred in 15%. Extremity bites accounted for 94%, with 66% in the lower extremities and 28% in the upper extremities. Snakebite victims who arrived at the hospital beyond 6 hours post bite had the greatest probability of death. 56% of patients consulted traditional healers, 12% managed their bites at home, and the remainder presented to a hospital for treatment.

**Discussion:** A study in Eastern Nepal by Sharma et al. in 2004, found similar demographics, with 75% cases in the age group 11–40 years, and likewise similar rates of snakebites occurring in those performing fieldwork, rates of upper and lower extremity bites, and the percentage use of traditional healers.

**Conclusion:** The largest number of snakebite cases was recorded from Nawalparasi. Farmers were the highest risk occupational group. The majority of snakebite victims consulted traditional healers, with a pre-hospital case-fatality rate of 27% and only a third seeking medical care. Most bites occur in those between 10 and 40 years of age and in those engaged in outdoor and agricultural activities, although 15% occur during sleep.

## THE MOLECULAR EVOLUTION OF LIZARD AND SNAKES VENOMS: CLINICAL AND EVOLUTIONARY IMPLICATIONS

*Fry BG*

*Australian Venom Research Unit, Level 8, School of Medicine, University of Melbourne, Australia. Email: bgf@unimelb.edu.au*

The venom clade within the squamate reptiles is composed of the Anguimorpha (Anguidae, Helodermatidae, Lanthanotidae, Shinisauridae, Varanidae and Xenosauridae), Iguania (agamids, chameleons and iguanids) and Serpentes. Subsequent to the single origin of venom at the base of this clade, the mandibular and maxillary pairs of glands have continued to derive, independently of each other. Major derived states include development of mandibular or maxillary glands into highly complex venom delivery systems, often accompanied by the reduction or even complete loss of the glands located on the other jaw. The venom itself has in parallel undergone tremendous changes, not only in relative expression levels of a particular toxin type but also including lineage specific additional toxin recruitment events. In the Iguania, the venom delivery systems are of the basal type (serial, lobular and non-compound). Helodermatids (Gila monsters and Beaded Lizards) and Varanids (Goannas, Monitor Lizards and the Komodo Dragon) have the most highly evolved venom delivery systems of the Anguimorpha. Venom evolution has been at its greatest within the advanced snake lineage (Colubroidea). This clade is comprised of numerous family level lineages and the venom delivery system varies tremendously. Only Pareatidae and Xenodermatidae lack any species that have been implicated in severe bites. Both lineages, however, have specialised ecologies. Advanced architecture includes the three independent evolutions of hollow hypodermic needle-like high-pressure systems. However, additional lineages have greatly enlarged dentition but without high pressure systems. Almost every colubroid snake family has at least one species shown to produce notable clinical

symptoms. The hollow-fanged Atractaspidae (Stiletto Snakes), Elapidae (Cobras, Mambas, Sea snake, Taipans) and Viperidae (Night Adders, Pitvipers and Vipers) account for the majority of lethal species. The other families contain species known to produce envenomations resulting in significant symptoms or even death, examples including: Colubridae (*Dispholidus, Thelatornis*), Dipsadidae (*Hydrodynastes, Xenodon, Waglerophis*), Homalopsidae (*Cerberus, Enydris, Homalopsis*), Natricidae (*Macropisthodon, Rhabdophis*), Psammophiidae (*Malpolon, Psammophis, Rhamphiophis*), Pseudoxyrhophiidae (*Leioheterodon, Madagascarophis*). Within these various lineages, different envenomation strategies have been evolved and niches colonies. Major variables include venom composition, venom quantity, relative toxicity, dentition and relative efficacy of venom delivery. In conjunction with the lack of antivenom for the vast majority of colubroid venoms, some combinations of these variables result in very dangerous envenomations.

## SNAKE BITE DIAGNOSIS AND MANAGEMENT IN TROPICAL COUNTRIES

*Warrell DA*

*Nuffield Department of Clinical Medicine, University of Oxford, UK. Email: david.warrell@clinicalmedicine.oxford.ac.uk*

**Background:** Most tropical countries have a diverse venomous herpetofauna, including Colubridae, Atractaspididae (Africa and Middle East), Elapidae (except Europe) and Viperidae (except Oceania). Snake bites are common where medical resources are limited.

**Diagnosis:** The history of snake bite is usually unambiguous but the snake responsible is rarely brought. Clinical diagnosis is based on the victim's description of the circumstances, appearance of the snake and evolution of symptoms. A syndromic approach may be helpful but there are many exceptions to the simple attribution of local swelling, shock and haemostatic abnormalities to viperid, paralysis to elapid and rhabdomyolysis to sea snake envenoming. In developed countries, immunodiagnosis has been underused as a tool for clinical management and research.

**First Aid:** Consists of reassurance, immobilization of the whole patient (especially the bitten limb with a splint or sling) and rapid transport to a medical facility. Pressure immobilization is recommended for neurotoxic but non-necrotic venoms (neurotoxic elapids).

**Medical treatment:** Antivenom is the only specific antidote.

**Indications for antivenom:** Include haemostatic abnormalities (coagulopathy or spontaneous systemic bleeding), shock, neurotoxicity, myotoxicity, nephrotoxicity and severe local envenoming. Antivenom reactions are common. 0.1% epinephrine is effective for treating early anaphylactoid reactions. Hypersensitivity tests do not predict these reactions which result from complement activation not IgE-mediated, type I hypersensitivity.

**Prophylaxis for hypersensitivity reactions:** Only epinephrine 2.5 ml of 0.1% solution (adults) by subcutaneous injection has proved effective. The initial dose of antivenom is repeated six hourly until coagulapathy, bleeding and other major signs of envenoming are controlled.

**Recurrent envenoming:** Results from continued absorption of venom from the bite site depot, or redistribution of venom especially with rapidly-eliminated Fab antivenoms (eg. CroFab®).

**Supportive treatment:** Anticholinesterases improve neuromuscular transmission in some paralysed patients. Assisted ventilation is needed for severe descending paralysis. Circulating volume repletion and vasopressors are used for treating hypotension, dialysis for renal failure, antimicrobials and tetanus toxoid for infected wounds and surgical débridement for necrotic wounds.

**Surgical intervention:** Absolutely contraindicated until blood coagulability has been restored. Fasciotomy is indicated only if there is objective evidence of raised intra-compartmental pressure (>40 mmHg).

## TESS-BASED CHARACTERIZATION OF NON-NATIVE SNAKE ENVENOMATION IN THE UNITED STATES

*Seifert SA*

*Nebraska Regional Poison Center, Omaha, NE. Email: sseifert@nebraskamed.com*

**Background:** Only scattered case reports and personal consultation case series are available to characterize non-native envenomations in the U.S.

**Methods:** Non-native envenomations in the Toxic Exposure Surveillance System (TESS) from 1995–2004 were reviewed.

**Results:** There were 1001 non-native envenomations reported to TESS between 1995 and 2004. However, 53% of the database represented probable erroneous inclusion secondary to coding error. After removal of probable erroneous cases, there were 471 non-native, venomous, snake exposures. 12% of the remaining cases represent possible coding error and case duplications but have been

retained in the analysis, pending confirmation. The following should be considered preliminary data: 408 (87%) were identified by genus and species and an additional 37 (8%) were identified by class or region of origin. Snakes with origins in all geographic areas were represented, involving at least 90 species. Viperids comprised 50%, elapids 39%, sea snakes 2%, and there was one colubrid and one atractaspid exposure, with 9% undeterminable. The vast majority of exposures were bites, but dermal, ocular, parenteral and ingestion exposures were also reported. 74% occurred in the victim's own residence. Males comprised 82% of victims. Children were frequently involved in exposures, with 7% occurring in children under 6 years of age, 4% in children aged 6 to 12 years, and 7% in children aged 13–19 years. 38% of patients were admitted to critical care units, 34% were treated with antivenom (58% of followed, admitted patients), and 7% required intubation. There were 3 (0.06%) deaths, 10% "Major," 24% "Moderate," 19% "Minor," and 4% "No effect" outcomes, but 29% of cases were not followed to outcome. The incidence of non-native, venomous exposures has increased by 33% comparing 1995–2000 and 2001–2004.

**Discussion:** There are numerous case reports and series of non-native envenomations in the U.S. and worldwide. This study confirms that the vast majority of exposures are from privately collected snakes and also confirms the large variety of genera and species involved. The number of envenomated children, the large number of elapids, and delays inherent in obtaining appropriate antivenom, pose serious public health risks. The relatively low percentage of patients receiving antivenom may reflect barriers to obtaining species-specific antivenom in a timely manner.

**Conclusion:** Currently, over 50 non-native, venomous snake exposures are reported to U.S. poison centers every year, involving a wide range of venomous snake genera and species. Numerous case series and reports attest to the worldwide nature of this problem. In the U.S., the vast majority of exposures occur from privately collected snakes. Almost 40% are to elapids, 18% of exposures occur in children, and 34% result in moderate clinical effects or greater, including deaths. The incidence of non-native envenomations in the U.S. appears to have increased significantly in the past four years, although this may be an artifact of changes in poison center coverage and access. Improvements in the TESS database are required for a more accurate and complete characterization of these exposures.

## EXOTICS IN THE HOMELAND

*Keyler DE*

*Minneapolis Medical Research Foundation, Minneapolis, Minnesota. Email: keyle001@umn.edu*

**Report:** Eyelash viper (*Bothriechis schlegelii*), Caucasus adder (*Vipera kaznakovi*), East African green mamba (*Dendroaspis angusticeps*), Rhinoceros viper (*Bitis nasicornis*), Egyptian cobra (*Naja haje annulifera*), Lowland swamp viper (*Proatheris supercilliaris*), White-lipped tree viper (*Trimeresurus albolabris*); just a sampling of exotic venomous species responsible for snakebites in the United States during the past ten years. From Fea's vipers (*Azemiops feae*) to Taipans (*Oxyuranus scutellatus*), ranging in cost from tens to thousands of dollars, today's zoos and amateur herpetologists can acquire nearly any species of exotic venomous snake they desire.

**Discussion:** In the United States, prior to 1950, exotic venomous snakes were primarily found in zoos and institutions, but were quite unique in private collections. Exotic venomous snakebites were a rare occurrence. However, there has been increased interest in reptiles and a consequent explosive surge in the importation and captive breeding of exotic reptiles during the latter 20th century. The U.S. is the major importer of live reptiles, representing 80% of the world trade, with approximately two million importations annually from Columbia, El Salvador, Vietnam, China, and Thailand as chief sources. Underground markets and highly successful breeding programs further contribute to the volume of exotic venomous snakes in the nation. The U.S. Dept. of Health and Human Services and the U.S. Dept. of Agriculture are the two major regulatory agencies responsible for monitoring importation activity. Individual state regulations vary widely. Alabama has no regulations concerning importation or possession of exotic venomous snakes, while California prohibits such activities without a permit, and Hawaii prohibits any and all snakes outside of zoos. Concerns have arisen from an apparent increase in exotic venomous snakebites in recent years. Toxic Exposure Surveillance System (TESS) data, for 2002, showed the number of exotic venomous snakebites (125 bites) surpassed native coral snakebites (88 bites). This increase in exotic envenomations is further complicated by the fact that amateur and private collectors do not routinely maintain exotic antivenoms, making zoos a default supplier in emergency circumstances. Antivenoms are expensive and stocks are limited. Availability has declined in recent years due to lack of economic return to manufacturers and some, such as Behringwerke, have terminated production, leaving no source of antivenom available for treating bites by some species.

**Conclusion:** Regulatory agencies are beleaguered in monitoring the flow, sale, and trade of exotic venomous snakes in the U.S. State regulations are inconsistent. The number of exotic venomous snakes and exotic snakebites in the United States has increased in recent decades, and exotic antivenom accessibility is limited.

## PREVENTION AND RESPONSE TO NON-NATIVE ENVENOMATIONS AT OMAHA'S HENRY DOORLY ZOO

*Krebs J, Morris DJ, Simmons LG*

*Omaha's Henry Doorly Zoo, Omaha NE. Email: jkrebs@omahazoo.com*

**Objective:** The Omaha Zoo houses 17 species and 49 specimens of venomous reptile. Most local emergency medical services (EMS) are not adequately experienced or equipped in terms of stocked antivenom for non-native envenmonation. The objective of this institution was to make sure adequate services and training would be available internally if such an envenomation occurred.

**Methods:** To safely house each specimen five concrete block rooms were constructed with one-inch thick tempered laminated glass windows to allow public viewing of the individual exhibits. Each exhibit is constructed of extruded, hollow core PVC plastic with 1/8-inch tempered glass fronts, built in screen tops and drop in safety screen lids. Each room is equipped with an alarm system and pull station indicator panel along with all necessary handling equipment and first-aid kits. Of the 17 species there is antivenom available for all but five species. The zoo properly maintains all recommended and available antivenom in accordance with the manufactures storage recommendations, along with specific on-site and off-site medical management protocols prepared by the Regional Poison Control Center (RPCC) staff for all the 17 species. A color-coding system of the antivenom insures proper antivenom accompanies the envenomated individual to the trauma center. On site "bite drills" are conducted quarterly with all essential building personal. Training courses are conducted by zoo personal on the premise with EMS personal. This insures familiarity with the location of the venomous reptiles and our in-house protocols. The RPCC is provided with an up-to-date inventory of all antivenom in the zoo's possession both expired and non-expired. If an envenomation occurs out of area that requires antivenom, the RPCC will contact zoo staff to arrange the delivery of the specific antivenom. A letter of agreement is signed by both zoo personal and the hospital receiving the serum stating that the receiving hospital will compensate the zoo for antivenom used.

**Results:** Because of our pro-active approach in training with EMS, RPCC and Omaha Zoo staff, the zoo has improved reaction times, and safety protocols for all envenomations.

**Discussion:** It is essential to maintain standardized procedures and clear communication with both RPCC and EMS to insure an effective handling of an envenomation.

**Conclusion:** Most EMS will not be prepared for all possible non-native envenomations. It is the responsibility of institutions and individuals that handle venomous reptiles to insure that all necessary safety precautions and procedures are in place.

## WHEN THE PHONE RINGS AT MIDNIGHT: EXOTIC SNAKEBITE IN THE UNITED STATES

*Wiley KL*

*Kentucky Reptile Zoo, Slade, KY. Email: kyreptil@pop.mis.net*

**Background:** In recent years exotic venomous snakes have become readily available to the private sector. This study attempted to characterize exotic snakebite in the private sector by surveying zoos, which are the primary repository and most used source of exotic antivenoms, as well as to explore the impact of antivenom requests on zoos.

**Methods:** Zoo curators at 30 zoos were surveyed by a written questionnaire. "Exotic" was defined as a species not occurring in the locale of the bite, including non-U.S. snakes and U.S. snakes out of their natural range. An effort was made to target large-scale zoos and also zoos that have significant herp collections, and thus significant antivenom stores. Curators were queried regarding the number of incidents, all aspects of the bite scenario, whether compensation had been received, whether their zoo had a policy or position regarding the provision of antivenom, and their personal position regarding private collectors.

**Results:** Responses were received from 15 of 30 queried zoo curators. There were 50 bite incidents with requests for antivenom, involving 11 zoos. Twenty-four of the bites were reported as occurring in the last five years. All of these requests involved a private sector bite. Twenty species of snakes were involved in these envenomations, and antivenom from 6 different manufacturers were used to treat the bites. A total of more than 480 vials of antivenom were shipped and used. The most common biting snakes were *Bitis* species and the most frequently used exotic antivenom was South African Vaccine Producer's polyvalent. Surprisingly, the second most requested and used antivenom was Wyeth Antivenin Crotalidae Polyvalent. This antivenom was frequently requested for North American species being housed out of their range, and thus the local hospitals did not carry any antivenom. Most bites occurred late at night and frequently involved alcohol or poor handling technique. Some zoo curators believe venomous snakes in the private sector are a problem, but no responding zoo has a policy not to provide antivenom when requested to treat an envenomation, though a few will only ship half of their supply and then attempt to contact other sources if more

antivenom is needed. Zoos were reimbursed in about 75% of cases for the cost of the antivenom, but not for the other expenses involved in acquiring it. Eight of 15 responding zoos have had difficulty in replacing antivenom, both after it was used and in the regular replacement process after expiry.

## THE QUALITY OF ENGLISH LANGUAGE REPORTS OF ENVENOMATIONS BY COBRA, MAMBA, BOOMSLANG, AND BITIS SNAKES

*Seifert SA*[a], *Boyer LV*[b]

[a] *Nebraska Regional Poison Center, Omaha, NE*

[b] *Arizona Poison and Drug Information Center, Tucson, AZ. Email: sseifert@nebraskamed.com*

**Background:** Very little primary research has been performed or published in English regarding common African and Asian snake envenomations. As part of an effort to develop management guidelines to assist U.S. clinicians caring for patients bitten by non-native species, English-language publications were searched regarding human envenomations from non-native snake genus' and species considered of importance. Additional manuscripts, referenced in the indexed articles, were also obtained, when available.

**Methods:** Literature searches were performed in Ovid, with specification of snake genus and species and limited to English language abstracts or manuscripts. To date, searches have been conducted for cobra, mamba, boomslang, and the *Bitis* genus.

**Results:** English language abstracts and manuscripts for the years 1966 to present were reviewed. There are nearly 500 cobra reports in the literature, but there is very little detailed or easily comparable clinical or management data. The vast majority of reports were small, retrospective case series, and individual case reports. There are fewer reports of African vipers, mambas and boomslang snakes. For *Bitis*, there are less than 150 cases, most contained in two large case series that combined all *Bitis* species. The largest series of *B. arietans* (puff adders) included 10 patients. For human envenomation by mambas and boomslangs, only a handful of small retrospective case series and individual case reports were located.

**Discussion:** There are few, high quality reports of common African and Asian human snakebites. The vast majority are small, retrospective case series or single case reports, which suffer from difficulties of snake identification, a lack of standardization in data collection and laboratory studies, lack rigor regarding antivenom administration and assessment of outcomes, and from which only limited conclusions may be drawn.

**Conclusion:** There is a dearth of high-quality scientific information in the English language medical literature on common African and Asian snake envenomations, which precludes the development of evidence-based management guidelines. Prospective data collection in non-native US envenomations, and well-conducted, prospective studies in endemic areas, are needed.

## ONLINE ANTIVENOM INDEX

*Boyer L,*[a] *Antivenom Index Advisors, AZA AI Development Team*

[a] *University of Arizona, Tucson, Arizona. Email: boyer@pharmacy.arizona.edu*

**Background:** The Antivenom Index (AI) is a publication, by the American Zoo and Aquarium Association (AZA) and the American Association of Poison Control Centers (AAPCC), upon which both organizations rely for information on, and quick access to, specific antivenoms for treatment of Americans envenomated by exotic animals. It includes lists of common and scientific names of venomous animals; guidelines for species-specific antivenom selection; and contact information for participating institutions and antivenom manufacturers. All of these data are subject to change over time, meaning that the published AI outdates rapidly. The last publication was in 1999. Nevertheless, the AI remains the most practical source of information available to the AAPCC in the provision of antivenom information for exotic snake envenomations.

**Objective:** Development of a web-accessible, interactive, instantaneously updateable Antivenom Index based on the existing dataset maintained by the AZA.

**Methods:** Existing data from the print version of the AI were transferred into an HTML-accessible relational database. Keywords were established limiting access to AZA and AAPCC affiliates. Programming was undertaken to link species (and their synonyms) with antivenoms claiming efficacy against specific animals and animal groups. Package inserts for antivenoms maintained in AZA collections were scanned into PDF format, and linked where appropriate. Links between specific antivenom products and contact information at AZA sites were made, and AZA members were encouraged to update their institution's data, including types and quantities of specific antivenoms, expiry data, and emergency contact information.

**Results:** Programming, linking species and antivenoms, and linking antivenoms and zoo contact data, has been completed. Editing and testing by AAPCC affiliates are now in the final stages, with release for poison center use anticipated in October 2005.

## FROM HELODERMA TO DENDROASPIS: VENOM PRODUCTION AND HUSBANDRY IN A LARGE, DIVERSE COLLECTION

*Wiley KL, Harrison JR*

*Kentucky Reptile Zoo, Slade, KY. Email: kyreptil@pop.mis.net*

**Discussion:** While venom extraction for specialized purposes may require one or a few species, providing venom for a wide clientele with diverse research needs demands diversity in a collection, with challenges in habitat diversity, and differences in handling. Kentucky Reptile Zoo (KRZ) has had success in providing venom from all families of venomous reptiles, with over 50 species (with about 1400 individuals) currently on our pricelist.

**Methods:** Careful attention is paid to the differing needs of each species. The biology of each animal is taken into consideration when designing the proper captive environment, attempting to replicate the significant ecological factors that influence their life history in the wild, including temperature, humidity, seasonal fluctuations, structural habitat characteristics, diet, and reproductive cycling. Knowledge of common diseases and parasitology is also imperative, especially when dealing with wild-caught animals or ectothermic or unusual food items. A rigorous quarantine program, which incorporates blood testing for virus, deworming, and establishment of steady feeding, avoids spreading disease in the collection. Necropsy of any suspicious deaths is performed in order to pinpoint contagious conditions. All equipment and cages are cleaned between each animal to minimize disease transmission. General handling for husbandry is as safe and stress free for both snake and handler as possible. Proper use of tools such as hooks, grabsticks, tubes, and trapboxes is mandatory. Any holding containers for snakes are locked and secured at all times. Minimizing the stress on the animal is not just ethically sound; it also results in a better yield and quality of venom and a longer life for the individual snake. VENOM YIELD between species obviously varies tremendously, however yield within species can also vary depending on the origin of the snake, the stress the snake is under, and time between extractions. KRZ uses different schedules for different species in order to maximize production. Safety considerations are paramount when dealing with any venomous reptile, and preparedness protocols attempt to address any possible bite scenario. Appropriate antivenom in-house for all species being kept is the most important safety measure. All keepers and handlers are trained in safe techniques and have proper equipment. Two people are present at all times when working with venomous snakes, and no hands-on techniques are used unless absolutely necessary. Different methods of handling during extraction are used in a species-specific manner. Hand position is extremely important, as is the substrate used for the actual grab. KRZ has also developed a technique for extracting from Helodermatidae. A snakebite protocol for any treating physicians is available, listing consulting physicians and backup antivenom locations.

**Conclusion:** Housing an extremely large number of venomous animals presents many challenges. However, following basic guidelines and methods prevents adverse events and results in animals supplying quality venom for many years.

## VENOMOUS SNAKE SHIFT TRAINING AT THE HENRY DOORLY ZOO

*Kipp SL, Krebs J, Simmons LG*

*Omaha's Henry Doorly Zoo, Omaha, NE. Email: kippsara@yahoo.com*

**Objective:** To condition large and potentially dangerous venomous snakes to shift from an exhibit to a secure holding box in a contact free manner.

**Methods:** A shift box with a clear double hinged lockable lid measuring $32 \times 12 \times 5$ inches was designed to universally fit on the back of several venomous reptile exhibits at the Omaha Zoo. There are corresponding shift holes measuring 5 inches on both the exhibit and the box with guillotine access doors that lock into place. The training process begins by placing a food item in the shift box and connecting the box to the exhibit. The vibrations caused by putting the shift box into place signal to the snake that it is time to shift. The guillotine doors are opened to allow the animal to shift in for a food reward. Once fully inside the box the animal can be secured and the exhibit can be safely accessed. Once exhibit maintenance is finished the guillotine doors are reopened and the animal returns to the exhibit without intentional reinforcement.

**Results:** Initial training sessions were done with two different species, though training sessions were time consuming with one species the coastal Taipan (*Oxyuranus scutellatus*) progressed rather quickly, achieving the desired behavior within 5 training sessions. The taipan is now easily shifted off exhibit in an average time of five minutes and rarely delays in shifting back onto exhibit. While food is typically offered as a reward for this behavior, the animal can be successfully shifted without it.

**Discussion:** Classical conditioning is used to form the association between the shift box and food. When the guillotine doors are opened and the snake begins to shift its tongue flicks continually in search of food. The ability to shift venomous snakes is beneficial in any captive situation. In times of inclement weather or other adverse situations that might delay treatment time in the case of an

envenomation, shifting allows the ability to safely work the animal and service the exhibit. Shifting also allows a time of close inspection of the animal inside the box.

**Conclusion:** Venomous shifting is a valuable asset that allows dangerous animals to be worked in a completely safe environment.

## THE USE OF A VENOMOUS REPTILE RESTRAINING BOX AT OMAHA'S HENRY DOORLY ZOO

*Krebs J, Curro TG, Simmons LG*

*Omaha's Henry Doorly Zoo, Omaha, NE. Email: jkrebs@omahazoo.com*

**Objective:** To improve upon safety measures and restraint methods for medical procedures with venomous reptiles by utilizing a versatile restraint device.

**Methods:** A clear, acrylic box measuring 24 × 7 × 5¼ inches, with twelve 1/8 inch holes drilled at each end, was constructed as a restraint device to accommodate many species of venomous reptiles during medical procedures. A semi-rigid, drop-in lid measuring 23½ × 6½ × ¼ inches offered the ability to restrain while allowing minimal animal movement during radiographic procedures. A second, more rigid lid measuring 23½ × 6½ × ⅜ inches with 3/16 inch holes drilled every two inches was used to restrain an animal to allow for injections. One-eighth inch metal retaining pins are inserted into the ⅛ inch end-holes above the lid to hold it in place. To utilize the box for procedures, the venomous reptile is placed in the box using a snake hook and the lid is placed in the box with an 18 or 24 inch feeding forceps. Slight pressure is applied to the lid until the ⅛ inch pins are placed into the desired end-holes producing significant immobilization.

**Results:** The restraint box has been utilized on several species of Rattlesnake (*Crotalus sp.*), Puff adders (*Bitis arietans*), and both species of the Heloderma family (*Heloderma sp.*). All procedures utilizing the restraint box have improved the safe handling of venomous reptiles by keepers by limiting their unrestrained contact with the reptiles. Using the ¼ inch flex lid, the animals have been held in place while the box is turned on its side to examine the ventrum of the animal, and to perform lateral radiographs.

**Discussion:** Though the dimensions of the box have suited the current needs of the Zoo, using the same basic design with increased widths and lengths may be useful for applications on larger species, such as the King cobras (*Ophiophaguse Hannah*), and reduced dimensions for small species like the Namaqua Dwarf Adder (*Bitis Schneideri*).

**Conclusion:** Handling venomous reptiles during medical procedures has inherent risks for both keepers and medical personnel. By limiting unrestrained animal contact utilizing the restraint box, the level of handling safety is improved.

## THE EARLY EVOLUTION OF VENOM IN LIZARDS AND SNAKES

*Fry BG*

*Australian Venom Research Unit, Level 8, School of Medicine, University of Melbourne, Australia. Email: bgf@unimelb.edu.au*

Amongst extant reptiles only two lineages are known to have evolved venom delivery systems, the advanced snakes and helodermatid lizards (Gila Monster and Beaded Lizard). Evolution of the venom system is thought to underlie the impressive radiation of the advanced snakes (2,500 out of 3000 snake species). In contrast, the lizard venom system is thought to be restricted to just two species, and to have evolved independently from the snake venom system. Here we report the presence of venom toxins in two additional lizard lineages (Monitor Lizards and Iguania) and show that all lineages possessing toxin secreting oral glands form a clade, demonstrating a single early origin of the venom systems in lizards and snakes. Construction of gland cDNA libraries and phylogenetic analysis of transcripts revealed that nine toxin types are shared between lizards and snakes (AVIT, BNP, CRISP, Cobra Venom Factor, Crotamine, Cystatin, Kallikrein, NGF and Vespryn). Toxinological analyses of venom components from the Lace Monitor *Varanus varius* showed potent effects upon blood pressure and clotting ability, bioactivities associated with a rapid loss of consciousness and extensive bleeding in prey. The Iguanian lizard *Pogona barbata* appears to retain characteristics of the ancestral venom system, i.e. serial, lobular non-compound venom secreting glands on both the upper and lower jaws, whereas the advanced snakes and anguimorph lizards (including Monitor Lizards, Gila Monster and Beaded Lizard) have more derived venom systems characterised by the loss of the mandibular (lower) or maxillary (upper) glands. Demonstration that the snakes, iguanians, and anguimorphs form a single clade provides overwhelming support for a single, early origin of the venom system in lizards and snakes. These results provide new insights into the evolution of the venom system in squamate reptiles and open additional new avenues for biomedical research and drug design using hitherto unexplored venom proteins.

## INHIBITION OF TWO NORTH AMERICAN CORAL SNAKE VENOMS BY THE UNITED STATES AND MEXICAN CORAL SNAKE ANTIVENOMS

*Sánchez EE, Pérez JC*

*Natural Toxins Research Center, Texas A&M University-Kingsville, Kingsville, TX. Email: kaees00@tamuk.edu*

**Objective:** To compare the efficacy of the Mexican (Coralymn MR) and United States North American Coral Snake antivenoms on North American coral snake venoms, *Micrurus tener tener* (Texas coral snake) and *M. fulvius* (Eastern coral snake).

**Methods:** Six groups of BALB/c mice (n = 8/group, 18–20 g, female) for every venom (n = 2) were injected in the lateral tail vein with various concentrations of venom. The endpoint of lethality (LD ) was determined after 48 hr. The LD was calculated by the Reed and Muench method. For the serum protection test (effective dose at 50%, ED), six doses of antivenom were used for each venom. The venom and antivenom were mixed and incubated at 37°C for 30 min prior to lateral tail vein injection. The mice were observed for 48 hr and the percent survival and ED were calculated by the Reed and Muench method. Control groups were injected with physiological saline. RESULTS: The LD for *M. t. tener* and *M. fulvius* venoms were 1.15 and 0.74 mg/Kg body weight, respectively. The Mexican antivenom had an ED of 172 and 145 mg/Kg body weight against *M. t. tener* and *M. fulvius*, respectively. The ED of the North American antivenom for these venoms were 395 and >395 mg/Kg body weight.

**Discussion:** The Mexican antivenom was capable of neutralizing both coral snake venoms more effectively than the North American antivenom. Effective neutralization of North American coral snake venoms by the Mexican antivenom suggest that lethal components in different species of coral snake venoms are similar; and perhaps, the difference in neutralization between antivenom could be the way in which these antivenoms are produced and processed.

**Conclusion:** Coral snake envenoming is far more dangerous than envenoming by a Crotalidae due to its neurotoxic effects blocking the neuromuscular synapse by a-neurotoxins leading to possible death by muscle paralysis and respiratory arrest. With the shortage of North American coral snake antivenom in the United States, it now crucial to begin considering other alternatives for the treatment of coral snake envenoming. Acknowledgement: This research is supported by NIH/RIMI: #5 PMD000216-02; NIH/Viper: #1 P40 RR018300-01; and NIH/SCORE: #5 S06 GM008107-29.

## A REVIEW OF IMPORTANT CONSIDERATIONS IN DEVELOPING ANTIVENOM

*Pérez JC & Sánchez EE*

*Natural Toxins Research Center, Texas A&M University-Kingsville, Kingsville, TX. Email: kfjcp00@tamuk.edu*

The venom delivery system of venomous snakes has evolved to insure efficient capturing of prey. Venomous snakes with a single injection release higher concentration of venom into its victim than any other animal, plant, or bacteria. The immediate action of the venom can create a medical emergency when humans are the victims. Current approaches to treatment of victims of snakebites rely on rapid injection of purified immunoglobulin antibodies (IgG) or its fragments, $F(ab')_2$ or Fab. There are numerous antivenoms manufactured throughout the world and each is produced in a different way. The intent of this article is to review areas of research that need to be further developed for the production of more effective antivenoms. One of the focal points of this paper is to discuss the clearance of venom from circulation by erythrocytes, which seems to be missing in the literature pertaining to the treatment of envenomation. The information presented will also touch upon the inadequacies of antivenom testing, reasons why the Fc region of the IgG should not be removed and the importance of new animal models for testing and producing antivenoms. Acknowledgements: Funding for this project was provided by; NIH/NCRR #1 P40 RR018300-01, NIH/RIMI #5 PMD000216-02, and NIH/SCORE #5 S06 GM008107-29.

## INHIBITION OF LUNG TUMOR FORMATION IN BALB/C MICE TREATED WITH DISINTEGRINS ISOLATED FROM *CROTALUS ATROX* (WESTERN DIAMONDBACK RATTLESNAKE) VENOM

*Sánchez EE, Galán JA, Pérez JC*

*Natural Toxins Research Center, Texas A&M University-Kingsville, Kingsville, TX. Email: kaees00@tamuk.edu*

**Objective:** To determine if disintegrins isolated from venom of *Crotalus atrox* (Western Diamondback Rattlesnake) inhibit lung tumor formation in BALB/c mice.

**Methods:** Two disintegrins (crotatroxin 1 and crotatroxin 2) were isolated from crude *C. atrox* venom using multidimensional liquid chromatography consisting of reverse phase, followed by size exclusion, and anion exchange. Control mice (n = 42) were

injected in the lateral tail vein with 2 ×105 murine mammary carcinoma cells (66.3p). A total of 0.2 mL containing a dose of 1000 μg/Kg of crotatroxin 1 incubated with 66.3p cells were tail vein injected in mice (n = 8). This procedure was repeated with crotatroxin 2. Mice were monitored for 18 days post injection. After 18 days, mice were sacrificed, lungs removed, and tumors were counted for the control, crotatroxin 1 and crotatroxin 2 treated mice.

**Results/Discussion:** Mice that received crotatroxin 1 and 2 had a 78 and 63% inhibition of tumors per mouse, respectively, as compared to the control group. Snake venom disintegrins could have therapeutic potential in the treatment of certain cancers; therefore, further analysis of crotatroxin 1 and 2 with other cancer cell lines including human cell lines is warranted. Acknowledgement: This research is supported by NIH/RIMI: #5 PMD000216-02; NIH/Viper: #1 P40 RR018300-01; and NIH/SCORE: #5 S06 GM008107-29.

## SMALL MAMMALS AS A NATURAL SOURCE OF SNAKE VENOM METALLOPROTEASE INHIBITORS

*Biardi JE*

*University of California, Davis CA. Email: jebiardi@ucdavis.edu*

**Objective:** To review the literature on snake venom metalloprotease inhibitors (SVMPIs) from mammals, identify ecological and evolutionary correlates of resistance, and identify new sources of protein or peptide inhibitors that may be effective against hemorrhagic venoms.

**Methods:** Possible molecular, ecological, and evolutionary patterns in the ability of mammals to neutralize snake venoms were identified from the literature. Several experiments were used to test these patterns. First, to test the hypothesis that resistance is due to endogenous mammalian protease inhibitors, SVMPI from California ground squirrels (*Spermophilus beecheyi*) were identified and characterized using LC, PMM and de novo MS/MS sequencing. Second, California ground squirrels were used to examine the influence of ecological and evolutionary factors on variation in resistance. Third, a community of desert rodents was sampled for SVMPIs to test the hypotheses that venom neutralization is likely in other small mammal prey, and may vary with body size or taxonomic group.

**Results:** California ground squirrels possess a potent SVMPI showing regions of homology to inter-alpha-trypsin inhibitor that was effective against venom from northern Pacific rattlesnakes (*Crotalus oreganus oreganus*), but less effective against other rattlesnake venoms. Variability in resistance among California ground squirrels was correlated with local snake density, independent of genetic relatedness. In a community of desert rodents there was no clear trend of increasing resistance with body size. However, plasma samples from the sole sciurid species within that community demonstrated complete neutralization of SVMPI activity.

**Discussion:** California ground squirrels possess a SVMPI from the same gene family as other known antihemorrhagic proteins; however, this factor shows specialization against specific crotalid venoms. Regional variation in resistance was related to local rattlesnake density, suggesting that natural selection may maintain SVMPI in other mammalian prey subject to intense predation by venomous snakes. Resistance in a community of small mammal prey was not correlated with body size, suggesting other ecological or taxonomic factors may be important in identifying resistant prey.

**Conclusion:** Small mammals present a useful resource of naturally occurring SVMPIs, and may vary in specificity and structure. Knowledge gained from these SVMPIs should be useful in a therapeutic context.

## ANTI AGGREGATION AND ADHESION ACTIVITIES OF PEPTIDE FRACTIONS ELUTED FROM BOTHROPS COLOMBIENSIS VENOM

*Salgueiro-Tosta LM,*[a,b] *Eedala S,*[a] *Garcia AM,*[a] *Martinez J,*[a] *Sánchez EE,*[a,b] *Rodríguez-Acosta FA,*[b] *Pérez JC*[a]

[a] *Natural Toxins Research Center (NTRC), Texas A&M University Kingsville, MSC 158, Kingsville, Texas 78363*

[b] *Universidad Central de Venezuela, Instituto de Medicina Tropical, Sección de Immunoquímica, Caracas 1041, Venezuela. kalms03@tamuk.edu*

**Objective:** To purify disintegrins from the *Bothrops colombiensis* venom and explore differences in their biological activity. Methods: Purification of disintegrins from *B. colombiensis* venom was carried out by combination of two chromatographic steps: An initial reverse phase chromatography step (Vydac, C18–218TP54 Column), followed by size exclusion chromatography (Waters, Protein–Pak 60 Column). Eluted chromatographic fractions were tested against platelet aggregation using a Chrono-Log whole blood aggregometer, and inhibition of T24 cells adhesion to fibronectin-coated wells.

**Results:** After the size exclusion chromatography, five fractions were obtained with retention times of 11.52, 15.50, 16.00, 17.38, and 18.50 min, and $IC_{50}$ values for platelet aggregation were 8.8, 0.079, 5.7, 2.94, and 10.19 μg/mL respectively. The $IC_{50}$ values for cell adhesion inhibition was effective at sample concentrations of 825, 1125, 156.7, 71.7, and 62.5 ng/mL.

**Discussion:** The difference in the retention time, and inhibitory activity suggest structural variations in the disintegrins. These structural variations affect the affinity of the disintegrin for its integrin target receptor and biological activity.

**Conclusion:** Significant correlations between structure and biological activity could be used as criteria of selection and/or design of disintegrins for biomedical research. New molecules for biomedical research could be discovered in *Bothrops colombiensis* venom by comparing the degree of homology between its peptides and adhesion proteins of cellular matrix.

## ANTIVENOMS: CLINICAL EFFICACY AND SAFETY—THE NEED FOR TRIALS DATA

*Warrell DA*

*Nuffield Department of Clinical Medicine, University of Oxford, UK. Email: david.warrell@clinical-medicine.oxford.ac.uk*

Following the discovery by Albert Calmette of "sérothérapie antivenimeuse" in Saigon in the 1890s, antivenom production was established in five continents. Efficacy was proved in animals and became clinically-convincing long before the era of randomised controlled trials. However, antivenoms now occupy a highly anomalous position in the pharmacopoeia. Formal efficacy/safety trials data are lacking for a large number of antivenoms which differ in their method and quality of preparation and range of claimed neutralising activity. Traditionally, potency of antivenoms (median effective dose, ED50) has been measured by protection of mice and other small animals from fatal challenge by several median lethal doses (LDs) of venom. In this test, venom and antivenom are usually incubated together before being injected intravenously. This procedure ignores the delay between bite and treatment and the diffusion barriers between venom and antivenom in a human victim of snake bite. Other problems in interpreting animal ED50 data are variations in 1) the susceptibility of different animal species to a particular venom, 2) the venom composition of snakes of the same species, and 3) the amount of venom injected into the patient. Predicting specific neutralisation of a particular venom by an antivenom raised against that same venom is difficult enough, but para-specific neutralisation or cross-neutralisation of venoms other than those used in raising the antivenom is even less certain and should never rely on *in vitro* immunological tests. After preclinical testing of a candidate antivenom, using the expanded range of WHO laboratory assays, there is no substitute for randomised, comparative clinical trials in patients envenomed by reliably-identified species of snakes. Power calculations must accommodate differences in the dose and composition of venom injected (always unknown but can be inferred from venom antigenaemia) and interval between bite and treatment. Appropriate dosage and safety can only be assessed by clinical studies. Many tropical countries, especially in Africa, lack appropriate, affordable and proven safe/effective antivenoms. India has many state and private antivenom manufacturers, some of which export their products, but there is no regulation of clinical safety or efficacy and no evidence-based guidelines to direct dosage.

## ADVANCES IN ANTIBODY THERAPY OF VENOM POISONINGS: REVIEW AND CONSIDERATIONS

*Straight R*

*Protherics, Inc., Depts. Medicine, Surgery, Univ. Utah, Salt Lake City, UT. Email: richard.straight@protherics.us*

**Objective:** Most of the early medical antibodies up until the late 1960s early 1970s were only slightly purified or modified, if at all. Their clinical use was impacted by concerns about purity, safety and effectiveness. This presentation examines new findings in the field of medical antibodies from 1950 to present. Review and consideration of these new findings about medical antibodies impact both their development and use for snakebite therapy now and in the future.

**Methods:** This review extracts and synthesizes information from the current state of knowledge about the development and use of medical antibodies in general to apply to the understanding, the development, and the use of antivenom (antitoxin) antibodies in particular.

**Results/Discussion:** It has been found that antibody therapy basically depends on three interrelated factors. These are (1) the purity and properties of the antibodies; (2) how and why the antibodies are being used clinically; and (3) how the human or other animal processes the antibody with and without its antigen (toxin) being present. It has been found that the properties of antibodies are determined by (1) their genetic source and (2) their structure and quality. Genetically, antibodies may be either polyclonal or monoclonal. Antibodies may be further genetically modified or humanized. The quality of a therapeutic antibody (monovalent or polyvalent) is determined by its specificity, binding affinity, avidity, size and titer. All else being equal, size does matter in medical

antibodies and ranges from about 150kDa (IgG) to about 15kDa (nanobodies). The relative importance of these properties guides the scientific and technical development; the clinical use of antibodies; and the scientific and societal cost/benefit of how and why they are being used. The in vivo, primary sites (intravascular) and relative rates of antibody interactions with antigens (toxins) determine and are determined by key processes including access, binding, neutralization, redistribution and elimination. These processes are of primary significance for successful antibody (antivenom/antitoxin) therapy.

**Conclusion:** New antibody medicines have advanced significantly and concepts of antibody use in snakebite therapy need to be re-examined. The principal findings on the properties, characteristics, and the use and misuse of medical antibodies suggest useful new guidelines for snakebite antibody therapy.

## MANUFACTURING ISSUES IN ANTIVENOM DEVELOPMENT AND PRODUCTION USING RECOMBINANT TOXINS: THE CASE OF *LOXOSCELES* SPIDERS

*Estévez J, Olvera A, Ramos B, Vázquez H., Odell G, Paniagua J, de Roodt A, Olvera Mancilla, RF, Salas M, Zavaleta A, Stock R, Alagón A*

*Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología-UNAM, Cuernavaca, Mor., Instituto Bioclon, S.A. de C.V., México, D.F.*

*Laboratorios Silanes, S.A. de C.V., México, D.F., Instituto Nacional de Producción de Biólogicos, Buenos Aires, Argentina y Universidad Peruana Cayetano Heredia, Lima, Perú. Email: alagon@ibt.unam.mx*

In Mexico, four antivenoms are currently used for the specific treatment of scorpion, viperid snake, coral snake, and *Latrodectus* spider envenomations. The scorpion antivenom alone is used in more than 200,000 cases per year. Instituto Bioclon also produces an antivenom useful in the envenomation of *Bothrops*, *Crotalus* and *Lachesis* species of South America. A polyvalent (*Echis*, *Bitis*, *Naja* and *Dendroaspis*) antivenom has been recently developed for its use in sub-Saharan African countries and a Phase III clinical trial of this antivenom is being carried out in the northern region of the Republic of Benin. The above antivenoms are obtained from plasma of hyperimmunized horses with natural venoms. Recently, we have developed a new anti-*Loxosceles* antivenom based on recombinant immunogens. The enzymatically-active sphingomyelinases D (SMDs) that occur in the venom of these spiders are the molecules responsible for the dermonecrosis found in the cutaneous form of loxoscelism and the hemolysis and kidney damage characteristics of systemic loxoscelism. Also, other venom molecules have been implicated in the pathophysiology of this type of envenomation but their roles appear to be much less important. We have cloned several forms of SMDs from the venomous glands of *L. reclusa* (Stillwater, OK), *L. boneti* (Tuxpan, Gro, Mexico) and *L. laeta* (Lima, Peru) and expressed the major necrotoxin from the North American species and two from the South American species. We identified the culture and expression conditions (in *E. coli*) to have them expressed with their enzymatic activity intact and in the soluble fraction of the bacterial lysates; four liters of cultivated cells yield from 15 to 25 mg of pure SMDs. The purified recombinant SMDs are highly immunogenic both in rabbits and horses, and induce very high titers of antibodies. The antibodies are able to completely neutralize the lethality (mouse model), the dermonecrotoxicity (rabbit model), and the enzymatic activity (in vitro) of both recombinant toxins and whole venoms. A polyvalent antivenom (Loxmyn®) that neutralizes (per vial) the SMD activity of at least 150 μg of each of the four recombinant toxins used as the immunogens has been already prepared; each vial of antivenom contains less than 10 mg of equine F(ab')2 fragments. As in the case of all antivenoms produced by Instituto Bioclon, Loxmyn® is composed of digested and highly purified equine F(ab')2 fragments with less than one percent of whole IgGs or serum albumin, it is presented as a lyophilysate that dissolves instantaneously when reconstituted with sterile water, and has been ultrafiltrated with validated procedures to remove viruses of all types that potentially could be present in horse plasmas.

## CROFAB™: TAKING ANTIVENIN MANUFACTURE INTO THE 21ST CENTURY

*O'Donovan K*

*Protherics UK Ltd. Email: Kieran.odonovan@protherics.com*

CroFab was approved by the FDA 5 years ago yet already work is underway to re-engineer the manufacturing process. The current operation, whilst able to meet market needs and produce safe and efficacious product, is very demanding on manufacturing capacity. This restricts opportunities to manufacture other products at the state of the art licensed cGMP facility in the UK. A new generic manufacturing platform which will enable the processing of any antibody based product is being developed using scaled down

process models backed with statistically powerful experimental design. Using these methods a greater understanding of the process can be obtained, a key requirement when working with biologics, leading to the implementation of a robust manufacturing process. The development of optimised IgG capture and the subsequent digestion stage, through the use of scaled down models coupled with experimental design response surface maps of the process stages, have been developed and these used to determine edges of failure and optimum process control parameters. The use of these techniques has enabled the rapid development of a contemporary generic manufacturing process, permitting a step change in manufacturing scale and freeing up capacity at Protherics.