Toxinology

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Carl-Wilhelm Vogel · Steven A. Seifert
Denise V. Tambourgi *Editors*

Clinical Toxinology in Australia, Europe, and Americas

Springer

Toxinology

Editor-in-Chief
P. Gopalakrishnakone

In recent years, the field of toxinology has expanded substantially. On the one hand it studies venomous animals, plants and microorganisms in detail to understand their mode of action on targets. While on the other, it explores the biochemical composition, genomics and proteomics of toxins and venoms to understand their interactions with life forms (especially humans), development of antidotes and exploring their pharmacological potential. Therefore, toxinology has deep linkages with biochemistry, molecular biology, anatomy and pharmacology. In addition, there is a fast-developing applied subfield, clinical toxinology, which deals with understanding and managing medical effects of toxins on human body. Given the huge impact of toxin-based deaths globally, and the potential of venom in generation of drugs for so-far incurable diseases (for example, diabetes, chronic pain), the continued research and growth of the field is imminent. This has led to the growth of research in the area and the consequent scholarly output by way of publications in journals and books. Despite this ever-growing body of literature within biomedical sciences, there is still no all-inclusive reference work available that collects all of the important biochemical, biomedical and clinical insights relating to toxinology. Composed of 12 volumes, *Toxinology* provides comprehensive and authoritative coverage of the main areas in toxinology, from fundamental concepts to new developments and applications in the field. Each volume comprises a focused and carefully chosen collection of contributions from leading names in the subject.

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Clinical Toxinology in Australia, Europe, and Americas

With 112 Figures and 31 Tables

Springer

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Series Preface

The term TOXIN is derived from the Greek word Toeikov and is defined as a substance derived from tissues of a plant, animal, or microorganism that has a deleterious effect on other living organisms. Studying their detailed structure, function, and mechanism of action as well as finding an antidote to these toxins is the field of TOXINOLOGY, and the scientists are called TOXINOLOGISTS.

In recent years, the field of toxinology has expanded substantially. On one hand, it studies venomous animals, plants, and microorganisms in detail to understand their habitat, distribution, identification, as well as mode of action on targets, while on the other, it explores the biochemical composition, genomics, and proteomics of toxins and venoms to understand their interaction with life forms (especially humans), the development of antidotes, and their pharmacological potential for drug discovery. Therefore, toxinology has deep linkages with biochemistry, molecular biology, anatomy, pharmacology and drug development. In addition, there is a fast developing applied subfield, clinical toxinology, which deals with understanding and managing medical effects of venoms and toxins on the human body following envenomations. Given the huge impact of envenomation-based deaths globally, and the potential of venom and toxin in the generation of drugs for debilitating diseases (e.g., diabetes, chronic pain, and cancer), the continued research and growth of the field is imminent.

Springer has taken the bold initiative of producing this series, which is not an easy task of generating 12 volumes, namely, biological toxins and bioterrorism, clinical toxinology, scorpion venoms, spider venoms, snake venoms, marine and freshwater toxins, toxins and drug discovery, venom genomics and proteomics, evolution of venomous animals and their toxins, plant toxins, and microbial toxins.

Singapore

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Editor-in-Chief

Acknowledgments

I would like to sincerely thank the section editors of this volume Carl-Wilhelm Vogel, Steven A. Seifert, and Denise V. Tambourgi for the invaluable contribution of their expertise and time and the authors who obliged with my request and provided a comprehensive review on the topics.

Springer provided substantial technical and administrative help by many individuals at varying levels, but special mention should go to Sarah Mathews, Sunali Mull, Meghna Singh, Mokshika Gaur, and Audrey Wong for their tireless effort in bringing these volumes to reality.

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Volume Preface

"*. . .I have now taken some pains to re-consider my subject. . .Neither have I been ashamed, on some occasions (as the Latins said) caedere vineta mea, to retrench or alter whatever I have judged to be wrong. Dies diem docet [One day telleth another]. I think truth never comes so well recommended, as from one who owns his error. . .*" – Richard Mead, Preface to the Fourth Edition, Corrected, A Mechanical Account of Poisons, in Several Essays.

Toxinology in Australia, Europe, and Americas constitutes a tremendous diversity in venomous animal types, their venoms, venom effects, envenomation management, issues of captive species, native and exotic, and includes the cutting edge in clinical management, public health systems, as well as antivenom development. As Volume Editors, our primary job was to locate and recruit the world's experts in their respective fields, as they related to this collection. This volume attempts to capture the diversity of toxinology in these regions, written by the experts – researchers, clinicians, zookeepers, veterinarians – in their respective fields. These chapters are based on evidence-based knowledge and practice as well as having rigorous peer review. Unlike a textbook, or a systematic review, we have chosen selected topics that are timely and important to advancing the science and practice of toxinology in these regions. Globalization has significantly modified some species' natural ranges, terrestrial and marine, introduced issues of venomous animal collection and management, created challenges in managing non-native envenomations, expanded to veterinary medicine, and provided both challenges and opportunities in antivenom development and other aspects of translation science. Although snakes are still a major factor in global morbidity and mortality, spiders, scorpions, Hymenoptera, caterpillars, ticks, aquatic animals, and others are increasingly recognized for their health impact. We have done our best to cover these tremendous range of toxinologic subjects.

The opening quotation is from Richard Mead, a London physician of the 1700s who wrote the first book in the English language devoted entirely to poisons and envenomations. Although he lived somewhat before the era of the modern scientific method, he was among the first proponents of evidence-based medical and public health practice. Aside from being unafraid to own his errors and revise his thinking based on new information – truly revolutionary ideas and practices for his time – he was so magnanimous of spirit that at his funeral Dr. Johnson proclaimed that he had

lived more in the broad sunshine of life than almost any man. Those are qualities worthy of aspiration in any era.

In the end, although a work by many collaborators, this series is primarily the result of the vision of Dr. Ponnampalam Gopalakrishnakone. We thank him for that vision and for the invitation to co-edit this volume. We would also like to thank our authors, whose expertise, scientific accuracy, and scintillating writing have made this volume a joy to read. Our thanks, as well, to Audrey Wong, Sarah Mathews, and the other staff at Springer for their support and efforts to keep to the volume moving ultimately toward publication. Any oversights, omissions, or other deficiencies are our own.

January 2018

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His research studies include structure function studies, toxin detection, biosensors, antitoxins and neutralization factors, toxinogenomics and expression studies, antimicrobial peptides from venoms and toxins, and PLA2 inhibitors as potential drug candidates for inflammatory diseases. The techniques he employ include quantum dots to toxinology, computational biology, microarrays, and protein chips.

Prof. Gopalakrishnakone has more than 160 international publications, 4 books, about 350 conference presentations, and 10 patent applications.

He has been an active member of the International Society on Toxinology (IST) for 30 years and was president from 2008 to 2012. He is also the founder president of its Asia Pacific Section, a council member, as well as an editorial board member of *Toxicon*, the society's official journal.

His research awards include the Outstanding University Researcher Award from the National University of Singapore (1998); Ministerial Citation, NSTB Year 2000 Award in Singapore; and the Research Excellence Award from the Faculty of Medicine at NUS (2003).

His awards in teaching include Faculty Teaching Excellence Award 2003/4 and NUS Teaching Excellence Award 2003/4. Professor Gopalakrishnakone also received the Annual Teaching Excellence Award in 2010 at both university and faculty levels.

Editors

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Carl-Wilhelm Vogel received his M.D. degree and Ph.D. degree in Biochemistry from the University of Hamburg in Germany. For three-and-a-half years, Dr. Vogel was a Postdoctoral Research Fellow in Molecular Immunology at Scripps Clinic and Research Foundation in La Jolla, California. He completed a medical internship in Germany and 4 years of residency training in clinical pathology at Georgetown University in Washington, D.C., and at Indiana University/Purdue University in Indianapolis. He is a licensed physician and a board-certified clinical pathologist both in the USA and Germany.

He was on the faculty of Georgetown University School of Medicine for approximately 9 years in the Departments of Biochemistry and Molecular Biology and Internal Medicine, and a member of the Lombardi Cancer Center, before assuming the chairmanship of the Department of Biochemistry and Molecular Biology at the University of Hamburg in 1990. In 1999, he became Director of the Cancer Research Center of Hawaii at the University of Hawaii, a position he held for about a decade. He currently is a Full Professor at the same institution.

His research interests have been in the area of basic biomedical research with particular emphasis on the immunological aspects of cancer as well as the development of novel therapeutic concepts for diseases with complement pathogenesis, based on the complement-depleting activity of cobra venom factor. His research has been supported continuously by peer-reviewed

grants since 1983, mainly from the National Institutes of Health. He is the author of well over 100 publications and patents.

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Among other toxinology-related activities, he has published original research, reviews, book chapters, editorials, and other work on envenomation, including chapters in Goldman-Cecil's *Textbook of Medicine*, Brent's *Critical Care Toxicology*, Conn's *Current Therapy*, Dart's *Medical Toxicology 3e*, and is an author of the "Crotalinae" and "Micrurus" chapters of *UpToDate*. He was a founding member and the first President of the North American Society of Toxinology.

Photo of Steven Seifert seen here with an amelanistic Burmese python (*Python bivittatus*), *courtesy Bob Myers* (Source: Bob Myers).

Denise V. Tambourgi Graduated with a biology degree, and with a Master's and Ph.D. in Immunology, from the University of São Paulo. She is currently Director of the Laboratory of Immunochemistry, Butantan Institute, and Scientist of Productivity in Research from the National Council of Scientific and Technological Development (CNPq) of Brazil. She has experience in the area of immunology, with emphasis on immunochemistry, acting on the following subjects: complement system, innate immune system, immunomodulators, venoms/toxins, and antivenoms.

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Part I

Clinical Toxinology in Australia, Europe, and Americas: Envenomation in the Americas

Snake Bites in Colombia

1

Rafael Otero-Patiño

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Abstract

Nearly 4,000 snakebites are annually reported in Colombia – 90–95% are inflicted by pit vipers from *Bothrops*, *Porthidium*, *Bothriechis*, *Bothriopsis*, and *Bothrocophias* genera, specially by *B. asper* (50–80%); 2% by the bushmaster *Lachesi*s spp.; 1% by the rattle snake *Crotalus durissus cumanensis*; 1% by coral snakes (*Micrurus* spp.); and the remaining 1–5% by snakes of aglyphous and opisthoglyphous dentition, mainly from Colubridae family. The Colombian fatality rate of snakebite envenoming is 1–3%, the highest percentage in the Orinochian and Amazonian regions, whereas 6% of patients suffer some type of sequelae, mainly as a result of dermonecrosis and myonecrosis. *B. asper* is responsible for 60–90% of deaths secondary to snakebites. In this chapter, the clinical and epidemiological aspects of bothropic, lachesic, crotalic, and elapidic envenomations are described, with specific features for the country, as well as the actual knowledge of venom composition and actions.

Introduction: Epidemiological Background

The incidence of snakebites, regardless of the species involved, varies between regions in a country depending on factors as diverse as climate, ecological parameters, biodiversity, distribution of venomous snakes, human population density, economic activities, and types of dwellings, among others (Otero-Patiño 2009). In Latin America, the overall incidence of snakebite envenomings ranges from 5 to 62 cases/100,000 population per year, depending on the country (roughly corresponding from 130,000 to 150,000 cases in the whole region, with an estimated number of 2,300 deaths) (Chippaux 2006; Kasturiratne et al. 2008). For Colombia, the incidence is 6–8.5 cases/100,000 population per year, being higher in the Orinochian and Amazonian regions (34.5 cases/100,000 population/year) as a result of the lower population density and abundant ophidian fauna (Grupo Zoonosis-INS 2011; Silva-Haad 1989; Otero-Patiño 2011).

Ten snake families have been described for Colombia, grouped in 70 genera with 248 snake species (49 venomous) and 292 taxons (Perez-Santos and Moreno 1988; Campbell and Lamar 1989, 2004; Castro et al. 2006; Ayerbe and López 2006). Ninety to ninety-five percent of the 3,000–4,400 snakebites annually reported in the country are inflicted by pit vipers (Viperidae, Crotalinae), specially from *Bothrops*, *Porthidium*, *Bothriechis*, *Bothriopsis*, and *Bothrocophias* genera, which inhabit lowlands and inter-Andean valleys located below 2,500 m.a.s.l. Two percent are by *Lachesis acrochorda* and *L. muta*, pit vipers known as "verrugoso/a" in all the country, which inhabit in the tropical rain forest; it is also named "piña" in the Amazonian region, "epere" in Wounaan, and "burrú" in Embera ethnic territories,

respectively, in the Pacific coast from the Chocó department. One percent of the bites are inflicted by the rattle snake *Crotalus durissus cumanensis* (cascabel), a snake inhabiting desertic, dry, or semidry lowlands in the Caribbean region, in the high valley of the Magdalena River, in the Orinochian region, and savannahs (Yarí River) of the Caquetá department. The other 1% are inflicted by coral snakes (*Micrurus* spp., Elapidae family) (Figs. 1, 2, 3, 4, 5, 6, 7, 8, and 9). The remaining cases (1–5%) are inflicted by snakes of aglyphous (Boidae, Colubridae) or opisthoglyphous (Colubridae) dentition, considered of great ecological value (Silva-Haad 1989; Otero-Patiño 2011).

As in Costa Rica, Nicaragua, Honduras, Panama, Venezuela, and Ecuador, a large number of cases in Colombia are inflicted by *Bothrops asper*, where it is responsible for 50–80% of the snakebites, in the Caribbean, Pacific, and Andean regions of the country (Fig. 1) (Otero et al. 1992a; Otero-Patiño 2009). It is widely distributed in humid lowland regions of Mexico, Central America, Venezuela, Colombia, and Ecuador (Campbell and Lamar 1989, 2004; Sasa et al. 2009) and readily adapts to agricultural settings, thus being in close contact with agricultural workers and human dwellings. This species is popularly known in Colombia as cuatro-narices, equis, mapaná, rabiseca, boquidorá, damá (Embera ethnic group), or tapa (Cuna ethnic group). In contrast, *B. atrox* (Fig. 2) inflicts 90% of the snakebites in the Orinochian and Amazonian regions (Silva-Haad 1989).

The sea snake (*Pelamis platurus*; Fig. 10) rarely inflicts bites, although two incidents were described in the Colombian Pacific coast, one of them without envenomation (Campbell and Lamar 2004; Otero 2007). It inhabits the waters of the Pacific Ocean from the south of California (USA) to north of Peru in South America.

Although a number of snakebites (10–20% of the cases) occur inside or around houses, the majority of these accidents are an occupational hazard occurring in agricultural fields in rural communities (85–90% of the cases), mostly affecting agricultural workers (Gutiérrez et al. 2006a, b; Otero et al. 1992a). These accidents mainly affect young adults (15–45 years old, 54% of cases), predominantly males (76%); however, a significant number of cases occur in children (30%). Regarding the anatomical region of the bites, 70% of the cases occur in the lower extremities (Gutiérrez et al. 2006a, b; Otero et al. 1992a).

As in the case of incidence, the fatality rate also varies between countries and between regions within a country. Such differences in mortality are due to ethno-anthropological factors, geographical difficulties for the early transfer of patients to hospitals, the availability of therapeutic resources and primary care programs in remote places where bites occur, the snake species causing the accidents, and the intraspecies variability in venom composition (related to age, size, sex, diet, and geographical parameters).

For Colombia, the fatality rate of snakebite envenoming is 1–3%, the highest percentage in the Orinochian and Amazonian regions, whereas 6% of patients suffer some type of sequelae, mainly as a result of dermonecrosis and myonecrosis. *B. asper* is responsible for 60–90% of deaths secondary to snakebites, in Central America and northern South America, thus having a heavy public health impact in these regions (Gutiérrez et al. 2006a, b; Otero 2007; Sasa and Vázquez 2003).

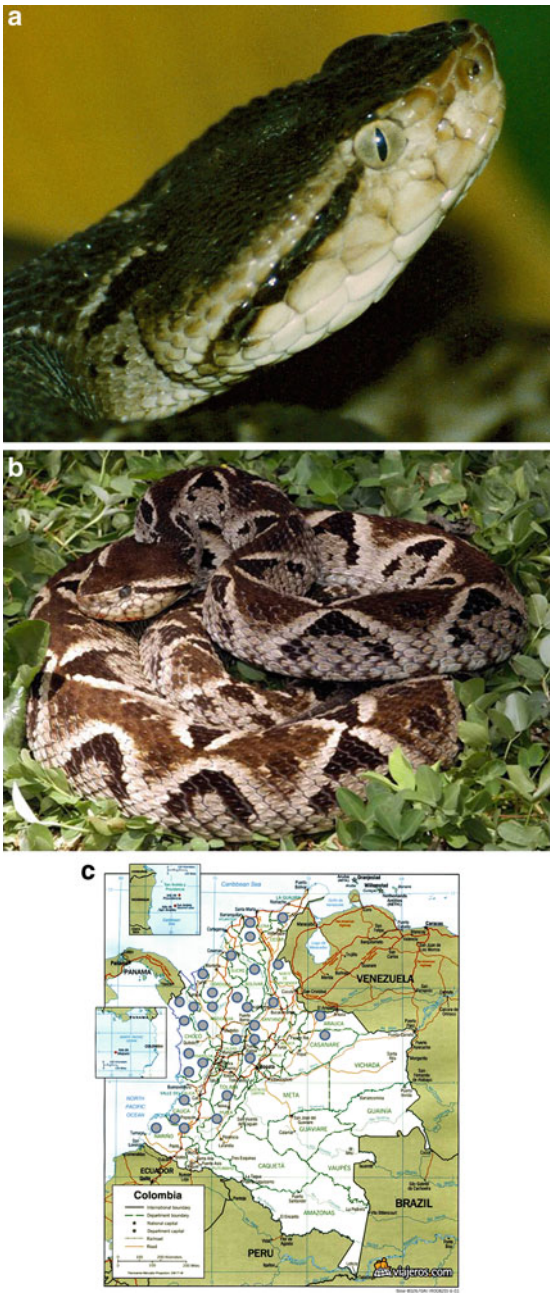

Fig. 1 (**a**, **b**) *Bothrops asper* (mapaná, equis, boquidorá, terciopelo, damá, tapa) from Antioquia/Chocó, Colombia (Photos: R. Otero-Patiño (**a**) and David Warrell (**b**)). (**c**) Distribution in the rain forest of the Caribbean, Pacific, and Andean regions of the country up to 1,400–1,500 m above the sea level (m.a.s.l) in northwestern Colombia (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Additionally, the antivenom supply in Colombia has also traditionally been insufficient, both in terms of quantity and quality (Otero et al. 1992a, b, c, 2000a, b, 2001a, b, 2002a, b; Otero 2004).

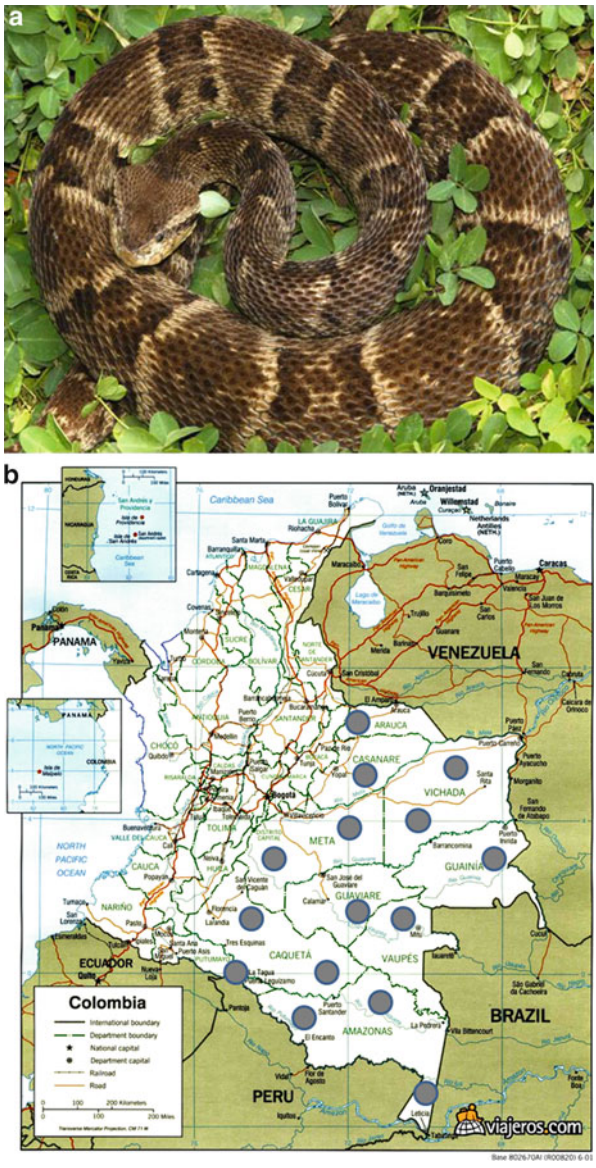

Fig. 2 (**a**) *Bothrops atrox* (cuatro-narices, jergón, jararaca) (Photo: Courtesy Dr David Warrell). (**b**) Distribution in the rain forest of the Orinochian and Amazonian regions of the country up to 1,500 m.a.s.l (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

More than 116 years after Calmette developed serotherapy for the treatment of snakebite envenoming, this resource remains the mainstay in the treatment (Bon 1996; Theakston et al. 2003; Otero-Patiño et al. 1998; Otero 2007). Unfortunately, antivenoms are often unavailable in remote rural places of developing countries, this being one of the factors responsible for the delayed treatment of envenomed patients (Otero et al. 1992c; Otero-Patiño et al. 1998; Gutiérrez et al. 2006a, 2013). In many

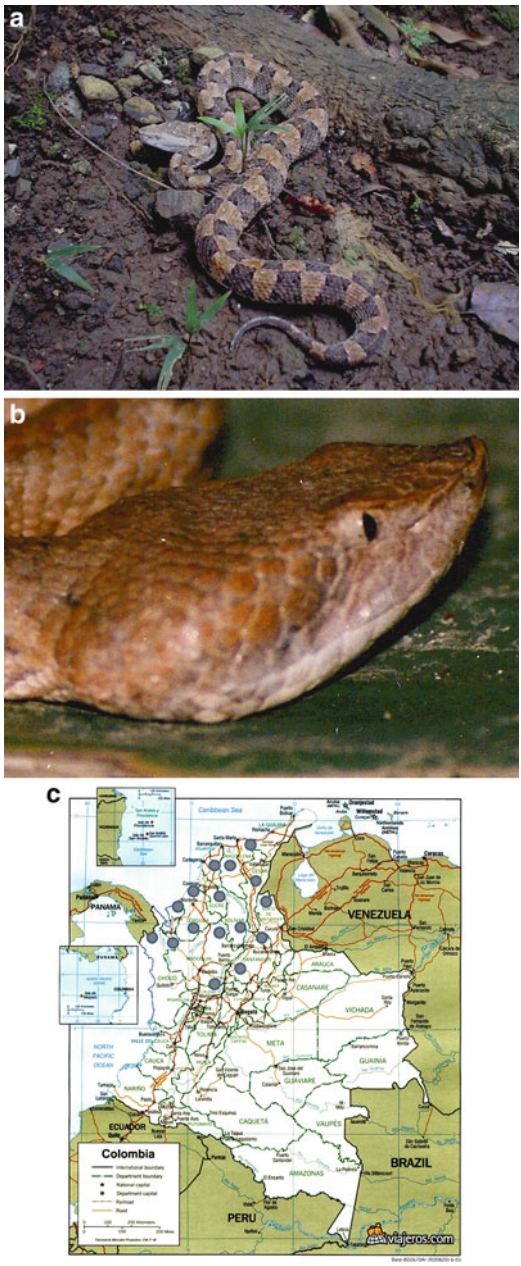

Fig. 3 (**a**, **b**) *Porthidium lansbergii* (patoco, patoquilla, veinticuatro, tugu) (Photos: R. Otero-Patiño). (**c**) Distribution in the humid and semidry lands of the Caribbean region, low Magdalena River Valley, northwestern and northeastern Colombia, up to 1,400 m. a.s.l. The very close species *P. nasutum*; it is distributed in the Pacific region and northwestern Colombia (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

tropical areas of Latin America, at least 40–50% of patients receive traditional medicine as first treatment (plants, chemical products, physical methods, prayers, etc.). This and other factors cause a delay in the arrival of those patients to the

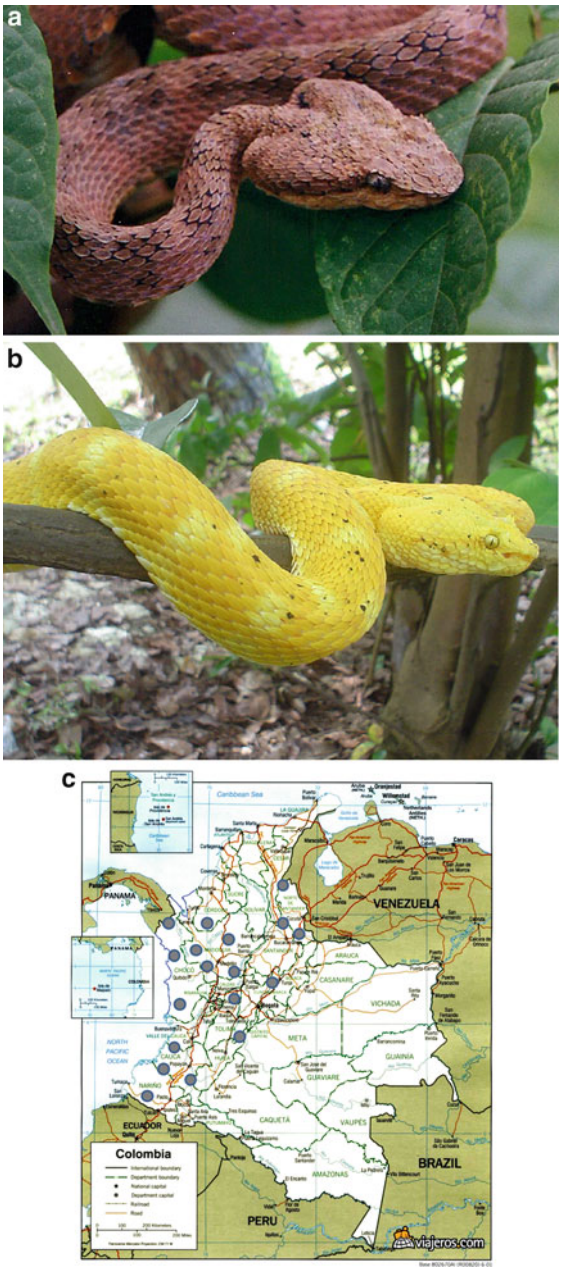

Fig. 4 (**a, b**) *Bothriechis schlegelii* (víbora de tierra fría, cabeza de candado, víbora de pestaña, colgadora, oropel) (Photos: R. Otero-Patiño). (**c**) Distribution in the humid lands of the Pacific and Andean regions and northwestern Colombia, up to 2,600 m.a.s.l (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014 and IATREIA 20 (3), Otero 2007)

hospitals; most of them seek medical attention 6 or more hours after the bite, thus affecting the prognosis of these cases (Otero et al. 1992a, 1996, 1999, 2000a, b; Otero-Patiño et al. 1998).

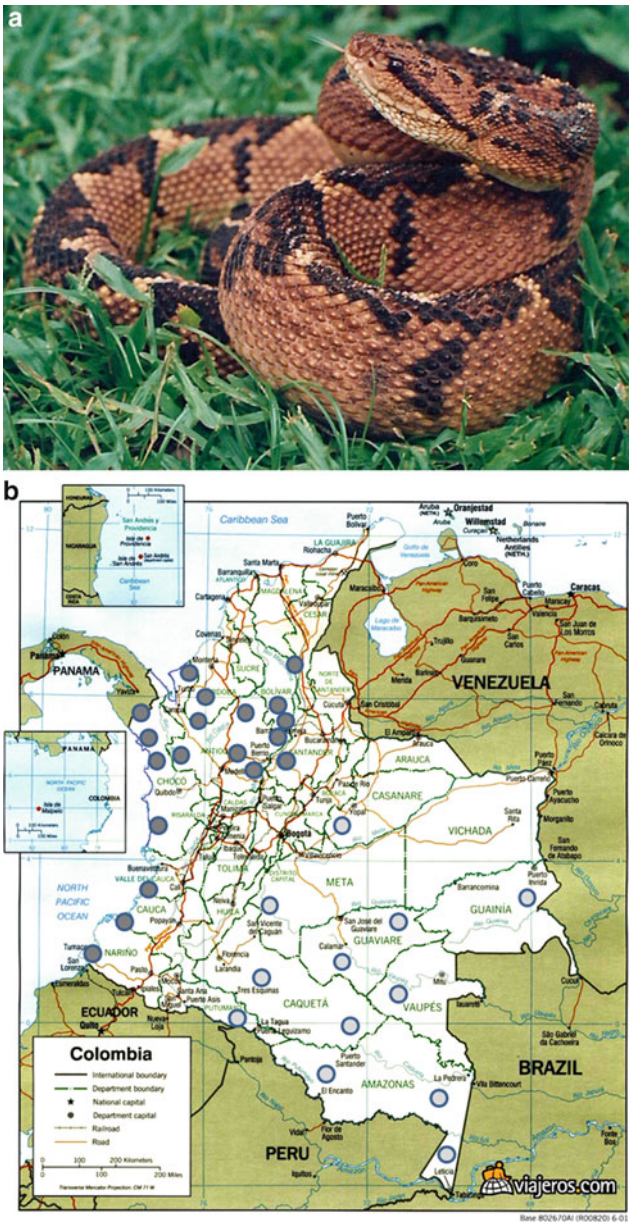

Fig. 5 (**a**) *Lachesis acrochorda/L. muta* (verrugoso/a, piña, epere, burrú) (Photo: R. Otero-Patiño). (**b**) Distribution in the rain forest of the Pacific and Andean regions and northwestern Colombia (*L. acrochorda*) and in the Orinochian and Amazonian regions (*L. muta*) (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

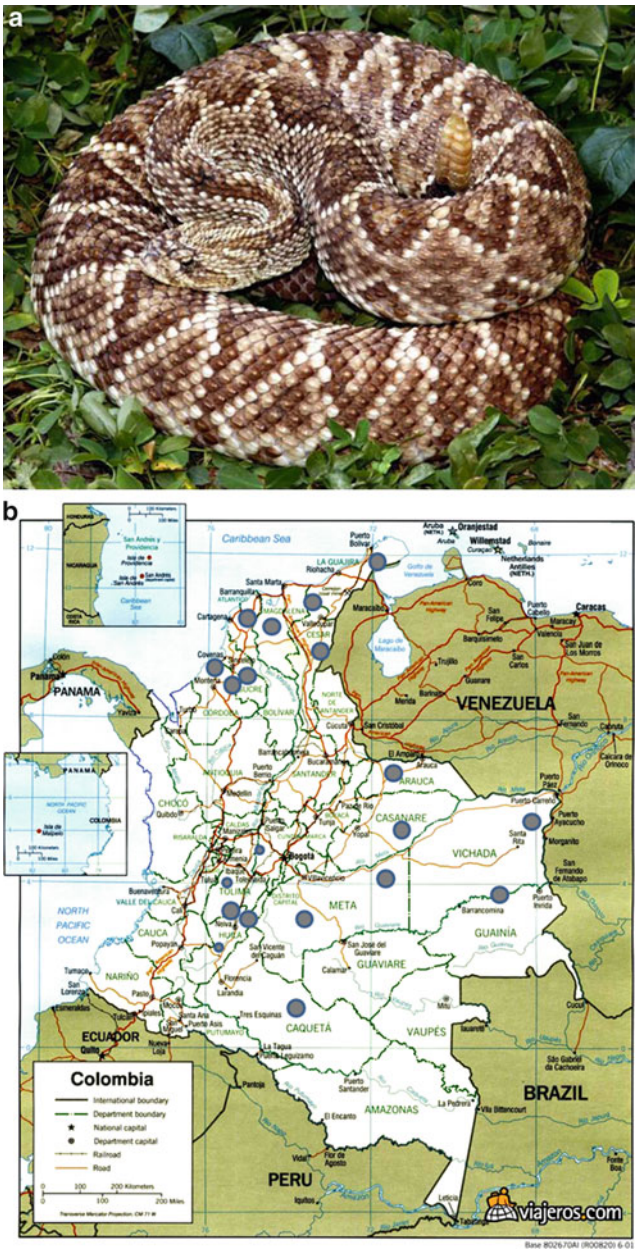

Fig. 6 (**a**) *Crotalus durissus cumanensis* (cascabel, rattle snake) (Photo: Courtesy Dr David Warrell). (**b**) Distribution in the dry or semidry lands of the Caribbean region, low and high Magdalena River Valley, in the Orinochian and north of the Amazonian regions (Yarí River Savannahs) of the country, up to 2,000 m.a.s.l (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

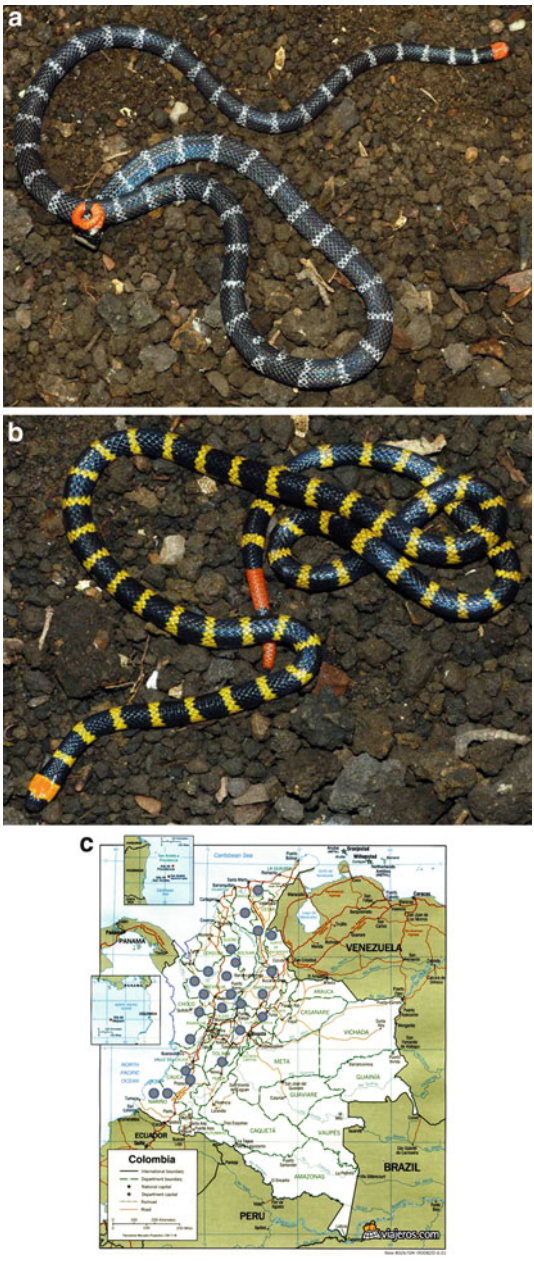

Fig. 7 (**a**, **b**) *Micrurus mipartitus* (coral rabo de ají, redtail coral snake), bicolor group (Photos: Courtesy Dr David Warrell). (**c**) Distribution in the Caribbean, Pacific, and Andean regions of the country, up to 2,200 m.a.s.l (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Death is usually prevented when the patients receive adequate antivenom therapy within the first 2 h of the bite. On the other hand, almost all cases ending in death or developing sequelae are associated with a delayed onset of specific treatment more than 2 h after the bite (RR = 2.5). Medical attention offered beyond this time interval

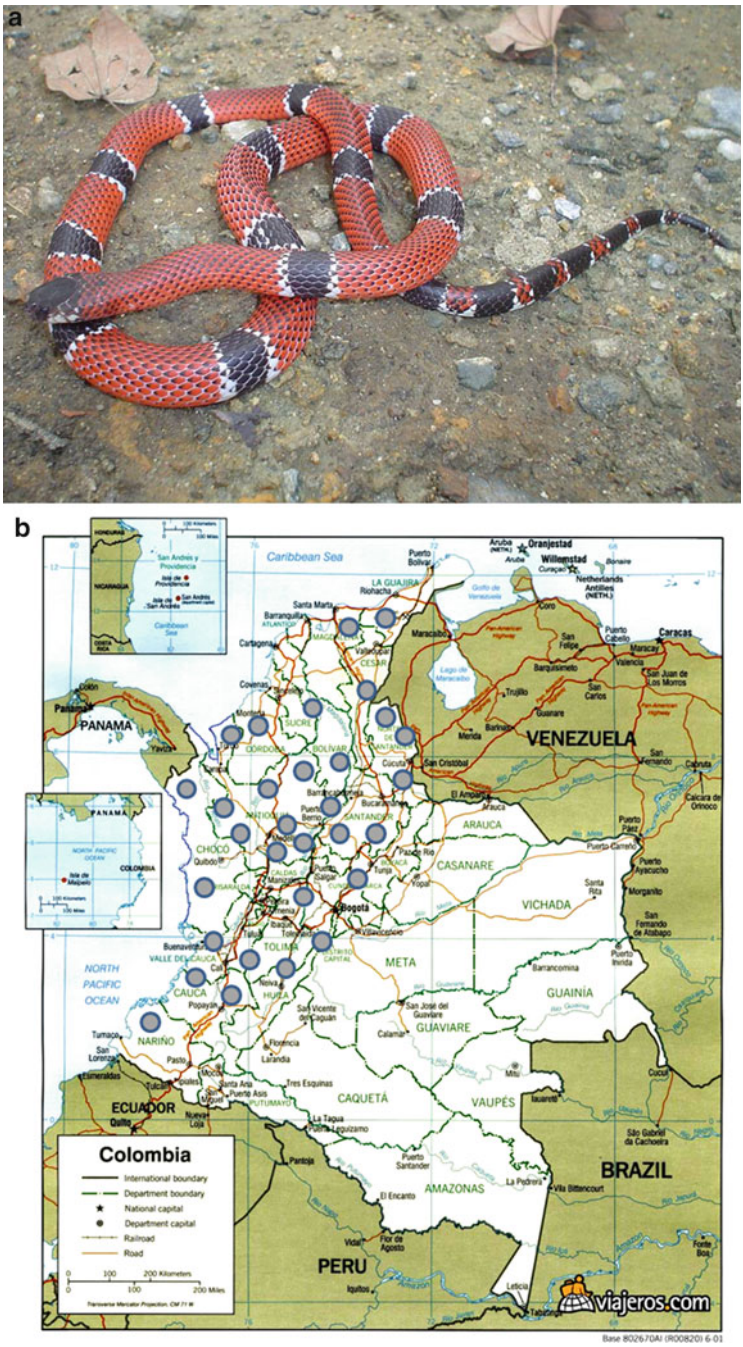

Fig. 8 (**a**) *Micrurus dumerilii* (coral), monadal group (Photo: R. Otero-Patiño). (**b**) Distribution in the Caribbean, Pacific, and Andean regions of the country, up to 1,800 m.a.s.l) (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

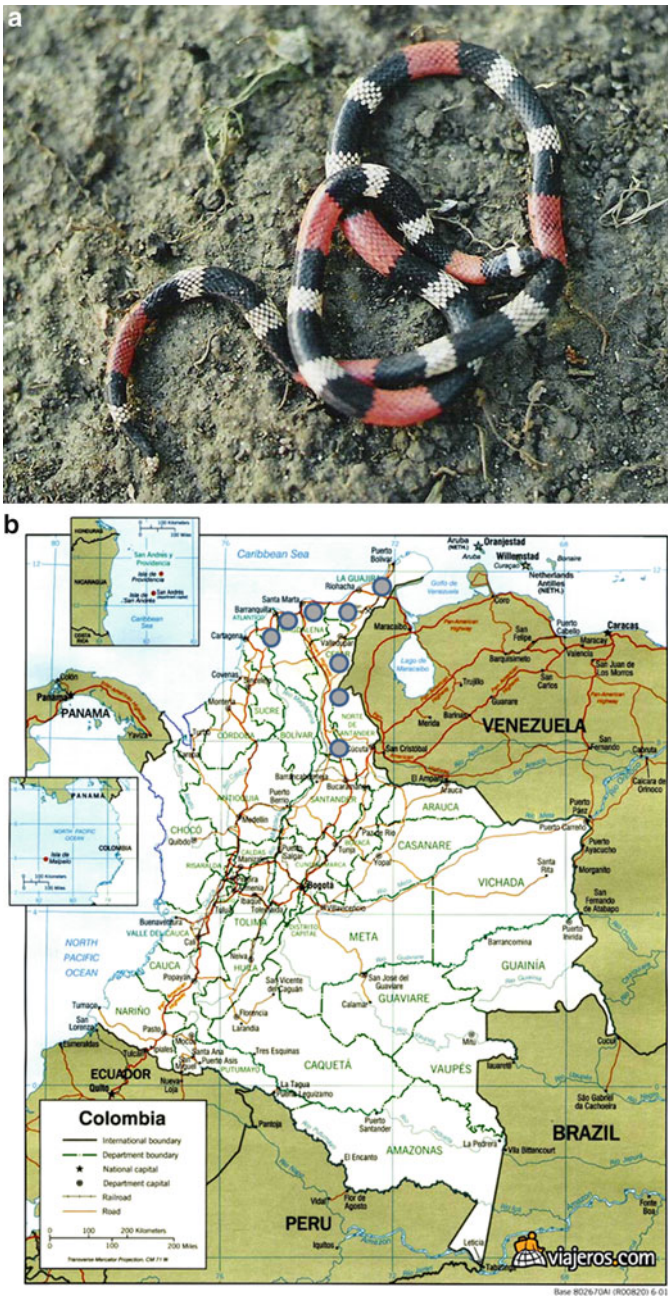

Fig. 9 (**a**) *Micrurus dissoleucus* (Coral de la Costa, Caribbean Coast coral snake), of *black* rings arranged in triads group (Photo: R. Otero-Patiño). (**b**) Distribution in the Caribbean region and northeastern Colombia, up to 1,000 m.a.s.l) (Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Fig. 10 *Pelamis platurus* (sea snake). Distribution in the Pacific Ocean from the south of California (USA) to north of Peru and in the Indian Ocean (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

is a critical factor since acute renal failure (ARF) and hemorrhage in the central nervous system (CNS) may develop in *B. asper* bites ($P = 0.02$), and it is also associated with an increased risk of developing compartmental syndrome, local necrosis, and other sequelae ($P < 0.001$) in patients bitten by adult, specially female, specimens (more than 1.0–1.1 m body length) (Otero 1994, 2007; Otero-Patiño 2009; Otero et al. 2002a; Sasa et al. 2009). Similarly, patients with coral snakebites who seek medical attention 2 or more hours after the bite have a higher risk of peripheral paralysis, respiratory arrest, and death (Otero-Patiño 2011).

Pathogenesis of Local and Systemic Effects of Snake Venoms

Snake venoms from *Bothrops*, *Porthidium*, *Bothriechis*, *Bothriopsis*, *Bothrocophias*, and *Lachesis* genera, with some intraspecific or intergenera differences, induce conspicuous local and systemic effects such as the following:

Edema

This is a real autopharmacological phenomenon. Autacoids as histamine are released and bradykinin is generated; eicosanoids as prostaglandins, leukotrienes, and thromboxanes are formed and released secondary to arachidonic acid metabolism. Edema is also secondary to the activation of lymphocytes or macrophages with the production of TNF and cytokines (IL). Additionally, there is a direct damage on microvessels, i.e., capillaries and venules, by the action of hemorrhagic toxins resulting in extravasation (Gutiérrez et al. 2009a; Otero 2014). *B. asper* PLA2 myotoxins have a direct effect on collecting lymphatic vessels inducing the perturbation of smooth muscle cell membranes and causing edema (Mora et al. 2008).

Hemorrhage

Viperid snake venoms contain a high proportion of hemorrhagin metalloproteinases (SVMPs) (11–65% of total protein content), currently classified in three groups according to their composition and molecular mass (group P-I, MP domain + zinc, 20–30 KDa; group P-II, MP domain + disintegrin domain, 30–60 KDa; and group P-III, MP domain + disintegrin-like and cysteine-rich structure, 60–100 KDa). Those of group P-III are the most potent hemorrhagic toxins (Gutiérrez et al. 2009a; Markland and Swenson 2013).

Group P-I MPs as those in *B. asper* venom only induce local hemorrhage, whereas those of groups P-II and P-III produce local and systemic hemorrhage. They are also responsible for blistering, myonecrosis, and dermonecrosis, the latter with the contribution of a fast rise in the local production of TNF and IL-6. Myonecrosis secondary to hemorrhagins enhances fibrosis as a sequel, with loss of muscle mass (Otero 2007, 2014; Laing et al. 2003; Gutiérrez et al. 2009a). Other activities attributed to SVMPs are fibrinolysis/fibrinogenolysis, prothrombin and factor X activation, apoptosis, platelet aggregation inhibition, proinflammation (chemotactic), and the inactivation of blood serine proteinase inhibitors. Group P-III SVMPs cause regional pulmonary alveolar damage, similar to acute respiratory distress of the adult (Gutiérrez et al. 2009b; Escalante et al. 2003). Hemorrhage may occur in any organ after an i.v. injection of total venom or MPs, but the lungs are the most affected because their microvessels are the first being in contact with those toxins (Gutiérrez et al., 2009b; Escalante et al. 2003).

B. asper venom from neonate specimens contains a higher proportion of group P-III MPs, whereas adult specimen venom contains a higher quantity of group P-I MPs, thus explaining the higher hemorrhagic activity of the former (Gutiérrez et al. 2009a, b; Fox and Serrano 2005; Alape-Girón et al. 2008). MPs enhance the development of secondary infection, impairing the local damage (Otero et al. 1992a, 2002a; Otero-Patiño 2009; Saravia-Otten et al. 2007).

Myonecrosis

This is a frequent manifestation of envenomings caused by snakes of Viperidae, Elapidae, and Hydrophiidae families and by Africanized bees (massive attack) (Gutiérrez and Ownby 2003; Harris 2003). In the first group, the hemorrhagins (MPs) are present in the *Bothrops*, *Agkistrodon*, *Porthidium*, *Bothriechis*, *Lachesis*, and other pit viper venoms, which induce hemorrhage, ischemia, and necrosis of muscle cells, with consequent fibrosis and denervation as a repairing process (Otero 2007; Gutiérrez and Lomonte 2003; Gutiérrez et al. 2009a). In the second group, the myotoxic PLA_2 is isolated from Viperidae, Elapidae, and Hydrophiidae family venoms (Gutiérrez and Ownby 2003; Harris 2003; Melo and Ownby 1999). These toxins are pore formers at the plasma membrane (PM) of skeletal muscle cells, causing its depolarization and degradation. They are classified into three groups: to class I belong those myotoxic PLA_2s from terrestrial elapids and the sea snake; to

class II belong those from viperids; and to class III belong those from bees and venomous lizards. The consequent high influx of calcium and macromolecules to the cytosol activates the enzymes that are calcium dependent and causes mitochondrial damage. The focal and morphological alteration in the periphery of muscle cells (myofibril) can be rapidly detected (15–30 min) as a delta lesion secondary to membrane damage (Otero 2007; Gutiérrez and Lomonte 2003; Gutiérrez et al. 2009a; Gutiérrez and Ownby 2003).

There are two subfamilies of class II myotoxic PLA_2 which locally exert its action: the enzymatically active Asp49 (D49) that binds to calcium and the Lis49 (K49) that loses the ability of binding to calcium, with low or undetectable enzymatic activity (Gutiérrez and Lomonte 2003; Gutiérrez et al. 2009a; Gutiérrez and Ownby 2003; Harris 2003; Melo and Ownby 1999). Those PLA_2s constitute 15–35% of the protein content of *B. asper* venom (Gutiérrez et al. 2009a).

Additionally, the myotoxic PLA_2s are divided into neurotoxic and non-neurotoxic. Those that are neurotoxic are myotoxins that act systemically, i.e., crotoxin (PLA_2 class II from *Crotalus durissus terrificus*) and from elapids (class I). All of them and that from bee venom (PLA_2 class III) induce systemic myotoxicity (rhabdomyolysis). They are also nephrotoxic and lethal. In comparison with MP-induced myonecrosis, the regeneration of the affected muscles by myotoxic PLA_2 is total within a total of 15 days, without sequelae (Otero 2007, 2014; Gutiérrez and Ownby 2003; Harris 2003).

In vitro, it has also been demonstrated that myotoxic PLA_2s have potent bactericidal activity against some gram-negative bacillus and *Staphylococcus aureus*; viperid venoms also contain low molecular weight peptides with antifungal activity. The effect of *Bothrops* venom myotoxic PLA_2 may also affect myocardial muscle cells, i.e., *B. asper*, inducing focal necrosis (Otero 2007; Gutiérrez et al. 2009b).

The following systemic effects of venoms are also intense or life-threatening:

Hypotension

This is one of the frequent and transitory signs (10–15%) of systemic envenoming in *Bothrops* and *Lachesis* spp. bites (Otero et al. 1992a; Otero-Patiño et al. 1993; Silva-Haad 1980/1981). The first mechanism involved is the presence of proteases as kallikreins in the venom which act on plasma kininogen obtaining bradykinin, a potent vasodilator. The second mechanism is that those venoms contain peptides (angiotensin-converting enzyme inhibitors (ACEi)) of low molecular weight, which additionally induce bradykinin-potentiating peptides (BPPs) by inhibiting its degradation (Ferreira 1965; Soares et al. 2005; McCleary and Kini 2013). The third mechanism is the presence of natriuretic peptides in the venom, natural inducers of diuresis and vascular relaxation (Soares et al. 2005). The fourth mechanism is that hypotension may be secondary to hypovolemia as a result of hemorrhage or loss of plasma to interstitial tissue by intense edema or by the lowering of plasma oncotic pressure. The fifth mechanism is the cardiotoxic effect of venom with hypokinesis of the left ventricle (Otero 2007, 2014; Gutiérrez et al. 2009b).

Hemostatic Disorders

Pit viper bites affect the three components of hemostasis (coagulation factors, platelets, and the blood vessel walls). *Bothrops*, *Crotalus*, and *Lachesis* snake venoms induce in vitro plasma coagulation by the activation of fibrinogen (I), prothrombin (II), or factor X and platelets in some cases. Basparin A is a group P-III metalloproteinase (SVMP) from *B. asper* venom, with action on prothrombin and forming meizothrombin. SVMPs are the most important *B. asper* venom components being in vitro coagulant and in vivo defibrinant, whereas serine proteases as asperase (thrombin-like) have a minor role probably by the low content of the latter in the venom (Gutiérrez et al. 2009b). Thrombin-like enzymes, after the activation of fibrinogen (a dimer), release only fibrinopeptide A or rapidly release fibrinopeptide A and slowly fibrinopeptide B from the fibrinogen molecule. The final result is the production of a friable and defective fibrin, the fibrinogen consumption named defibrination/defibrinogenation syndrome and the fibrinolysis activation (Otero 2007; Sano-Martins and Santoro 2003; Gutiérrez et al. 2009b; Reid et al. 1963a, b). Prothrombin or factor X activators generate thrombin, which hydrolyzes fibrinogen to fibrin; the final event may be disseminated intravascular coagulation (DIC) with additional fibrinogen consumption (Otero 2007; White 2005).

Experimentally, the i.v. injection of total venom of *B. asper* or *B. atrox*, or of their procoagulant components, at high doses causes animal death (mice, rabbits) as a consequence of DIC or pulmonary thromboembolism (Gutiérrez et al. 2009b; Silva-Haad 1989). Conversely, sublethal doses of these enzymes (asperase, a thrombin-like serine proteinase, or basparin A, a P-III SVMP that activates prothrombin) cause defibrination/defibrinogenation with drops of plasma fibrinogen concentration and increments in D-dimer and fibrinogen degradation products (FDPs) (Gutiérrez et al. 2009b). No factor X activator has been isolated in the *B. asper* venom. *B. lanceolatus* from Martinica and *B. caribbaeus* venom from Santa Lucia Island frequently cause thrombosis in limb veins or thromboembolism in the pulmonary or cerebral arteries of their bitten victims, with hemiplegia as a sequel (White 2005; Thomas et al. 1995, 2006). In *Bothrops* bites, inflicted at the dorsal part of the hands or feet, mainly by *B. asper*, the i.v. injection of venom or its rapid diffusion to veins may occur, with little local signs and fast induction of pulmonary thromboembolism (15 min), unconsciousness, and convulsions secondary to anoxia. This dramatic clinical picture may finish in death (Otero 2014).

There are venom fraction activators of platelet aggregation, i.e., thrombocytin in *B. atrox*, convulxin in *C. d. terrificus*, and thrombolectines in *B. atrox* and *L. muta* venoms. Aspercetin (lectin type C) in *B. asper* venom is an inductor of platelet aggregation dependent on the von Willebrand factor, with the production of clinical and experimental thrombocytopenia (Gutiérrez et al. 2009b; Rucavado et al. 2001). Conversely, disintegrins coupled to P-III MPs from *B. asper* venom inhibit platelet aggregation induced by collagen. Inhibition of platelet aggregation can also be observed as a result of fibrinogen consumption.

Bothrops and *Lachesis* snake venoms activate fibrinolysis, mainly by MPs (hemorrhagins) or by means of plasminogen activators. Additionally, fibrin microthrombi

formation stimulates the endogenous fibrinolytic system (Sano-Martins and Santoro 2003; Gutiérrez et al. 2009b). Nevertheless, coagulopathy is a rare sign in *Porthidium nasutum*/*P. lansbergii* bites because it is recognized as a non-coagulant venom, but it is a high inducer of local and systemic hemorrhage (Otero et al. 1992b; Lomonte et al. 2012). Recently, it was demonstrated that *P. nasutum* venom from neonate specimens has coagulant and defibrinating activities, with variations according to dose (Rey and Otero 2009). Additionally, *B. schlegelii* venom induces mild or moderate local and systemic effects, experimentally as well as in patients bitten by this species (Otero et al. 1992b; Otero 2014).

Anemia

This is a systemic sign which occurs in 50% of patients with *Bothrops* bites in Colombia. It may be the result of local and/or systemic hemorrhage or because of the presence of microangiopathic hemolytic anemia: or by transitory medullar cessation of erythrocyte production secondary to infection. Indirect hemolysis, which depends on additional lecithin, is an effect that all the snake venoms from northwestern Colombia have in vitro, with higher activity in *M. mipartitus* venom (Otero et al. 1992a, b; Otero 2007; Gutiérrez et al. 2009b).

Nephrotoxicity

This is a frequent systemic manifestation or complication which occurs in 10–17% of patients with pit viper envenomings in Colombia, specially in those inflicted by *Bothrops* spp. (*B. asper* and *B. atrox*, including neonate/juvenile specimens), *Porthidium* spp., *Lachesis* spp., and *Crotalus d. cumanensis* (Otero et al. 2002a; Otero-Patiño 2009). Acute renal failure (ARF) can be prerenal secondary to hypovolemia or shock or renal by acute tubular necrosis (ATN) as a consequence of prolonged hypovolemia, or by nephrotoxins affecting epithelial cells of proximal tubules, or by vasospasm related to the activation of the renin-angiotensin system. The most severe form of ARF is acute cortical necrosis (ACN), mainly by ischemia as a result of DIC because 90% of nephrons are located in the renal cortex (Otero 1994, 2007; Otero and Mesa 2001; Gutiérrez et al. 2009b; Otero-Patiño 2009).

Crotoxin is the main nephrotoxic component in the venom of *C. d. cumanensis* or *C. d. terrificus*, causing ATN with or without myoglobinuria secondary to rhabdomyolysis (Amaral et al. 1986; Pinho et al. 2005; De Azevedo-Marques et al. 2003; Martins et al. 2002). Some myotoxins in *Bothrops* venoms also cause increment in the synthesis of prostaglandins (PGEs) by means of cyclooxygenase (COX) enzymes 1 and 2. The PGEs are important mediators of vascular tonus and of water and salt balance in the kidney. Additionally, those myotoxic PLA_2s are COX activators through endothelin (ET), an endogenous vasoconstrictor peptide whose target is also the kidney (Otero 2007).

Neurotoxicity

In South America, the snake species from *Micrurus* (coral snakes), *Crotalus* (rattle snakes), and *Pelamis* (sea snake) genera have potent neurotoxins. Those toxins act either at presynapse (β-neurotoxins) or at postsynapse (α-neurotoxins). To the first group belong crotoxin from *C. d. cumanensis* and other neurotoxic PLA_2s present in some *Micrurus* spp. venoms, with combined action (pre- and postsynaptic) such as those of *M. corallinus* from Brazil, *M. nigrocinctus* from Costa Rica, and *M. dumerilii* and *M. mipartitus* from Colombia. They inhibit acetylcholine release by interfering with Ca^{++} metabolism (Brazil 1987; Da Silva Jr and Bucaretchi 2003; Rey-Suárez et al. 2011).

On the other hand, the main mechanism of action for neurotoxins presented by *Micrurus* spp. and *Pelamis platurus* venoms is the postsynaptic blockade by competitive inhibition of acetylcholine (α-neurotoxins). This group of toxins belongs to the three-finger toxin superfamily (3FTx) (Otero 2007, 2014; Rey-Suárez et al. 2012; Da Silva and Bucaretchi 2003; Brazil 1987; Mori et al. 1989). The venom of *M. dissoleucus* from Colombia also has postsynaptic action combined with myotoxic effect (Renjifo et al. 2012), the latter being similar to that previously described for *M. mipartitus* venom from Colombia (Otero et al. 1992b).

The myotoxicity and neurotoxicity of total venom and of a D49 PLA_2 isolated from *L. muta venom* were searched; in addition to the myotoxic effect, at low doses in avian muscle-nerve preparations, PLA_2 had presynaptic blocking actions in similar form to those of β-neurotoxins as crotoxin from *C. d. terrificus* venom. At high doses in the same preparations, it had postsynaptic actions similarly to α-neurotoxins as those from coral snakes. In *L. muta* human bites, neurotoxicity is exceptional (divergent strabismus, dysarthria, dysphagia). Other vagal signs observed in clinical studies (generalized sweating, hypotension, bradycardia, diarrhea) can be induced by vasodilatation secondary to the release of autacoids, prostanoids, and bradykinins (Otero 1994, 2007, 2014; Silva-Haad 1980/81; Otero-Patiño 2011).

C. d. cumanensis venom induces mild or moderate local effects, without dermonecrosis. In contrast, systemic signs are intense: neurotoxicity, nephrotoxicity, defibrination, thrombocytopenia, myotoxicity (rhabdomyolysis), and myoglobinuria (Otero 1994, 2007, 2014; Amaral et al. 1986; Pinho et al. 2005; De Azevedo-Márques et al. 2003). It is the most lethal snake venom among terrestrial Colombian snakes (Otero et al. 1992b).

Pelamis platurus, as other sea snakes, may bite and leave fang marks, failing to inject enough venom to cause any significant effects in human victims. So the incidence of dry bites (non-envenoming or only trivial envenoming) is high (80%) (Reid 1975). Its bite is usually painless, without local swelling, although its venom is myotoxic (rhabdomyolytic), causing muscle pains; besides, it is nephrotoxic and postsynaptically neurotoxic (Tu and Fulde 1987; Mori et al. 1989; Otero 2007).

Micrurus spp. (coral snake) venoms are essentially neurotoxic, although experimentally in mice and also in patients, they are myotoxic, slightly hemorrhagic, and edema inducing, with high PLA_2 activity expressed as indirect hemolysis (Otero

et al. 1992b; Otero 2007; Da Silva and Bucaretchi 2003; Gutiérrez et al. 1980a; Kitchens and Van Mierop 1987; Pettigrew and Glass 1985). Recently, there were two cases of bites by *Micrurus* spp. from the Amazonian region (the first inflicted by *M. lemniscatus* in Ecuador; the second was probably inflicted by the same snake species in Putumayo, Colombia), presenting in both cases mild coagulopathy without bleeding (PT and PTT moderately prolonged), severe thrombocytopenia, hemolytic microangiopathic anemia, and rise of serum CK concentrations secondary possibly to rhabdomyolysis (Otero 2007, 2014; Otero-Patiño 2007; Manock et al. 2008).

Clinical Features and Diagnosis

Bothrops Bites

The clinical characteristics of local bothropic envenomation include edema (95% of the cases), the classical sign of local envenoming, detectable as early as 5 min after the bite; local hemorrhage (34%), evident as bleeding or ecchymosis during the first 5–30 min; blisters (12%); and dermonecrosis and myonecrosis (10%), apparent only 6 or 8 h after the bite. Defibrinogenation (60–70%) is the classic sign of systemic envenoming, which usually occurs within 30–60 min and in rare cases may be delayed up to 6 h. Thrombocytopenia (15–30% of cases) and hypotension (10–14%) are also observed, as well as systemic bleeding (mainly gingival bleeding and hematuria (25–30%) or through recent wounds). As described for bites induced by various *Bothrops* species, other systemic hemorrhagic manifestations include vaginal, rectal, venipuncture site, or CNS bleeding, epistaxis and hemoptysis (França and Málaque 2003; Gutiérrez and Lomonte 2003; Otero 1994, 2007; Otero et al. 1992a, 2002a; Otero-Patiño 2008, 2009, 2011).

The local envenoming grade is defined by the progression and extension of edema and the presence of necrosis. The alteration of blood coagulation, bleeding, and the presence of life-threatening complications, such as shock, ARF, and damage of vital organs, define the systemic envenoming grade (Table 1; Figs. 11, 12, and 13) (Otero 2007, 2014; Otero-Patiño 2008, 2009). In *B. asper* bites, 15% of patients present severe systemic envenoming and 10% severe local envenoming (necrosis, edema extending to the trunk). On the other hand, 40% of cases have mild and 45% have moderate local envenoming, together with systemic envenoming in 23% and 25% of them, respectively. Additionally, 36% of all patients with *Bothrops* bites only present local envenoming, without systemic manifestations (Otero et al. 1992a; Otero 1994, 2007, 2014; Otero-Patiño 2009). On the basis of previous studies performed in Colombia in *B. asper* bites, only patients with severe envenoming are at risk of death and sequelae (Otero et al. 1992a, 1996, 1999, 2006; Otero 2014; Otero-Patiño et al. 1998, 2007; Otero-Patiño 2009). Nevertheless, the clinical grading herein described has a temporal validity on admission because the envenoming is a dynamic process which progresses in 10–15% of the cases after the beginning of antivenom therapy (Otero 2014; Otero-Patiño 2009).

Table 1 Clinical gradation and specific treatment of envenoming inflicted by *Bothrops*, *Porthidium*, *Bothriopsis*, *Bothriechis*, and *Bothrocophias* spp. bites

Grade of envenoming	Local signs	Systemic signs	Neutralization of venom (mg) – vials of antivenom (n)
Non-envenoming	Mild pain, negligible edema, and hemorrhage	Normal vital signs and blood coagulation	No antivenom needed
			Observation 6 h
			Repeat coagulation test
Mild envenoming	Swelling involving one or two segments of the bitten limb (e.g., foot and leg), circumference of extremity increased <4 cm, ecchymosis; scarce or no bleeding in the bite site, no necrosis	Incoagulable or normal blood, no systemic bleeding, no hemodynamic alterations	No less than 100 mg, 2 vials of A or 4 of B
Moderate envenoming	Swelling involving two or three segments of the bitten limb (e.g., foot, leg, thigh), circumference of extremity increased >4 cm; local bleeding, no local necrosis, blisters in a few cases	Incoagulable blood, systemic bleeding (gingival, hematuria, recent wounds, etc.) no renal failure, no hemodynamic affection	No less than 200 mg, 4 vials of A or 8 of B
Severe envenoming	Swelling extending beyond the bitten limb (to trunk), blisters, local bleeding, necrosis or compartmental syndrome. See text for cases of bites by specimens >1.0–1.10 m body length	Incoagulable blood, multiple systemic bleeding, hypotension or shock, DIC or renal failure, cerebral hemorrhage, or multisystemic failure	No less than 300 mg, 6 vials of A or 12 of B

Adapted from Amaral et al. (1991), França and Málaque (2003), Silva (1989), Wingert and Wainschel (1975), and Reid et al. (1963a, b)and as recommended by Otero (1994, 2007, 2014) and Otero-Patiño (2009, 2011)

A = polyvalent antivenom from INS, Bogotá, Colombia; B = polyvalent antivenoms from Probiol, Bogotá, or from Instituto Bioclon, Mexico

The ontogenetic variations in venom composition have implications in the clinical manifestations of these envenomings. As the venom of adult specimens has a higher content of myotoxic PLA_2 and P-I MPs, snakebites by *B. asper* specimens larger than 100–110 cm body length (adult specimens) involve a higher risk of severe envenoming with dermonecrosis and myonecrosis (Alape-Girón et al. 2008; Gutiérrez et al. 2009a; Otero-Patiño 2009; Sasa et al. 2009; Otero 2014). Similar observations were made by Hardy (1994). In contrast, the venoms of juvenile specimens and offsprings induce higher lethal, hemorrhagic, and defibrinogenating activities at the experimental level (Gutiérrez et al. 1980b; Saldarriaga et al. 2003) in agreement with their higher content of P-II and P-III group metalloproteinases (Alape-Girón et al. 2008; Gutiérrez et al. 2009a, b). Thus, bites inflicted by juvenile

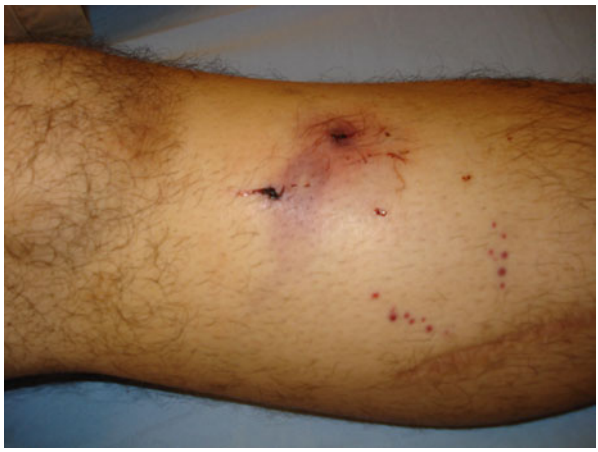

Fig. 11 Mild local bothropic envenomation after *B. asper* bite, combined with moderate systemic envenomation (defibrination, gingival bleeding, microscopic hematuria). See the marks of the fangs and teeth (two bites), edema in the leg, and local ecchymosis (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

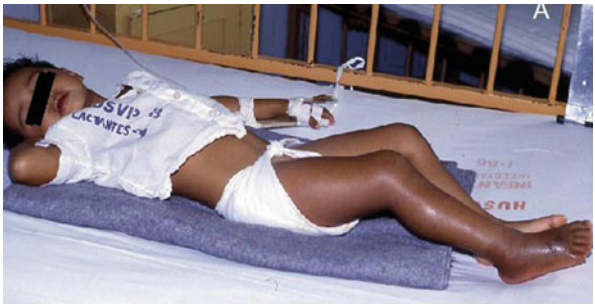

Fig. 12 Moderate local and systemic bothropic envenomation after *B. asper* bite. See edema in tree segments of the bitten limb, with a few blisters on the right foot, without necrosis. Additionally, the patient had macroscopic hematuria and defibrination (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

specimens tend to induce more frequently severe coagulopathy and bleeding in patients, with relatively low serum venom-antigen concentrations as demonstrated by ELISA. Additionally, there have been documented cases of ARF secondary to acute tubular necrosis (ATN) in Colombia in bites inflicted by juvenile (<50 cm) *B. asper* specimens, both in children and adults (Otero et al. 2006; Otero-Patiño 2009).

Mean venom yield of *B. asper* in Colombia corresponds, in terms of dry weight, to 130 mg, with a maximum of 1,000 mg by manual milking of a 175 cm female adult specimen (Otero-Patiño 2009). On the other hand, as has been described for *B. atrox* and *B. jararaca* from Brazil, females of *B. asper* are larger and heavier than males and present a higher venom yield (five times higher in the case of *B. jararaca*) (Belluomini et al. 1991; Furtado et al. 2006).

According to the complexity level and the available resources of health centers, clinicians must rely on laboratory examinations, such as blood coagulation tests

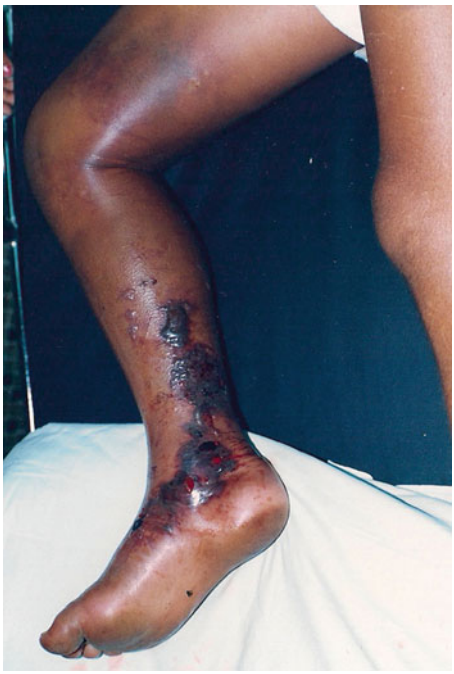

Fig. 13 Severe local and systemic bothropic envenomation after *B. asper* bite. See edema in three segments of the bitten limb, extended to trunk, hemorrhagic blisters, local necrosis, hematuria, defibrination and ARF (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

[whole blood clotting test (WBCT) or the 20-min WBCT], prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen concentration, D-dimer concentration, and fibrinogen/fibrin degradation product (FDP) concentration on admission and at 6, 12, 24, 48, 72, and 96 h after the onset of antivenom therapy (Otero et al. 1992a; Otero-Patiño 2009; Sano-Martins et al. 1994; Warrell 2004). Nevertheless, owing to its low cost, simplicity, and good correlation with plasma fibrinogen concentration, the 20-min WBCT is an effective and reliable method for the evaluation of coagulation status in the primary care centers without laboratory resources (Franca and Málaque 2003; Otero 2007, 2014; Otero-Patiño 2009). In addition, platelet and leukocyte counts are indicated, since 15–30% of envenomed patients present thrombocytopenia and since leukocytosis and neutrophilia (60–75% of patients) are common findings (Otero et al. 1992a). Determination of hemoglobin concentration and hematocrit is also indicated, together with the assessment of acute-phase reactants (C-reactive protein, erythrocyte sedimentation rate), plasma creatine kinase activity (a myonecrosis indicator which remains elevated for several days, mainly in severe cases), serum electrolyte concentration, urinary sediment and serum creatinine concentration (an indicator of renal function) at 24-h intervals at least during 2–4 days. Moreover, monitoring of arterial oxygen saturation (pulse oximetry) is recommended in severe cases instead of arterial blood sampling for pH and arterial gases (Otero-Patiño 2009) due to the risk of hematomas.

Alteration in coagulation tests occurs in moderate and severe envenomings, but not in all mild cases, since only one-half of them develop a concomitant systemic

envenoming. Acute-phase reactants usually rise within the first 48 h of treatment; if the elevation persists on the third day, clinicians must correlate this alteration with leukocyte counts (persistent leukocytosis and neutrophilia) and platelet count (persistent thrombocytopenia) to rule out infection as a likely complication (Otero 1994, 2007; Otero-Patiño 2009; Otero and Mesa 2006). Macro- or microhematuria may occur in moderate and severe cases, whereas the finding of erythrocyte casts in urinary sediment examination is an indicator of acute glomerulonephritis, a typical complication of severe envenoming. The fractional excretion of urinary sodium (FeNa), a good indicator of the proximal renal tubule function, can help to discriminate between prerenal (hypovolemia) or renal failure secondary to ATN, which induces the loss of the ability of the proximal tubule to reabsorb sodium, with the consequent natriuresis. Additionally, an endogenous creatinine clearance of <60 ml/min/1.73 m^2 indicates ARF (Otero 2007; Otero-Patiño 2009; Pinho et al. 2005).

The use of immunoassays (ELISAs), for the determination of serum venom-antigen and antivenom concentrations in patients bitten by *Bothrops* snake species, currently only have value for retrospective studies, as they have not been adapted at the hospital setting to estimate venom-antigen concentration in blood at the time of admission. ELISA has been successfully used in various studies to quantify venom-antigen and antivenom concentrations in clinical samples. Such studies have demonstrated that serum venom-antigen concentrations are higher in patients with severe envenoming (~128–192 ng venom/ml) than in patients with moderate (~39–142 ng venom/ml) or mild envenomings (~8–15 ng venom/ml) (Otero-Patiño 2009; França et al. 2003).

Lachesis spp. Bites

Clinical features are similar to those of *Bothrops* bites: edema (100%), local hemorrhage (80%), blisters (40%), dermonecrosis and myonecrosis (60%), defibrination (100%), mild thrombocytopenia (20%), epistaxis (20%), nausea/vomiting/sweating (60–80%), hypotension (20%), hematuria (40%), and rise of serum creatinine. Vagal excitement signs as severe hypotension, bradycardia, abdominal pain, and diarrhea have also been observed (Silva-Haad 1980/81; Jorge et al. 1997; Otero-Patiño et al. 1993; Málaque and França 2003; De Souza et al. 2007). Some of the complications are ARF (60%), mesenteric thrombosis (5%), central nervous system (CNS) hemorrhage (10%), and compartment syndrome.

The lachesic envenoming grade is established in a similar way as the bothropic envenoming (Table 1). As more than 60% of the cases are severe, all the patients must be treated with the maximal antivenom dose recommended for those cases (to neutralize not less than 300 mg of venom), using a polyvalent antivenom containing adequate specific antibody titers against *Lachesis* spp. venom (Table 2). Defibrination induced by that venom is not efficiently neutralized by one anti-bothropic antivenom, with the consequent prolonged hypofibrinogenemia for up to 10–14 days (Otero 2002; Bard et al. 1994; Escalante et al. 2000). Deaths occur in 10% of patients.

Table 2 Neutralizing potency of anti-pit viper polyvalent antivenoms produced or registered or used in clinical trials in Colombia

Antivenom	Neutralizing potency mg venom/10 ml antivenom			Presentation
	Bothrops	*Crotalus*	*Lachesis*	
Polyvalent INS	70	10	25	Liquid
Polyvalent PROBIOL	25	5	10	Lyophilized
Polyvalent Antivipmyn-Tri®, BIOCLON	30	7	15	Lyophilized
Polyvalent ICP[a]	30	20[a]	30	Liquid

Potency registered at INVIMA, Colombia (Instituto Nacional de Vigilancia de Medicamentos y Alimentos) for the period 2012–2013

INS Instituto Nacional de Salud, Bogotá; *PROBIOL* Laboratorios Probiol, Bogotá; *BIOCLON* Instituto Bioclon S.A. de C.V., Mexico; *ICP* Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica

[a]Polyvalent antivenom from ICP neutralizes *C. simus* venom from Central America. It does not have neutralizing potency against South American crotalic venom (*C. d. terrificus* or *C. d. cumanensis*) (Otero-Patiño 2011; Otero 2014)

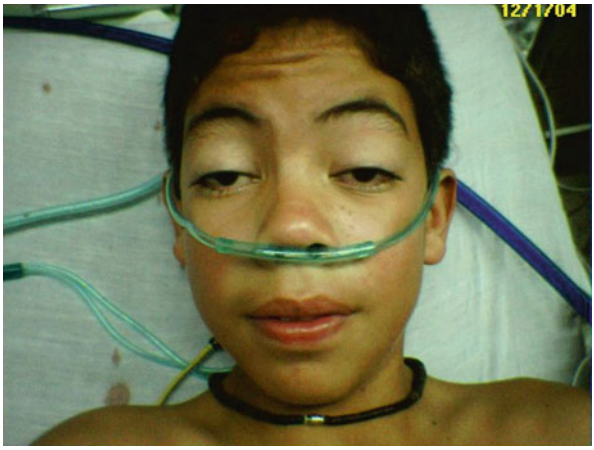

Fig. 14 Palpebral ptosis, 8 h after *M. mipartitus* bite. The patient, 12 years old, additionally presented cephaloplegia, dysphagia, and mild quadriparesis, without affection of respiratory muscles (Photo: Courtesy Dr Rafael Agudelo. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Micrurus (Coral Snake) Bites

This group of venomous snakes has short (2–3 mm) front fangs (proteroglyphous). As a consequence, in human bites, the snake bites for several seconds to inject the venom and the victim has to pull it or to shake off the extremity to detach the snake. Neurotoxicity of early initiation (within the first 2–14 h after the bite) is the main characteristic of this envenomation. This latency period is lower (2 h) in infants and children than for adolescents and adults (4–14 h) (Otero 1994, 2007, 2014; Otero-Patiño 2011; Da Silva and Bucaretchi 2003).

Local signs are negligible: minimum edema around the bite site and paresthesia. The first systemic envenoming sign is palpebral ptosis (Fig. 14), followed by ophthalmoplegia, blurred vision, dysphagia, sialorrhea, cephaloplegia, and paresis

Table 3 Clinical and epidemiological aspects of coral snakebites in Colombia (n = 24)

Age (years old)	No.	%
<15	8	33.3
>15	16	66.7
Mean ± S.D.	21.3 ± 15.5	–
Coral snake species		
M. mipartitus	9	37.5
M. dumerilii	10	41.6
M. nigrocinctus	2	8.3
M. dissoleucus	1	4.2
M. isozonus	1	4.2
Unknown	1	4.2
Time elapsed after bite (hr)		
≤2	8	33.3
3–5	8	33.3
6–12	8	33.3
Mean ± S.D.	5.3 ± 3.3	–
Envenoming signs		
Negligible edema	18	75.0
Palpebral ptosis/ophthalmoplegia	16	66.7
Sialorrhea/dysphagia	16	66.7
Paresis respiratory muscles	15	62.5
Mild quadriparesis	10	41.7
Quadriplegia	6	25.0
Other signs[a]	1	4.2
None paralytic sign[b]	6	25.0
Respiratory support	13	54.2
Deaths	4	16.7

[a]Other signs: one patient presented coagulopathy (PT and PTT increased) and thrombocytopenia, without bleeding, with elevated serum CK and hemolytic anemia

[b]Six patients bitten by different coral snakes (*M. mipartitus*, two cases; *M. dumerilii*, two cases; *M. isozonus*, one case; and *M. dissoleucus*, one case) sought medical attention within 2 h of the bite; all of them received antivenom and did not have signs of envenoming (neurotoxicity) (Otero-Patiño 2011)

of respiratory muscles and of the four extremities which may precede apnea and death (Otero-Patiño 2011). Nevertheless, there is polymorphism in the clinical presentation (Table 3). Fifty to sixty-five percent of patients present respiratory muscle affection with or without apnea and hypoxemia (Fig. 15). Consequently, respiratory support and strict monitoring in the intensive care unit (ICU) constitute an essential part of the treatment, mainly when there is absence of antivenom or when it was administered too late (Otero-Patiño 2011). Atelectasis is a frequent complication (Fig. 16). Thus, almost 50% of patients do not present paralysis signs, or they are mild, either by an early specific treatment or by a dry bite (without venom injection).

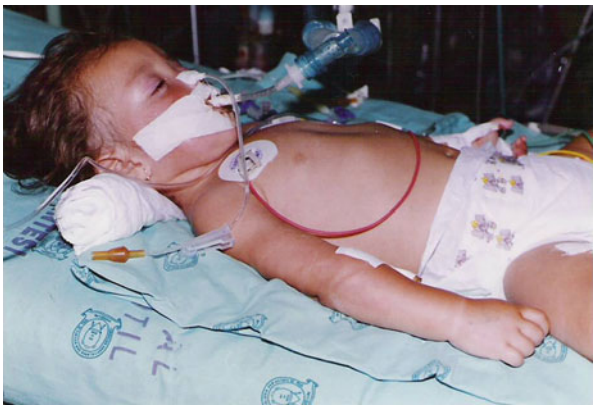

Fig. 15 Respiratory support in a patient (15 months of age), bitten on the hand by a *M. mipartitus* specimen 25 cm body length, in a rural setting. The patient presented apnea and quadriplegia 2 h after the bite. The paralysis continued for 3 days, despite the specific serotherapy administered after the paralysis appearance (see text) (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

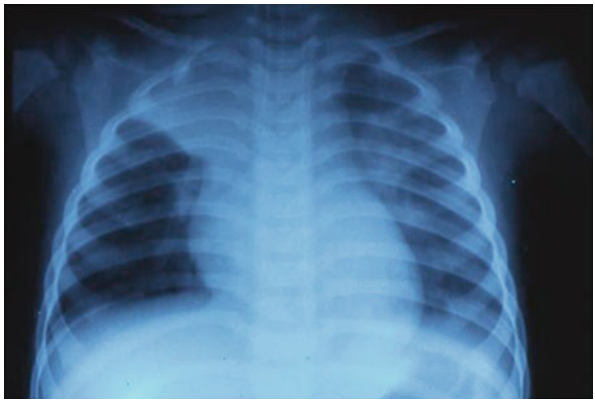

Fig. 16 The same patient suffered atelectasis of the upper right pulmonary lobe and bronchoaspiration (pneumonia at the left lung), before the beginning of mechanical respiration (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Experimentally, *Micrurus* spp. venoms are of low ability to induce edema, hemorrhage, and proteolysis, but they are indirectly hemolytic and some of them are myotoxic (Otero et al. 1992b; Gutiérrez et al. 1980a; Rey-Suárez et al. 2012). The latter effect was then documented in human bites inflicted by *M. fulvius* in the USA and *M. laticollaris* from Mexico (Kitchens and Van Mierop 1987; Pettigrew and Glass 1985) and by coral snakes from the Amazonian region in Colombia (Otero-Patiño 2007, 2011; Otero 2007), as it was also described for *M. lemniscatus* in Peru (Manock et al. 2008). Mortality in Colombia is near 20%, mainly secondary to respiratory insufficiency or complications as atelectasis and pneumonia (Otero 1994, 2007; Da Silva Jr and Bucaretchi 2003; Otero-Patiño 2011).

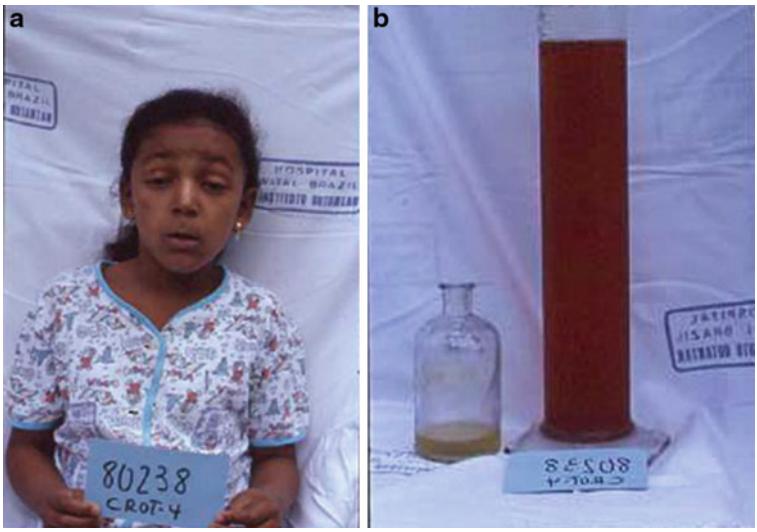

Fig. 17 Moderate crotalic envenomation induced by *C. d. terrificus*. (**a**) See the palpebral ptosis. (**b**) Myoglobinuria without ARF (Photos: Courtesy Dr Joao Luiz C. Cardoso. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

Crotalus d. cumanensis (Rattle Snake) Bites

Crotalic venom from South America is nephrotoxic, neurotoxic, myotoxic (rhabdomyolytic), defibrinant, platelet aggregating, of low capacity to induce hemorrhage and edema, and without dermonecrosis. In moderate envenomation, edema may affect two or three segments of the extremity, with defibrination, thrombocytopenia, myasthenic facies secondary to neurotoxicity, and dark urine by myoglobinuria, without ARF (Fig. 17). In severe cases, ARF occurs in 20–30% of patients, with or without respiratory insufficiency. ARF is the first cause of death (10% of the cases), mainly in patients less than 12 years old or in those receiving specific treatment after 2 h of the bite (Pinho et al. 2005; Otero-Patiño 2011; Otero 2014).

Complications of Bothropic Envenoming

The most frequent complications described in patients envenomed by *B. asper* are:

(a) Soft-tissue infection (11–30% of the cases), characterized by impetigo/cellulitis/abscesses/fasciitis, predominantly cellulitis and abscesses caused by gram-negative rods (*Morganella morganii*, *Proteus rettgeri*, *Klebsiella* spp., *Enterobacter* spp., *Aeromonas hydrophila*) or *Staphylococcus aureus* (Fig. 18). Infection occurs most frequently in moderate/severe local envenomings when extensive edema, hemorrhage, myotoxicity, and necrosis occur, which may

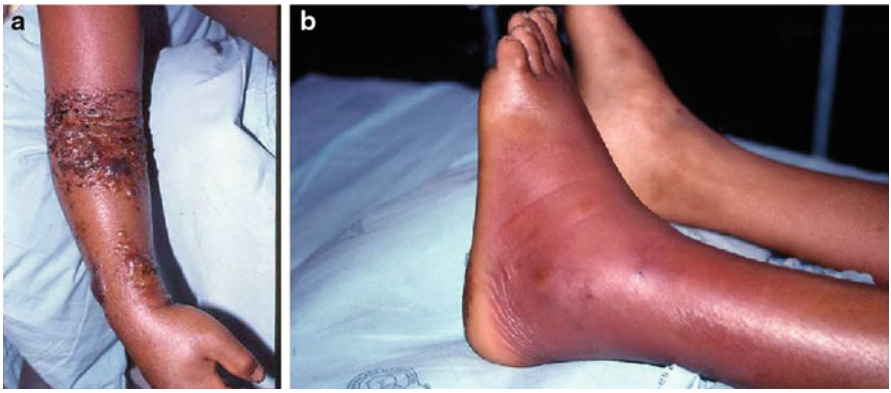

Fig. 18 (**a**) Impetigo and cellulitis after *B. asper* bite. (**b**) Cellulitis and abscess secondary to *Marganella morgagni*, after *P. nasutum* bite (Photos: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

create a favorable environment for the multiplication of bacteria present in the mouth, fangs, and venom of the snake (Jorge et al. 1998, 2004; Jorge and Ribeiro 1997; Saravia-Otten et al. 2007; Otero-Patiño 2009; Saldarriaga and Otero 1995). Culture of aspirates is a valuable guide to identify infecting organisms. Arthritis, osteomyelitis, sepsis, pneumonia, and meningitis are less frequent complications but have been described in *B. asper* envenomings (Fig. 19) (Otero-Patiño 2009).

(b) Acute renal failure (ARF), which develops in 11–17% of patients, sometimes without oliguric phase (<400 ml/day/1.73 m^2), hypertension, hyperkalemia, and metabolic acidosis. Clinically and pathophysiologically, ARF can be prerenal or can be associated with acute glomerulonephritis, acute tubular necrosis, or acute cortical necrosis (Soe et al. 1990), the latter being unresponsive to dialysis during 3–4 weeks (Amaral et al. 1986; Otero 2007; Otero et al. 2002a; Otero-Patiño 2009). These types of renal damage have been demonstrated in autopsies in Costa Rica in patients bitten by *B. asper* and constitute, along with CNS hemorrhage, the main causes of death in those patients (Otero et al. 2002a; Otero-Patiño 2009).

(c) Compartmental syndrome (CPS), which occurs in 3% of patients. In severe local envenoming, extensive swelling can lead to CPS, mainly when the bite is on a finger, hand, and foot or in the anterior tibial compartment (Fig. 20) (Garfin et al. 1979; Hayden 1983; Otero 2002a; Otero-Patiño 2009). If CPS is suspected, the intracompartmental pressure (IP) must be documented by means of the Stryker® pressure monitor or a similar equipment. It is elevated and associated with CPS if the IP is >30 mmHg in children or >45 mmHg in adults (Gómez and Dart 1995; Otero et al. 2002a). Since a needle (No. 16–18) is used in this procedure, it is contraindicated in patients with nonclotting blood (within the first 6–24 h of treatment) or with severe thrombocytopenia, owing to the risk of a large hematoma, thus increasing the morbidity of this intervention (Otero 2014; Otero-Patiño 2009).

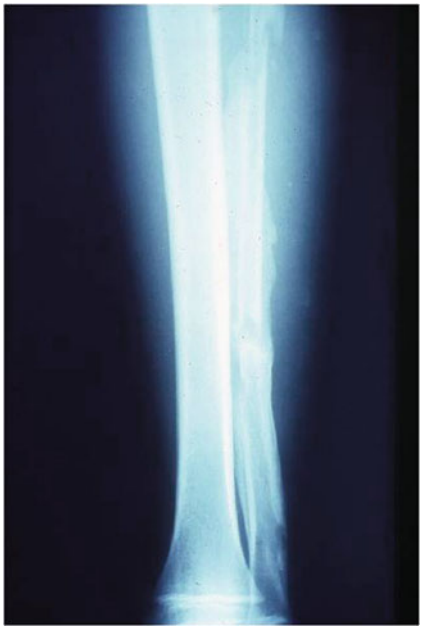

Fig. 19 Chronic osteomyelitis of fibula 4 weeks after *B. asper* bite (Photo: R. Otero-Patiño. Reprinted from Toxicon, 54/7, Otero-Patiño R., epidemiological, clinical and therapeutic aspects of *B. asper* bites, 998–1011, Copyright 2009, with permission from Elsevier)

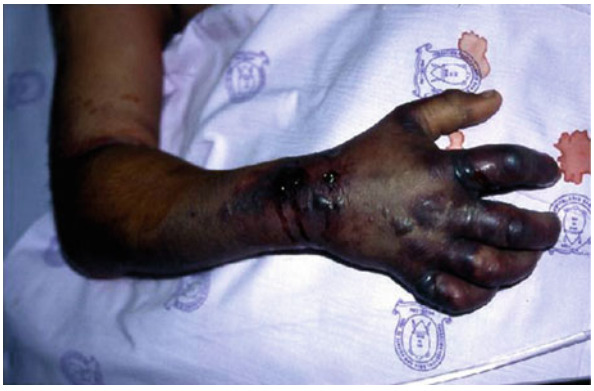

Fig. 20 Compartmental syndrome 36 h after bite inflicted by a *B. asper* specimen 150 cm body length. He suffered ARF and necrotizing fasciitis in the extremity, pneumonia, and pleural effusion. The extremity was amputated when his clinical condition improved (Photo: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

(d) CNS hemorrhage, which occurs in 2–3% of *B. asper* bite victims and constitutes one of the most serious systemic complications of these envenomings. It can be intracerebral, intraventricular (Fig. 21), intramedullar (hematomyelia), subarachnoid, cerebellar, subdural, or extradural, as described also for *B. moojeni*

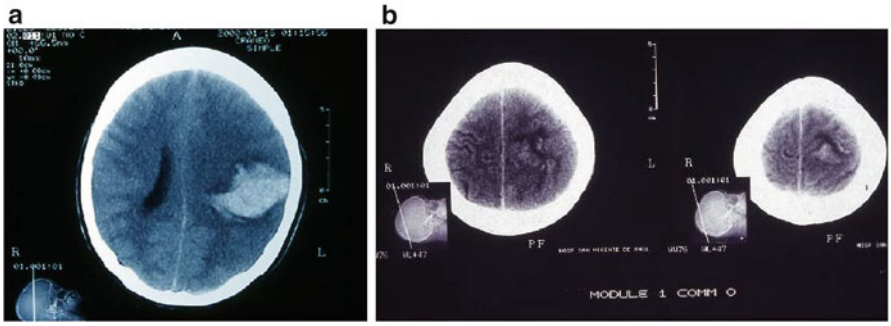

Fig. 21 (**a**) Cerebral hemorrhage affecting the left parietal lobe and ventricle (fluid level), with midline deviation, cerebral edema, and coagulopathy, 48 h after *B. asper* bite in an 18-year-old patient. (**b**) Simple cerebral tomography in a 19-year-old patient bitten by *P. nasutum*. See the left parietal lobe and subarachnoid hemorrhage (Photos: R. Otero-Patiño. Reprinted with permission from Fundamentos de Pediatría, Tomo V (Fondo editorial CIB, Medellín), Otero 2014)

(Kouyoumdjian et al. 1991), *B. atrox*, *Echis ocellatus*, and *Calloselasma rhodostoma* (Kerrigan 1991; Warrell 1995; Otero et al. 2002a). It can be spontaneous or may occur after a cephalic trauma following the bite. Clinical symptoms correspond to the localization of bleeding. For instance, depending on the site of hemorrhage, manifestations can be as diverse as focalization (palsy) and sensitive deficit signs, convulsions, intracranial hypertension, unconsciousness, and meningeal irritation signs. Hemorrhage in the pituitary gland, with secondary panhypopituitarism, has been described for *Daboia russelli* bites in Asia (Warrell 1995), but no cases of this complication have been reported for *B. asper* bites.

(e) Other systemic bleeding complications, such as hemarthrosis (finger joints and knee, with a risk of septic arthritis) and any hematoma distant from the bite site (sublingual, submandibular, subcapsular in the liver, retroperitoneal), have also been described in *B. asper* envenomings (Otero et al. 1992a, 2002a; Otero-Patiño 2009). Diagnosis can be established by means of appropriate imaging (Rx, computed tomography, magnetic resonance, angiography, ultrasound, etc.).

(f) Other complications of *B. asper* bites are hematic or serous pleural effusions in severe cases affecting the chest and upper extremities (Otero-Patiño 2009; Otero-Patiño et al. 2012) (Fig. 22). On the basis of the molecular mass of snake venom toxins and on the potential effect of metalloproteinases and other toxins on the placenta, it is highly likely that various venom components can cross the placental barrier in patients bitten by *B. asper*, causing fetal envenoming and pregnancy complications such as abortion, fetal wastage, and *abruptio placentae* ($\leq$1% of cases). Nevertheless, there are reports of envenomed patients who have completed pregnancy in good condition without complications after *B. asper* bites, certainly requiring strict control of the mother and also a complete

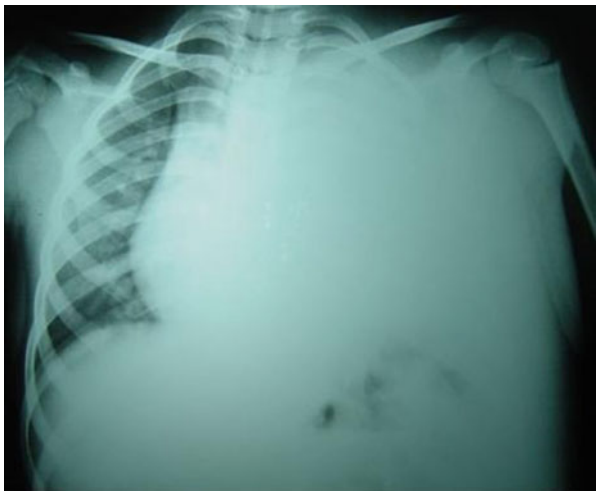

Fig. 22 Hematic pleural effusion with mediastinum deviation, after a *B. asper* bite on the left thorax (Photo: Courtesy Dr Carlos Paredes, Colombia. Reprinted from Toxicon, 54/7, Otero-Patiño R., epidemiological, clinical and therapeutic aspects of *B. asper* bites, 998–1011, Copyright 2009, with permission from Elsevier)

evaluation (renal, CNS, hematological) of the newborn (Zugaib et al. 1985; Pantanowitz and Guidozzi 1996; Otero et al. 1992a, 2002a, 2006; Otero-Patiño 2009).

Thromboses in cerebral or other arteries (femoral, mesenteric, etc.) have been described for *B. lanceolatus* bites in the Lesser Antillean island of Martinique (Thomas et al. 1995, 2006) for *Daboia russelli* in Asia (Ameratunga 1972) and for *Porthidium lansbergii* (one case) in Colombia (Otero 2002a) but are rare (<0.1%) in *B. asper* bites (Otero-Patiño 2009). Pulmonary thromboembolism may occur in *Bothrops* bites inflicted at the dorsal part of the hands or feet, mainly by juvenile or adult specimens of *B. asper* as described above. In these cases, there is an i.v. injection of venom or its rapid diffusion to veins, with little local signs and fast induction (15 min) of unconsciousness and convulsions secondary to anoxia (Otero 2014).

Specific Treatment

Bothropic Envenomation

First aid measures must be limited to immobilization of the extremity and rapid transfer of the patient to the hospital. Tourniquets, suction devices, multiple punctures around the wound, or incisions increase the risk of ischemia and necrosis (the former two) and of hemorrhage and infection (the latter two) (Busch et al. 2000; Hardy 2003). Therefore, these interventions are strongly contraindicated. The black stone, a traditional treatment very common in Africa and in some Afro-American communities from northwestern South America (Colombia), is not an adequate

treatment for snakebites, as indicated by clinical and experimental evidence (Otero et al. 1992a; Chippaux et al. 2007; Otero-Patiño 2009). Patients with severe envenoming should be preferably treated in the ICU. Those with mild or moderate envenoming can be treated in the emergency room during the first 24 h, the latter with monitoring of vital signs. Then, the treatment is followed and completed in a hospitalization ward (Otero-Patiño 2009).

The rational basis for specific antivenom therapy (selection of antivenom and dosage) in a particular region or country depends on the following: (a) the biology, epidemiology, and clinical manifestations of snakebites; (b) the venom yield of different snake species; (c) the toxicological profile of venoms; (d) the in vitro/in vivo evaluation of the neutralizing ability of the antivenoms available against those venoms; and (e) the information generated by controlled clinical trials performed to evaluate the efficacy and safety of antivenoms, together with immunoassays (ELISA) to quantify serum venom-antigen and antivenom concentrations (Theakston et al. 1992; Otero 1998; Otero-Patiño 2009). Three kinds of antivenoms, based on the type of active substance, are currently produced in the world: (a) equine-derived, whole IgG antivenoms obtained by ammonium sulfate or caprylic acid fractionation of plasma; (b) equine-derived $F(ab')_2$ fragments, obtained by pepsin digestion of IgG and ammonium sulfate precipitation; and (c) Fab fragments obtained by papain digestion of ovine-derived IgG, only produced in North America, Europe, and Australia (Otero 2002; Lalloo and Theakston 2003; Theakston et al. 2003).

Following recommendations by the World Health Organization (WHO 1981; Theakston et al. 2003), every country or region must perform preclinical studies to evaluate the neutralizing ability of the available antivenoms, either locally manufactured or imported, against the most relevant toxicological effects of the snake venoms of highest epidemiological importance in those countries and regions. In Colombia, two whole IgG polyvalent antivenoms, manufactured by Instituto Nacional de Salud (INS; neutralizing potency 70 mg *B. asper* venom per 10 ml vial) and Probiol (neutralizing potency 25 mg *B. asper* venom/vial), and one $F(ab')_2$ antivenom imported from Mexico (Bioclon; neutralizing potency 30 mg *B. asper* venom/vial) are produced and/or distributed (Table 2). In addition, antivenoms from Brazil, Costa Rica, and Venezuela, with differences in potency, have neutralizing capacity against *B. asper* venom from Colombia, as demonstrated experimentally (Otero et al. 1995, 1997, 2002b) and clinically (Otero-Patiño et al. 2012).

During the last 19 years, six collaborative randomized/controlled clinical trials (phase III) were performed in patients envenomed by *Bothrops* spp. bites, including the determination of serum venom-antigen and antivenom concentrations by ELISA, using the antivenoms mentioned above. Additionally, a post-marketing study also evaluated the total needs of antivenom (quantity, safety) for the country (Otero et al. 2001b). Taken together, these studies improved the design of rational protocols for the treatment of *Bothrops* spp. bites, in order to evaluate the safety of those products, to elucidate some factors involved in the early adverse reactions (EARs) to antivenoms, and to improve the production and distribution of these immunobiologicals (Otero-Patiño 2009).

As recommended in Brazil for *B. jararaca* and *B. atrox* bites (Amaral et al. 1991; França and Málaque 2003), and the acquired experience in the country after six clinical trials, antivenom doses currently suggested in Colombia for the treatment of *B. asper* bites should neutralize no less than 100, 200, or 300 mg of venom in case of mild (two vials of polyvalent antivenom from INS; four vials of polyvalent antivenom from Probiol or Bioclon), moderate (four vials INS; eight vials Probiol or Bioclon), and severe envenomings (6 vials INS; 12 vials Probiol or Bioclon), respectively (Table 1) (Otero 2007; Otero-Patiño 2009). Additionally, all patients bitten by *B. asper* larger than 1.0–1.1 m (adult specimens), who seek medical attention within the first 2 h, must receive the highest antivenom dose (no less than 6–12 vials, independently of the envenoming grade at this time) due to the risk of severe local/systemic envenoming (Otero et al. 2002a; Otero 2007; Otero-Patiño 2009). For the polyvalent antivenom from Costa Rica, similar doses to those of Probiol and Bioclon products are indicated for *B. asper* bites (Otero-Patiño 2009).

Results of efficacy obtained in Colombia, with the antivenoms described above, were similar in various clinical trials. Briefly, cessation of local and systemic bleeding (different of hematuria) should occur within the first 6–12 h (mainly within 2–3 h) of treatment in 100% of patients, and recovery of blood coagulability (WBCT, PT, PTT, fibrinogen) within 12–24 h after the onset of antivenom therapy in 90–100% of the patients (Otero-Patiño 2009; Otero-Patiño et al. 2012). **These simple clinical and laboratory parameters are recommended as criteria of the efficacy of the initial antivenom dose**. Without specific antivenom administration, coagulopathy can persist during 8–11 days (Kornalik and Vorlová 1990). **If these criteria are not fulfilled, an additional dose of three vials of the same antivenom should be administered at 12 or 24 h of treatment, respectively, or at any time when there is evidence of recurrence of coagulopathy or bleeding. Hematuria stops in 90% of patients who present this sign within 12–24 h and in 100% of patients in 48–72 h** (Otero 2007, 2014; Otero-Patiño 2009, 2011).

In spite of adequate venom neutralization by antivenom, **edema may continue to progress during 12–48 h, with the possible development of CPS. Thus, edema progression stops within 6–12 h in 60–70% of patients, within 12–24 h in other 25–35% of patients, and in 24–48 h in the remaining 5%**. For this reason, it is better to exclude edema progression as a criterion to assess therapeutic success and to determine whether additional antivenom doses should be administered, at least during the first 24 h (Otero et al. 1996). **If edema progression persists during the second day, after excluding secondary infection, an additional dose of three vials of antivenom should be administered** (Otero-Patiño 2009).

Besides the clinical and laboratory criteria of efficacy described, immunoassays also indicate that serum venom-antigen concentrations significantly decreased at the end of the antivenom infusion and remained undetectable during 48–96 h in most patients. Nevertheless, 10–30% of patients had recurrence of antigenemia, without clinical significance (Otero et al. 2006; Otero-Patiño et al. 1998, 2007, 2012). Interestingly, there were some patients (5–10%) who corrected WBCT and fibrinogen levels at a faster rate than PT, or vice versa.

Skin or conjunctival sensitivity tests should not be performed because they have no predictive value for the occurrence of early adverse reactions (EARs) (Malasit et al. 1986; Cupo et al. 1991). Antihistamines or other premedications should not be used, and all patients have to be regarded as potentially reactive to antivenom therapy. Antivenom must be diluted in 0.9% NaCl solution (100 ml for children and 250 ml for adults), and the intravenous infusion should be completed within 30–60 min. Patients should be carefully observed for 24 h for the development of EARs. As previously described (WHO 1981; Lalloo and Theakston 2003), these appear within the first 24 h of antivenom therapy, predominantly during the infusion and within the first 2 h of treatment (Otero-Patiño et al. 2012). Antivenom administration by means of bolus injection is not recommended, because it may induce a rapid hypotension, an effect attributable to the presence of phenol as preservative in many antivenoms (García et al. 2002; Otero-Patiño 2009).

Lachesic Envenomation

As described above, more than 60% of the cases are severe. Consequently, all the patients must be treated with the maximal antivenom dose recommended for those cases (to neutralize not less than 300 mg of venom), using a polyvalent antivenom (12 vials) containing adequate specific antibody titers against *Lachesis* spp. venom (Table 2).

Elapidic Envenomation

As in Colombia there is no production of specific antivenoms against all the epidemiologically important *Micrurus* spp. venoms, it is necessary to import adequate products from other producer countries of the region, after the preclinical evaluation of their neutralizing ability against Colombian *Micrurus* spp. venoms. Thus, Soro Antielapidico® from Instituto Butantan, São Paulo, Brazil, a bivalent product against the venoms of *M. corallinus* and *M. frontalis* from Brazil, has demonstrated neutralizing capacity against the venoms of the three groups (bicolor, monadal, black rings arranged in triads) of coral snakes from Colombia (Campbell and Lamar 2004; Otero and Mesa 2006; Barona et al. 2005; Prieto da Silva et al. 2001; Otero-Patiño 2011). Nevertheless, it is advisable to provide antivenoms with good cross-reactivity against the venoms of the Colombian monadal group of coral snakes, as those produced in Mexico and Costa Rica by immunizing horses with *M. nigrocinctus* venom (Table 4).

An i.v. infusion of five vials is the recommended antivenom dose for coral snakebites from the Caribbean, Pacific, and Andean regions of the country, diluted in 100–250 ml of isotonic saline solution, administered within 30–60 min, or ten vials for patients bitten by coral snakes of black rings arranged in triads, in the Orinochian and Amazonian regions, snakes with higher venom

Table 4 Neutralizing potency of anti-elapidic antivenoms available in Colombia, against coral snakebites

Antivenoms	Neutralizing potency mg venom/10 ml antivenom			Commercial presentation
	M. mipartitus	*M. dumerilii*	*M. nigrocinctus*	
Suero antiofídico ANTI-CORAL, ICP (Whole IgG)[a]	N.N[b]	3.0	3.0	Liquid (cage × 1 vial 10 ml)
Soro antielapídico, Instituto Butantan [F(ab′)$_2$][c]	2.7	11.2	Unknown	Liquid (cage × 5 vials 10 ml)
Coralmyn, Instituto Bioclon S.A. de C.V., Mexico [F(ab′)$_2$][d]	N.N[b]	9.2	5.0	Lyophilized (cage × 1 vial 10 ml)

[a]ICP: Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica; whole IgG antivenom produced immunizing horses with *M. nigrocinctus* venom from Costa Rica
[b]N.N: coral snake venom (*M. mipartitus*) not neutralized
[c]Instituto Butantan, Sao Paulo, Brazil; [F(ab′)$_2$] antivenom produced immunizing horses with *M. frontalis* (black rings arranged in triads group) and *M. corallinus* (monadal group) from Brazil
[d][F(ab′)$_2$] antivenom produced immunizing horses with *M. nigrocinctus* venom from Mexico. References: Barona et al. 2005; Otero-Patiño 2011; Otero 2014. Additionally, Laboratorios Probiol, Bogotá, Colombia, produces Suero Antiofídico Anticoral Liofilizado whose potency against coral snake venoms from different regions of the country is unknown

production (Silva-Haad 1994). The highest value of the antivenom administration is within the first 2 h of the bite, before paralysis appearance (Otero 2007; Otero-Patiño 2011).

Crotalic Envenomation

The three polyvalent antivenoms registered in Colombia, with some differences in potency, have antibodies against *C. d. cumanensis* venom (Table 2). Being a very lethal venom, in mild cases, it is recommended to neutralize at least 50–75 mg of venom (5–10 vials); in moderate cases, 100–150 mg of venom (10–15 vials); and in severe cases, 200–300 mg of venom (20–30 vials) (De Azevedo-Márques et al. 2003; Pinho et al. 2005; Otero-Patiño 2011; Otero 2014).

Adverse Reactions to Antivenom Therapy

EARs might correspond to type I hypersensitivity reactions, which involve IgE-mediated degranulation of mast cells and basophils, with rapid release (within minutes) of preformed mediators, such as histamine, TNF-α, and IL-4. Arachidonic acid metabolites, such as leukotriene D_4 and prostaglandin D_2, are synthesized and released more slowly (30 min to hours). The cross-linking of two molecules of IgE antibodies specific for the equine IgG (their relevant allergen) on the specific

receptors of cell surface (FcER1) is the triggering mechanism involved in anaphylactic reactions, for degranulation of mast cells and basophils. Nevertheless, histamine release can also be triggered by agents that act on other receptors of the mast cell/basophil plasma membrane; for example, the complement-derived fragments C3a and C5a (anaphylatoxins) generated through complement activation by heterologous IgG may induce mast cell/basophil degranulation. Such acute reactions to these agents, which do not involve IgE antibodies, are referred to as anaphylactoid reactions (Fan and França 1992; Otero-Patiño 2009). The majority of EARs (>90%) after antivenom infusion are anaphylactoid, whereas less than 10% of EARs correspond to true anaphylactic reactions. Pyrogenic reactions may occur after the administration of antivenoms containing bacterial endotoxins (Otero-Patiño et al. 1998).

The incidence of EARs varies depending on the product. It is relatively low using some $F(ab')_2$ preparations (12–36%) and caprylic acid-fractionated IgG antivenoms (11–29%). The former antivenoms are produced in Brazil, Mexico, and Venezuela, and the latter are produced in Costa Rica (Otero-Patiño 2009).

The incidence of EARs is also associated with the physicochemical characteristics of antivenoms. Factors such as albumin contamination, high load of protein, and the presence of protein aggregates are likely to contribute to EARs (Otero-Patiño 2009), thus stressing the need for good fractionation protocols that yield highly purified $F(ab')_2$ or IgG products. It has been demonstrated that antivenoms made of whole IgG obtained by ammonium sulfate precipitation have higher in vitro anticomplementary activity and, accordingly, induce a relatively high incidence of EARs (25–82%). In contrast, a lower incidence of EARs has been described after the administration of caprylic acid-fractionated whole IgG and $F(ab')_2$ preparations (11–29%) (Otero-Patiño 2009; Otero-Patiño et al. 2012).

EARs are classified as mild (characterized by cutaneous reactions, as urticaria or rash, or gastrointestinal reactions, chills, fever), moderate (associated with mild hypotension, facial angioneurotic edema), and severe (characterized by airway angioedema, shock, cardiac arrest, bronchospasm) (Otero-Patiño et al. 1998, 2012). These reactions should be treated as recommended by Fan and França (1992) and Otero-Patiño et al. (1998, 2007, 2012). Briefly, the antivenom infusion has to be stopped, and adrenaline (0.01 mg/kg in children and 0.3–0.5 mg in adults) should be administered subcutaneously for mild/moderate EARs and intravenously for severe EARs. Although some authors recommend intramuscular (i.m.) rather than subcutaneous (s.c.) adrenaline in terms of bioavailability, the risk of deep hematomas associated with i.m. injections in patients having coagulopathy must be kept in mind. Additionally, patients must receive hydrocortisone i.v. 100–200 mg every 6 h during 24 h (5–10 mg/kg/day in children), or any equivalent corticosteroid, and one i.v. dose of antihistamine (clemastine 0.0125–0.025 mg/kg in children and 2.0 mg in adults or diphenhydramine 1.0–2.0 mg/kg in children and 50 mg in adults). Once EAR symptoms have subsided or ameliorated (usually within 15 min), antivenom infusion should be continued with caution. If EARs repeat, a parallel continuous infusion of adrenaline (1.0 mg diluted in 250 ml 0.9% NaCl solution) should be administered during the antivenom infusion (Otero 2007; Otero-Patiño 2009; Otero-Patiño et al. 2012).

After discharge from the hospital, patients should be asked to return for reevaluation within the next 4 weeks and need to be instructed about the possible development of serum sickness within 3–24 days after antivenom infusion (Otero-Patiño et al. 1998; Otero 2007; Otero-Patiño 2009). This complication occurs in 30–75% of patients receiving heterologous immunoglobulins, as a result of the development of antibodies by the patient against equine IgG or $F(ab')_2$ with the formation of antigen-antibody complexes and complement activation (Lalloo and Theakston 2003). The circulating immune complexes are deposited in the walls of microvessels in various tissues (skin, lymphatic nodes, kidney, peripheral nerves, serous membranes) leading to increased vascular permeability and thus to a type III hypersensitivity reaction or immune complex disease. Fever, arthralgia, pruritus, urticaria, enlargement of lymphatic nodes, proteinuria, and a drop in serum complement activity are the usual symptoms and signs of serum sickness; in rare cases, glomerulonephritis and peripheral neuropathy can also occur (Otero-Patiño 2009).

The development of serum sickness is associated with the antivenom dose administered, i.e., with the total load of heterologous proteins received by the patient (LoVecchio et al. 2003; Otero 2007). This late adverse reaction is treated with antihistamines and, if necessary, with a short cycle of corticosteroids. As most of patients affected by snakebites live in remote rural communities, there is under-registration of this adverse reaction in tropical areas of Latin America, where only 20–30% of patients come back for reevaluation (Otero 2007; Otero-Patiño 2009).

Ancillary Treatment

Bothropic Envenomation

The ancillary treatment of *Bothrops* spp. envenomings includes the following: (a) the correction of hypovolemia; (b) the early administration of wide-spectrum antibiotics in severe local envenomations or whenever there is overinfection; the tetanus prophylaxis at the second day of treatment (after normalization of blood coagulation tests due to the risk of i.m. hematoma in patients with unclottable blood); (c) the diagnosis and treatment of CPS; (d) pain management; (e) the treatment of wound and complications (infection, surgical procedures, rehabilitation); and (f) the definition of the traditional (folk) medicine role (Otero-Patiño 2009):

(a) For the adequate correction of hypovolemia, two peripheral venous lines should be canalized: one for antivenom administration and the other to infuse crystalloids (normal saline solution or Ringer's lactate solution), as needed (15–30 ml/kg or more in 30–60 min) to restore circulating volume. The measurement of the arterial oxygen saturation is recommended, in order to assess the need to provide oxygen therapy. Then, i.v. liquids have to satisfy 1–2 times the daily needs of water, glucose, and electrolytes. In all cases, urinary output should be measured hourly, in severe cases, by means of a bladder catheter. Normally, adolescents and adults eliminate more than 0.5 ml/kg/h (30–40 ml/h) and

children more than 1.0–2.0 ml/kg/h. In severe envenomings, especially when there is anuria, the management of i.v. liquids is more difficult, and preferably, the patient should have a central venous catheter for continuous measurement of the central venous pressure (Otero 1994, 2007; Otero and Mesa 2001, 2006; Otero-Patiño 2009). If, after a second bolus of i.v. liquids, urinary output does not normalize, furosemide (1–2 mg/kg i.v., maximal dose 5 mg/kg/day) should be administered. If anuria persists, an i.v. infusion of dopamine (2.5–5.0 μg/kg/min) within 6 h may be recommended to normalize renal plasmatic flow and glomerular filtration (Otero 2007; Otero and Mesa 2001; Otero-Patiño 2009). Finally, if an adequate response is not achieved, concentrations of serum creatinine and electrolytes, and an electrocardiogram, should be assessed because the patient is likely to develop ARF in these circumstances, possibly secondary to ATN. Some criteria to perform dialysis (20% of patients with ARF secondary to *Bothrops* spp. bites need dialysis) are metabolic acidosis, persistent and unresponsive anuria with hypervolemia, elevation of serum creatinine concentration above 5 mg/dl, and hyperkalemia (>6 mEq/l) (Otero et al. 1992a, 2002a; Pinho et al. 2005; Otero 2007; Otero-Patiño 2009).

(b) The early administration of antibiotics in severe local bothropic envenoming, specially by *B. asper* (not prophylaxis, a term that implies the use of antibiotics before the trauma), is controversial for several reasons. Among these reasons are as follows: (1) bacteria of high pathogenicity might have been injected with the venom in the affected tissues for hemorrhage and necrosis, enhancing the development of infection; (2) the groups of patients of different studies are not comparable; (3) some clinicians prefer to wait for the appearance of infection signs; (4) almost all the patients with severe local envenoming after a *B. asper* bite suffer some type of local infection (impetigo, cellulitis, abscess, fasciitis), sometimes complicated with bacteremia and sepsis (pneumonia); (5) the indiscriminate use of broad-spectrum antibiotics promotes the development of bacterial resistance (Goldstein 1992; Blaylock 1999; Jorge et al. 1994, 1998, 2004; Jorge and Ribeiro 1997; Otero-Patiño 2009, 2011). However, despite the uncertainty derived from these considerations, envenomings by *B. asper*, and other large snakes distributed in Latin America such as *B. atrox*, *B. jararacussu*, and *L. muta*, are often associated with severe local effects and infection. Thus, the antibiotic schedule must include one of the following combinations against gram-negative rods and *S. aureus*: clindamycin + ceftriaxone, clindamycin + ciprofloxacin, or sulbactam + ampicillin. This antibiotic treatment in severe local envenomings inflicted by *B. asper* should be followed during hospitalization of the patient and thereafter, whenever required (Otero 2007; Otero et al. 2002a; Saravia-Otten et al. 2007; Otero-Patiño 2009). Adequate samples for cultures (blood, exudates, pus, blister contents) should be collected in order to identify the bacteria and to assess its corresponding antibiotic sensitivity (Otero et al. 2002a; Otero-Patiño 2009).

(c) CPS is clinically suspected by the presence of tight edema, dysesthesia, alteration of deep sensitivity (proprioceptive) in the extremity, and limitation of movement, with or without slow capillary filling. It occurs during the first

2 days of envenoming, mainly within the first 12 h (phase of rapid increment of edema) (Otero 2007; Otero-Patiño 2009). Diagnosis must be confirmed by measurement of IP, as described above. However, during the first 12 h of treatment, the measurement of IP is not recommended owing to the risk of hematoma under the fascia in a coagulopathic patient. Therefore, an alternative method is the administration of an i.v. infusion of the osmotic diuretic mannitol (1–2 g/kg) in 30–60 min (half-life 1.7 h), which generally induces a fast reduction of IP within 2 h. This has been reported to reduce the need of fasciotomy in many cases (Gómez and Dart 1995; Warrell 1995; Otero et al. 2002a; Otero-Patiño 2009).

Mannitol is contraindicated if the following are present: (1) persistent anuria with hypervolemia, (2) oliguria with hypovolemia, (3) active bleeding into the CNS, and (4) edema or pulmonary hemorrhage (Otero 2007; Otero-Patiño 2009). The use of fasciotomy has been a controversial subject. Morbidity, disfiguration/scars, and complications (infection, bleeding) increase with this surgical intervention; it must be deferred only for those patients with high IP which does not decrease after mannitol administration (Garfin et al. 1979; Warrell 1995, 2004; Otero et al. 2002a; Dart 2004; Otero-Patiño 2009).

(d) For an analgesic effect, tramadol or meperidine (1–2 mg/kg i.v. every 6–12 h) or acetaminophen per oral route is recommended. Morphine should not be used because it causes a decrease of venous return and hypotension, which are particularly problematic in hypovolemic patients. Nonsteroidal anti-inflammatory drugs (NSAIDs) are not recommended, because they inhibit platelet aggregation and may exert renal, hepatic, and gastrointestinal deleterious actions (Otero 2007; Otero and Mesa 2006; Otero-Patiño 2009). An adequate antivenom dose administered early in the course of envenoming usually induces a fast reduction of pain, because it interferes with the activation that venom toxins induce on inflammatory cascades (Otero 2007; Otero-Patiño 2009). Intramuscular injections should be avoided during the first 24–48 h of treatment or while coagulopathy persists.

(e) The affected extremity must rest at the level of the bed, i.e., neither elevated nor pendant. Wound cleaning may be performed with saline solution and a soft antiseptic every day; then, it can be covered with sterile gauzes moistened with saline solution without bandage. Blister contents must be aspirated with sterile syringe at 12- to 24-h intervals, because they contain high venom antigen concentrations that can be reabsorbed. The search for infection is recommended, ordering cultures when necessary. Careful debridement of necrotic soft tissue, as well as amputations, must be performed under anesthesia and in a sequence indicated by clinical evolution, usually after the third day (Otero 2007; Otero-Patiño 2009). At this time, covering with gauzes, antibacterial or healing ointments, and bandages is indicated. Surgical drainage of soft tissue abscesses and necrotizing fasciitis should be as extensive as necessary, especially in the latter. Drainage should be followed by cultures for aerobic and anaerobic bacteria. After the second week, a rehabilitation program is essential for some patients who develop local complications (skin grafts, physical exercises,

prosthesis, etc.) (Otero 2007; Otero-Patiño 2009, 2011). Finally, in patients who presented soft tissue infection, especially in moderate and severe cases, and when the bite was on anatomical sites covered by a thin layer of soft tissue, i.e., tibial or peroneal compartment, fingers, and head, a radiographic assessment of the affected limb or anatomical part is recommended at 2 and at 4 weeks after the bite, in order to rule out osteomyelitis (Otero 2007; Otero-Patiño 2009).

(f) Despite the demonstration of significant neutralization of pharmacological and enzymatic effects (lethality, edema, hemorrhage, defibrination, coagulant) of snake venoms in experiments performed by preincubating venom and plant extracts (in vitro), before the administration to mice, this protective effect disappeared or lowered significantly when both substances were independently administered by separate routes. For example, when the plant extract was administered after the venom injection as occurs in natural conditions. In consequence, snakebites will not be treated with plant extracts as a unique and definitive therapy to replace the antivenom. Additionally, there are no clinical trials (phase III studies) neither designed nor approved by ethical reasons which allow to recommend their use (plant extracts) in patients (Otero et al. 2000a, b).

Lachesic Envenomation

The ancillary treatment is similar to that recommended for *Bothrops* bites.

Elapidic Envenomation

The support or ancillary treatment includes the following: (a) strict monitoring of vital signs and oximetry, including those patients with early admission without paralysis; (b) availability of the ICU for those patients who need respiratory support; (c) intravenous liquids have to satisfy 1–2 times the daily needs of water, glucose, and electrolytes; (d) antimicrobials usually are not needed, whenever overinfection (pneumonia secondary to mechanical ventilation) or bronchoaspiration does not occur; (e) pharmacological treatment with anticholinesterases (neostigmine + atropine), currently not used because many coral snake venoms have combined presynaptic and postsynaptic actions (containing both α-and β-neurotoxins) (Otero 2007; Da Silva Jr and Bucaretchi 2003; Otero-Patiño 2011).

Crotalic Envenomation

The ancillary treatment includes the following: (a) the maintenance of adequate diuresis (2.0 ml/kg/h in children; 30–40 ml/h in adolescents and adults); (b) prevention of the renal damage secondary to rhabdomyolysis and myoglobinuria by alkalinizing urine by means of hydrating the patients with a solution of 5% glucose (500 ml), 20% mannitol (25 ml), 20% sodium chloride (10 ml), and 8.4%

sodium bicarbonate (20 ml), two times the daily maintenance during 48–72 h (Pinho et al. 2005); (c) if diuresis does not improve using this protocol, i.v. furosemide (2.0–4.0 mg/kg) must be administered; (d) antimicrobials are only indicated if infection signs appear; tetanus prophylaxis should be indicated after coagulation normalization. Dialysis will be required for 25% of patients complicated by ARF in this envenomation. Respiratory support must be available (ICU) for the rare cases of hypoxemia secondary to paresis of respiratory muscles (Otero 2007, 2014; Otero-Patiño 2011).

Conclusions and Future Directions

The strategy of primary health care is the most viable and the best method to decrease the high impact of snakebites in morbidity, mortality, and sequelae in Latin America and for prevention programs. Some of the essential components for the success of this strategy are as follows: (a) the education offered to the community and health workers by multiple media about snakes, venoms, snakebites, treatment, and prevention; (b) to have adequate channels for the early supply and distribution of antivenoms and other basic medicines which are necessary for the attention of patients at health institutions of different complexity; (c) the interdisciplinary work; (d) the participation of the communities in different components of the strategy; (e) an expedite network of communications and transportation media to transfer patients opportunely; (f) a suitable distribution of antivenoms and delegation of functions for the health personnel according to the grade of envenoming of patients and the resources available in the attendant institution; (g) the epidemiological surveillance; (h) the strategic alliance university/health authorities to develop research and educational programs; and (i) an adequate financial support, mainly from the budget of the Ministry of Health, who must be outlining policies and evaluating the program (Otero et al. 1992c, 2001a; Gutiérrez et al. 2006a; Otero-Patiño 2009, 2011).

Cross-References

► Snakebite Envenoming in Latin America and the Caribbean

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Snakebite Envenoming in Latin America and the Caribbean

2

José María Gutiérrez

Contents

Abstract

Envenomings induced by snakebites constitute a serious public health problem in Latin America. This condition affects predominantly vulnerable rural populations and has a high impact in regions where the provision of health services is deficient. Most envenomings are provoked by species of the genera *Bothrops* and *Crotalus*, classified in the family Viperidae, whereas about 1% of cases are

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due to *Micrurus* species (family Elapidae). There are laboratories in several countries in the region which manufacture antivenoms. Scientific and biotechnological research has generated a significant body of knowledge on snakes and their venoms and on antivenoms. Despite important advances in the control of these envenomings in Latin America, it is necessary to strengthen regional efforts in order to (a) improve the knowledge on snakes and their venoms; (b) acquire information on the incidence and mortality of snakebite envenomings; (c) increase the volume of antivenom produced and, in some cases, the quality of antivenoms; (d) improve the regulatory work of national quality control laboratories; (e) develop knowledge-based strategies of distribution of antivenoms; (f) consolidate continuous education programs for the health staff in charge of the treatment of these envenomings; (g) ensure support to people that suffer physical or psychological sequelae as a consequence of these envenomings; and (h) strengthen community programs aimed at improving the prevention and adequate management of snakebites. The development of interprogrammatic and inter-sectorial projects in this field should be promoted in the region, involving multiple actors and institutions, within a frame of regional cooperation programs.

Introduction

Snakebite envenoming constitutes a highly relevant public health problem, particularly in Africa, Asia, and Latin America (Kasturiratne et al. 2008; Gutiérrez et al. 2010a). This pathology, which largely affects impoverished populations in rural settings (Harrison et al. 2009), has been largely neglected by health authorities, research agendas, and pharmaceutical companies. As a consequence, the World Health Organization (WHO) has included snakebite envenoming in its list of neglected tropical diseases (www.who.int/neglected_diseases/diseases/en/). A renewed interest in this subject has been raised in the last years, resulting in a number of initiatives; publications; scientific events; regional workshops; the publication of the WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins (WHO 2010); and the birth of the Global Snakebite Initiative (GSI; www.snakebiteinitiative.org) (Williams et al. 2010). In Latin America, a number of initiatives have been developed aimed at increasing the awareness of the seriousness of this pathology and at promoting regional cooperative efforts to confront it (www.paho.org/spanish/ad/dpc/vp/poisonous-animals.htm; Gutiérrez et al. 2007).

An effective attention to this public health problem demands concerted efforts in the frame of an integrated and holistic strategy, taking into consideration its complexity and multifactorial nature (Williams et al. 2010; Gutiérrez et al. 2010a). This demands actions in scientific research, technological development and innovation, production of sufficient volumes of safe and effective antivenoms, programs for acquisition and distribution of antivenoms to regions where they are needed, permanent education programs for health personnel, effective prevention platforms, and adequate attention to people suffering from sequelae secondary to snakebites. This

chapter discusses snakebite envenomings in Latin America and the Caribbean and highlights some of the tasks that need to be undertaken in this region in order to significantly reduce the impact of this disease.

Epidemiology

Incidence and Mortality

Information on the incidence of snakebite envenomings is rather incomplete in many regions of the world (WHO 2010). The majority of the studies have been based on hospital statistics, which are often incomplete, for various reasons. In some cases, only a fraction of snakebitten patients attend hospitals and health centers for attention, and also the collection of hospital records by epidemiological surveillance units is often deficient. When more precise estimations have been performed, such as in community- and household-based studies, the actual dimension of incidence and mortality is evident. The problem of poor or incomplete epidemiological records on snakebites also occurs in Latin America (Gutiérrez 2011). Therefore, it is necessary to develop national and regional efforts in this region aimed at gathering robust epidemiological data on snakebite envenomings by integrating hospital-based information with community-based studies, in order to have a more precise estimation of the magnitude of this public health problem. An aspect that will contribute to this goal is the introduction of compulsory report of snakebites; this has been achieved in some countries (Gutiérrez et al. 2007), but needs to be generalized to the whole region.

Despite the limitations of currently available data, a rough estimation of the incidence and mortality of snakebite envenomings in Latin America and the Caribbean can be obtained by using records emanating from hospitals and ministries of health. Such estimations indicate that there are at least 70,000 cases of snakebites in the region (Table 1). Such estimation corresponds to the lower limit of a study in which the global burden of snakebites was investigated (Kasturiratne et al. 2008). In this work, the annual number of snakebites in the region was estimated to be in the range of 80,329–129,084. Likewise, data on mortality are also incomplete, although there is reliable information for some countries. The mortality rates (expressed per 100,000 population per year) described for various countries are Costa Rica, 0.02–0.15; Panama, 0.5 (Hildaura Acosta, personal communication); Venezuela, 0.1–0.2; Brazil, 0.05; and Ecuador, 0.05 (Gutiérrez 2011 and references therein). Kasturiratne et al. (2008) estimated the total number of deaths due to snakebite envenomings in Latin America to be in the range of 540–2,298, although this is likely to represent an underestimation due to the problems discussed above.

Snakebites affect predominantly young adult agricultural workers, especially males, although a significant number of cases also occur in women, as well as in children and adolescents, most of whom are affected when working in the fields (de Oliveira et al. 2009). Most of the accidents occur when people are performing agricultural duties (de Oliveira et al. 2009; Gutiérrez 2010, 2011). Incidence varies

Table 1 Estimated number of snakebites per year per country in Latin America and the Caribbean and species of highest medical impact in each country (From Gutiérrez (2011) and references therein)

Country	Estimated number of snakebites per year	Species of highest medical impact[a]
North America		
Mexico	27,000	*Agkistrodon bilineatus*
		Agkistrodon taylori
		Bothrops asper
		Crotalus atrox
		Crotalus scutulatus
		Crotalus simus
		Crotalus totonacus
Central America		
Belize	50	*Bothrops asper*
Costa Rica	500–600	*Bothrops asper*
		Crotalus simus
El Salvador	50	*Crotalus simus*
Guatemala	500	*Bothrops asper*
		Crotalus simus
Honduras	500	*Bothrops asper*
Nicaragua	600	*Bothrops asper*
		Crotalus simus
Panamá	1,300–1,800	*Bothrops asper*
The Caribbean		
Aruba	Information not found	*Crotalus durissus*
Martinique	20	*Bothrops lanceolatus*
Saint Lucia	12	*Bothrops caribbaeus*
Trinidad and Tobago	Information not found	*Bothrops atrox*
South America		
Argentina	270	*Bothrops alternatus*
		Bothrops diporus[b]
		Crotalus durissus
Bolivia	1,000	*Bothrops atrox*
		Bothrops mattogrossensis[b]
		Crotalus durissus
Brazil	26,000–29,000	*Bothrops atrox*
		Bothrops jararaca
		Bothrops jararacussu
		Bothrops leucurus
		Bothrops moojeni
		Crotalus durissus
Colombia	3,000	*Bothrops asper*
		Bothrops atrox
		Bothrops bilineatus
		Crotalus durissus

(*continued*)

Table 1 (continued)

Country	Estimated number of snakebites per year	Species of highest medical impact[a]
Ecuador	1,400–1,600	*Bothrops asper*
		Bothrops atrox
		Bothrops bilineatus
		Lachesis muta
Guiana	200	*Bothrops atrox*
		Bothrops bilineatus
		Bothrops brazili
		Crotalus durissus
French Guiana	100	*Bothrops atrox*
		Bothrops bilineatus
		Bothrops brazili
		Crotalus durissus
Paraguay	400–500	*Bothrops alternatus*
		Crotalus durissus
Peru	1,400–1,500	*Bothrops atrox*
		Bothrops bilineatus
		Bothrops pictus
		Crotalus durissus
		Lachesis muta
Suriname	Information not found	*Bothrops atrox*
		Bothrops bilineatus
		Bothrops brazili
		Crotalus durissus
Uruguay	50–60	*Bothrops alternatus*
		Crotalus durissus
Venezuela	7,000	*Bothrops atrox*
		Bothrops colombiensis
		Bothrops venezuelensis
		Crotalus durissus

[a]The species having the highest medical impact are those classified within category 1 in the *WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins* (WHO 2010). In the case of Venezuela, the species *Bothrops colombiensis* is added
[b]Formerly classified as subspecies of *Bothrops neuwiedi*

during the year, with peaks generally occurring during the rainy season, associated with agricultural work (Sasa and Vázquez 2003; de Oliveira et al. 2009). For some snake species, the invasion of natural habitats by agricultural activities and the development of human settlements provokes a close contact between snakes and people, which increases the likelihood of accidents, as occurs in the case of the viperid species *Bothrops asper* (Gutiérrez 2010) and other species that also adapt to altered environments. Likewise, the effect of natural disasters on snakebite incidence needs to be considered, as floods or other natural phenomena might increase the

incidence of snakebites. However, presenting general data on incidence per country does not allow the identification of specific regions where the magnitude of this problem is very high. Thus, there is a need to assess incidence and mortality on a regional basis within countries, in order to detect highly vulnerable regions and human groups that require particular attention of public health programs, such as indigenous populations. The use of Geographical Information Systems (GIS) methodologies should be fostered, with the aim of analyzing spatial patterns of distribution of incidence, snake species, location of health services, and transportation facilities, among other parameters. These technologies have been used in Argentina (Leynaud and Reati 2009) and Costa Rica (Hansson et al. 2013) and have allowed the identification of vulnerable regions in which provision of health care for snakebitten patients should be improved.

Sequelae of Snakebite Envenomings: The Need to Know Their Impact

The impact of snakebite envenomings in Latin America should be viewed from a wide perspective, considering the consequences in terms of permanent sequelae and of social and economic implications. An unknown percentage of viperid snakebite cases end up in permanent physical sequelae associated with tissue loss or dysfunction (Warrell 2004; Cardoso et al. 2009; Gutiérrez et al. 2010a). Moreover, psychological sequelae occur after snakebite envenomings, as has been described in Sri Lanka (Williams et al. 2011). Although this subject has not been investigated in Latin America, the severity and complications of many of these envenomings strongly suggest that psychological effects occur. The issue of physical and psychological sequelae after snakebite envenomings should be analyzed in terms of DALYs (disability adjusted life years) lost, a valuable tool to assess the impact of diseases. Likewise, the social and economic impacts of this disease have not been properly assessed. Since the large majority of cases occur in young agricultural workers, including women and children, the impact of this pathology in household and community economics and social life is considerable, especially since snakebites occur predominantly in impoverished rural areas. There is an urgent need to assess the impact of snakebite envenoming from this broader perspective, using research tools of the social sciences. The adequate understanding on the physical, economic, social, and psychological consequences of snakebites is required for a knowledge-based allocation of resources and for developing robust advocacy to combat this neglected problem in Latin America and elsewhere in the world.

Snake Species Responsible for the Highest Burden of Envenomings

The medically most important snake species in Latin America and the Caribbean belong to the families Viperidae and Elapidae (Cardoso et al. 2009). Species of the family Colubridae (*sensu lato*) cause a number of bites and are able to inject venom,

although the severity of these cases is generally mild (Prado-Franceschi and Hyslop 2002). The vast majority of snakebites in the region are inflicted by species of the family Viperidae, especially of the genus *Bothrops*, followed by species of the genus *Crotalus* (Warrell 2004; de Oliveira et al. 2009; Gutiérrez 2010, 2011). Envenomings caused by coral snakes (family Elapidae, genus *Micrurus*) represent 1% of the total number of bites (de Oliveira et al. 2009; Gutiérrez 2010, 2011). The species causing the highest number of bites vary depending on the country and are enlisted in Table 1. Some species having a high impact are *Bothrops asper* in Central America and northern regions of South America, *Bothrops atrox* in the Amazon, *Bothrops jararaca* and *B. alternatus* in southern South America, and the rattlesnake *Crotalus durissus* in South America (Warrell 2004; Cardoso et al. 2009; Gutiérrez 2010; WHO 2010) (Table 1; Fig. 1).

Clinical Aspects of Envenomings

Envenomings by Species of the Family Viperidae

The majority of envenomings provoked by species of the family Viperidae are characterized by a complex combination of local and systemic pathological and pathophysiological alterations. Local effects are characterized by edema, pain, hemorrhage, dermonecrosis, blistering, and myonecrosis (Warrell 2004; Cardoso et al. 2009; Otero-Patiño 2009; Gutiérrez 2010) (Fig. 2). The magnitude of these effects varies according to the severity of envenoming. Mild cases are characterized mostly by local edema and pain, whereas severe envenomings are associated with prominent necrosis which might result in tissue loss resulting in permanent sequelae (Otero et al. 2002; Warrell 2004; Cardoso et al. 2009). Local infection often occurs in the affected tissue, and venom-induced muscle tissue damage promotes colonization by bacteria (Otero et al. 2002; Otero-Patiño 2009). Systemic manifestations of envenomings by viperid snakes are characterized by bleeding; coagulopathy associated with defibrinogenation and incoagulability, together with thrombocytopenia and platelet hypoaggregation; hypovolemia leading to hypotension and cardiovascular shock; and acute kidney injury (Warrell 2004; Cardoso et al. 2009; Otero-Patiño 2009; Gutiérrez 2010). The severity of viperid envenomings depends on a number of factors, mostly the volume of venom injected, but also the site of the bite and the size and physiological constitution of the victim. Snake venoms present notorious inter- and intraspecies variations in their composition due to geographical and ontogenetic factors (Calvete 2011); such variation might influence the clinical outcome of envenomings.

There are several exceptions to this predominant clinical picture of viperid envenomings. Bites by South American rattlesnakes classified within the species *Crotalus durissus*, as well as by other species of rattlesnakes distributed in North America, such as the Mojave rattlesnake *C. scutulatus*, induce envenomings characterized by the absence of local tissue damage, and instead by neurotoxic manifestations resulting in respiratory paralysis. In addition, these venoms provoke systemic

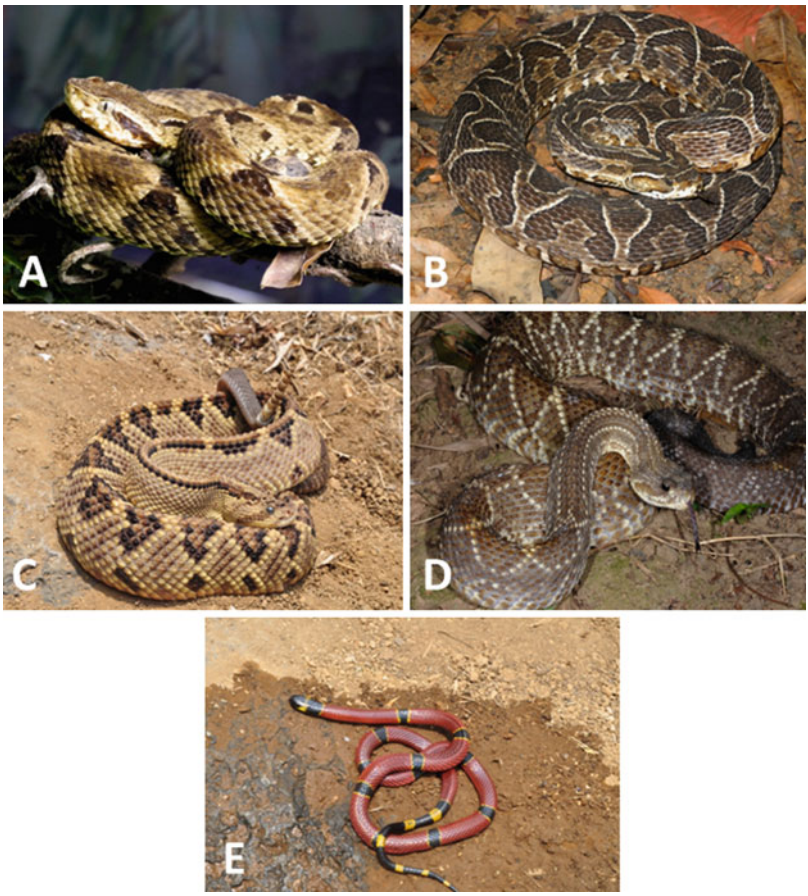

Fig. 1 Representatives of venomous snake species in Latin America. (**a**) *Bothrops atrox* (family Viperidae), responsible for a large number of snakebites in South America. It induces drastic local and systemic effects associated with tissue necrosis (see Fig. 2) and systemic bleeding and hemodynamic disturbances. Specimen from Brazil (Photo by Giuseppe Puorto). (**b**) *Bothrops alternatus* (family Viperidae), distributed in the southern parts of South America. Specimen from Brazil (Photo by Giuseppe Puorto). (**c**) *Crotalus simus* (family Viperidae), a rattlesnake distributed in Mexico and Central America. Envenomings provoked by adult specimens are characterized by local tissue damage, coagulopathy, systemic hemorrhage, and cardiovascular alterations, although venoms of adult specimens of the subspecies *C. s. simus* from Mexico and of neonate specimens of *C. s. simus* from Central America induce neurotoxic effects. Specimen from Mexico (Photo by Edgar Neri Castro). (**d**) *Crotalus durissus terrificus* (family Viperidae), the medically most important rattlesnake in South America, which induces severe envenomings associated with neurotoxicity, myotoxicity, and acute kidney injury. Specimen from Brazil (Photo by Giuseppe Puorto). (**e**) *Micrurus diastema* (family Elapidae), a coral snake distributed in Mexico and northern Central America, which provokes neurotoxic envenomings. Specimen from Mexico (Photo by Edgar Neri Castro)

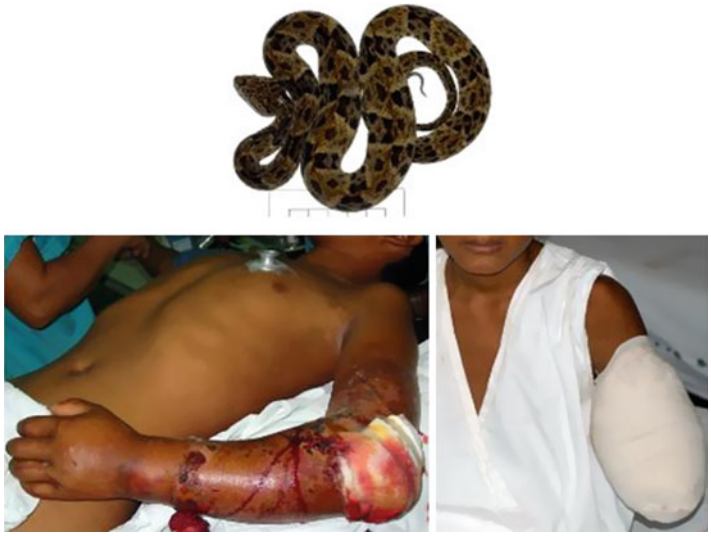

Fig. 2 Local tissue pathology characteristic of envenomings by *Bothrops* sp. snakes. This 12-year-old boy was bitten by a specimen of *Bothrops atrox* (upper photograph) in a rural area of Peru. Severe local tissue damage developed, and the necrotic arm was amputated. The delay in medical attention of snakebitten people in many rural regions of Latin America results in complications which might lead to permanent sequelae, like in this case. Deployment of antivenom to rural health posts and proper use of this immunotherapeutic agent by trained health staff should be strengthened in the region. Photos by David A. Warrell (Reprinted from Gutiérrez et al. (2010a) Toxicon 56: 1223–1235, with permission from Elsevier)

myotoxicity, i.e., rhabdomyolysis, and coagulopathy (Warrell 2004; Azevedo-Marques et al. 2009). On the other hand, envenomings by *Bothrops lanceolatus* and *B. caribbaeus*, endemic species in the Lesser Caribbean islands of Martinique and Saint Lucia, respectively, are characterized, in addition to local tissue pathology, by severe thrombotic effects, often resulting in myocardial or cerebral infarctions (Thomas and Tyburn 1996). Patients suffering envenomings by bushmasters (*Lachesis* sp.) develop, in addition to local tissue damage and systemic hemorrhage, coagulopathy and cardiovascular shock, a unique syndrome characterized by bradycardia, hypotension, abdominal colic, diarrhea, sweating, and vomiting of possible autonomic or autopharmacological origin (Warrell 2004).

Envenomings by Species of the Family Elapidae

Envenomings by coral snakes (genus *Micrurus*) are scarce (approximately 1% of the cases in Latin America). These envenomings are characterized by the absence of local effects, except for pain, and by a predominant neurotoxic picture secondary to neuromuscular blockade induced by neurotoxins present in these venoms. Thus,

signs and symptoms include palpebral ptosis, diplopia, ophthalmoplegia, dysarthria, and, eventually, respiratory paralysis (Warrell 2004; da Silva and Bucaretchi 2009). However, few clinical reports suggest that some coral snake venoms might induce additional effects, including myotoxicity and mild clotting disturbances, which might complicate the differential diagnosis in snakebite cases. Bites by the only species of the subfamily Hydrophiinae distributed in the Americas, the yellow-bellied sea snake *Pelamis platurus*, are very infrequent, and antivenoms against its venom are not produced in the region. On the basis of clinical observations performed in bites by other sea snakes, it would be expected that envenomings by *P. platurus* would be characterized by neurotoxicity and myotoxicity.

Bites by Species of the Family Colubridae (*sensu* lato)

Colubrid snakes are diverse and abundant in Latin America and induce bites in humans. In South America, cases inflicted by species of the genus *Philodryas* have been reported to induce mostly local effects, i.e., bruising, edema, and pain, with a low frequency of systemic manifestations. Predominantly local effects have been also described after bites by species of other genera (Prado-Franceschi and Hyslop 2002). Regardless of the low severity usually associated with colubrid bites, care should be taken since there is growing evidence on the biochemical and pharmacological complexity and toxicity of colubrid venoms.

Snake Venoms: Unveiling their Biochemical and Pharmacological Complexity

Since the first decades of the twentieth century, a large body of knowledge on the composition and toxicological profile of snake venoms has developed in Latin America. Many venom components have been isolated and characterized, and the last decade has witnessed the application of proteomic tools in the study of venoms, a field known as "venomics" (Calvete 2011). Proteomic analysis of viperid venoms in the region has unveiled the great complexity of these toxic secretions (Gutiérrez et al. 2009a; Calvete 2011). Viperid venoms are comprised by many different proteins, grouped in a relatively limited number of families. Predominant components are zinc-dependent metalloproteinases (SVMPs), phospholipases A_2 (PLA_2s), and serine proteinases, followed by other types of proteins which are present in lower amounts, such as C-type lectin-like proteins, disintegrins, cysteine-rich secretory proteins (CRISPs), L-amino acid oxidases, and a number of vasoactive peptides, among others (Alape-Girón et al. 2008; Calvete 2011) (Fig. 3). P-III SVMPs play a key role in local and systemic pathology and pathophysiology associated with hemorrhage, coagulopathy, and cardiovascular disturbances (Gutiérrez et al. 2010b). PLA_2s and PLA_2 homologues are responsible for the local myonecrosis characteristic of these envenomings, as well as for inflammation and pain (Gutiérrez and Lomonte 2009; Teixeira et al. 2009). In the case of the venoms of

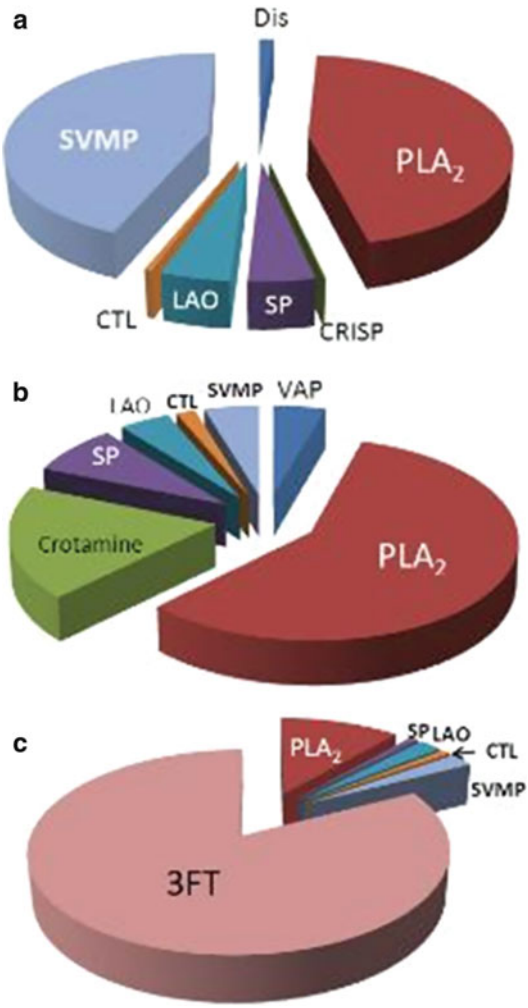

Fig. 3 Relative occurrence of components from different protein families in the venoms of the viperid snakes *Bothrops asper* from Costa Rica (**a**), *Crotalus durissus terrificus* from Brazil (**b**), and of the elapid snake *Micrurus corallinus* from Brazil (**c**). The proteomic analyses of these venoms were described in detail by Alape-Girón et al. (2008), Calvete et al. (2010), and Corrêa-Neto et al. (2011), respectively. *PLA_2* phospholipases A_2, *SVMP* snake venom metalloproteinases, *SP* serine proteinases, *LAO* L-amino acid oxidases, *Dis* disintegrins, *CRISP* cysteine-rich secretory proteins, *CTL* C-type lectin-like proteins, *VAP* vasoactive peptides, *3FT* neurotoxins of the three-finger family

some rattlesnakes, such as the South American species *Crotalus durissus*, a PLA_2 heterodimer, known as "crotoxin," induces neurotoxic and myotoxic effects and is responsible for the predominant alterations characteristic of envenomings by this species (Bon 1997; Gopalakrishnakone et al. 1984) (Fig. 3). Serine proteinases induce clotting disturbances, especially enzymes exerting a "thrombin-like"effect, and also contribute to hemodynamic alterations (Serrano and Maroun 2005). In addition, some C-type lectin-like proteins induce thrombocytopenia (Rucavado et al. 2001), and there are other venom components exerting deleterious actions in viperid envenomings. In the case of *Micrurus* sp. venoms, proteomic analyses have identified predominantly low molecular mass neurotoxins of the three-finger family, together with abundant PLA_2s which contribute to the pathophysiology of envenoming (Corrêa-Neto et al. 2011) (Fig. 3). Short-chain neurotoxins bind to the

cholinergic receptor at the motor end plate in muscle fibers and induce neuromuscular blockade, whereas PLA_2s induce myotoxicity and, in some cases, neurotoxicity. The venom of *Pelamis platurus*, the only sea snake in the Americas, contains a postsynaptically acting neurotoxin of the three-finger family (Tu et al. 1975). In resemblance to other sea snakes, it is likely that the venom of *P. platurus* also presents myotoxic PLA_2s.

Proteomic and pharmacological analysis has shown evidence of a conspicuous pattern of inter- and intraspecies venom variability. The venoms of some species having a wide geographical distribution range, such as *Bothrops atrox* and species of *Crotalus* sp., are characterized by conspicuous intraspecies variation (Alape-Girón et al. 2008; Calvete et al. 2010; Calvete 2011). In addition, a complex pattern of ontogenetic venom variation has been also described for various species, such as *Crotalus simus* (Saravia et al. 2002; Calvete et al. 2010), *Bothrops asper* (Alape-Girón et al. 2008), *Bothrops jararaca* (Zelanis et al. 2011), and *Lachesis stenophrys* (Madrigal et al. 2012). Some venoms present a "paedomorphic" pattern in which the characteristics of the venoms of neonate specimens are maintained in the adults (this is the case of *Crotalus durissus* in South America), whereas other species are characterized by an "ontogenetic" pattern of venom development in which prominent changes occur during the maturation of individuals to become adults, as occurs in the venom of *Crotalus simus* from Central America (Calvete et al. 2010). The large variability in venom composition should be considered when designing venom mixtures for immunization of animals for antivenom production, as to ensure that representative venom pools are prepared (Gutiérrez et al. 2009a).

Antivenoms in Latin America: Production and Quality Control

The parenteral administration of antivenoms constitutes the only scientifically validated therapy for snakebite envenoming on a worldwide basis (WHO 2010; Gutiérrez et al. 2011). Vital Brazil and coworkers were the pioneers in the production of these immunobiologicals in Latin America by generating bothropic and crotalid antivenoms at Instituto Butantan in the first decade of the twentieth century. Further developments in the region have resulted in a conglomerate of antivenom manufacturers, both in the public and private realms, in Argentina, Uruguay, Brazil, Peru, Ecuador, Bolivia, Colombia, Venezuela, Costa Rica, and Mexico (Gutiérrez et al. 2007). Detailed information on manufacturers, types of products, and species coverage are included in the WHO webpage devoted to antivenoms (http://apps.who.int/bloodproducts/snakeantivenoms/database/).

The majority of antivenoms produced in the region are polyspecific, i.e., they are generated by immunizing animals (mostly horses) with mixtures of venoms from two or more snake species. In some cases, monospecific antivenoms are produced by immunizing animals with a venom pool from only one snake species (Gutiérrez et al. 2011). Various polyspecific venoms are produced against venoms of *Bothrops* sp. For instance, in Brazil, a bothropic antivenom of wide distribution and use is prepared by immunizing horses with a mixture of the venoms of *Bothrops jararaca*,

B. jararacussu, *B. moojeni*, *B. neuwiedi*, and *B. alternatus*. In Central America and in Mexico, polyspecific antivenoms are prepared by immunization with a mixture of venoms of *Bothrops* sp. and *Crotalus* sp. and in some cases including *Lachesis* sp. venoms. In addition, monospecific crotalic antivenoms are manufactured in South America, to treat envenomings by the rattlesnake *Crotalus durissus*. Moreover, various laboratories in Brazil, Colombia, Costa Rica, and Mexico manufacture either monospecific or polyspecific antivenoms for the treatment of envenomings by coral snakes (*Micrurus* sp.). In addition to these antivenoms manufactured in the region, Sanofi Pasteur produces a monospecific anti-*Bothrops lanceolatus* antivenom which is used in Martinique for the treatment of envenomings by this endemic species (Thomas and Tyburn 1996).

Antivenoms manufactured in Latin America are of two basic types, depending on the nature of the active neutralizing substance. Some laboratories generate antivenoms composed of whole I_gG molecules. These are produced either by salting-out procedures using various concentrations of ammonium sulfate or, alternatively, by caprylic acid precipitation of non-I_gG plasma proteins (Rojas et al. 1994; WHO 2010; Gutiérrez et al. 2011). Other laboratories produce antivenoms made of $F(ab')_2$ antibody fragments, generated by pepsin digestion of plasma proteins, followed by ammonium sulfate precipitation of antibody fragments; in few cases, ion-exchange chromatography is used to further purify the active substance(WHO 2010; Gutiérrez et al. 2011). The WHO has issued guidelines for the production, regulation, and control of antivenom, which constitutes a highly useful document for manufacturers and regulators (WHO 2010). After fractionation of hyperimmune plasma with the methods described, antivenoms are formulated and standardized as to have a specific neutralizing potency against the venoms for which they are produced. The vast majority of antivenom manufacturers use horses for immunization, although donkeys and llamas are used in La Paz, Bolivia (Gutiérrez et al. 2007).

Some countries produce the volume of antivenom required to fulfill the national needs, such as the case of Mexico, Costa Rica, Brazil, and Argentina. On the other hand, Colombia, Venezuela, Peru, Bolivia, and Ecuador have laboratories that generate a volume of antivenom which covers the national demand only to a partial extent, thus having to rely on producers from other countries to fill their national needs. In the cases of countries which do not have antivenom-producing laboratories, their requirements for this product are fulfilled by importing antivenoms from other countries in the region. Antivenom requirements for the Martinique are covered by a French manufacturer.

The quality control of antivenoms is performed both by manufacturer laboratories and regulatory bodies in the ministries of health. They involve a set of biological, chemical, and physical tests, such as neutralizing potency tests in mice, pyrogen test, sterility test, determination of the concentration of protein, preservatives, sodium chloride, excipients of various sorts, and pH, together with tests for turbidity and visual inspection of the product. A complete description of the methodologies for the quality control of antivenoms is provided in the WHO guidelines (WHO 2010). The quality control of locally produced or imported antivenoms is weak in the ministries of health of some countries in Latin America; therefore, it is necessary to promote

regional programs and workshops aimed at improving the regional capacity to ensure the efficacy and safety of antivenoms being produced or imported in every country.

The Preclinical Assessment of Antivenom Efficacy

Owing to the large variation in the composition of snake venoms, both within and between species, the assessment of the efficacy of antivenoms to neutralize medically relevant snake venoms is highly relevant to ensure that antivenoms to be used in a specific setting are indeed effective. In general, preclinical testing of antivenoms involves the incubation of a "challenge dose" of venom with various dilutions of the antivenom, following by assessing the toxicity of the mixtures in standard laboratory tests. The single most important test to confirm antivenom efficacy is the neutralization of lethality using mice (WHO 2010; Gutiérrez et al. 2013). However, due to the complexity of the pathophysiological manifestations induced by viperid snake venoms, it has been proposed that a more comprehensive evaluation of preclinical efficacy should include the neutralization of additional effects, such as hemorrhagic, myotoxic, coagulant, and defibrinogenating activities (WHO 2010; Gutiérrez et al. 2013). In the case of coral snake (*Micrurus* sp.) venoms, the neutralization of lethality properly evaluates the most relevant toxic effect, i.e., neuromuscular paralysis.

Many studies have been performed in Latin America to assess the preclinical efficacy of antivenoms produced in various countries (see, e.g., de Roodt et al. 1998; Bogarín et al. 2000; Camey et al. 2002). Recently, a large collaborative regional project evaluated several antivenoms against the venoms of the medically most important *Bothrops* species in the region (Segura et al. 2010). In general, these studies have shown a notorious cross-neutralization by antivenoms against heterologous viperid snake venoms, thus supporting the use of some antivenoms in countries different from where they are produced, facilitating regional cooperation in antivenom distribution. On the other hand, there are cases where antivenoms are not effective against venoms from different geographical settings. For example, crotalic antivenoms manufactured in Central America do not neutralize lethality of *Crotalus* sp. venoms from South America, and antivenoms prepared against *C. durissus* from South America are not effective in the neutralization of hemorrhagic activity of *C. simus* from Central America (Saravia et al. 2002). Such observations are explained by the different venom composition, since *C. durissus* is rich in the neurotoxic PLA_2 complex crotoxin, which is largely absent in the venoms of adult specimens of *C. simus*. On the other hand, the latter contains hemorrhagic metalloproteinases, which are absent in South American *C. durissus* (Calvete et al. 2010). Likewise, bothropic antivenoms are not effective in the neutralization of coagulant and defibrinogenating effects induced by *Lachesis* sp. venoms (Colombini et al. 2001). Moreover, there is limited cross-reactivity between species in the case of antivenoms against *Micrurus* sp. venoms.

Evaluation of Antivenom Efficacy and Safety at the Clinical Level

After the demonstration of efficacy at the preclinical level, the introduction of a new antivenom for clinical use in a particular geographical setting should be preceded by appropriate clinical assessment of its efficacy and safety, as established by the WHO (2010). In many instances in Latin America, clinical evidence in support of the use of some antivenoms derives from nonsystematic observations of many years on the efficacy and safety of antivenoms. In the last decades, however, efforts have been implemented to perform controlled, randomized clinical trials (see, e.g., Cardoso et al. 1993; Otero et al. 1999; Otero-Patiño et al. 1998). The efficacy of antivenoms manufactured in Brazil, Colombia, Ecuador, México, and Costa Rica has been demonstrated, and novel findings concerning antivenom safety have been made. For instance, it has been shown that ammonium sulfate-fractionated whole I_gG antivenoms induce a higher incidence of early adverse reactions than caprylic acid-fractionated whole I_gG antivenoms (Otero-Patiño et al. 1998; Otero et al. 1999). Likewise, some of these studies demonstrated that the incidence of early adverse reactions is similar in whole I_gG antivenoms manufactured by caprylic acid precipitation of plasma and in $F(ab')_2$ antivenoms prepared by pepsin digestion and ammonium sulfate fractionation (Otero-Patiño et al. 1998). It is necessary to further explore the clinical profile of safety and efficacy of antivenoms produced in Latin America through international cooperative projects, on the basis of the expertise developed in some countries in the region. Moreover, it is important to assess specific aspects of the therapy of snakebite envenomings, such as the time required, after administration of the antivenom, to correct the main clinical manifestations of envenoming, i.e., bleeding and coagulopathies in the case of viperid snakebite envenomings (Cardoso et al. 1993; Otero et al. 1999).

Beyond Science and Technology: The Issue of Antivenom Distribution

Even if antivenom production in Latin America is improved, with the consequent increment in the volume of antivenom available for public health systems, and with the generation of products if high efficacy and safety, this does not ensure that antivenoms will be accessible to people suffering snakebite envenomings. Additional factors within the public health realm determine whether these products are available and accessible to the people that need them. Some factors relevant for the distribution of antivenoms, and which should be considered by health authorities, are the following:

1. The distribution of antivenoms should be based on a meticulous knowledge of the epidemiology of envenomings. A proper understanding on the incidence of these accidents in different regions, and the species of snakes responsible for the accidents, is necessary to estimate the number of antivenom doses that need to be deployed to various regions in a country. Unfortunately, such information is scarce in many countries; moreover, even when the information is available, the

decisions on antivenom distribution are not necessarily based on these data. It is therefore necessary to improve the epidemiological records of snakebite envenomings in the region and to ensure that this information is properly used for the design of antivenom distribution policies. The use of novel tools, such as geographical information system (GIS) methods, should contribute to a better understanding of vulnerable areas that demand attention regarding antivenom accessibility (Leynaud and Reati 2009; Hansson et al. 2013). Likewise, distribution systems must ensure that antivenoms will be allocated to the rural health posts where the majority of snakebites occur.

2. The policies and procedures for antivenom acquisition by public health authorities are often cumbersome, bureaucratic, and slow, thus precluding a rapid response to cope with antivenom needs. Novel schemes for the purchase of antivenoms should be devised to ensure that the required volumes of effective antivenoms are available. Advocacy should be promoted to ensure that governments will allocate the necessary resources for the purchase of the needed volume of antivenom to avoid shortages of this precious drug in some regions or some times of the year. Moreover, it is necessary to keep the antivenom prices at a level that guarantees the acquisition of the required volumes to cover the needs of the various countries.
3. The maintenance of a functional "cold chain" has to be guaranteed to ensure that liquid antivenoms, which should be kept at 2–8 °C (Gutiérrez et al. 2009b; WHO 2010), are properly transported and stored. This issue is of concern in many regions of Latin America, where power supply often fails and where conditions to keep the cold chain are not always present. Investment in the cold chain system should be promoted, together with the use of cold chain channels already developed for vaccines in the region. In addition, the staff in charge of antivenom transportation and storage should be trained in the basic aspects of the cold chain. Several antivenoms manufactured in Latin America are freeze-dried, thus avoiding the need of a cold chain (Gutiérrez et al. 2009b); however, the majority of the products available in the region are liquid antivenoms. There have been efforts to increase the thermal stability of liquid antivenoms, for example, by using excipients such as sorbitol (Segura et al. 2009). Technological development projects in this subject should be promoted.
4. The lack of health centers in many rural regions of Latin America where snakebites are frequent represents a serious drawback in the efforts to reduce the impact of this pathology. The deficient investment, over several decades, in public health systems in many countries, has had serious implications for an effective attention of health problems. This structural constraint demands renewed political efforts at many levels, from the central government to local community organizations and health advocacy groups of various sorts.

How to Ensure the Adequate Use of Antivenoms

Even if antivenoms are available and accessible at the health posts where most snakebites occur in Latin America, there is a need to guarantee that the health personnel in charge of attending snakebite victims is well trained in the diagnosis

of envenomings and in the proper treatment of this pathology, including the use of antivenoms (correct dose, management of adverse reactions, need of an additional dose, etc.) and the ancillary treatment of snakebite envenomings. This task includes the coverage of this subject in the programs of study of medicine and nursing in universities. Moreover, continuous education programs for health personnel, especially in rural areas, should be designed and implemented. These activities should come together with the publication and distribution of national and regional guidelines for the diagnosis and management of envenomings, such as the ones that have been prepared in Brazil, Costa Rica, Panamá, Argentina, Venezuela, and Paraguay, among other countries (Gutiérrez 2011 and references therein). It is also necessary to develop novel methodologies, taking advantage of the possibilities offered by communication and information technologies, aimed at extending the scope of training programs for health personnel in the region.

People Suffering from Sequelae: A Poorly Attended Aspect of Snakebite Envenoming

Viperid snakebites in Latin America are often characterized by prominent tissue damage at the site of venom injection, i.e., necrosis, blistering, and hemorrhage (Gutiérrez and Lomonte 2009). Antivenom is only partially effective in the neutralization of these effects, since they develop very rapidly, thus generating significant tissue damage before antivenom is administered. As a consequence, people suffering from severe viperid bites often end up with permanent tissue damage, which have a notorious impact in their quality of life. Since the majority of affected people are agricultural young workers or children, these sequelae have evident deleterious effects from the economic and social standpoints. Moreover, it is very likely that people suffering from snakebite envenomings in Latin America develop psychological sequelae, as has been described in Sri Lanka (Williams et al. 2011). In the vast majority of cases, there is no follow-up of snakebitten patients after they leave hospitals and other health facilities, and, consequently, there is a lack of attention to the sequelae that affect them. This demands renewed efforts to understand the magnitude of this aspect of the problem and to establish intervention programs aimed at providing these people with resources to confront the long-term consequences of envenomings.

Prevention of Snakebites and Improvement of the Early Attention of Victims

Public campaigns aimed at the prevention of snakebites constitute a key component of the regional strategy to reduce the impact of this health problem. Such campaigns should involve diverse stakeholders, including health authorities, community groups of various sorts (health advocacy associations, art groups, youth groups, teachers, etc.), and other actors. The participation of local authorities and community organizations is required to ensure that the campaigns will be designed and performed on

the basis of local cultural, social, economic, and political contexts. Since the large majority of snakebites occur in the feet and hands, preventive measures such as wearing shoes while doing agricultural duties, and using a stick to avoid hand exposure, can reduce the incidence of snakebites. Information campaigns on snakebite prevention in primary and high schools in rural areas, as well as in agricultural associations and other groups at risk, need to be reinforced. Particular attention has to be given to vulnerable groups often excluded from the provision of health services, such as indigenous communities and remote rural localities. In this regard, the involvement of both public and private sector organizations, in addition to governmental agencies, is necessary through diverse innovative and cooperative programs.

A key aspect for the reduction of the impact of these envenomings is the appropriate early attention to snakebite victims. Once a person has suffered a snakebite, he or she should be immediately transported to the nearest health post to receive antivenom and other aspects of medical care. Thus, communities should be organized in such a way that people receive rapid attention after a snakebite; this involves implementing transport systems, which have to be designed on the basis of the local contexts. In some cases, this can be achieved by ambulance transportation, whereas in others by the use of private cars, motorcycles, boats, or other means. In this regard, a common problem in the region is the implementation of actions at the local level which might worsen the cases, such as the use of harmful first aid interventions (application of tourniquets or ligatures, incisions, administration of toxic substances, etc.). Besides their direct deleterious effects, these interventions result in a delay in the transportation of the patient to health facilities. It is necessary to develop campaigns to promote a dialogue between health staff and people working in traditional medicine, with the aim of reducing the use of harmful practices and promoting the rapid deployment of patients to health facilities. A successful project, supported by the Pan American Health Organization (PAHO), was developed in Nicaragua, in which traditional healers and staff from the Ministry of Health established a fruitful dialogue and agreed on policies of intervention for the benefit of people affected by snakebites (Luz Marina Lozano, personal communication).

Conclusions and Future Directions

Snakebite envenoming represents a serious public health problem in Latin America and in few Caribbean islands. The majority of cases are inflicted by species of the family Viperidae, which provoke envenomings characterized by local and systemic pathological alterations that may provoke lethality or permanent tissue damage and psychological sequelae. A large body of knowledge has been built in the region on the biochemical and pharmacological characteristics of snake venoms, as well as on the clinical manifestations of envenomings. Toxinological research, both basic and clinical, should be fostered in the region. There are antivenom manufacturing laboratories in many Latin American countries. Regional cooperative networks are

necessary to improve the regional production and quality control of antivenoms, in order to guarantee the availability of safe and effective products throughout Latin America. Despite important advances in confronting this problem, there are still vulnerable regions where the provision of health services and the proper medical attention of snakebitten patients, including the administration of antivenoms, are deficient. Likewise, there is a need to improve the epidemiological information on snakebites in order to design knowledge-based policies of antivenom distribution, training of health personnel, and deployment of medical services. The medical management of cases has to be also improved through the provision of health services to the population, the training of health staff in the diagnosis and treatment of snakebite envenomings, and the access to safe and effective antivenoms. The social, psychological, and economic consequences of snakebite envenomings are largely unknown, and therefore, renewed efforts should be implemented to gain a better understanding of these aspects of the problem. Finally, people suffering from permanent sequelae, both physical and psychological, as a consequence of envenomings should receive proper attention, and preventive campaigns have to be implemented and strengthened, with the involvement of diverse participants, including local community organizations. All these pending tasks demand the development of integrated multisectorial strategies at the national and regional levels, with the long-term goal of reducing the impact of this neglected pathology in the region.

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Snakebites in the Brazilian Amazon: Current Knowledge and Perspectives

3

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Abstract

Although important efforts were carried out during the past decades in Brazil to understand and control snakebite envenomings, important gaps remain for the fulfillment of these goals, particularly in the Amazon region. *Bothrops atrox* is the most important venomous snake in the Brazilian Amazon, causing 80–90% of the snake envenomings in the region. In the Brazilian Amazon, *Bothrops* envenoming shows pain, swelling, regional lymphadenopathy, ecchymosis, blistering, and necrosis as the most common local clinical manifestations. Secondary bacterial infections were observed in around 40% of the *Bothrops* snakebites. Spontaneous systemic bleeding and acute renal failure are common systemic complications after *Bothrops* envenomings. It is difficult for riverine and indigenous populations to reach health centers for treatment of snakebites. As a result, the number of cases detected officially is probably underestimated. Current antivenoms (AVs) require conservation in adequate facilities, which are not always available in remote settings. In addition, training of multidisciplinary teams is not always appropriate for indigenous health services regarding AV administration, side effect management, and case monitoring and surveillance. Although clinical research related to venomous animal injuries has increased, most publications are based on case reports and lack methodological rigor. Moreover, outcome definitions, such as severity ranking criteria, were empirically established, making the results even less generalizable. Clinical research from hospital-based studies and community observational studies are needed. In addition to all the above recommendations, the importance of international cooperative efforts toward the control of these neglected health problems through international partnerships, namely, with other Amazonian countries, is highlighted.

Introduction

Snakebites impose a high burden worldwide and result in considerable social and economic impact. It is estimated that snakebite rates are as high as over 1.8 million cases per year, with associated deaths reaching more than 90,000 cases annually. However, snakebites are a neglected condition with no associated World Health Organization (WHO) programs for control and prevention. Countries most affected by snakebites are those located in the tropical zone with areas of high rates of field use for agriculture where the main affected populations are adult men working in agricultural activities. In Brazil, the Ministry of Health implemented the National Program for Snakebites Control in 1986, extended to other poisonous animals in 1988. Since then, antivenom (AV) production has been standardized, and all the AV

production from the three national laboratories (Instituto Butantan, Fundação Ezequiel Dias, and Instituto Vital Brazil) has been acquired by the Ministry of Health for free-of-charge distribution to patients. Five types of snake AVs are currently available in Brazil: *Bothrops* AV (main one), *Crotalus* AV, *Bothrops-Crotalus* AV, *Bothrops-Lachesis* AV, and *Micrurus* AV.

The Amazon rainforest covers several countries such as Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname, and French Guiana. Studies conducted in these countries linking snakebites cases with species distribution have described similar epidemiological features. In the Brazilian Amazon, snakebites appear among the most important envenomations with the higher incidence (52.6/100,000 inhabitants). In 2013, the Brazilian Ministry of Health reported about 27,181 cases of snakebites (Saúde 2014). In the northern state of Roraima, eastern Pará, and Amapá, incidences higher than 100 cases per 100,000 inhabitants have been showed (Fig. 1). *Bothrops atrox* is largely responsible for bites in this region, with more than 80% of the reported cases, while *Lachesis*, *Crotalus*, and *Micrurus* species are secondary agents of

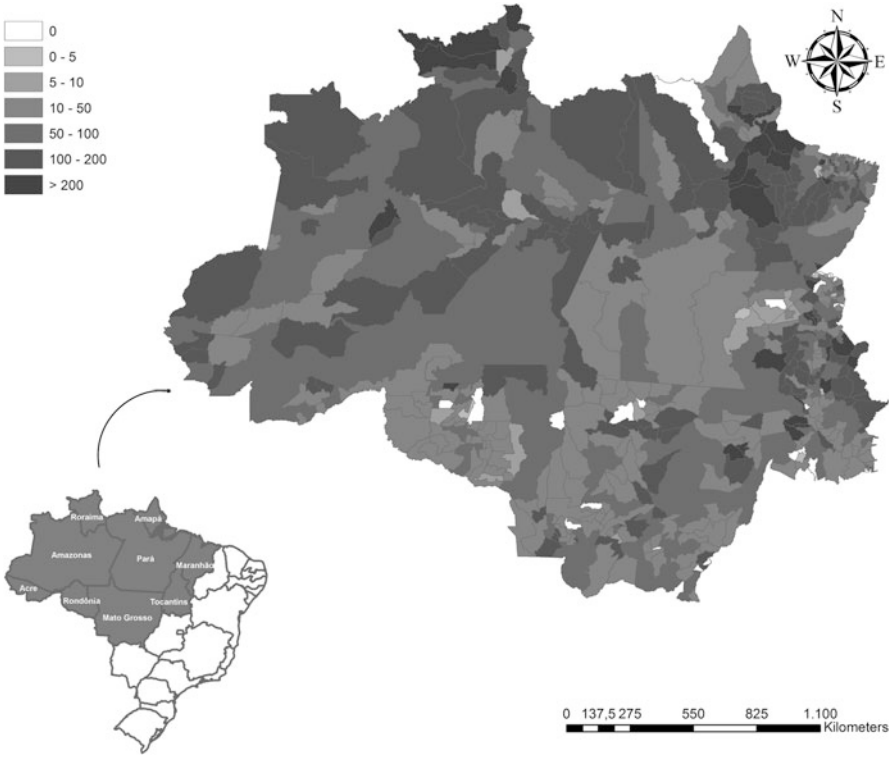

Fig. 1 Spatial distribution of snakebites in the Brazilian Amazon. Map were created using incidence per 100,000 inhabitants. Snakebites are largely distributed in the Amazonian states, with several counties presenting incidences higher than 100 cases per 100,000 inhabitants, especially in northern Roraima, eastern Pará, and Amapá and in unevenly distributed municipalities across all states

envenomings (Feitosa et al. 2015). Spatial distribution of snakes is strongly influenced by Amazon ecosystem diversity. Surveys on species distribution and frequency, ecological structure, and other particular features about those venomous species must be carried out in order to improve health services (Fan et al. 2015). Incidence increase has been suggested to be associated with the rainy season. Since water volume is large, snakes use to seek for drier shelters, generally closer to human settlements in rural areas. In addition, urban sprawl along with deforestation causes major influence on the natural habitat of these animals, exerting pressure for animal migration and consequently leading to accidental snakebites (Bernarde and Gomes 2012).

A few studies concerning this issue are available in the Amazon region; nevertheless, such results indicate the following profile of the affected population: victims of snakebite are predominantly men, in working age, rural residents, riverine and indigenous, linking a major cause as occupational hazard for many of these work in agriculture, hunting, and forest activities, as in the case of rubber tappers (Feitosa et al. 2015). A study conducted with indigenous and riverine populations showed that 13% of them had experienced snakebite during their lifetime (Pierini et al. 1996). Severity has been mostly classified from mild to moderate cases, although severe cases had been reported in around 8% of the cases (Feitosa et al. 2015). The most affected parts of the body are usually the lower limbs (Feitosa et al. 2015; Pierini et al. 1996). The Amazon region has a reduced coverage of highways and roads, with much of the human displacement happening through river transportation, leading to a delay in medical care. Time increasing to more than 6 h to care has been associated with undesirable outcomes such as severity and mortality. Other risk factors associated with poor outcomes are older age and bites related to work activities (Feitosa et al. 2015).

Injury outcomes are sometimes used to be influenced by cultural behaviors. Riverine, indigenous, and rural people often use such devices as tourniquet and even chemicals such as alcohol (ingested and applied to the bite) attempting to reduce the venom effects. Herbal extracts are also largely used in snakebite episodes, especially where AVs are scarce or not available. Puncture and suction of the injury in an attempt to remove the venom, although not recommended, are common practices among patients. Although AV availability is recommended for high-incidence areas, there are some drawbacks regarding this issue. The lack of healthcare facilities in remote rural areas impairs the access to AV because it requires low temperatures for conservation in addition to skilled health professionals. Such difficulties, besides those already mentioned, lead people to seek alternative therapies.

Bothrops Envenomings in the Brazilian Amazon

Bothrops Snakes in the Brazilian Amazon

In the Brazilian Amazon region and surrounding *cerrado* areas, there are 12 species of pit vipers, belonging to the *Bothrops* and *Bothrocophias* genera. Five of them (*Bothrops lutzi*, *B. marmoratus*, *B. mattogrossensis*, *B. moojeni*, and *B. pauloensis*)

are present only in *cerrado* areas, while the others are characteristic of the Amazon rainforest environments (*Bothrops atrox*, *B. bilineatus*, *B. brazili*, *B. marajoensis*, *B. taeniatus*, *Bothrocophias hyoprora*, and *B. microphthalmus*). *Bothrops atrox* also occurs in deforested areas (pastures and crops) and in urban environments (Bernarde 2014).

Bothrops atrox is the most important venomous snake in the Brazilian Amazon, causing 80–90% of the snake envenomings in the region (Fan et al. 2015). This species is widely distributed in the Amazon and is the most abundant venomous snake in this region. The size of adult specimens ranges from 1 to 1.5 m, with a record of up to 1.72 m. This species is present both in forested areas as well as in disturbed areas, such as pastures, crops, and urban areas. *Bothrops atrox* is active especially during the night, when adults often occur on the ground for expected hunting, while juveniles hunt on vegetation (up to 1.5 m height). Regarding food, this snake is generalist, feeding on centipedes, fishes, amphibians, lizards, other snakes, rodents, marsupials, and birds. Juveniles prefer to prey on ectothermic animals (frogs, lizards, and centipedes), and adults prefer to prey on endothermic animals, namely, rodents. As a viviparous species, this snake may give birth between 11 and 43 offsprings of 28–35 cm, found between December and February (Martins and Oliveira 1998). This snake is commonly known by different common names according to the area (*jararaca*, *surucucu*, *surucucu-do-barranco*, *boca-podre*, and *comboia*). However, there is a possible confounding factor in snake identification by the local population since both *Bothrops atrox* and *Lachesis muta* receive the same popular name "*surucucu*" in certain Amazonian areas (Fan et al. 2015).

Popularly known as green pit viper or parrot's beak *jararaca*, *Bothrops bilineatus* stands out for being relatively abundant in some regions and to present arboreal habits, which contributes to their bite that reaches the upper regions of the body of the victim. Two subspecies of the green pit viper are present in the Amazon: *B. b. bilineatus* is present in the states of Amazonas, Roraima, Amapá, Pará, south of Rondônia, and northern Mato Grosso; *B. b. smaragdinus* occurs in the states of Acre, Amazonas (Purus River basin), and Rondônia (northern state) (Bernarde et al. 2011a). Adult specimens are between 70 cm and 1 m and, same as the juveniles, have arboreal habits. This snake inhabits primary and secondary forests, especially near water courses, and is less common in anthropogenic environments (Bernarde 2014). Their prey consists mainly of rodents, amphibians, birds, snakes, and lizards. It is viviparous like *Bothrops* species, giving birth between 6 and 16 offsprings.

Bothrops brazili, the red pit viper, and *B. taeniatus*, the gray pit viper, occur less frequently in the Amazonian biome. *Bothrocophias* pit vipers are little frequent and have few records in the Brazilian Amazon. *Bothrocophias hyoprora*, the big-nose pit viper, has few records from the states of Acre, Amazonas, Rondônia, Mato Grosso, and Pará (Bernarde et al. 2011b), and *B. microphthalmus* has a single record for Brazil in the state of Rondônia (Bernarde 2012).

Main *Bothrops* species involved in biting humans in the Brazilian Amazon are shown in Fig. 2.

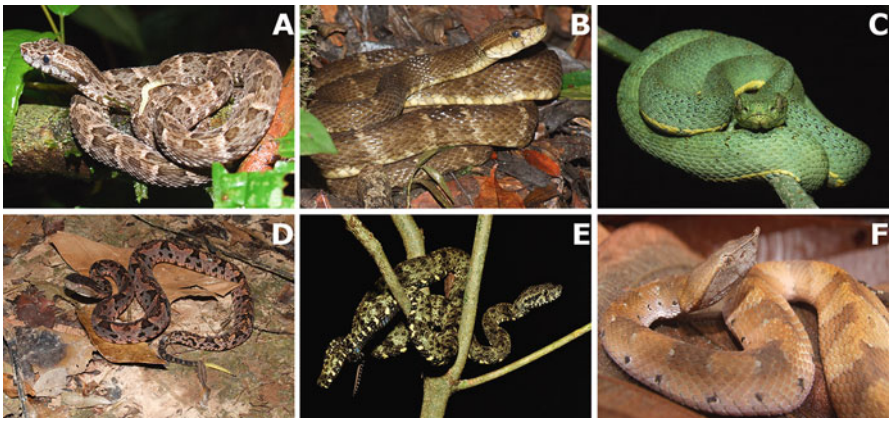

Fig. 2 Main *Bothrops* species involved in biting humans in the Brazilian Amazon (a–f). *Bothrops atrox* (**a** and **b**) is implicated in most of the human snakebites registered in the Brazilian Amazon region (80–90 %). The species *Bothrops bilineatus* (**c**), *Bothrops brazili* (**d**), *Bothrops taeniatus* (**e**), and *Bothrocophias hyoprora* (**f**) present secondary medical importance in the region

Toxinology

Despite the wide geographic distribution in the Amazon, *B. atrox* venoms share the same family of toxins, such as PIII and PI snake venom metalloproteinase, phospholipase A2, serine proteinase, cysteine-rich secretory protein, L-amino acid oxidase, and C-type lectin-like (Calvete et al. 2011; López-Lozano et al. 2002). A bradykinin-potentiating peptide from *B. atrox* venom was also identified (Coutinho-Neto et al. 2013). The variability in venom composition of *B. atrox* from different geographical origins is mostly related to the expression level of each family of toxins than to the presence or absence of major families of toxins (Calvete et al. 2011; López-Lozano et al. 2002). *B. atrox* venoms from Colombia and Venezuela show an ontogenetic toxin profile, with PI metalloproteinases and phospholipases representing the most abundant toxins (Calvete et al. 2011). Venoms from Brazil, Ecuador, and Peru show a pedomorphic phenotype, with PIII metalloproteinases being the most abundant toxins (Núñez et al. 2009). Transcriptomic analysis of the *B. atrox* venom gland from Brazil indicates a predominance of transcripts encoding mainly metalloproteinases (Neiva et al. 2009). The biological activities of *B. atrox* venom can be correlated with geographical distribution and ontogenetic stage of the snake (newborn, juvenile, and adult snakes), as has been observed in venoms from Colombia and Brazil (López-Lozano et al. 2002). One speculates that differences in the expression level of each family of toxins and biological activities of *B. atrox* venom could explain the clinical manifestations observed in victims of bite caused by this species in different regions of the Amazon (Sousa et al. 2013).

The composition of venom from other *Bothrops* snakes in the Brazilian Amazon should be further elucidated. However, some components with coagulant activity have been described in the venom of *B. brazili*, *B. marajoensis*, and *B. moojeni* (Assakura et al. 1992).

Pathophysiology

Bothrops venom is characterized by three main pathophysiological activities: coagulant, hemorrhagic, and proteolytic or acute inflammatory effects. The coagulating activity of the *B. atrox* venom results from components of the venom with thrombin-like activity, which directly hydrolyzes fibrinogen in fibrin and procoagulant activity, which activate factors II and X of the coagulation, resulting in the formation of endogenous thrombin. Other clotting factors activated by components isolated of *B. atrox* venom are the factors XIII and V (Assakura et al. 1992; López-Lozano et al. 2002). *B. marajoensis* and *B. hyoprora* venoms have coagulant activity on plasma and fibrinogen (Assakura et al. 1992). Components with thrombin-like activity were isolated from the *B. brazili* venom. Components that act on platelets function were also isolated from the *B. atrox* venom (Freitas-De-Sousa et al. 2015).

Hemorrhagic activity has been observed in the *B. atrox* and *B. marajoensis* venoms (Assakura et al. 1992; Freitas-De-Sousa et al. 2015; Sousa et al. 2013). A PI metalloproteinase, called batroxase, isolated from *B. atrox* venom, has fibrinolytic and thrombolytic activities and induces weak bleeding through the digestion of the extracellular matrix components such as laminin, type IV collagen, and fibronectin (Jacob-Ferreira et al. 2016). The batroxrhagin, isolated from *B. atrox* venom, also induces bleeding (Freitas-De-Sousa et al. 2015), as well as atroxlysin-I, a PI metalloproteinase (Sanchez et al. 2010). Compounds with thrombolytic activity were found in the *B. atrox* venom (Jacob-Ferreira et al. 2016).

The proteolytic or acute inflammatory activity induced by *B. atrox* venom causes plasma extravasation; migration of leukocytes; vascular wall lesion, which results in bleeding; and musculoskeletal disruption (Moreira et al. 2012). Phospholipases A2, namely, BaPLA2I and BaPLA2III, which cause edema and myonecrosis, were isolated from *B. atrox* venom (Kanashiro et al. 2002). Besides these, a myotoxin isolated from *B. atrox* venom also induces edema and myonecrosis (Núñez et al. 2004). A genotoxic potential has been observed in *B. atrox* and *B. brazili* venoms (Marcussi et al. 2013). Nephrotoxic compounds were identified in the *B. marajoensis* venom (Dantas et al. 2015).

In victims of *B. atrox*, envenoming can be observed with coagulation disorders, such as hypofibrinogenemia, fibrinolytic system activation, and intravascular thrombin generation, resulting in blood incoagulability (Otero et al. 1996; Pardal et al. 2004). A study carried out in Belém, Pará State, Brazil, verified that approximately 10% of the victims have thrombocytopenia (Pardal et al. 2004). On the other hand, aggregating activity on rabbit's washed platelet was not observed in *B. atrox* venom experimentally (Francischetti et al. 1998). The platelet function disorders in envenomings caused by *B. atrox* snake are not well described. Coagulation disorders, edema, and hemorrhage appear in envenomings as a result of the biological activities of *B. atrox* venom (Otero et al. 1996; Pardal et al. 2004).

Clinical Aspects

Bothrops envenomings cause local and, in a significant proportion, systemic manifestations, depending on the snake involved, characteristics of the victim, and circumstances of the injury. Snakebite diagnosis in general should consider epidemiological, clinical, and laboratorial aspects, which, when analyzed together, can lead clinicians to the probable perpetrating snake genus and to the correct interpretation of severity status and further therapeutic approach. Epidemiological diagnosis should consider the habitat, habits, and other information leading to snake identification as well as time, region of occurrence, and circumstances related to the accident. Importantly, clinical examination should be initiated by assessing the affected region to search for bite signs, especially fang marks that can be double or possibly only when only one prey is introduced. *Bothrops* snakebite may result in negligible or no envenoming, even if fang marks are visible ("dry bite"). This issue is important to be considered at the time of patient's admission at the hospital to avoid giving antivenom when specific therapy may not be necessary. This may occur when a nonvenomous snake is implicated.

Local envenomation ranges from a painless reddened injury to intense pain and swelling at the site of bite, starting minutes after the event. Bleeding caused by traumatic injury due to fang introduction may be present. Local manifestations may increase progressively and may affect the whole limb. Enlargement of the regional lymph nodes draining the site of bite and bruising can also be observed some hours after bite, especially if patient delayed in reaching a health service (Pardal et al. 2004; Otero et al. 1996) (Fig. 3). In the first 24 h, blistering and tissue necrosis may be evident. Cellulitis or abscess occurs mostly in the moderate or severe cases, generally as a polymicrobial infection. Gram-negative bacteria have been implicated in secondary bacterial infection, which frequency may vary according to region. In Manaus, secondary bacterial infections were observed in around 40% of the *Bothrops* snakebites (Souza 2002) (Fig. 4). Necrosis of variable extension is more frequent when tourniquet is applied, associated with initial treatment with traditional healers, and delayed hospital admission resulting from problems with transportation. Although uncommon, compartment syndrome is a dangerous complication because of the potential ischemia, tissue necrosis, and neuropathy. Nonspecific symptoms such as headache, lethargy, weakness, nausea, and vomiting are often observed (Pardal et al. 2004; Souza 2002).

Symptoms and signs of systemic envenoming are mainly due to the incoagulable blood. Hemorrhage from venipunctures, other sites of trauma or healed wounds, gengivorrhagia, hemoptysis, macrohematuria, and hematemesis are observed in 16–18% of *Bothrops* snakes (Pardal et al. 2004; Souza 2002). In the Amazon, cases of hemorrhagic stroke have also been described (Machado et al. 2010). An important systemic complication of *Bothrops* snakebites is acute kidney injury (AKI), which has a great impact on morbidity and mortality. Oliguria or anuria may develop within the first 24 h of the bite. If patient is not treated, blood pressure rises within a few days of the onset of oliguria, and signs of uremia (drowsiness, irritability, vomiting, hiccups, convulsions) develop within 3–7 days after bite. AKI

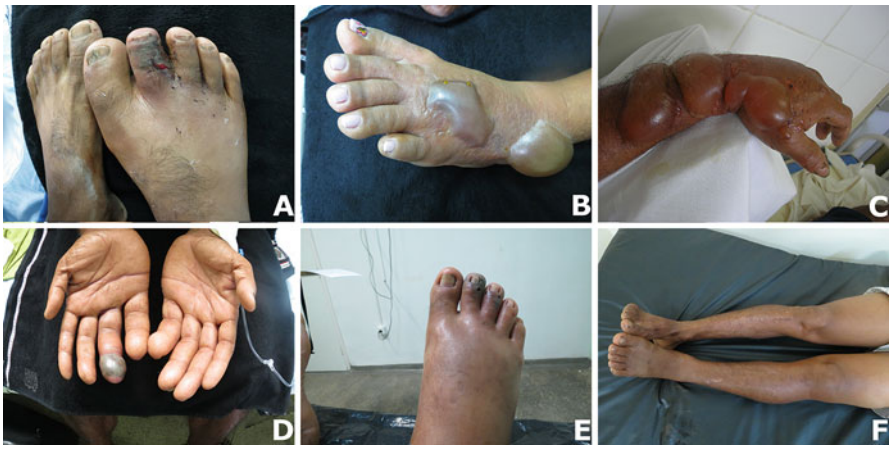

Fig. 3 Local manifestations resulting from *Bothrops* snakebites. (**a**) Ulceration and local bleeding of the first finger of right foot with less than 12 h after envenoming. (**b**) Envenoming with blisters around the snakebite in the dorsal area of the right foot. (**c**) Envenoming on the hand; this patient arrived 12 h after the bite at the hospital, with swelling and serohemorrhagic blisters on left upper limb and incoagulable blood. (**d**) Envenoming in distal finger of the right ring finger with serohemorrhagic blisters 24 h after the bite. (**e**) Envenoming with intense swelling on right foot, local bleeding, and ecchymosis with purplish coloration on the first and second finger. (**f**) Severe snakebite with extensive swelling of the five segments of the left leg (from foot to thigh), less than 20 h after envenoming

was observed in 10.9% of the patients in Manaus (Souza 2002) and in 20.5% of the cases in Colombia (Otero et al. 1996) (Fig. 5). Ischemia, hemorrhage, and direct nephrotoxic action of the venom may be implicated in the development of AKI. In the Brazilian Amazon, severe systemic complications were independently associated to age $\leq$15 years, age $\geq$65 years, and time to medical assistance >6 h. Lethality rates were 0.7% for *Bothrops* snakebites, associated with age $\geq$65 years and time to medical assistance >6 h (Feitosa et al. 2015). These features of victims of snakebite demand adequate management according to well-defined protocols, including prompt referral to tertiary centers when necessary, as well as an effective response from surveillance systems and policy makers for these vulnerable groups.

The blood is commonly incoagulable in patients with systemic envenoming. There is a variable hypofibrinogenemia associated with reduction of D-dimer. Levels of fibrin/fibrinogen degradation products are high. Thrombocytopenia is usual in severe cases. The total peripheral white blood cell count is usually elevated, and hematocrit may be increased initially as a result of hemoconcentration but falls subsequently depending on the occurrence of hemorrhage or liquid infusion. Coagulation tests are valuable in the initial investigation of snakebites, since incoagulable blood is present in about 50% of *Bothrops*-bitten patients in the Brazilian Amazon (Pardal et al. 2004; Souza 2002). In Colombia, incoagulable blood was found in 77% of *Bothrops atrox* envenomings (Otero et al. 1996).

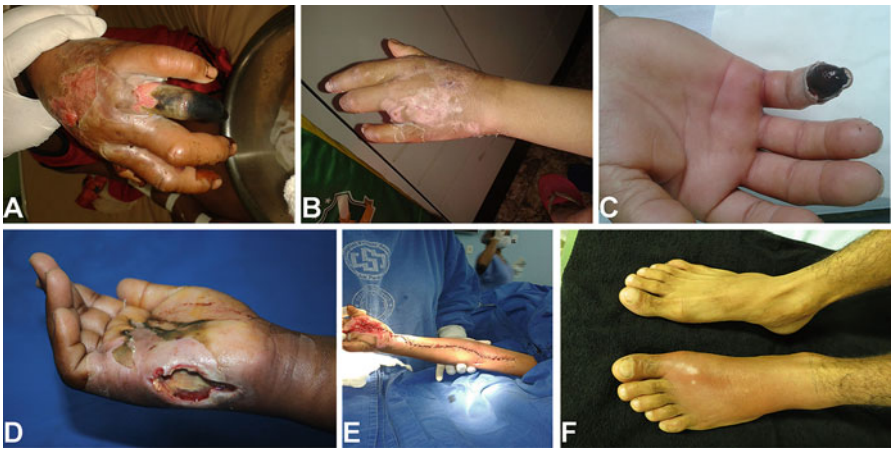

Fig. 4 Local complications resulting from *Bothrops* snakebites. (**a**) Envenoming on the left hand, patient with more than 48 h after the bite, with an extensive area of edema and necrosis in the left upper limb and gangrene of the fourth finger. (**b**) The same patient shown in A, after amputation of the fourth finger (in the healing phase). (**c**) Envenoming in the distal part of the little finger of the right hand with evolution to necrosis after 48 h after the bite. (**d**) Envenoming on dorsal region of the right hand spreading to hand palm, requiring surgical debridement of the necrosis area on 5 day after the bite. (**e**) Severe envenoming on the left hand; this patient arrived 24 h after the bite, presenting compartmental syndrome in the left upper limb, requiring fasciotomy. (**f**) Patient presenting abscess and cellulitis due to secondary bacterial infection, on the dorsal area of the left foot, 48 h after the bite

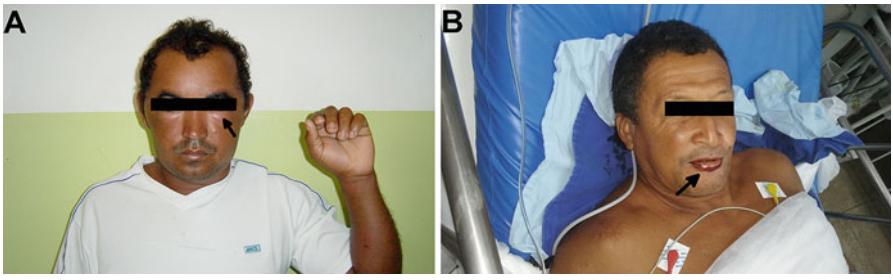

Fig. 5 Systemic complications resulting from *Bothrops* snakebites. (**a**) Patient bitten on the dorsal part of the left forearm showing uremic face because of acute renal failure, 72 h after of the envenoming. (**b**) Patient presenting systemic hemorrhage, evidenced in the picture by bleeding in the lower lip 24 h after the bite

Therapeutics

The specific treatment of *Bothrops* envenomations in the Brazilian Amazon follows the protocol established by the Ministry of Health according to the severity of the envenomation (Ministério da Saúde 2001). As soon as indications are fulfilled, antivenom should be administered. Delay in antivenom therapy is the main determinant for poor prognosis, where failure of antivenom in reversing clinical and

laboratory effects are more likely to occur in patients admitted more than 6 h after bite. However, even a long delay between bite and admission to hospital should not exclude the indication of antivenom therapy if symptoms and signs of systemic envenoming are still evident. Antivenom should be administered by intravenous route, diluted in isotonic fluid, and infused over approximately 60 min. Preferably, patients should be hospitalized for antivenom therapy and be monitored in the first 24 h for early anaphylactic reactions. Clinical studies have shown that antivenoms are highly effective in reversing hematological disturbances and stopping local and systemic bleeding caused by *Bothrops* snake venoms (Pardal et al. 2004; Otero et al. 1996). Usually, coagulation disturbance is reversed in the first 24 h after antivenom therapy, and no relapse occurs if the recommended dose is given. On the other hand, the efficacy of antivenom in reducing local tissue damage shows to be limited, unless antivenom is given within a few hours of the bite.

The *Bothrops*, *Bothrops-Lachesis*, and *Bothrops-Crotalus* antivenoms, which are used in the treatment, are produced in horses immunized with a mixture of the snake venoms. The mixture of the *Bothrops* venom is composed for *B. jararaca* (50%), *B. moojeni* (12.5%), *B. alternatus* (12.5%), *B. jararacussu* (12.5%), and *B. neuwiedi* (12.5%). Thus, *B. atrox* venom, which is from a medically important snake in the Brazilian Amazon, is not part of the immunization pool used for the production of antivenoms used in the treatment of these envenomations (Fan et al. 2015). Experimentally, the *Bothrops* antivenom neutralizes the main biological activities, i.e., bleeding, lethality, and defibrinating, of *B. atrox* venom from Manaus, Amazonas State, and São Bento, Maranhão State, requiring, however, different doses of antivenoms for the distinct geographical areas (Furtado et al. 2010). On the other hand, a clinical study in Belém, Pará State, shows specific *B. atrox-Lachesis*, and standard *Bothrops-Lachesis* antivenoms were equally effective in reversing clinical manifestations and laboratories abnormalities observed in victims of *Bothrops* envenomations. Venom-induced hemostatic abnormalities were resolved with 24 h after the start of antivenom treatment (Pardal et al. 2004).

The victims of *Bothrops* envenomation in the Brazilian Amazon receive antivenom between 2 and 11.7 h on average after the snakebite (Pardal et al. 2004; Souza 2002). The delay between the elapsed time of the envenomation and the administration of antivenom in health services is the result of the vast territory and difficulty in access to health services, since often the transport used by the victims is by boat. Moreover, the practice of traditional treatments, such as the use of medicinal plants, could also delay the search for victims for specific treatment in the health services (Ministério da Saúde 2001). Indeed, various plant species are used to treat snakebites without any scientific validation. However, studies have shown the effectiveness of anti-snakebite plants to inhibit the local effects, i.e., edema, of *B. atrox* venom in the west of the state of Pará, Brazil (Moura et al. 2015).

Early adverse effects of antivenoms vary in frequency and severity, whose mechanisms can involve type I hypersensitivity, which seems not to be responsible in most cases, since acute reactions often occur in patients with no history of previous exposure to equine proteins; anti-complementary activity has been suggested; and human heterophilic antibodies toward equine immunoglobulins

have been described. Independently of the mechanisms involved, clinical symptoms of early reaction are undistinguishable and have been reported in variable frequencies. Most of the early reactions are mild, including pruritus, urticaria, nausea, vomiting, and abdominal pain. Dyspnea and hypotension indicate severe reaction and demand interruption and the antivenom infusion and specific treatment. The risk of early reaction depends on the dose and speed of administration. There is a widespread practice in the antivenom therapy to recommend the slow infusion of antivenom, often achieved by diluting the antivenom in isotonic fluid, although clinical studies have not provided full support that the speed of antivenom infusion correlates with the frequency of acute reaction. Intradermal/conjunctival hypersensitivity tests have no longer been indicated since they do not predict antivenom reactions.

Early hypersensitivity reactions to the use of antivenom may occur even after the use of premedication with corticosteroids and antihistamines, which are between 16% and 28%. (Pardal et al. 2004; Souza 2002). The frequency of delayed reactions (serum sickness) needs to be better known (Fan et al. 2015). Only one victim from 212 of *Bothrops* envenomation treated at a reference hospital in the Amazonas State had serum sickness (Souza 2002). In rural areas of the Brazilian Amazon without electricity to conserve the liquid antivenom in cold temperatures (2 °C to 8 °C), the use of lyophilized antivenom could be strategically used. Studies show that the frequency of adverse reactions observed in victims of *Bothrops* envenomations in the Amazonas State who received lyophilized *Bothrops-Lachesis-Crotalus* antivenom was not statistically different when standard *Bothrops* antivenom was used (Silva and Tavares 2012).

Regarding treatment of local complications such as necrosis and compartment syndrome, there have been described cases of amputation and fasciotomy, respectively (Fan et al. 2015). In the treatment of secondary infections, such as abscesses, it is necessary to use broad-spectrum antibiotics. Studies on the microorganisms present in these infections and sensitivity to antibiotics need to be performed. Moreover, it is important to get information on the vaccination status of the victim against infection with tetanus bacilli and proceed according to the guidance of the Ministry of Health. Thus, in the Brazilian Amazon, a region that shows peculiar characteristics, the frequent training of professionals in the management of envenomation is necessary, especially in small towns.

Lachesis Envenomings in the Brazilian Amazon

Envenomings caused by *Lachesis* snakes, the bushmasters, popularly known in Brazil as *surucucu*, *surucucu-pico-de-jaca*, and *surucutinga*, are unusual events due to their nonaggressive behavior. These snakes are found in dense, preserved, and rainy tropical forest environments, with high temperatures, in the countries of South and Central America (Souza et al. 2007). In the Amazon region, there are two main *Lachesis* species: *L. acrochorda*, present in the northwest of Colombia and Ecuador, and *L. muta*, found in Venezuela, Suriname, Guyana, French Guiana,

Fig. 6 Specimens of *Lachesis muta*, the species responsible of *Lachesis* envenomings in Brazilian Amazon, highlighting details in the tail

Brazil, Ecuador, Peru, Bolivia, and the eastern Andes. *L. muta* is the bigger venomous snake in the Americas and may exceed 3 m in length (Fig. 6); it is a species of forest environment, with large body size (easier to be seen), no aggressive behavior, and low population density, thus contributing to the lower incidence of *Lachesis* bites in relation to *Bothrops* bites (Bernarde 2014). *L. muta* has nocturnal habits, hunting in the stalking ground and feeding on rodents and marsupials. It is the only oviparous species of viperid in Brazil, with records of up to 20 eggs. Female curls up next to the eggs to protect them.

In the Brazilian Amazon, *Lachesis* envenomings accounted for 6.6% of cases (Saúde 2014). In a study conducted in the city of Manaus, this genus was involved in 17% of cases (Bard et al. 1994). In Belém, state of Pará, through enzyme immunoassay or by examination of the dead snake, only one *Lachesis* envenoming was shown among 46 bitten patients (Pardal et al. 2004). In the state of Acre, from 45 previously bitten individuals, 14% tested positive for *Lachesis* antibodies using enzyme immunoassay (Pierini et al. 1996).

Although *L. muta* has a wide geographical distribution, *Lachesis* venoms from Brazil, Costa Rica, and Colombia share similar pathophysiological characteristics (Pla et al. 2013), with four major pathophysiological activities: coagulant, proteolytic, hemorrhagic, and neurotoxic (Torres et al. 1995). The coagulant action of the

Lachesis venom is due to the presence of serine proteases, also called thrombin-like proteins, which act directly on fibrinogen-to-fibrin reaction transformed without thrombin participation (Pla et al. 2013), causing an acceleration in blood coagulation and consumption of clotting factors, resulting in blood incoagulability and prolongation of bleeding time (Torres et al. 1995). In the Amazonas State, Brazil, coagulant activity of the *L. muta* venom showed more intensity than the *B. atrox* (Bard et al. 1994). Other toxins found in *Lachesis* venom are the disintegrins, acting as platelet aggregation inhibitors, even though some components have been found that induce aggregation (Francischetti et al. 1998).

Regarding proteolytic activity, the venom has many enzymes that help in an acute inflammatory process in the first hours post-envenoming, namely, phospholipases and serine proteases (Jorge and Ribeiro 1997). The hemorrhagic activity of the venom occurs through metalloproteinase action, also called hemorrhagins, that compromise the vascular integrity and increase fibrinolytic activity, not only locally but systemically (Estêvão-Costa et al. 2000). The neurotoxic activity is characteristic of *Lachesis* envenomings caused by vagal stimulation by the action of phospholipases which act as potent neuromuscular presynaptic blockers (Jorge et al. 1997). The kininogenases also play an important role in the clinical picture with the release of kinins, which affect neuromuscular conduction process. Experimental models show that neurotoxic action of *L. muta* venom possesses presynaptic effects at low doses and postsynaptic in high doses (Damico et al. 2006). Besides these activities, *L. muta* venom has a minimum myotoxic action (Damico et al. 2006).

Clinical manifestations at the bite site are similar to those caused by *Bothrops*, with an intense tissue damage evidenced by pain, restricted edema or affecting the member, blisters, bleeding, and ecchymosis (Torres et al. 1995). Chronic ulcers are reported in patients bitten by *Lachesis* (Fig. 7). Systemic manifestations are characterized by coagulation disorders, nausea, frequent vomiting, intense sweating, and hypersalivation or oral dryness (Souza et al. 2007). Classic signs and symptoms of vagal stimulation are dizziness, blurred vision, diarrhea, abdominal cramps, sinus bradycardia, severe hypotension, and shock (Souza et al. 2007; Torres et al. 1995). Occasionally, divergent strabismus, dysarthria, and dysphagia may occur (Torres et al. 1995). Main local complications are secondary infection, functional impairment, and acute renal failure (Souza et al. 2003, 2007). Due to stimulation of the autonomic nervous system, *Lachesis* envenomings can still present with shock and death (Souza et al. 2007). Serological tests to distinguish between *Bothrops* and *Lachesis* in the absence of vagal manifestations are available only for research purposes (Pardal et al. 2004).

The *Lachesis* antivenom is the specific treatment for this type of envenoming, especially effective in the occurrence of inoculation of large amounts of venom. Despite the wide geographic distribution of *Lachesis* snakes, antivenom seems to give good coverage in different geographical areas of the Amazon (Theakston et al. 1995). Despite some similarities between *Bothrops* and *Lachesis* venom components, the *Bothrops* antivenom is not recommended for neutralization of the coagulant action of the *Lachesis* venom (Bard et al. 1994).

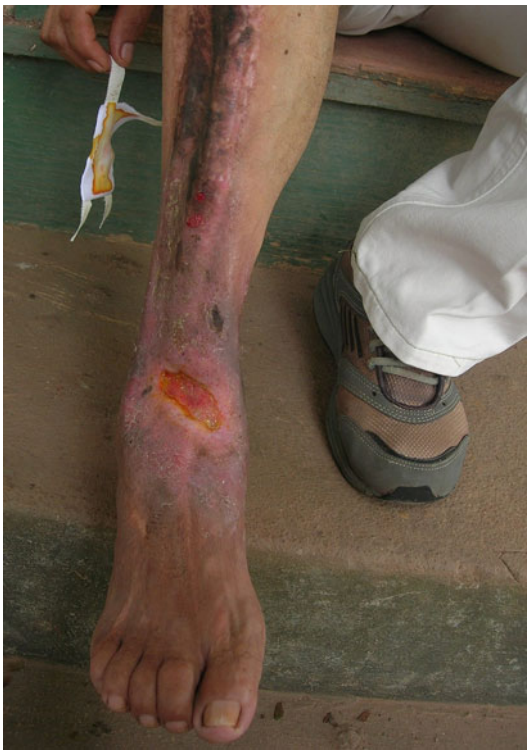

Fig. 7 Local complications resulting from a *Lachesis* snakebite. Chronic ulcer and scar in a patient bitten by a *Lachesis* snake 3 years after the envenoming

Crotalus Envenomings in the Brazilian Amazon

In the Brazilian Amazon, *Crotalus durissus*, the rattlesnake, is present in relictual *cerrado* spots in the states of Rondônia (Vilhena, Chupinguaia, Rolim de Moura, Alta Floresta d'Oeste, and Guajará-Mirim), Amazonas (Humaitá), Roraima, Amapá, and Pará (Serra do Cachimbo, Santarém, and Marajó Island) and is probably absent in the state of Acre (Bernarde 2014). Adults range between 1 and 1.5 m long (Fig. 8). This snake has nocturnal and terrestrial behavior, feeding on rodents. It is a viviparous species, giving birth between 11 and 33 offsprings.

In Brazil, the *Crotalus* bites accounted for 9.2% of cases in 2015. In the Brazilian Amazon, there were 341 recorded cases, representing 23.9% of notifications from the country (Saúde 2014). Epidemiological studies show that *Crotalus* cause 0.7% of accidents by snake envenomings in Amapá (Lima et al. 2009), 13.4% in Roraima (Nascimento 2000), and 0.5% in the Amazonas State (Feitosa et al. 2015). In the upper Juruá River, state of Acre, snakebites were classified as *Crotalus* bites in 2% of the patients, but it is believed that in this region, rattlesnakes are commonly named *surucucu*, a name also used for *Bothrops* and *Lachesis* (Bernarde 2014). In a case series from Rio Branco, also in the state of Acre, no *Lachesis* envenomings were recorded (Moreno et al. 2005).

Fig. 8 Two specimens of *Crotalus durissus*, the species responsible of *Crotalus* envenomings in relictual *cerrado* spots within the Brazilian Amazon

Crotalus durissus venom has three main biological activities: neurotoxic, myotoxic, and coagulant (Azevedo-Marques et al. 2009). The venom of *Crotalus durissus ruruima*, a snake found in the northern state of Roraima in Brazil and southern Venezuela, has shown phospholipase, hemorrhagic, and edematogenic activities, with a notable intrapopulation variation (Dos-Santos et al. 2005). The venom of *C. d. ruruima* may vary in their composition and biological activity in accordance with the color yellow or white; white *C. d. ruruima* venom has activities similar to that of *C. d. terrificus* (Dos Santos et al. 1993). The major component of *Crotalus* venoms is the crotoxin, a neurotoxin with presynaptic activity that acts on motor nerve endings by inhibiting the release of acetylcholine. This inhibition may result in neuromuscular blockade and therefore motor and respiratory paralysis. The myotoxic activity produces injury of skeletal muscle fibers, resulting in rhabdomyolysis. The coagulant action is attributed to the presence of thrombin-like components in the venom, which can lead to hypofibrinogenemia and blood incoagulability (Azevedo-Marques et al. 2009). A potent platelet-aggregating protein, called convulxin, was also isolated from the venom of *C. d. terrificus*, but thrombocytopenia has not been observed in the *Lachesis* envenoming (Azevedo-Marques et al. 2009).

Clinical manifestations at the bite site generally are little evident, with fang marks, paresthesia, and discrete edema and erythema. Systemic manifestations include drowsiness, ptosis, ophthalmoplegia, sagging face muscles, blurred vision, diplopia, myalgia, arthralgia, and myoglobinuria. Swallowing difficulties and changes of smell and taste may occur in some patients. The major complications that can arise after *Crotalus* bites are acute renal failure and acute respiratory failure. *Crotalus* bites patients may present with increased serum levels of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and aldolase. Clotting time can be abnormal in some cases (Azevedo-Marques et al. 2009). In the Brazilian Amazon, few clinical descriptions of *Crotalus* envenomings have been reported in the state of Pará, evolving to acute renal failure (Pardal et al. 2003).

Treatment of *Crotalus* envenomings consists of the administration of the specific antivenom, which is produced in Brazil from the immunization of horses with *C. d.*

terrificus and *C. d. collilineatus* venoms (Fan et al. 2015). In the presence of systemic complications, patient may require supportive treatment, using renal replacement therapy in case of acute renal failure, artificial ventilation in case of respiratory failure, and corticosteroids, antihistamines, and epinephrine in case of anaphylactic reactions following antivenom administration.

Micrurus Envenomings in the Brazilian Amazon

In Brazil, Elapidae snakes are called *coral snakes* because most species have colored rings along the body extension (black, red, or orange and white or yellow) (Bernarde 2014). However, there are exceptions of species presenting no colored rings (for instance, *Micrurus albicinctus*) (Fig. 9). Most coral species usually will not exceed 1 m in length, with no record of *Micrurus spixii* with 1.6 m. Since there are several species of false coral snakes, it is prudent only experts capture these animals. General population must treat all snakes with coral pattern as "possible true coral snakes," thus avoiding accidents with these snakes. These snakes occur in primary forests or in disturbed areas of crops and pasture, including records of some species (e.g., *M. lemniscatus* and *M. surinamensis*) in urban areas. Most species have fossorial or terrestrial habits, but two species (*M. lemniscatus* and *M. surinamensis*) have aquatic habits. *Micrurus* mainly feed on elongated vertebrates (other snakes, amphisbaenians, lizards, and caecilians) but also on fishes (*Callichthys*, *Gymnotus*, and *Synbranchus marmoratus*, predated by *Micrurus lemniscatus* and *Micrurus surinamensis*) and velvet worms (recorded for *M. hemprichii*) (Bernarde 2014; Martins and Oliveira 1998). These snakes are oviparous, with a record of 2 to 15 eggs, which varies between species and the size of the snake (Martins and Oliveira 1998). In the Brazilian Amazon, it is estimated that 0.3% of the snakebites

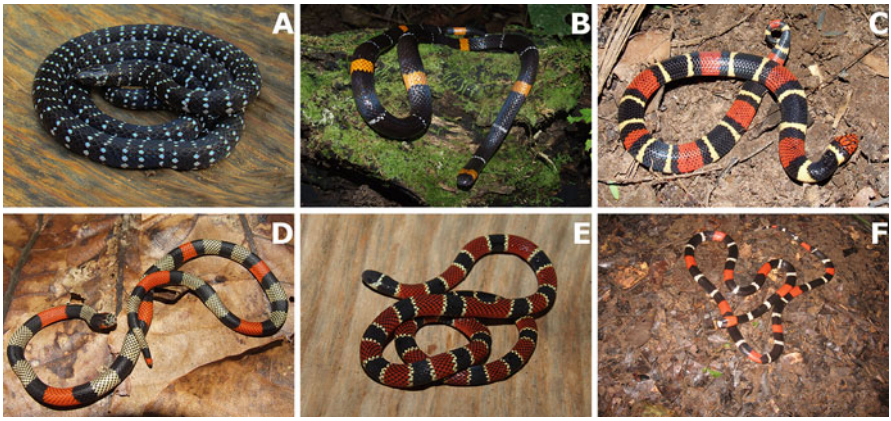

Fig. 9 Some *Micrurus* species involved in biting humans in the Brazilian Amazon: (**a**) *Micrurus albicinctus*, (**b**) *Micrurus hemprichii*, (**c**) *Micrurus surinamensis*, (**d**) *Micrurus spixii*, (**e**) *Micrurus remotus*, and (**f**) *Micrurus lemniscatus*

are caused by *Micrurus*. Clinical data from *Micrurus* envenomings are scarcely reported in this region, and *M. hemprichii* and *M. lemniscatus* are involved in such envenomings (Fan et al. 2015).

Micrurus venom primarily induces neurotoxic effects due to the presence of neurotoxins with pre- and postsynaptic activity. The neurotoxins can competitively bind with acetylcholine receptors causing postsynaptic blockage of neuromuscular transmission or act at the neuromuscular junction blocking the presynaptic release of acetylcholine (Ministério da Saúde 2001). *M. surinamensis* has a presynaptic neurotoxin (Dos-Santos 2009). Venoms of *Micrurus spixii*, *M. averyi*, *M. lemniscatus*, *M. surinamensis*, and *M. hemprichii* from the Brazilian Amazon region have no coagulant activity. However, with the exception of the *M. surinamensis* venom, the venoms of the other species have edematogenic and myotoxic activities (Terra et al. 2015). Myotoxic effect of *Micrurus* venom of the Brazilian and Colombian Amazon is evidenced experimentally by the increase in plasma levels of creatine kinase and acute muscle damage on histology (Gutiérrez et al. 1992). Toxin and crude venoms of *M. spixii*, an endemic species of South America and northern states of Brazil, show phospholipase activity. In a mouse phrenic nerve-diaphragm preparation, *M. spixii* venom and $MsPLA_2$-I induced the blockage of both direct and indirect twitches (Terra et al. 2015).

Victims of *Micrurus* bites have ptosis; ophthalmoplegia; jaw, laryngeal muscles, and pharynx paralysis; drooling; and paralysis of the neck and limbs as a result of neurotoxic venom activity (Ministério da Saúde 2001). Acute respiratory failure has been observed in accidents caused by *M. surinamensis* that occurred in the state of Pará, as a result of paralysis of the respiratory muscles. In this state, *Micrurus filiformis* also causes envenomings with pain and mild edema at the bite site, epigastric pain, and vomiting (Pardal et al. 2010). In the Amazonian Ecuador, unusually, there was a case of *M. lemniscatus helleri* bite with severe local pain, slow evolution of neurological manifestations, thrombocytopenia, and mild coagulopathy (Manock et al. 2008).

Treatment of *Micrurus* bites is made with the use of specific antivenom. In Brazil, *M. corallinus* and *M. frontalis* venoms are used for the production of *Micrurus* antivenom. In cases of acute respiratory failure, intubation and mechanical ventilation are required (Manock et al. 2008; Pardal et al. 2010).

Prevention Measures

Snakebites are considered preventable injuries and most of the envenomings occur by lack of preventive habits, including individual protection equipment, especially for workers in rural activities. As lower and upper limbs are the most affected areas of the body, the use of jackboots, leggings, and gloves is supposed to be the major primary prevention measure. Some simple additional measures such as keeping clean household surroundings and closing garbage cans help to keep away small rodents, which are part of some snakes' diet. In the Amazonian context, it should be

noted that many Amerindian and traditional riverine individuals are habitually barefoot populations, representing a challenge for preventing snakebites. Education about safe habits for the most affected groups is essential. For instance, there is no systematic program of interventions for primary prevention of snakebites as an occupational hazard in the Amazon.

Secondary prevention of snakebites aims to reduce the impact of an already occurred envenoming, by detecting and treating patients as soon as possible to prevent severe complications such as local necrosis and secondary bacterial infections, systemic bleeding, and renal failure, commonly observed after *Bothrops atrox* snakebites (Souza 2002). In the Brazilian Amazon, more than 30% of patients took more than 6 h to receive medical assistance, and such delay was an independent risk factor for severe complications and associated mortality (Feitosa et al. 2015). Moreover, underdosing of antivenom in the region seems to be common. Improvement in the access to health facilities and systematic professional training on diagnosis, specific therapy, and clinical management of complications could have a significant impact in preventing poor outcomes, long-term disabilities, and lethality. The Brazilian Ministry of Health additionally recommends not doing tourniquets, cutting or sucking the bite site, or applying substances such as alcohol, coffee, kerosene, mud, and other traditional "medicines" (Ministério da Saúde 2001).

The burden of function loss associated to snakebites on vulnerable populations remains as a major research gap, both from the health system and society perspective. In the state of Acre, in the Western Brazilian Amazon, functional impairment of the bitten limb was recorded in 10% of the indigenous and riverine population surveyed, including permanent loss of function and sensibility, amputations, and permanent scarring (Pierini et al. 1996). In order to improve as much as possible their ability to function, their quality of life, and their life expectancy, public policies aiming to identify incapacitated victims and provide them socio-economical support and physical rehabilitation should be part of integrated national programs for chronic pathologies.

Conclusions and Future Directions

Despite important efforts carried out during the past decades in Brazil to understand and control the problem of snakebites, important gaps remain for the fulfillment of these goals, particularly in the Amazon region. A workshop was held in Manaus, Amazonas, in 2013 with representatives of Health Departments of Amazonian states, AV producers, universities, reference hospitals, and the Ministry of Health to identify research bottlenecks. A proposal to create the research network Snakebite and Scorpionism Network in the Amazon (*Rede de Ofidismo e Escorpionismo da Amazônia* (ROdA)) emerged from researchers at the Butantan Institute and the Tropical Medicine Foundation Dr. Heitor Vieira Dourado. The general aim of the network is to enhance implementation of collaborative work and multicenter studies

resulting in integration of services, research institutions, and health professionals. Identified research gaps are listed below (Fan et al. 2015).

Burden on Vulnerable Populations

It is difficult for riverine and indigenous populations to reach health centers for treatment of snakebites. As a result, the number of cases detected officially is probably much lower than the real number. Current AVs require conservation in adequate facilities (2°–8 °C), which are not always available in these remote settings. In addition, training of multidisciplinary teams is not always appropriate for indigenous health services regarding AV administration, side effect management, and case monitoring and surveillance.

Recommendations:

1. Assess disease burden through population- and hospital-based field studies in remote areas.
2. Seek innovation in the network for efficient distribution of immunobiologicals, especially interaction with other networks such as those providing vaccines.
3. Integrate different sectors (Health Surveillance Secretariat (SVS), Indigenous Health Special Secretariat (SESAI), National Agency of Sanitary Surveillance (ANVISA)) for articulation of common strategies to be pursued with other ministries (Agriculture, Environment, Science, and Technology).
4. Review the skills of professionals assisting injured patients in areas without infrastructure and doctors according to international guidelines.

Venom Research and Revision of the AV Spectrum

Currently, AV immunoglobulins are the only treatment available for snake envenomings. The WHO List of Essential Medicines includes them in the basic package of healthcare in affected countries. There is an urgent need to ensure availability of effective AVs and to improve their manufacture regulation. However, the possible interspecific venom variation associated with the geographical distribution of snakes may affect the effectiveness of therapeutic AVs against the Amazon *Bothrops* venom.

Current AV production methods, based on studies conducted in the 1980s, need updating in light of new technologies. Antivenom recommendations are based on experimental studies of cross-neutralization between specific venoms and AVs. These excluded venom from *Bothrops atrox*, the main cause of snakebites in the Amazon. Efficacy of Brazilian AVs against venom from some Amazon *Bothrops* species has been investigated. *Bothrops* AV showed neutralization of *B. atrox* venom major toxins (Pardal et al. 2004). Thus, new studies are a needed investment in technological development to assess different AV candidate formulations.

A major concern relates to the failure in AV distribution. Antivenoms are usually available in the municipal hospitals, as opposed to being distributed to peripheral health clinics. The lack of adequate cold chain impairs AV distribution to rural areas.

Also, inadequate storage and transportation may result in loss of material. Freeze-dried AVs are available, and one of the national producers (Butantan Institute) has been working to provide both liquid and freeze-dried products.

Recommendations:

1. Revise toxicity of snake venoms, including proteomics, as well as the potential for AV neutralization against major venom activities.
2. Study seroneutralization in experimental models to support the venom pool used to immunize animals for AV production, considering the absence of *Bothrops atrox* venoms in these pools. Experimental studies should indicate the need for inclusion of new venoms; the new product needs to be validated by clinical and epidemiological data.
3. Perform stability studies of liquid AVs considering the Amazonian environmental conditions. Decisions on AV distribution, either liquid or freeze-dried products, should be based on careful and detailed analysis of the epidemiology of snakebites, the prevailing conditions, and health facilities available.
4. Study the mechanisms of venom action for different populations of snakes in the Amazon (inter- and intraspecies variations).
5. Study supporting action or herbal drugs with specific activity on certain venom components to enable complementary or alternative treatments.

Priorities in Clinical Research

Although clinical research related to venomous animal injuries has increased, most publications are based on case reports and lack methodological rigor. Moreover, outcome definitions, such as severity ranking criteria, were empirically established, making the results even less generalizable. Clinical research from hospital-based studies (patient follow-up for evaluation of the frequency of events related to envenoming and their risk factors) and community observational studies (verbal autopsy studies and seroepidemiological surveys, group population cohorts, and qualitative studies) is needed.

Delays in patient care, along with the use of substances that may aggravate the conditions at the bite site, lead to a high frequency of local complications resulting from *Bothrops* and *Lachesis* envenomings. However, severity is also possibly related to the composition of Amazon venoms. Medical management of secondary infection, abscess, necrosis, and compartmental syndrome has been the subject of controversy, partly because of the lack of standardization regarding concepts and management protocols. The possibility of reducing local effects by means of drugs with anti-inflammatory activity, early antibiotic therapy for secondary infection, cross-neutralization of AVs for different types of accidents, and new complementary treatments needs to be further investigated while observing good clinical practice and, preferably, in multicenter studies.

Systemic complications such as sepsis and acute kidney injury are less well known, and apparently less frequent, than local complications, but most frequent across the series from other regions in Brazil. The lack of patient follow-up, including laboratory tests, appears to be related to this observation.

Recommendations:

1. Perform multicenter studies by standardization of clinical protocols for estimating independent risk factors for complications and also assessing AV efficacy (choice of outcomes) and defining objective criteria for recommending AV dosage.
2. Identification of the species responsible for snakebites in the Amazon requires the establishment of a gold standard method and determination of levels of antigenemia, preferably by means of rapid diagnostic tests.
3. Evaluate phase IV studies for adverse reactions (from three Brazilian manufacturers) under AV pharmacovigilance.
4. Submit phase II/III protocols simultaneously to assess feasibility of comparative studies on efficacy and safety.
5. Plan training in good clinical practices, as well as establish a link to the National Clinical Research Network (RNPC) from teaching hospitals.
6. Inform the regulatory agencies about the limitations and peculiarities of research involving animal envenomings, paying clarification and consultation to the National Human Research Ethics Council and the National Regulatory Agency.

Adverse Reactions and Pharmacovigilance

Early adverse reactions (EAR) to AV therapy are expected events of varied frequency according to the type of AV used and individual hypersensitivity to heterologous proteins. Clinically, patients may present urticaria, itching, tachycardia, nausea, vomiting, abdominal colic, bronchospasm, hypotension, and angioedema. Over time, both the frequency and severity of early reactions have decreased due to the improvement of the AV purification process in Brazil. Frequency of delayed reactions (serum sickness) seems to be lower than EAR, but the true frequency is unknown. AV pharmacovigilance has been implemented, but reliable efficacy and safety data are still lacking.

There are no accurate predictive factors for side effect occurrence, and preventing them is not always possible, even with the use of premedication containing corticosteroids and/or antihistamines. Existing studies do not include controls for the intervening variables, and samples are of insufficient size, limiting the validity of the results.

Recommendations:

1. Perform multicenter phase IV studies, identifying sentinel hospitals for monitoring cases for both early and late reactions.

Professional Training

Despite the high incidence of injuries from venomous animals, there is a lack of systematic professional training on diagnosis, specific therapy, and clinical management of complications. Thus, AV misuse is not infrequent, either in quantity (number of ampoules administered) or the specific AV. Current training programs seek to link medical knowledge with the snakes' biology and surveillance. However, this

approach often does not reflect the need for professional diagnosis algorithms and coherent and responsive medical management. Thus, adherence to medical training and courses in this area has been a major challenge. Furthermore, there is a high turnover of health professionals in small Amazon cities. Although communication technologies that greatly facilitate knowledge dissemination have proliferated in the area, these are still barely harnessed. The use of electronic media for training professionals in the management of envenomations is increasing and may be an alternative to classroom courses.

Recommendations:

1. Investments in training should cover all health professionals, including nurses who are critical to initial management of the patient and follow-up of possible complications.
2. Update systematically all relevant diagnosis and treatment guidelines.
3. Encourage the use of technological resources for communication and other electronic media used in training programs and distance learning.
4. Include the topic in the undergraduate curriculum of health professionals with regionalized approaches to issues involving venomous animals.
5. Design new postgraduate and other courses, as well as interaction between graduate programs to increase the critical mass of professionals and researchers involved,
6. Implement nonformal education activities for science communication, particularly aimed at school audiences.

Fauna Surveys and Capture of Animals for Venom Production

Traditionally, institutions producing AVs get animals caught from nature that are kept in vivariums and used to obtain venoms. However, environmental legislation restricts the collection and transport of wild animals. There is a requirement to establish specific policies to capture animals, and captive breeding is not done satisfactorily.

Field work is not limited to animal collection; it also includes studies of behavioral patterns such as diet, reproduction, and activities to establish phylogeny patterns for identifying risk factors of the envenomings. Increased knowledge on biodiversity of Amazon animals has applications on their use and species conservation. Zoological collections of invertebrates are also informative regarding their geographical distribution and diversity.

Recommendations:

1. Establish partnerships for capture of *Lachesis* snakes and transport to vivariums of laboratories producing AVs.
2. Guide *Lachesis* reproduction in captivity, which should be led by professionals familiar with their conditions both in nature and supportive maintenance environment.
3. Follow shared standard operating procedures (SOPs) for the collections of venomous animals in order to facilitate exchange of information.

In addition to all the above recommendations, international cooperative efforts toward the control of this neglected health problem through international partnerships are needed, namely, with other Amazonian countries.

Cross-References

► Snake Bites in Colombia
► Snakebite Envenoming in Latin America and the Caribbean

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Envenomation by Wandering Spiders (Genus *Phoneutria*)

4

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Abstract

Spiders of the genus *Phoneutria*, commonly known as wandering or banana spiders, are found in southern Central America (Costa Rica) and throughout South America east of the Andes into northern Argentina. Eight species, classified as Amazonian (*P. fera*, *P. reidyi*, and *P. boliviensis*) and non-Amazonian (*P. keyserlingi*, *P. pertyi*, *P. eickstedtae*, *P. bahiensis*, and *P. nigriventer*), have been described. Most of the clinically important bites by this genus occur in Brazil (~4,000 cases per year), with only 0.5% being severe. Local pain is the major symptom reported after most bites and involves peripheral (tachykinin (neurokinin NK_1 and NK_2) and glutamate receptors) and central (spinal) mechanisms (neurokinins, excitatory amino acids, nitric oxide, proinflammatory cytokines, and prostanoids). Other local features observed in envenomed patients include edema, erythema, radiating pain, sweating, fasciculation, and paresthesia. Systemic manifestations are less common and may include diaphoresis, tachycardia, arterial hypertension, agitation, prostration, sialorrhea, vomiting, tachypnea, pallor, hypothermia, cyanosis, diarrhea, and priapism. Shock and pulmonary edema, the main severe complications, are uncommon and possibly related to increased sympathetic activity and a systemic inflammatory response, although no sequential serum catecholamine, nitric oxide, and interleukin levels have been measured in prospective case series of human envenomations by *Phoneutria* spp. Most cases are treated symptomatically, with antivenom being recommended only for patients who develop important systemic clinical manifestations; such manifestations occur in ~3% of cases and involve mainly children <10 years old and adults >70 years old. Fifteen deaths attributed to *Phoneutria* spp. have been reported in Brazil since 1903, but in only two of these cases are there sufficient details to confirm a causal nexus.

Introduction

Wandering spiders (genus *Phoneutria*) are among the most medically important venomous spiders in the world (Bücherl 1985; Lucas 1988; Isbister and Fan 2011). Despite the large number of *Phoneutria* spp. bites in Brazil, there are in fact few detailed clinical reports involving these spiders in this country or indeed for other countries where these spiders occur. This chapter reviews the clinical toxinology of bites by *Phoneutria* species (spp.) in humans in Central and South America, particularly Brazil, reported from 1925 to 2016, with particular emphasis on the species involved, the geographical region where the bite occurred, the clinical

features described, the treatment used, including antivenom, and the outcome. An analysis of fatal cases is also included. Electronic databases [EMBASE, PubMed/Medline, SciELO, and LILACS (the latter two covering collections of Brazilian and other Latin-American scientific journals)] were searched for relevant reports using key words in Portuguese, Spanish, and English (including some MeSH terms), such as "mordidas/picadas de aranhas" (spider bites), "aranha armadeira" ("armed spiders" or *Phoneutria* spp. spiders), "arañas del banana," "arañas del plátano" or "arañas de las bananeras" (banana spiders), envenenamento (envenomation), "foneutrismo" or "phoneutrismo" (bites in humans by *Phoneutria* spp.), *Ctenus* (an earlier taxonomic designation for the genus *Phoneutria*), *Phoneutria*, banana spiders, wandering spiders, envenoming, and envenomation. In addition, standard (text)books on toxicology/toxinology, abstracts published in congress proceedings and academic dissertations, were searched manually, as were the reference lists of all the publications that were consulted. One personal communication and two reports of severe envenoming based on cases monitored at two Brazilian Poison Control Centers (PCC) are also included. Information on the taxonomy, geographical distribution, and biology of Amazonian and non-Amazonian *Phoneutria* spp. and on the physiopathology of envenomation is also provided, in the expectation that this will be useful to clinical toxinologists and doctors treating persons bitten by these spiders.

Taxonomy, Geographical Distribution, and Biology

Wandering or banana spiders (so-called because they are frequently found in crates of transported bananas) of the genus *Phoneutria* Perty 1833 (family Ctenidae, suborder Araneomorphae, and order Araneae) are popularly known in Brazil as "aranha armadeira" in reference to their characteristic display when threatened (in Spanish-speaking Latin-American countries, these spiders are commonly known as "arañas del banana," "arañas del plátano," or "arañas de las bananeras," i.e., banana spiders). *Phoneutria* spiders are very agile, medium-to-large-sized (17–48 mm in total body length and up to 180 mm with outstretched legs) nocturnal hunters that do not construct webs to trap their prey. When molested, these spiders may assume a very characteristic and intimidating defensive posture by standing on their hind legs with the front legs raised to expose their fangs while directly facing the enemy; this posture is frequently accompanied by lateral movements of the body. *Phoneutria* spiders bite readily and may jump distances of up to 30–40 cm (Bücherl 1953a; Lucas 1988, 2003; Schenberg and Pereira-Lima 1978; Martins and Bertani 2007; Hazzi 2014).

The taxonomy of *Phoneutria* spp. has had a long and checkered history, primarily because of the great similarity between these spiders and those of other closely related genera in the Ctenidae, especially the genus *Ctenus* that contains >200 species (World Spider Catalog 2016). Although the genus *Phoneutria* was originally diagnosed based on the recurved median row of eyes (Perty 1833), the 2-4-2 ocular arrangement and the oval anterior lateral eyes (Fig. 1b) were later considered to be

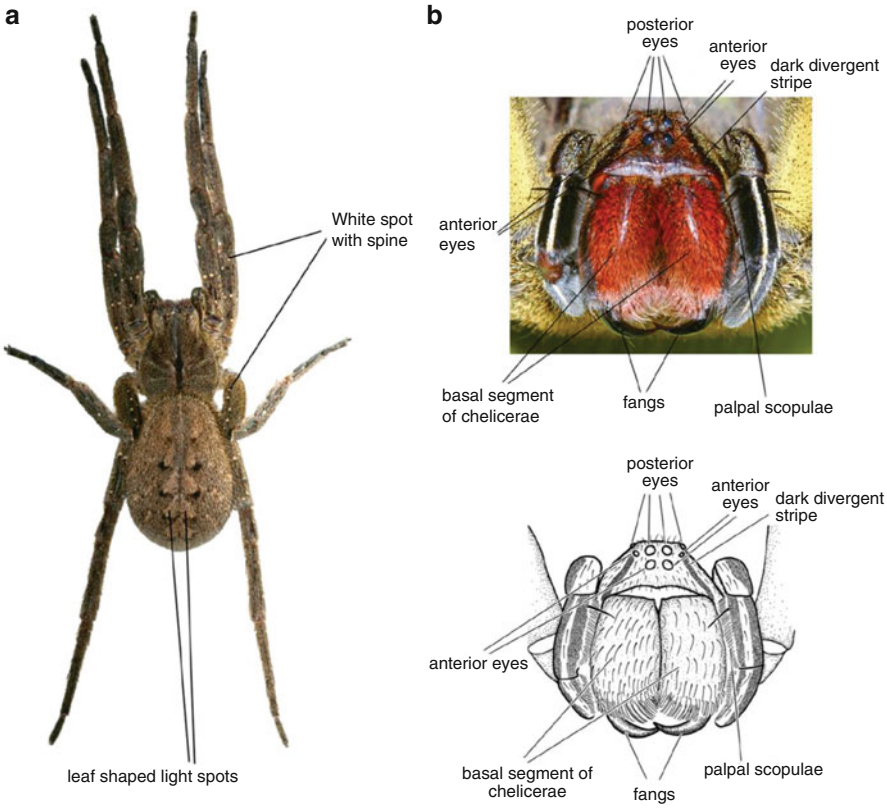

Fig. 1 Key morphological characters for the identification of *Phoneutria* spp. (**a**) – Dorsal aspect of a female *P. nigriventer* showing the abdominal folium and white dots with spines. (**b**) – Face of a female *P. reidyi* showing the diagnostic scopulae (brush of hairs) on the internal surface of the palps, the dark divergent stripe and the eyes arranged in a 2-4-2 pattern (Photographs: R. Bertani©)

characteristic of the Ctenidae in general (Lehtinen 1967, as cited in Simó and Brescovit 2001). In this regard, von Eickstedt and Bücherl (1969) reported considerable variation in the arrangement of the second ocular row in 50 individuals of *Phoneutria nigriventer* from the same geographical region (São Paulo City and surroundings). Since *Phoneutria* spp. share several morphological characters with other related genera, they are frequently misidentified as belonging to the ctenid genus *Cupiennius* in Central America and northwestern South America (Vetter and Hillebrecht 2008) and to *Ancylometes* and *Ctenus* in South America. These large spiders are sporadically found in fruits, mainly bananas, exported to the United States and Europe, with their arrival often being a cause for concern (Vetter and Hillebrecht 2008; Jäger and Blick 2009; Vetter et al. 2014). For this reason, Vetter and Hillebrecht (2008) and Vetter et al. (2014) provided information on how to distinguish *Phoneutria* spp. from *Cupiennius* spp. from Central America and northwestern South America.

The best way to identify a *Phoneutria* specimen is by the presence of dense scopulae (a brush of hairs) on the internal margin of the two distal palpal segments of males and females, as stated by Mello-Leitão (1936); this feature is still used to distinguish this genus from other genera of the Ctenidae (Martins and Bertani 2007) (Fig. 1b). Another possibility is to note the presence of a combination of characters that, although not being exclusive, can be useful in identification. These include an eye arrangement in a 2-4-2 pattern (Fig. 1b); the presence, on the legs and palps, of small white spots with a spine inserted in the centrum (Fig. 1a); an abdominal dorsum with folium (light spots resembling leaves) (Fig. 1a); the presence or absence of white dots, depending on the species; and the occurrence of two divergent lines on the face (Fig. 1b). The defensive display of raising the anterior legs is common to other spiders as well, but only *Phoneutria* spp. maintain the anterior legs totally stretched with the body supported on the four hind legs (Figs. 2b, e, h and 3b), a posture that allows lateral movements of the body.

Phoneutria spp. live on vegetation, mainly in palm trees, plants with large leaves (Torres-Sánchez and Gasnier 2010), and bromeliads in forested areas from southern Central America (Costa Rica) throughout South America east of the Andes into northern Argentina (Martins and Bertani 2007; Hazzi 2014). Eight species have been described and broadly classified as Amazonian (*P. fera*, *P. reidyi*, and *P. boliviensis*) and non-Amazonian (*P. keyserlingi*, *P. pertyi*, *P. eickstedtae*, *P. bahiensis*, and *P. nigriventer*) species (Martins and Bertani 2007).

The non-Amazonian species are restricted to northern Argentina, Paraguay, Uruguay, and Brazil, including areas of Brazilian Atlantic rainforest and in forest fragments in the Cerrado (savannah) (Simó and Brescovit 2001; Martins and Bertani 2007; Peralta 2013), but show little overlap in their distribution. Although in many cases definitive species identification requires examination of the genitalia by a specialist, the color patterns and geographical distributions shown in Figs. 2 and 3 and Table 1A can be useful indicators of the possible identity where there is no specialist. In contrast, the Amazonian species show strong overlap in their distribution (Fig. 4d, h, and l), and it is common for two and, more rarely, three species to occur in the same area. *Phoneutria reidyi* is more common in the eastern Amazon (Fig. 4d), whereas *P. fera* is common from central to western Amazon (Fig. 4h), and *P. boliviensis* from the extreme western Amazon to southern Central America (Fig. 4l). The color patterns of these species are readily distinguished and even immature individuals can be identified, as shown in Fig. 4 and Table 1B.

Phoneutria nigriventer is the most important species of the genus, based on its clinical significance and geographical distribution (the species is also found in large urban areas). This species is restricted to central-western, southeastern, and southern Brazil, northern Argentina, Uruguay, and Paraguay (Simó and Brescovit 2001; Martins and Bertani 2007) (Fig. 2c). According to Simó (1984) and Simó and Brescovit (2001), this species was probably introduced into Uruguay and parts of Argentina along with imported bananas. Previous taxonomic denominations such as *Ctenus ferus*, *C. nigriventer*, *C. medius* and *P. fera* are considered synonyms of *P. nigriventer* (Schenberg and Pereira-Lima 1978; Lucas 2015). The venom of *P. nigriventer* is highly toxic and is the most studied of *Phoneutria* spp. Apart

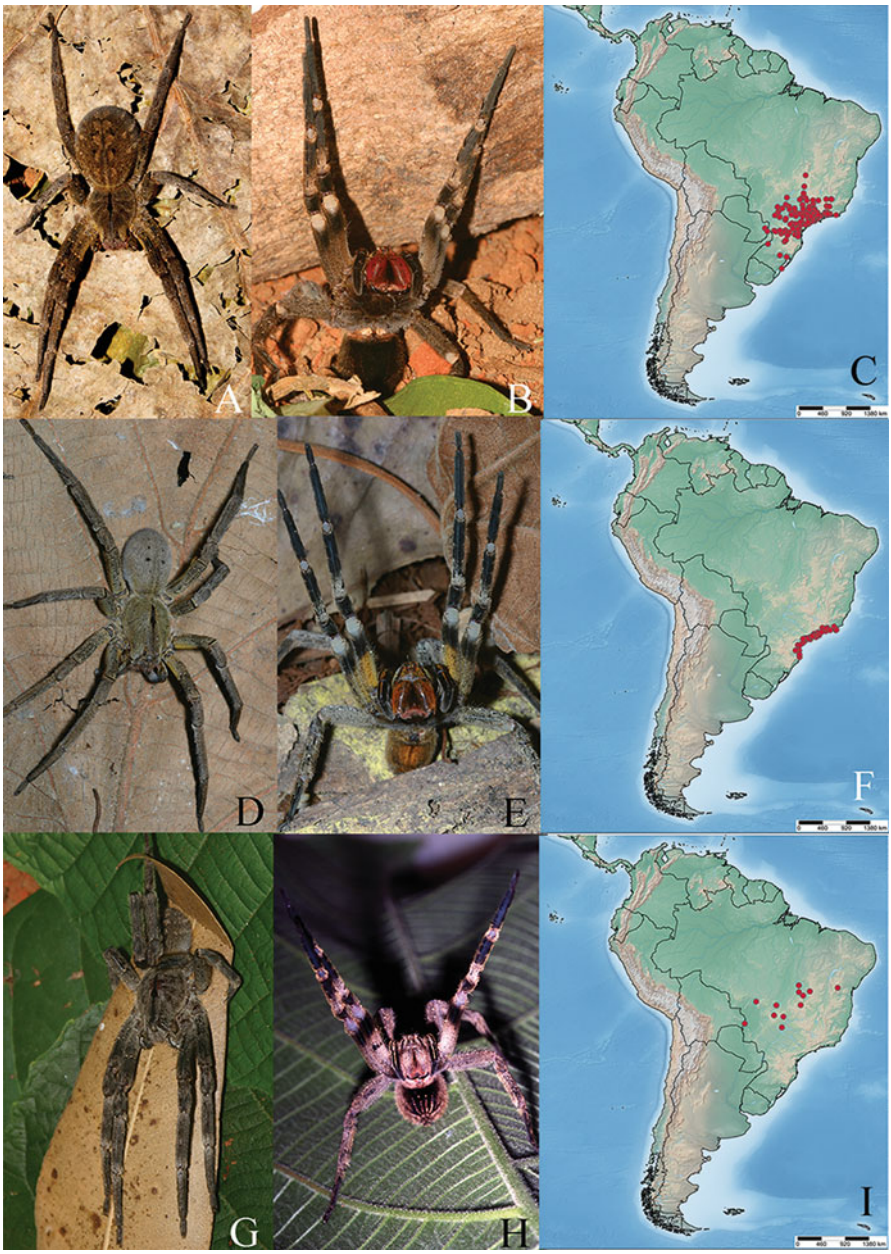

Fig. 2 Non-Amazonian *Phoneutria* spp. (**a**–**c**) *P. nigriventer*, (**a**–**b**) – female and (**c**) – map with records (red dots). (**d**–**f**) *P. keyserlingi*, (**d**–**e**) – female and (**f**) – map with records. (**g**–**i**) *P. eickstedtae*, (**g**–**h**) – female and (**i**) – map with records (Photographs: R. Bertani©)

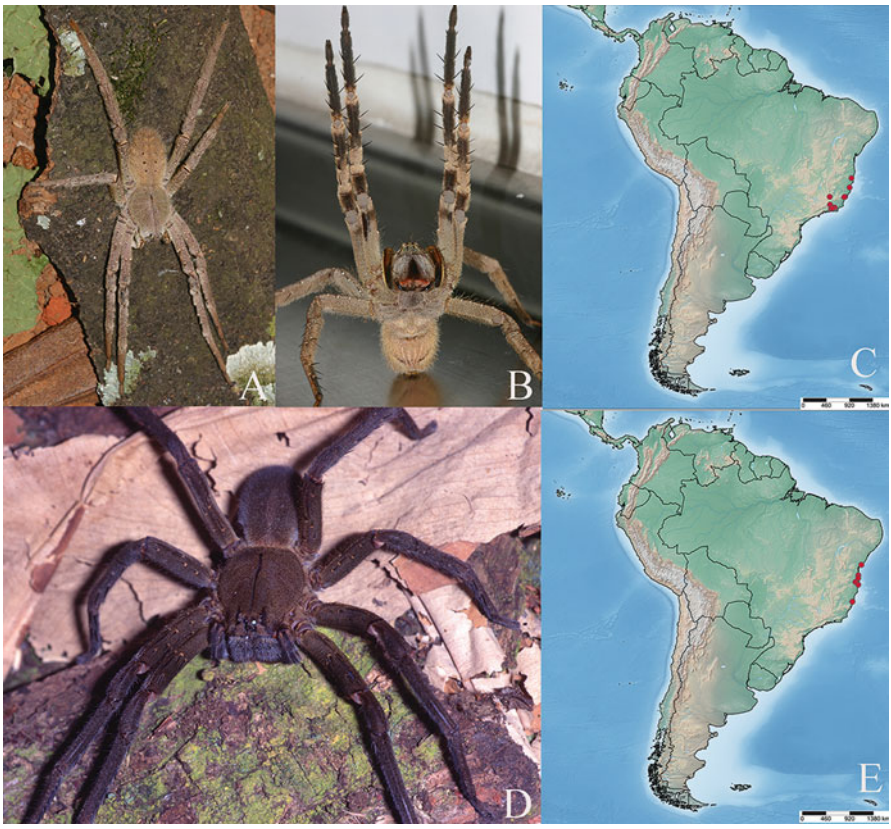

Fig. 3 Non-Amazonian *Phoneutria* spp. (**a**–**c**) *P. pertyi*, (**a**–**b**) – female and (**c**) – map with records. (**d**–**e**) *P. bahiensis*, (**d**) – female and (**e**) – map with records (Photographs: R. Bertani©)

from *P. nigriventer*, two other *Phoneutria* species have been identified in southern and southeastern Brazil, namely, *P. keyserlingi* (southern and southeastern region) and *P. perty* (southeastern region) (Martins and Bertani 2007); however, no confirmed bites by these species have been described.

Phoneutria nigriventer is native to the Brazilian Atlantic Rainforest but can assume synanthropic habits in disturbed areas close to forest remnants. Consequently, this species is frequently encountered near human dwellings, where it finds shelter and abundant prey such as other spiders and insects (e.g., cockroaches, crickets) (Lucas 1988). These spiders live beneath fallen trees, in gaps in walls, in bundles of sticks, and in rubbish dumps, banana bunches, palm trees, and bromeliads. Indoors, these spiders hide during daytime in dark places such as shoes, chests, and under doorknobs (Lucas 1988). This behavior may help to explain the large number of bites in humans that occur inside houses during the day in Brazil, in both urban and rural settings, with most bites involving the feet and hands (Rosenfeld 1972;

Table 1 Color patterns that aid in the identification of non-Amazonian (A) and Amazonian (B) *Phoneutria* spp.

A

Species	Abdominal ventral pattern	Abdominal dorsal pattern
P. bahiensis	Brown	Homogeneous, lacking dots or folium
P. eickstedtae	Black with a longitudinal series of white dots	Folium
P. keyserlingi	Orange[a]	Black dots
P. nigriventer	Black[a]	Folium
P. pertyi	Light brown with two short black areas and a series of light dots	Folium

B

Species	Dorsal dark stripe on palp	Abdominal dorsal pattern	Color of female anterior legs ventrally	Color of male anterior legs ventrally
P. boliviensis	Double	White dots	Femur and other appendages golden	Yellow
P. fera	Single	Folium	Light brown	Light brown
P. reidyi	Double	White dots	Only femur yellow	Very dark

[a]Males of *P. nigriventer* and *P. keyserlingi* have an orange abdomen ventrally

Bucaretchi et al. 2000; Antunes and Málaque 2003; Cardoso et al. 2011), and also during work activities, such as civil construction, farming, or handling and trading bananas (Belluomini et al. 1987; Bucaretchi et al. 2000).

In southern Brazil, mating in *P. nigriventer* occurs from April to July, when mature males and females are more active and most frequently observed; females with egg sacs appear in mid-July (Bücherl 1969, 1985; Ramos et al. 1998). This reproductive behavior may help to explain the greater number of *Phoneutria* spp. bites from March to May in Brazil, with a peak in April, at least in southern and southeastern regions (Bucaretchi et al. 2000; Cardoso et al. 2011; Brasil, Ministério da Saúde 2016. *Phoneutria nigriventer* females construct 1–4 egg sacs and produce up to 3,000 eggs (Bücherl 1971; Lucas 1988). Despite the large number of eggs, no more than 2–3 pairs of spiders survive to adulthood (Bücherl 1985). Depending on the availability of food, young *Phoneutria* molt five to ten times, three to seven times, and one to three times in the first, second, and third year of life, respectively. The spiders become adults in the third year and the maximum longevity is around 6 years; the male/female ratio varies from 1:3 to 1:5 (Bücherl 1969; Lucas 1988).

Phoneutria nigriventer feeds mainly on insects and other spiders, but large individuals can potentially prey on small vertebrates (mammals, lizards, and amphibians); Santana et al. (2009) recorded *P. nigriventer* preying on a frog. On the other hand, *Phoneutria* spp. may be preyed upon by various animals, including some species of mammals, birds, frogs, lizards, insects, and other spiders. The same predator species may also be prey of *Phoneutria* when the spider is larger than the predator, i.e., the prey-predator relationship depends on the relative size in

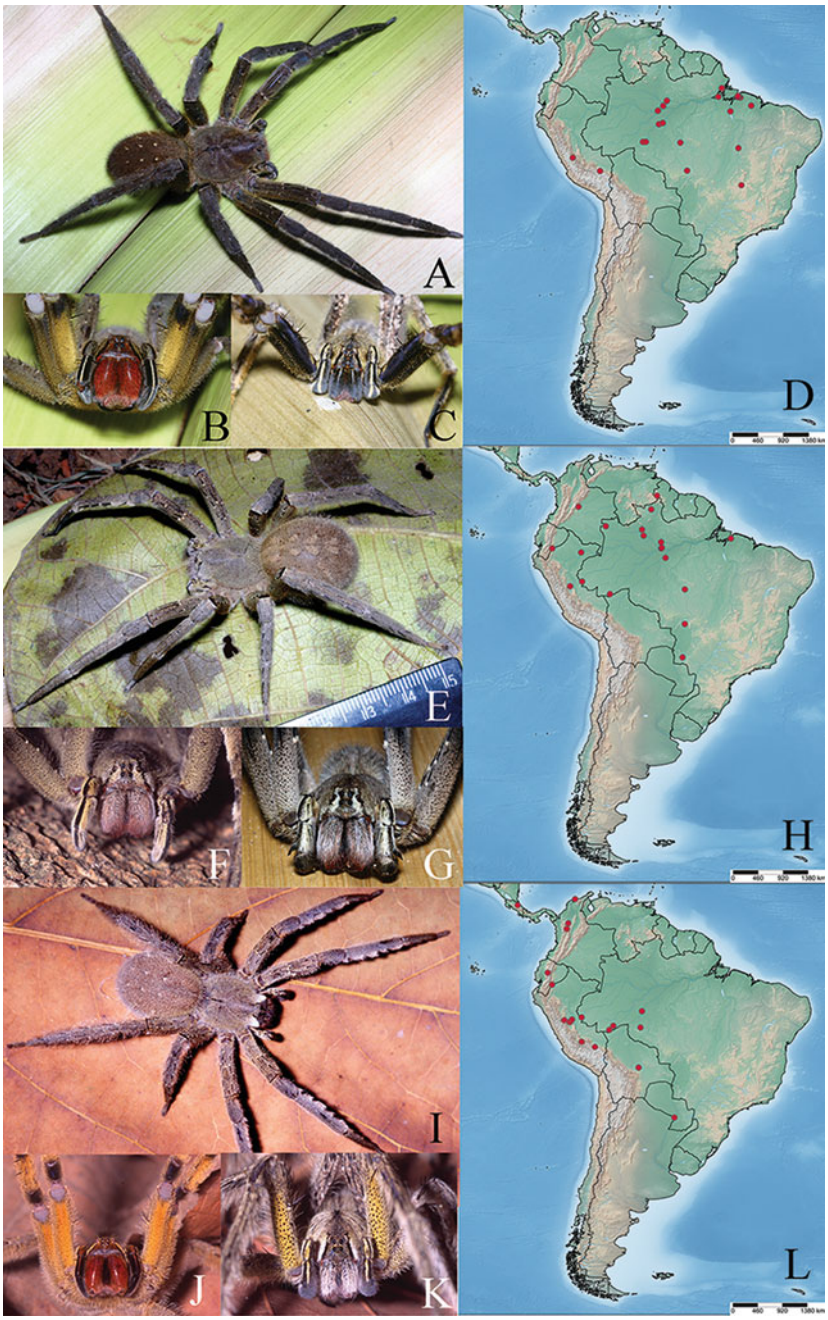

Fig. 4 Amazonian *Phoneutria* spp. (**a**–**d**) *P. reidyi*, (**a**–**b**) – female, (**c**) – male and (**d**) – map with records. (**e**–**h**) *P. fera*, (**e**–**f**) – female, (**g**) – male and (**h**) – map with records. (**i**–**l**) *P. boliviensis*, (**i**–**j**) – female, (**k**) – male and (**l**) – map with records (Photographs: R. Bertani©)

Fig. 5 Predation of *P. nigriventer* by a wasp of the family Pompilidae in a remnant area of Atlantic Forest (22°51′11″S 47°1′56″W) in the region of Campinas, SP, southeastern Brazil. In the left panel, the foraging wasp has not yet encountered the spider (lower left of the photograph), while in the right panel the wasp is seen attacking and stinging the spider. (Photographs: E.M. De Capitani©)

relation to the spider. As large specimens of *Phoneutria* tend to have fewer predators, most predation on these spiders occurs when they are young (Torres-Sánchez and Gasnier 2010). Some specialized predators, e.g., wasps of the family Pompilidae, are known to prey on large spiders such as tarantulas and ctenids, including *Phoneutria* (Fig. 5).

The minimum, mean, and maximum dry venom yields obtained by electrical stimulation (3–4 shocks of 6 V) delivered to the cephalothorax of lightly anesthetized *P. nigriventer* were reported to be 0.3, 1.25, and 8.0 mg, respectively (Bücherl 1971; Schenberg and Pereira-Lima 1978). Bücherl (1953a), using dry venom from *P. nigriventer*, reported subcutaneous (s.c.) and intravenous (i.v.) LD_{50} values in mice of 0.7 mg/kg and 0.34 mg/kg, respectively. In addition, Bücherl (1953b) reported that the mean dry venom yield for *P. nigriventer* in the region of São Paulo (southeastern Brazil) was greater on hot days (mean: 2.0–2.5 mg; maximum of 8 mg) than on cold days (mean: 0.4–0.71 mg; maximum of 8 mg). Ontogenetic development (Herzig et al. 2004) and sex-related (Herzig et al. 2002) and seasonal (Bücherl 1953b) variations may affect venom yield and composition. During growth, the venom yield increases according to a fourth order function of the prosoma size that mainly reflects an increase in venom gland volume. In addition, venom from young instars is not lethal in mice up to a dose of 3.28 mg/kg, i.v., compared to an LD_{50} of 0.63 mg/kg and 1.57 mg/kg for venom from adult females and males, respectively (Herzig et al. 2002, 2004). These features suggest that ontogenetic changes in the venom protein composition of growing *P. nigriventer* may involve an increase in lethality in vertebrates (Herzig et al. 2004).

The practical relevance of these differences in venom composition and lethality is unclear as there has been no systematic assessment of the influence of spider sex or age on the clinical manifestations of envenomation. Although it is tempting to suggest that because of their greater venom yield and lethality bites by adult female *P. nigriventer* could place more vulnerable groups, such as children and elderly people, at greater risk of more serious manifestations after envenomation, there is little evidence that this is the case. Only two abstracts have reported the frequency of bites in relation to the sex of the spider, but the findings were not consistent. Coelho and Gonçalves Jr (1993) stated that most of the *Phoneutria* bites in a case series in the southern Brazilian state of Santa Catarina were caused by males, whereas Ribeiro et al. (2001) reported that most of the bites in São Paulo City and surroundings were caused by adult female *P. nigriventer*. In agreement with the latter finding, in two well-documented cases of severe envenomation, the spiders responsible were adult female *P. nigriventer* (Zannin et al. 2005; Bucaretchi et al. 2008) (see Tables 2, 3, and 4).

Clinical Envenoming

Epidemiology

Most of the clinically important bites involving *Phoneutria* spp. are from Brazil (Brazil and Vellard 1925, 1926; Vellard 1936; Fonseca 1949; Rosenfeld 1972; Ribeiro et al. 1984, 2001; Coelho and Gonçalves Jr. 1993; Brasil, Ministério da Saúde 1998; Bucaretchi et al. 1993, 2000, 2008; Miranda et al. 2000; Antunes and Málaque 2003; Zannin et al. 2005; Cardoso et al. 2011) (Table 2). According to the Brazilian Notifiable Diseases Information System (SINAN), *Phoneutria* spp. are the second most important cause of bites by venomous spiders in this country, with 20,132 cases being reported from 2010 to 2014 (~4,000 cases per year, corresponding to ~2 cases/100,000 inhabitants), mainly in southern (61.8%) and southeastern (32.7%) Brazil (Chippaux 2015; Ministério da Saúde 2016) (Fig. 6). As also shown in Fig. 6, most patients (~75%) sought health facilities within 3 h post-bite, probably in search of alleviation for the immediate, persistent, and intense pain at the bite site. The Brazilian states with the highest incidences of *Phoneutria* spp. bites are Santa Catarina (15 cases per 100,000 inhabitants), Paraná (10 cases per 100,000 inhabitants), and Rio Grande do Sul (3.5 cases per 100,000 inhabitants), all of them in southern Brazil (Fig. 7). However, the true incidence is probably lower than that suggested here since some cases reported as bites by *Phoneutria* spp. were probably misdiagnosed and likely to have been caused by *Ctenus* spp.

Most of the bites by *Phoneutria* spp. in Brazil occur in April (Bucaretchi et al. 2000; Cardoso et al. 2011; Brasil, Ministário da Saúde 2016), which coincides with the beginning of the mating season for *P. nigriventer* in southern and southeastern regions of the country (Bücherl 1985; Ramos et al. 1998) (Fig. 8). The majority of cases involve males bitten during the day, at home or during work activities (Bucaretchi et al. 2000; Antunes and Málaque 2003; Cardoso et al. 2011) (Fig. 9).

Table 2 Clinical studies of bites by wandering spiders (*Phoneutria* spp.) in Brazil and other Central and South American countries, showing the author(s), year and type of publication, period, description of the clinical manifestations of envenoming (number of cases), deaths, use of antivenom, and identification of the offending spiders, including species where applicable

Authors (year)	Type of publication (language; type of study)	Service (period)	New cases (deaths)	Partial clinical description	No clinical description	Use of antivenom	Offending spiders brought for identification
Brazil and Vellard (1925)	Article (P; R)	IB (1925)	3 (2)	1	0	0	2; *Ctenus nigriventer* (1), *Ctenus ferus* (1)
Brazil and Vellard (1926)	Article (P; R and CS)	IB (1925–1926)	17 (0)	0	0	16	11; *Ctenus nigriventer*
Vellard (1936)	Article (F; R and CS)	IVB (NR)	7 (1)	5	0	2	4; *Ctenus nigriventer* (2), *Ctenus ferus* (2)
Fonseca (1949)	Book chapter (P)	IB (1925–1945)	415 (1)	0	415	400	415; *Ctenus nigriventer*
Trejos et al. (1971)	Article (S; CR)	Costa Rica (1971)	3	1	0	0	1; *Phoneutria* spp., probably involved *P. boliviensis*
Simó (1983)	Article (S)	Uruguay (NR)	5	1	1	0	0
Ribeiro et al. (1984)	Abstract (P; CE)	HVB (1981)	543	543	0	49	331; *Phoneutria* spp., probably involved *P. nigriventer* and *P. keyserlingi*
Coelho and Gonçalves Jr. (1993)	Abstract (E; CE)	Santa Catarina PCC (NR)	68	68	0	0	68; *Phoneutria* spp.
Bucaretchi et al. (1993)	Abstract (P; CR)	Campinas PCC (1993)	1	0	0	1	0

(*continued*)

Table 2 (continued)

Authors (year)	Type of publication (language; type of study)	Service (period)	New cases (deaths)	Partial clinical description	No clinical description	Use of antivenom	Offending spiders brought for identification
Bucaretchi et al. (2000)	Article (E; CE)	Campinas PCC (1984–1996)	422 (1)	0	0	10	422; *Phoneutria* spp., probably involved only *P. nigriventer*
Miranda et al. (2000)	Article (P; CE)	SJRP PCC (1989–1998)	116	116	0	2	Not described. Probably involved only *P. nigriventer*
Ribeiro et al. (2001)	Abstract (P; CE)	HVB (1988–1991)	1235	1235	0	NR	1165; *P. nigriventer*
Castillo and Otero Patiño (2002)	Article (S; R)	Colombia	3	0	3	NR	NR
Zannin et al. (2005)	Abstract (P; CR)	Santa Catarina PCC (NR)	1	0	0	0	1; *P. nigriventer*, adult female
Bucaretchi et al. (2008)	Article (E; CR)	Campinas PCC (2007)	1	0	0	1	1; *P. nigriventer*, adult female
Vargas et al. (2008)	Article (S; CR)	HNCH, Peru (2005–2008)	2	2	0	0	2; *P. nigriventer*[a]
Cardoso et al. (2011)	Book chapter (P)	HVB (1989–1998)	3135	0	0	105	Not described. Probably involved *P. nigriventer* and *P. keyserlingi*
Mena-Muñoz et al. (2016)	Article (S; CR)	IHC, Peru (2015)	1	1	0	0	1; *P. nigriventer*[a]

E, English; F, French; P, Portuguese; S, Spanish. CR, case report; CS, case series; CE, Clinico-epidemiological study; R, review; HNCH, Hospital Nacional Cayetano Heredia, Lima, Peru; HVB, Hospital Vital Brazil, Instituto Butantan, São Paulo, SP, Brazil; IB, Instituto Butantan, São Paulo, SP, Brazil; IHC, Ingenio Health Center, Piura, Peru; IVB, Instituto Vital Brazil, Niterói, RJ, Brazil; PCC, Poison Control Center; SJRP, São José do Rio Preto, SP, Brazil. NR, not reported. Taxonomically, previous denominations such as *Ctenus ferus*, *C. nigriventer*, *C. medius*, and *P. fera* are considered synonyms for *P. nigriventer*

[a]*P. nigriventer* is a nonindigenous *Phoneutria* spp. in Peru; see the comments about these bites in the main text

Table 3 Clinical studies of severe (n = 10) and fatal (n = 2) envenomation caused by wandering spiders (*Phoneutria* spp.) in Brazil, showing the author(s), year of publication, identification of the offending spiders, clinical manifestations, and treatment

Authors (year)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Vellard (1936)	Bucaretchi et al. (2000)	Bucaretchi (PC) (1986)	Bucaretchi et al. (1993)	Bucaretchi et al. (2000)	Zannin et al. (2005)	Bucaretchi et al. (2008)	SC/PCC (2007), UD	Campinas/ PCC (2014), UD
Age, sex	45 years, M	22 years, F	16 years, M	31 years, F	3 years, F	11 months, F	55 years, M	9 months, M	15 years, M	52 years, M	4 years, M	75 years, M
Year of the bite	1926	1926	1926	NR	1985	1986	1993	1996	2005	2006	2007	2014
Service	IB	IB	IB	IVB	UTH/ UNICAMP	UTH/ UNICAMP	UTH/ UNICAMP	UTH/ PUCC	Local hospital	UTH/ UNICAMP	Local hospital	Local hospital
Offending spider	*Ctenus nigriventer*	*Ctenus nigriventer*	*Ctenus nigriventer*	*Ctenus ferus*	*Phoneutria* spp.	NBI	NBI	*Phoneutria* spp.	*Phoneutria nigriventer*	*Phoneutria nigriventer*	NBI	NBI
Bite site	Foot	Hand	Hand	Finger	Finger	Wrist	Neck	Not detected	Hallux	Neck	Foot	Neck
Clinical manifestations												
Local												
Pain	+	+	+	+	+	+	+	+	+	+	+	+
Edema	–	–	–	–	–	+	–	–	+	–	–	–
Erythema	–	–	–	–	–	–	–	–	+	–	–	+
Radiating pain	+	+	+	+	–	–	–	–	–	–	–	–
Fasciculation	–	–	–	–	–	–	+	–	–	–	–	–
Radiating spasms	+	–	–	–	–	–	–	–	–	–	–	–
Systemic												
Blurred vision	+	–	+	+	–	–	+	–	–	+	–	+
Grunting	–	+	–	+	–	–	–	–	–	–	–	–
Trismus	–	–	–	+	–	–	–	–	–	–	–	–
Tachycardia	+	+	+	+	+	+	+	+	+	+	+	+
Bradycardia	–	–	–	–	–	–	–	–	–	–	+	–
Arrhythmic pulse	+	+	+	+	–	–	–	–	–	–	–	+

(*continued*)

Table 3 (continued)

Authors (year)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Vellard (1936)	Bucaretchi et al. (2000)	Bucaretchi (PC) (1986)	Bucaretchi et al. (1993)	Bucaretchi et al. (2000)	Zannin et al. (2005)	Bucaretchi et al. (2008)	SC/PCC (2007), UD	Campinas/ PCC (2014), UD
Filiform pulse	+	+	–	–	–	–	–	–	–	–	–	–
Poor peripheral perfusion	–	–	–	–	+	–	+	+	–	+	+	+
Hypertension	–	–	–	–	–		+	–	+	+		+
Prostration	+	+	–	–	+	+	–	+	–	–	+	–
Agitation	–	–	–	–	+	+	–	–	+	+	–	+
Inability to stand up	+	+	–	–	–	–	–	–	–	–	–	–
Inability to walk	–	+	–	–	–	–	+	–	–	–	–	–
Hypothermia (<36 °C)	+	–	–	+	–	–	–	–	–	–	–	–
Pallor	–	–	–	–	+	+	+	–	–	–	–	–
Cyanosis	–	–	–	–	+	+	+	+	+	–	+	–
Diaphoresis	+	+	+	+	+	+	+	–	+	+	–	+
Tremors	+	–	+	+	–	+	+	–	–	+	–	–
Generalized cramps	–	+	+	–	–	–	–	–	–	–	–	–
Seizures	–	–	–	–	–	–	–	+	–	–	–	–
Hypertonia	–	–	–	–	–	+	–	–	+	–	–	–
Diarrhea	–	–	–	–	+	–	–	–	–	–	–	–
Vomiting	–	–	–	–	+	+	–	–	+	+	+	+
Rhinorrhea	+	–	–	–	–	–	–	–	–	–	–	–
Sialorrhea	+	–	–	–	–	+	–	–	+	–	–	+
Priapism	–	–	–	–	–	–	–	+	+	+	–	–
Abdominal pain	–	–	–	–	+	+	–					–
Thoracic pain	–	–	–	–	+	–	–	–	–	–	–	–

(*continued*)

Table 3 (continued)

Authors (year)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Brazil and Vellard (1926)	Vellard (1936)	Bucaretchi et al. (2000)	Bucaretchi (PC) (1986)	Bucaretchi et al. (1993)	Bucaretchi et al. (2000)	Zannin et al. (2005)	Bucaretchi et al. (2008)	SC/PCC (2007), UD	Campinas/ PCC (2014), UD
Tachypnea	–	–	–	–	+	+	–	–	+		–	–
Dyspnea	–	–	–	–	+	–	–	+	+	–	+	+
Hypotension/ shock	–	–	–	–	–	–	–	–	–	–	–	+
Pulmonary edema	–	–	–	–	+	–	–	+	+	–	+	+
Treatment												
Antivenom (mL)	15/10	5	5	5	25/15	40	25	40	No	25	No	25
Route	i.m.and s.c.	NR	NR	s.c.	i.v.	i.v	i.v	i.v	NA	i.v	NA	i.v
Anti-*Ctenus* AV	+	+	+	+	–	–	–	–	NA	–	NA	–
Aar AV	–	–	–	–	+	+	+	+	NA	+	NA	+
Hours post-bite	1/1.5	1	1.5	1	3/8	2	3	6	NA	4	NA	2.6
Early AV reactions	No	No	No	No	No	No	No	No	NA	No	NA	No
Mechanical ventilation	–	–	–	–	–	–	–	+	–	–	+	–
Electric cardioversion	–	–	–	–	–	–	–	–	–	–	–	+
Fatal envenomation	–	–	–	–	+	–	–	–	–	–	+	–

Note that publications are listed according to the year in which the bite occurred. +, present; –, absent; Aar AV, Antiarachnid antivenom; AV, antivenom; Campinas/PCC, Campinas Poison Control Center, Campinas, SP, Brazil; F, female; IB, Instituto Butantan, São Paulo, SP, Brazil; i.m., intramuscular; i.v., intravenous; IVB, Instituto Vital Brazil, Niterói, RJ, Brazil; M, male; NA, not applicable; NBI, not brought for identification; NR, not reported; PUCC, Pontifical Catholic University of Campinas, Campinas, SP, Brazil; PC, personal communication published report; SC/PCC, Santa Catarina Poison Control Center, Florianópolis, SC, Brazil; s.c., subcutaneous; UNICAMP, State University of Campinas, Campinas, SP, Brazil; UD, unpublished data; UTH, University Teaching Hospital. Taxonomically, previous denominations such as *Ctenus ferus* and *C. nigriventer* are considered synonyms of *P. nigriventer*

Table 4 Laboratory (blood samples, first or single analysis) and bedside pulse oximetry results for seven patients bitten by wandering spiders (*Phoneutria* spp.) in Brazil, classified as severe (n = 5) and fatal (n = 2) envenomation

Authors (year)	Bucaretchi et al. (2000)	Bucaretchi (1986), PC	Bucaretchi et al. (1993)	Zannin et al. (2005)	Bucaretchi et al. (2008)	Santa Catarina PCC (2007), NP	Campinas PCC (2014), NP
Age, sex	3 years, F	11 months, F	55 years, M	15 years, M	52 years, M	4 years, M	75 years, M
Year of the bite	1985	1986	1993	2005	2006	2007	2014
Offending spider	*Phoneutria* spp.	NBI	NBI	*P nigriventer*	*P. nigriventer*	NBI	NBI
Service	UTH/ UNICAMP	UTH/ UNICAMP	UTH/ UNICAMP	Local hospital	UTH/ UNICAMP	Local hospital	Local hospital
Hours post-bite	7	2	4	4	4	7	12
Bedside pulse oximetry (RV, 95–100%)	ND	ND	ND	90	96	ND	NR
Arterial gases							
pH (RV, 7.35–7.45)	7.28	7.2	7.31	ND	7.43	ND	7.22
paO_2 (RV, 83–108 mmHg)	38.8	32.7[a]	87.1	ND	73.7	ND	119
$paCO_2$ (RV, 32–45 mmHg)	27.2	51.3[a]	31.2	ND	35.6	ND	22
Bicarbonate (RV, 18–23 mEq/L)	10.7	19.5	15.9	NR	23.5	ND	9
Blood count							
WBC (RV, 4,000–10,000/ mm^3)	14,000	25,000	10,600	18,500	ND	26,360	21,400

(*continued*)

Table 4 (continued)

Authors (year)	Bucaretchi et al. (2000)	Bucaretchi (1986), PC	Bucaretchi et al. (1993)	Zannin et al. (2005)	Bucaretchi et al. (2008)	Santa Catarina PCC (2007), NP	Campinas PCC (2014), NP
Biochemical tests							
Glucose (RV 70–100 mg/dL)	226	169	87	212	163	ND	172
Lactate (RV, 4.5–19.8 mg/dL)	ND	ND	ND	ND	ND	ND	49.5
CK (RV, 39–308 U/L)	ND	ND	ND	ND	ND	ND	802
CK-MB (RV, <25 U/L)	ND	ND	ND	ND	ND	ND	90
Troponin	ND	ND	ND	ND	ND	ND	negative
Amylase (RV, 28–100 U/L)	ND	ND	ND	ND	ND	ND	207
Venom (ng/mL) (ELISA, cutoff = 17.1 ng/mL)	ND	ND	ND	ND	47.5	67.8[a]	ND
Pulmonary edema	+	–	–	+	–	+	+
Shock	–	–	–	–	–	–	+
Fatal envenomation	+	–	–	–	–	+	–

+, present; –, absent; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; ELISA, enzyme-linked immunosorbent assay; F, female; M, male; NBI, not brought for identification; ND, not determined; PC, personal communication; PCC, Poison Control Center; UNICAMP, State University of Campinas, Campinas, SP, Brazil; RV, reference value; UTH, University Teaching Hospital; WBC, white blood cells. [a]Blood sample for ELISA was collected postmortem and was probably a venous sample. Both ELISA analyses were done in the Laboratory of Toxicology at the Campinas PCC

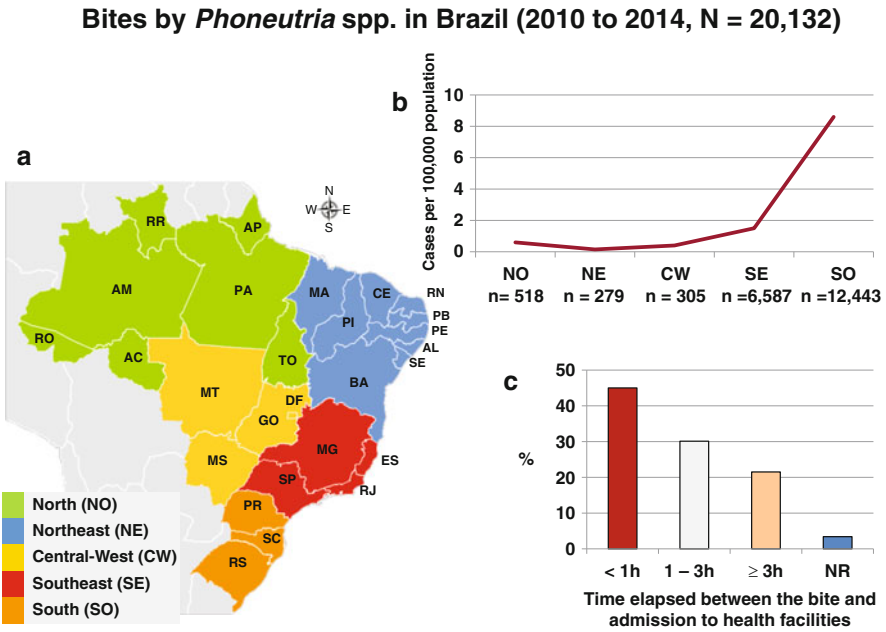

Fig. 6 Bites by *Phoneutria* spp. in Brazil (2010–2014). (**a**) Geographic regions of Brazil. (**b**) Incidence of bites by *Phoneutria* spp. in relation to geographic region (cases per 100,000 population). (**c**) Time elapsed between the bite and admission to health facilities (Based on data from the Brazilian Notifiable Diseases Information System, SINAN, Brasil, Ministério da Saúde 2016). *NR* not reported. Region abbreviations: *NO* North, *NE* Northeast, *CW* Central-West, *SE* Southeast, *SO* South. State abbreviations: *AC* Acre, *AL* Alagoas, *AM* Amazonas, *AP* Amapá, *BA* Bahia, *CE* Ceará, *DF* Distrito Federal (Federal Capital Territory), *ES* Espírito Santo, *GO* Goiás, *MA* Maranhão, *MG* Minas Gerais, *MS* Mato Grosso do Sul, *MT* Mato Grosso, *PA* Pará, *PB* Paraíba, *PE* Pernambuco, *PI* Piauí, *PR* Paraná, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *RO* Rondônia, *RR* Roraima, *RS* Rio Grande do Sul, *SC* Santa Catarina, *SE* Sergipe, *SP* São Paulo, *TO* Tocantins

According to Antunes and Málaque (2003), an analysis of 345 patients bitten by *Phoneutria* spp. admitted to the Hospital Vital Brazil at the Instituto Butantan in São Paulo City showed that most bites occurred while house cleaning (37.4%), putting on shoes (20.5%), and handling bananas or other fruits or vegetables (17%). This may explain why the hands and feet are the most commonly bitten anatomical sites (75–88%) (Bucaretchi et al. 2000; Antunes and Málaque 2003) (Fig. 9).

There is little epidemiological information for bites by *P. nigriventer* in other South American countries of this species' range. At least 150 bites by *Phoneutria* spp. in humans are reported yearly in Argentina, essentially in the northeastern region (province of Misiones), and are probably caused by *P. nigriventer* (de Roodt et al. 2011; Argentina, Ministerio de Salud 2012).

There have been few published reports of bites by Amazonian species of *Phoneutria*, and most of them have provided few clinical details. In a written communication (1982) to Dr. Vera von Eickstedt (a spider taxonomist working at the Instituto Butantan, São Paulo, SP, Brazil), a missionary working in a village of

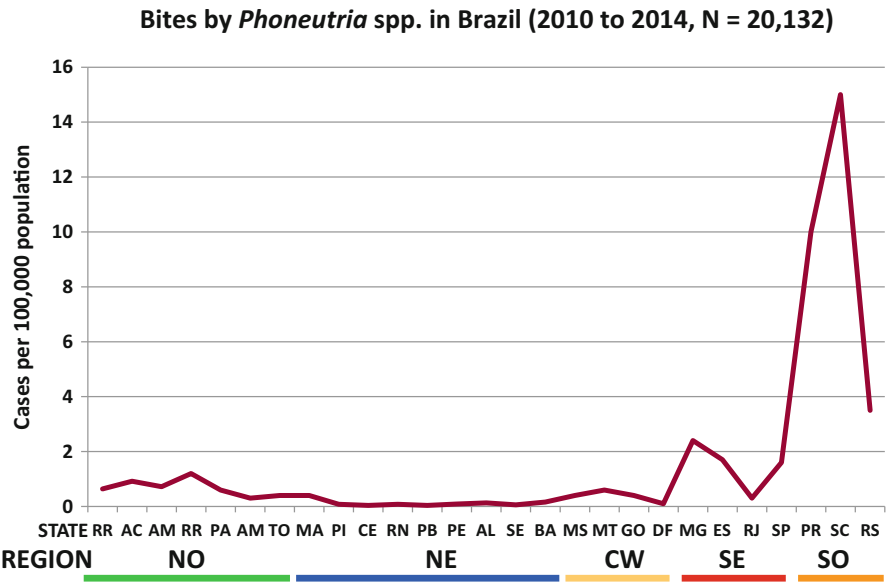

Fig. 7 Incidence of *Phoneutria* spp. spiders bites in Brazil (cases per 100,000 population) in relation to the geographical region and state (Based on data from the Brazilian Notifiable Diseases Information System, SINAN, Brasil, Ministério da Saúde 2016). Region abbreviations: *NO* North, *NE* Northeast, *CW* Central-West, *SE* Southeast, *SO* South. State abbreviations: *AC* Acre, *AL* Alagoas, *AM* Amazonas, *AP* Amapá, *BA* Bahia, *CE* Ceará, *DF* Distrito Federal (Federal Capital Territory), *ES* Espírito Santo, *GO* Goiás, *MA* Maranhão, *MG* Minas Gerais, *MS* Mato Grosso do Sul, *MT* Mato Grosso, *PA* Pará, *PB* Paraíba, *PE* Pernambuco, *PI* Piauí, *PR* Paraná, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *RO* Rondônia, *RR* Roraima, *RS* Rio Grande do Sul, *SC* Santa Catarina, *SE* Sergipe, *SP* São Paulo, *TO* Tocantins

the Amerindian tribe Xikrin located ~200 km from Marabá in the northern Brazilian state of Pará claimed that *Phoneutria reidyi* caused five bites a year, with "significant symptomatology," and provided a specimen of this species to back up this claim (von Eickstedt 1982). Trejos et al. (1971) described three bites by wandering spiders in Guápiles, a region of banana plantations in northeastern Costa Rica. One spider brought for identification was later identified as a *Phoneutria* spp. by Dr. Wolfgang Bücherl (Instituto Butantan). All three bites occurred while those bitten were handling bananas, and, in two cases, the clinical features and outcome were detailed. The first patient (65-year-old male), bitten on the hand, developed intense local and radiating pain, edema of the hand and forearm, nausea, dizziness, and diaphoresis; he was admitted to a local hospital and discharged after 3 days with no symptoms. The second case involved a 43-year-old female, also bitten on the hand. The patient showed intense, radiating local pain, local edema, sweating at the bite site, nausea, dizziness, and one episode of vomiting and tremors; there was marked improvement within 36 h post-bite. Considering the geographical region where the bites occurred, the three cases were probably caused by *P. boliviensis* (see Fig. 4l).

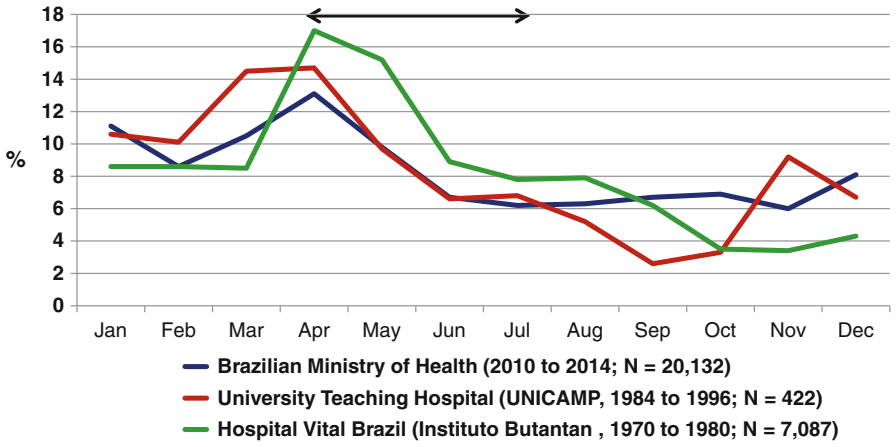

Fig. 8 Distribution of bites by *Phoneutria* spp. in humans according to the month of the year (Based on data from two referral hospitals in southeastern Brazil (Bucaretchi et al. 2000; Cardoso et al. 2011) and from the Brazilian Notifiable Diseases Information System (SINAN, Brasil, Ministério da Saúde 2016)). The *black double-headed arrow* indicates the mating season of *P. nigriventer* in the southeastern Brazilian states of São Paulo and Rio de Janeiro (Bücherl 1969, 1985; Ramos et al. 1998)

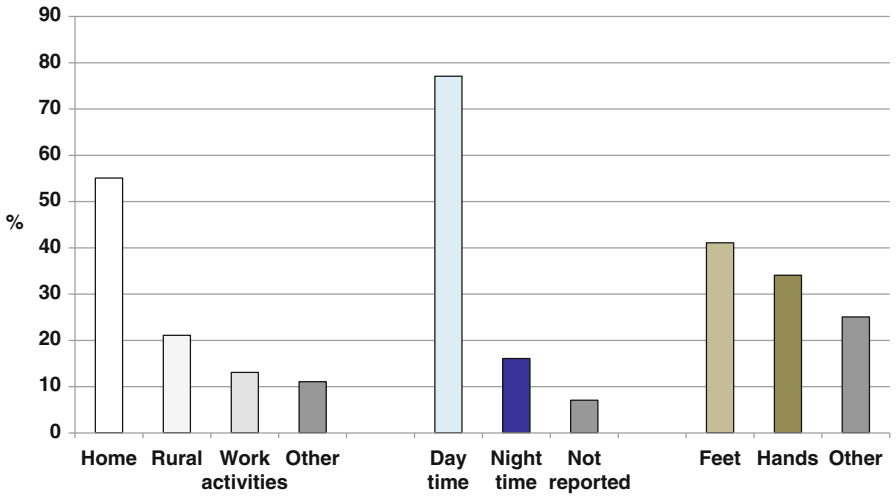

Fig. 9 Setting, period of day, and bite site for 422 bites by *Phoneutria* spp. in Campinas, SP, southeastern Brazil (Based on data from Bucaretchi et al. 2000)

Castillo and Otero Patiño (2002) mentioned three bites by *Phoneutria* spp. in the 1990s, in the state of Antioquia, northern Colombia, which they classified as mild (n = 1) and moderate (n = 2) envenomation; no further details were provided. More recently, Vargas et al. (2008) and Mena-Muñoz et al. (2016) described three

cases from two distinct geographical regions of Peru: two from Lima (central Pacific coast of Peru) and one from Piura (northern Peru). The first two patients (a 30-year-old female and 55-year-old female) had been working in a fruit market in Lima, with both being bitten on the hands while handling bananas from the region of Tingo Maria (central Peruvian jungle). Both patients developed similar clinical features, such as intense local pain and local edema, with transient mild arterial hypertension also being detected in the younger patient. Tramadol was used for pain relief in both cases (Vargas et al. 2008). The third case, from Piura, involved an 11-year-old boy bitten on the second right toe (the spider was inside his shoe) who developed intense local pain and paresthesia, mild foot edema, transient priapism, and arterial hypertension (Mena-Muñoz et al. 2016). According to the authors, in the three cases, the offending spiders were identified as *P. nigriventer*, a nonindigenous *Phoneutria* spp. in Peru. However, close examination of Fig. 1 in Vargas et al. (2008) suggests that the spider is a female *P. reidyi* rather than *P. nigriventer*. In addition, based exclusively on the remaining photographs of the spiders provided in these two reports, it is not possible to definitively identify the other two species involved. A literature search turned up no further reports of bites by *Phoneutria* spp. in Central and South American countries.

Bites by *Phoneutria* spp. generally cause only signs/symptoms of local envenomation. Life-threatening envenomation is uncommon (~0.5%), with children and elderly people representing the most vulnerable groups (Bucaretchi et al. 2000; Cardoso et al. 2011) (Fig. 10 and Table 3). Fifteen deaths attributed to *Phoneutria* spp. have been reported in Brazil since 1903. Based on the data provided in the corresponding reports, two deaths were probably caused by *Phoneutria* spp., one was very probably caused by this genus, and in only two cases was there sufficient detail to provide a causal nexus (see the section "Fatal Cases" below and Table 6). Although four additional deaths have been reported in the Brazilian Notifiable

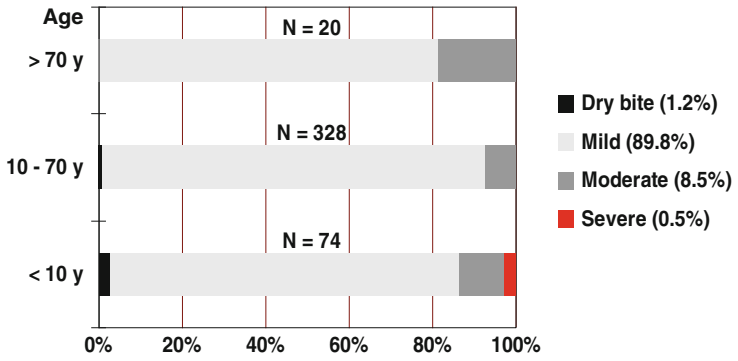

Fig. 10 Distribution of bites by *Phoneutria* spp. according to age subgroup and severity (N = 422; Based on data from Bucaretchi et al. 2000). Dry bite, no envenomation; mild, local symptoms exclusively; moderate, systemic envenomation; severe, life-threatening systemic envenomation, including one fatal case

Table 6 Reported cases of fatal envenomation caused by wandering spiders (*Phoneutria* spp.) in Brazil

Authors (service, period)	Year of publication	Year of death(s)	Number of cases	Age, sex	Case description	Offending spider	Causal nexus
Brazil and Vellard, Vellard[a]	1925, 1936	1903, NR	2	7 years M; 45 years M	Few clinical details	NBI	No
Vellard[a]	1936	NR	4	10 years M	Some clinical details for one case	NBI	Very probably (one case)
Fonseca (IB, 1925–1945)	1949	NR	1	NR	No	*Ctenus nigriventer*[b]	Not enough details
Bücherl[a]	1972	NR	2	6 months M; 18 months M	No clinical details	*P. nigriventer*	Probably, in both cases
Rosenfeld (HVB-IB, 1954–1965)	1972	1958, 1959, 1962 and 1965	4	NR	No	NR	Not enough details
Bucaretchi et al. (UTH/UNICAMP)	2000	1985	1	3 years F	Yes (pulmonary edema)	*Phoneutria* spp.	Confirmed
Santa Catarina PCC	NP	2007	1	4 years M	Yes (pulmonary edema)	NBI (positive ELISA)[c]	Confirmed
Total			15				

HVB, Hospital Vital Brazil, São Paulo, SP; IB, Instituto Butantan; IVB, Instituto Vital Brazil, Niterói, RJ; NBI, not brought for identification; NR, not reported; NP, no published report; PCC, Poison Control Center; UNICAMP, State University of Campinas, Campinas, SP; UTH, University Teaching Hospital

[a]The fatal cases were reported by the primary sources (medical doctors and others). A critical analysis of the original report in which Guimarães (1903) described the death of a 7-year-old child [cited by Brazil and Vellard (1925)] indicated that the clinical picture (local necrosis, jaundice, and dark urine, suggesting, perhaps, intravascular hemolysis) was more compatible with fatal systemic envenomation by a brown spider (*Loxosceles* spp.) or a necrotizing local infection evolving to sepsis caused by gram-negative bacteria, e.g., *Pseudomonas aeruginosa*

[b]Taxonomically, the previous denomination *Ctenus nigriventer* is considered a synonym of *P. nigriventer*

[c]Although the offending spider was not brought for identification, high levels of *P. nigriventer* venom were detected by ELISA in a blood sample collected postmortem, thus confirming the envenomation (see Table 4); this case was partially cited in the case report published by Bucaretchi et al. (2008).

Diseases Information System from 2010 to 2014 (Brasil, Ministério da Saúde 2016), unfortunately, in view of the non-obligatory nature of careful review of the clinical charts, it is impossible to confirm the causal nexus of these fatal cases.

Clinical Manifestations

Local Features

Intense local pain is the major symptom reported after most *Phoneutria* spp. bites, with the frequency ranging from 83% (Ribeiro et al. 2001; n = 1,235) to 96% (Miranda et al. 2000; n = 116; Antunes and Málaque 2003; n = 345); this pain is described as distressing or excruciating in several cases. Other local signs observed at the bite site include swelling, erythema, sweating, muscle fasciculation, presence

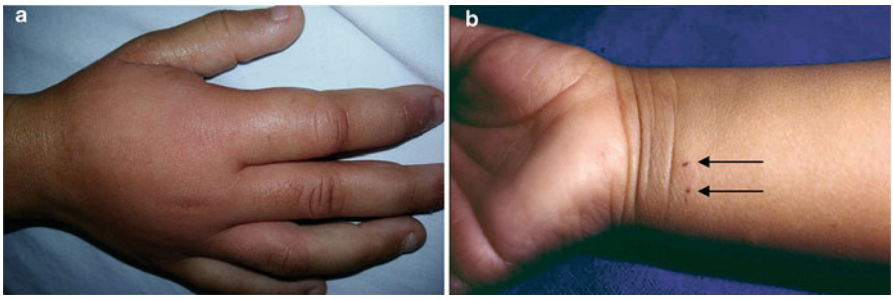

Fig. 11 Local effects observed in two children bitten by wandering spiders (*Phoneutria* spp.). (**a**) Edema of the second right finger and hand and slight erythema of the hand and fingers, in a 6-year-old girl bitten by *P. nigriventer* who was admitted to a local emergency department and monitored by the Campinas Poison Control Center in 2016. (**b**) Two fang marks (*arrows*) on the wrist of an 11-month-old girl admitted to the University Teaching Hospital at the State University of Campinas (UNICAMP), Campinas, SP, Brazil in 1986 and who developed severe envenomation (see Tables 3 and 4). The offending spider was not brought for identification, but according to the patient's mother, a large (~10 cm long) spider jumped onto the child's wrist and bit her; the spider subsequently assumed a defensive posture with outstretched anterior legs while supporting the body on the four hind legs (Photographs provided by (**a**) the doctor who attended the patient at the local emergency department (the photograph was e-mailed to the Campinas Poison Control Center) and (**b**) F. Bucaretchi©)

of fang marks, and paresthesia (Brazil and Vellard 1925, 1926; Vellard 1936; Trejos et al. 1971; Ribeiro et al. 1984, 2001; Coelho and Gonçalves Jr. 1993; Bucaretchi et al. 1993, 2000, 2008; Miranda et al. 2000; Antunes and Málaque 2003; Vargas et al. 2008; Cardoso et al. 2011; Mena-Muñoz et al. 2016). Figure 11 shows the local edema, erythema and fang marks in two children bitten by *Phoneutria* spp. Pain starts immediately after the bite, radiates to other regions associated with the bite site in ~25% of the cases, and generally lasts 24–48 h; analgesics and local infiltration with anesthetics (lidocaine) are generally required for relief (Fleury 1964; Coelho and Gonçalves Jr. 1993; Miranda et al. 2000; Bucaretchi et al. 2000; Antunes and Málaque 2003; Cardoso et al. 2011).

Figure 12 shows the frequency of local features in 422 patients bitten by *Phoneutria* spp. compared to those of 1,327 patients stung by scorpions (*Tityus serrulatus* and *Tityus bahiensis*), admitted to the same emergency department (Bucaretchi et al. 2000, 2014). There is considerable similarity in the local manifestations and their frequency in both types of envenomation. However, local edema is apparently more intense in patients bitten by *Phoneutria* spp., as are sweating and the presence of fang marks, when compared to scorpion stings.

Systemic Features

Systemic manifestations are considerably less common than local effects, occurring in 9% (Bucaretchi et al. 2000) to 27% (Ribeiro et al. 2001) of case series; the latter authors only mentioned the severity of the bites (N = 1,235 cases recorded at the Hospital Vital Brazil, Instituto Butantan, São Paulo, 1988–1991) in their abstract

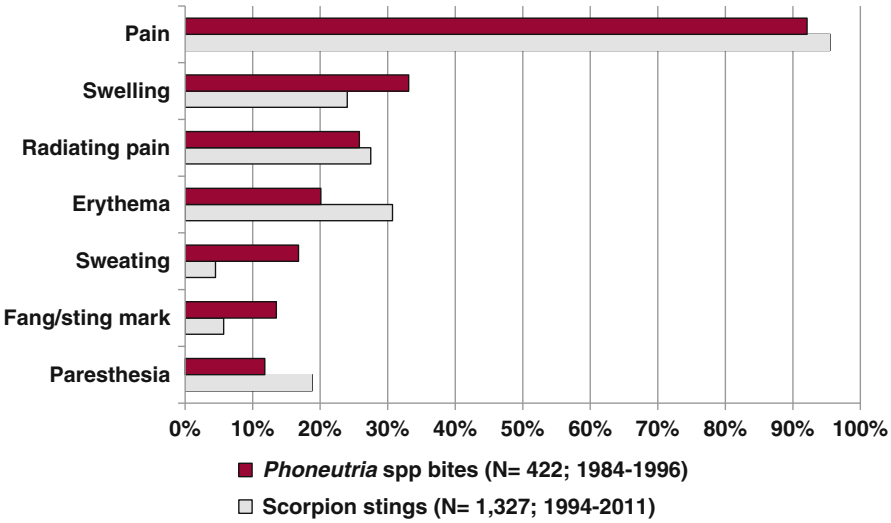

Fig. 12 Local features in 422 patients bitten by *Phoneutria* spp. compared to 1,327 patients stung by scorpions (*Tityus* spp.), admitted to the same emergency department (University Teaching Hospital at UNICAMP) and monitored by the Campinas Poison Control Center (Based on data from Bucaretchi et al. 2000, 2014)

(moderate envenomation = 25.4%, severe envenomation = 1.6%) without describing the systemic clinical features involved. The signs/symptoms described in case reports and case series include blurred vision, diaphoresis, arterial hypertension, tachycardia, bradycardia, sialorrhea, rhinorrhea, agitation, prostration, vomiting, tachypnea, bradypnea, pallor, hypothermia, abdominal pain, thoracic pain, trismus, cramps, diarrhea, and priapism (Brazil and Vellard 1925, 1926; Vellard 1936; Fonseca 1949; Coelho and Gonçalves Jr. 1993; Bucaretchi et al. 1993, 2000, 2008; Zannin et al. 2005; Cardoso et al. 2011).

In an analysis of 46 patients bitten by *Phoneutria* spp. who developed systemic manifestations and were treated at the Hospital Vital Brazil, Instituto Butantan (1989–1998), Cardoso et al. (2011) classified the cases in three age groups (<15 years old, n = 21; 15–60 years old, n = 16; >60 years old, n = 9) and noted that priapism (n = 12), vomiting (n = 6), and diaphoresis and sialorrhea (n = 5) were the most frequent signs in children <15 years old, whereas arterial hypertension (15–60 years old, n = 13; >60 years old, n = 10), diaphoresis (15–60 years old, n = 14; >60 years old, n = 4), and tremors (15–60 years old, n = 5; >60 years old, n = 2) were the most frequent signs detected in these older groups; only one patient in the 15–60-year-old group developed priapism. Table 3 summarizes the local and systemic manifestations observed in 10 severe cases and two fatal cases of envenomation.

Life-threatening manifestations include filiform pulse, poor peripheral perfusion (capillary refill time >2 s), and hypotension, indicative of shock, whereas dyspnea, crepitant rales in pulmonary bases, wheezing, or cough with frothy sputum tinged

with blood are associated with pulmonary edema. Shock and pulmonary edema are the main severe complications of envenomation by *Phoneutria* spp. (Bucaretchi et al. 2000; Zannin et al. 2005; Cardoso et al. 2011). Other very uncommon features of severe envenomation include arrhythmias with low cardiac output and seizures (Fonseca 1949; Bucaretchi et al. 2000; unpublished data from the Campinas Poison Control Center (Table 3). As indicated in Table 3, there is a report of a 75-year-old male who was bitten on the neck by a wandering spider that was not brought for identification. The patient developed pulmonary edema, atrial fibrillation with rapid ventricular rate, and hypotension/shock (BP = 80/40 mmHg) and was successfully treated at two regional hospitals with antivenom, electric cardioversion, oxygen, furosemide, and a continuous infusion of dopamine/noradrenaline.

Classification of the Severity of Envenomation

Based on the clinical manifestations, the Brazilian Ministry of Health guidelines (Brasil, Ministério da Saúde 1998, 2014) have classified envenomation by *Phoneutria* spp. as:

Mild: involves essentially local effects/responses such as pain, edema, hyperemia, radiating pain, local sweating, and/or paresthesia. The pain felt by the patient may lead to tachycardia and agitation.

Moderate: local manifestations coupled with systemic features such as hypertension, tachycardia, "blurred" vision, occasional vomiting, agitation/restlessness, and/or sweating.

Severe: in addition to the clinical features associated with moderate envenomation, severe cases also show diaphoresis, diarrhea, frequent vomiting, somnolence/lethargy, bradycardia, hypertonia, seizures, priapism, sialorrhea, cardiac arrhythmias, cyanosis, shock, and/or pulmonary edema.

However, this classification is not totally satisfactory and requires revision. For example, should a patient with excruciating pain who requires more than two local infiltrations with anesthetics and opioids and who may also require several hours of treatment in an emergency department be classified as a case of mild envenomation? Using a standardized validated scale for grading the severity of poisoning (Poisoning Severity Score – PSS), a patient with pronounced and prolonged symptoms, as in the example above, should be classified as moderate envenomation, i.e., PSS = 2 (Persson et al. 1998). Based on the clinical features observed during envenomation and the associated outcome, a modified system for classifying envenomation that includes five categories is proposed here, namely:

Dry bites: no envenomation

Local envenomation: pain, edema, hyperemia, radiating pain, local sweating, local fasciculation, and/or paresthesia

Systemic envenomation: in addition to local manifestations, hypertension, tachycardia, "blurred" vision, vomiting, agitation/restlessness, somnolence/lethargy, diaphoresis, priapism, diarrhea, hypertonia, sialorrhea, rhinorrhea, abdominal pain, thoracic pain, pallor, and/or hypothermia

Life-threatening systemic envenomation: pulmonary edema, seizures, bradycardia, or severe arrhythmias that potentially may evolve to low cardiac output, cardiac failure, and shock

Fatal: death caused directly by envenomation or secondary to venom-related complications such as respiratory failure

This proposed new classification is similar to that used for scorpionism based on an international consensus regarding the natural history of scorpion stings (Khattabi et al. 2011). Although this proposed classification might be useful for assessing outcomes and for comparing studies from different regions, it still requires rigorous validation.

Laboratory Alterations

There is very little information on the changes in clinical laboratory parameters in patients bitten by *Phoneutria* spp. Indeed, such information has been reported in only seven cases, five classified as severe envenomation and two as fatal envenomation due to pulmonary edema (Table 4). Leukocytosis (n = 6), hyperglycemia (n = 5), hypoxemia (n = 2), and metabolic acidosis (n = 4) were the main changes. These findings are compatible with increased sympathetic activity (hyperglycemia and leukocytosis), poor peripheral perfusion (metabolic acidosis and lactate increase), systemic inflammatory response (leukocytosis), and pulmonary edema (hypoxemia). In two cases, blood venom levels were quantified with an enzyme-linked immunosorbent assay (ELISA) and found to be above the cutoff in both patients, with one sample collected postmortem (Table 4).

Treatment

Antivenom Therapy

Antiarachnid antivenom.has been produced in Brazil since 1924–1925 (Instituto Butantan), with the first report of its therapeutic use dating from 1926, when it was used to treat severe envenomation in a 45-year-old male bitten by *Ctenus* (= *Phoneutria*) *nigriventer* (Brazil and Vellard 1926) (Tables 2 and 3). The chronological order of spider antivenom production by the Instituto Butantan is indicated below (based on Lucas 2015):

1924–1925: *Ctenus* antivenom and *Lycosa* antivenom produced on a small scale from 1926 onward

1958: *Lycosa* antivenom and *Ctenus* antivenom

1963: *Ctenus-Lycosa* antivenom
1964: *Ctenus* antivenom, *Lycosa* antivenom, and *Loxosceles* antivenom (produced until 1984)
1984: *Loxosceles* antivenom and *Phoneutria* antivenom

From 1925 to the 1950s, macerated desiccated venom glands of *P. nigriventer* were used for antivenom production (Brazil and Vellard 1926; Lucas 2015). In 1953, with the aim of improving the quality and amount of venom collected for antivenom production, Bücherl (1953a) developed a method to obtain pure venom from *Phoneutria* spp. that involved using two long glass pipettes joined at their ends by a thin elastic rubber tube. This device was introduced into the cage containing the spider, and, when the tube was bitten by the spider, small drops of venom were deposited in the tube lumen. Subsequently, in the same year, Bücherl (1953b) introduced the use of electrical stimulation to obtain *Phoneutria* venom, a method that is still used to milk these spiders (Lucas 2015).

Currently, Brazilian antiarachnid/antiscorpionic antivenom is obtained by immunizing horses with a pool of venoms from the spiders *P. nigriventer* (21.5%) and *Loxosceles gaucho* (brown spider; 21.5%) and the scorpion *T. serrulatus* (57%) (Barbaro et al. 2005). According to the official Brazilian manufacturer (Instituto Butantan), 1 mL of antivenom [soro antiaracnídico (*Phoneutria* e *Loxosceles*) e antiescorpiônico; Fab_2, 1 vial = 5 mL] neutralizes 1.5 minimum lethal doses (1.5 LD_{100}) of *P. nigriventer* reference venom in guinea pigs, which means that 1 mL of antiarachnid/antiscorpionic antivenom neutralizes 0.45 mg of *P. nigriventer* reference venom in guinea pigs. With the exception of *P. nigriventer*, no studies have analyzed the cross-neutralization of Brazilian antiarachnid antivenom against other non-Amazonian and Amazonian *Phoneutria* spp. This is an unusual situation given that this antiarachnid/antiscorpionic antivenom is the only one available in the world for treating patients bitten by *Phoneutria* spp. Recent experimental results have raised the possibility that Argentina may produce an Fab_2 antivenom raised against the venom of *P. nigriventer* from the province of Misiones in northeastern Argentina (de Roodt et al. 2011).

The benefit of using antiarachnid antivenom for envenoming by *Phoneutria* spp. in Brazil, as recorded in case series and case reports, has been based exclusively on personal clinical impressions (Table 3). From 1926 to the 1940s, antiarachnid antivenoms in Brazil were commonly administered subcutaneously or intramuscularly (Brazil and Vellard 1926; Vellard 1936; Fonseca 1949), whereas from 1945 to the 1980s, they were administered by two routes (subcutaneously and intravenously) simultaneously (São Paulo, Secretaria de Estado da Saúde 1982). Analysis of the early case series showed that most patients envenomed by *Phoneutria* spp. from 1926 to 1945 and monitored by the Instituto Butantan were treated with antivenom (Table 2). From 1981 onwards, there was a decrease in the use of antivenom to treat envenomation by *Phoneutria* spp., primarily because of stricter criteria for antivenom administration (antivenom indicated only for patients with important systemic manifestations) and the exclusive use of the intravenous route for injection (Tables 2 and 3). According to Cardoso et al. (2011), an important therapeutic

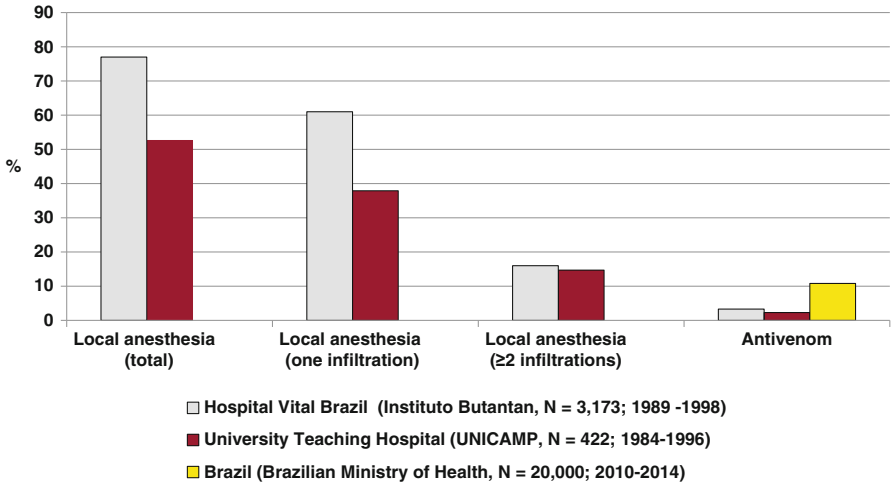

Fig. 13 Therapeutic approaches (local anesthesia, number of local anesthetic infiltrations, and antivenom administration) used in patients bitten by *Phoneutria* spp (Based on data from two referral hospitals in southeastern Brazil (Bucaretchi et al. 2000; Cardoso et al. 2011) and from the Brazilian Notifiable Diseases Information System (SINAN, Brasil, Ministério da Saúde 2016))

measure that led to a widespread reduction in the use of antiarachnid antivenom for envenomations by *Phoneutria* spp. treated at the Hospital Vital Brazil in the Instituto Butantan was the successful introduction of local anesthesia for treating the intense local pain caused by these bites. The use of local anesthetics was introduced in 1953 but was first reported by Fleury in 1964 and has been used ever since.

Since 1992, the Brazilian Ministry of Health has recommended the use of antiarachnid antivenom given intravenously and only for patients with important systemic manifestations, mainly children (Brasil, Ministério da Saúde 1992, 1998, 2014). Figure 13 shows the frequency of therapeutic approaches (local anesthesia and antivenom) used by two referral services in the state of São Paulo, southeastern Brazil (Hospital Vital Brazil, Instituto Butantan, São Paulo, SP, and the University Teaching Hospital of the State University of Campinas (UNICAMP), Campinas, SP), to treat patients bitten by *Phoneutria* spp., as well as the frequency of antivenom use reported by the Brazilian Notifiable Diseases Information System (Brasil, Ministério da Saúde 2016). These data indicate that the frequency of antiarachnid antivenom use was similar in the two referral hospitals (3.3% for Hospital Vital Brazil and 2.3% for the University Teaching Hospital at UNICAMP). However, for Brazil as a whole, the data from the Brazilian Notifiable Diseases Information System (2010–2014) indicate that the frequency of antivenom use in patients bitten by *Phoneutria* spp. is significantly greater than observed at these two hospitals (2,155/20,000 notifications, i.e., 10.8%), a finding suggestive of the wasteful use of a limited resource.

Early adverse reactions to antiarachnid antivenom have not been described in case series and case reports. Bucaretchi et al. (2000) reported no reactions in ten patients with systemic envenomation by *Phoneutria* spp. who were treated with

antivenom i.v. (eight classified as moderate and two as severe, with one fatal outcome; eight of the cases involved patients <10 years old; median of five vials/patient). In addition, none of the other eight severe cases treated with antivenom described in Table 3 showed adverse reactions to antivenom. Based on the physiopathology of envenomation by *Phoneutria* spp., it is possible that increased adrenergic activity associated with systemic envenomation may have protected the patients against adverse reactions to the antivenom, in a manner similar to that postulated for scorpion envenomation (Amaral et al. 1994).

Ancillary Measures

Immediate and continued pain at the bite site is the major complaint reported by most patients bitten by *Phoneutria* spp., sometimes described as distressing or excruciating. Therefore, effective symptomatic efforts to relieve pain are essential. As mentioned above, a new paradigm was established in Brazil in 1953 when Dr. Fleury, working at the Hospital Vital Brazil (Instituto Butantan), started to treat local intense pain with infiltrations of local anesthetic (Fleury 1964). According to two case series studies (Bucaretchi et al. 2000; Cardoso et al. 2011), local anesthesia (2% lidocaine without vasoconstrictors) was used in 52.6% and 77% of the patients bitten by *Phoneutria* spp., respectively, with ~15% requiring two or more local infiltrations (Fig. 13). Although the frequency of local pain detected in patients bitten by *Phoneutria* spp. or stung by scorpions (*T. serrulatus* or *T. bahiensis*) is similar (Fig. 12), the patients bitten by wandering spiders required greater doses of local anesthetic for effective pain relief, suggesting that this symptom is more intense after *Phoneutria* spp. bites (Bucaretchi et al. 2000, 2014) (Fig. 14). In contrast, severe

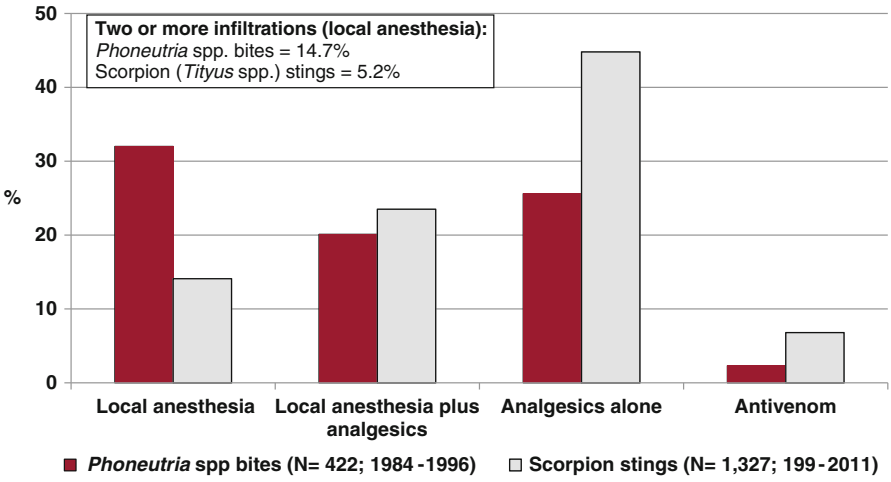

Fig. 14 Therapeutic approaches used to treat local pain in 422 patients bitten by *Phoneutria* spp. compared to 1,327 patients stung by scorpions (*Tityus* spp.) admitted to the same emergency department (University Teaching Hospital, UNICAMP) and monitored by the Campinas Poison Control Center (Based on data from Bucaretchi et al. (2000, 2014))

envenomation is more frequent after scorpion stings, with a higher frequency of antivenom use and a greater need for life-support measures.

In addition to local anesthetics, analgesics have also been used to control local pain. The most frequently used have included dipyrone for oral or intravenous use, paracetamol or paracetamol/codeine preparations, and other opioids such as morphine or tramadol; preparations of paracetamol for intravenous use are not available in Brazil (Fig. 14).

Although uncommon, supportive treatment is essential in patients with life-threatening systemic envenomation and includes the use of inotropes/vasopressors for myocardial depression and shock, mechanical ventilation in individuals with respiratory failure, and cardioversion for arrhythmias with low cardiac output (Table 3). It is worth pointing out that in two reported deaths caused by *Phoneutria* spp., antiarachnid antivenom had already been used (Fonseca 1949; Bucaretchi et al. 2000). This finding indicates that, in addition to antivenom, life-support measures may be necessary to achieve a better prognosis for uncommon life-threatening envenomation caused by *Phoneutria* spp.

In the last 5 years, the Campinas PCC has adopted an algorithm to provide a more rational approach for treating local pain in patients bitten by *Phoneutria* spp., as shown in Fig. 15. According to this algorithm, local anesthesia is indicated for patients suffering from intense local pain, i.e., score >7 on a pain rating scale of 0–10. Figure 16 shows a proposed algorithm for the treatment of patients bitten by *Phoneutria* spp. in Brazil based on the clinical manifestations (local, systemic, and life-threatening envenomation), with the doses of antivenom in agreement with the latest recommendation of the Brazilian Ministry of Health (Brasil, Ministério da Saúde 2014).

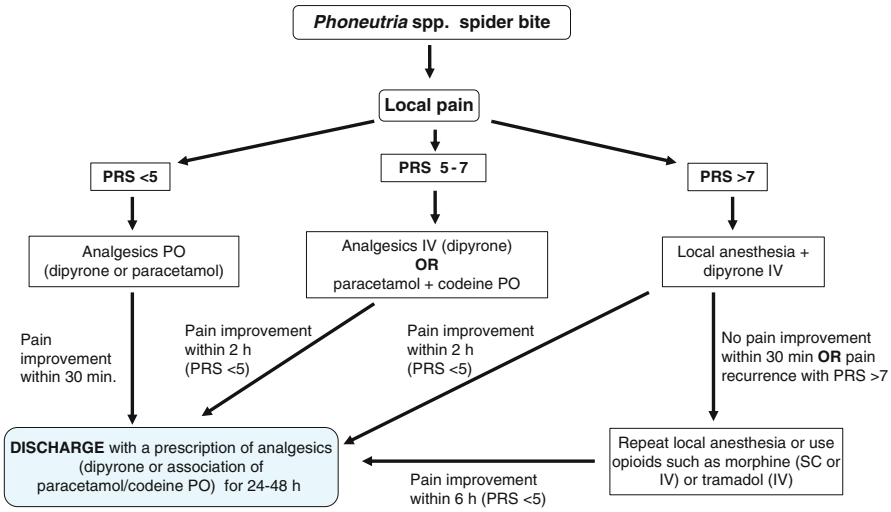

Fig. 15 Algorithm for the treatment of local pain in patients bitten by *Phoneutria* spp. as used by the Campinas Poison Control Center (Campinas, SP, Brazil). *PRS* pain rating scale of 0–10, *IV* intravenous, *SC* subcutaneous, *PO* per oral

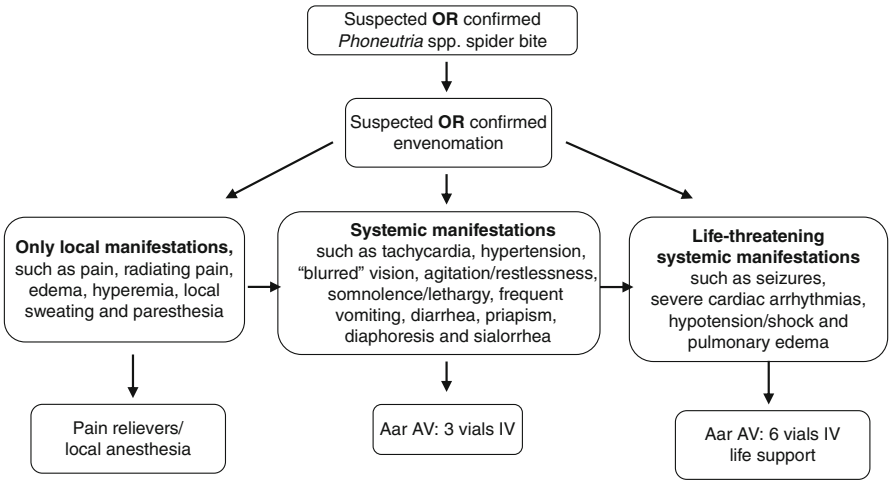

Fig. 16 Algorithm for the treatment of patients bitten by *Phoneutria* spp. in Brazil (Modified from the Brazilian Ministry of Health 2014). *Aar AV* antiarachnid antivenom (Instituto Butantan, São Paulo, Brazil; Fab_2, 1 vial = 5 mL, equine origin), *IV* intravenous

Physiopathology of Envenomation

Phoneutria spp. venoms contain proteins (e.g., hyaluronidase, phospholipase, and serine proteases) (Richardson et al. 2006; Okamoto et al. 2009; Gewehr et al. 2013), numerous peptides (ion channel activators and blockers and tachykinin-like peptides) (Pimenta et al. 2005; Richardson et al. 2006; Gomez et al. 2002; De Lima et al. 2016), and low molecular substances such as amines (histamine, serotonin) (Schenberg and Pereira-Lima 1966, 1971; Pimenta et al. 2005; Gewehr et al. 2013). As with clinical investigations, the best characterized venom is that of *P. nigriventer*, although the venoms of other species of this genus (*P. reidyi*, *P. keyserlingi*) have been studied (Richardson et al. 2006). Although *P. nigriventer* venom (PNV) has been studied for ~90 years (Brazil and Vellard 1925, 1926), relatively few of the estimated >150 components of this venom (Richardson et al. 2006) have been investigated in detail (De Lima et al. 2016). In addition, one of the challenges in studying these toxins is determining to what extent the biological activities of purified toxins observed in mammalian vertebrates, primarily rodents, are directly applicable to humans. Thus, while several toxins have been identified that, in rodents, can reproduce the clinical manifestations seen in humans, it is unclear whether these same toxins are responsible for the corresponding phenomena in human envenomation. Interspecies variations in the responses to venom have been observed in the case of skin edema induced by PNV in rabbits and rats: in rabbits this edema is essentially mediated by activation of the tissue kallikrein-kinin system (KKS), with bradykinin playing a major role in the responses

(Marangoni et al. 1993a; Antunes et al. 1993b), whereas in rats, the venom-induced edema is mediated by neuropeptides (tachykinins/neurokinins) rather than the KKS (Palframan et al. 1996; Costa et al. 1997, 2000); this divergence reflects differences in the relative importance of the tissue KKS between species (Farsky et al. 2005). Despite the limitations indicated above, studies of venom and purified toxins in animal models have provided important information on several phenomena associated with envenomation in humans, as discussed below.

Table 5 summarizes the principal toxin groups identified so far in PNV, with an indication of their primary mode of action and corresponding physiological manifestations during envenomation. Most of the toxins in the first four groups are ion channel modulators that act on Na^+ channels to affect neuronal excitability and on Ca^{2+} and K^+ channels to enhance or attenuate the release of neurotransmitters such as acetylcholine and glutamate. The most lethal toxins belong to the first three groups (PhTx1, PhTx2, and PhTx3) indicated in the table. Of these, the PhTx2 group contains the most active toxins, with the two best characterized being PnTx2-5 and PnTx2-6, both of which cause scratching, lachrymation, hypersalivation, sweating, agitation, priapism, and spastic paralysis. Toxins in the remaining groups either affect ion channels (4.0 kDa peptides) and nonvascular smooth muscle activity (PhM toxins) or show little or no toxicity in mammals (rodents) (PnTx4 toxins and 3.5 kDa peptides). Nigriventrine, a 422 Da nonprotein neurotoxin, produces convulsions and tonic-clonic crises in mice by poorly understood mechanisms, while the three types of enzymes (hyaluronidase, phospholipase A_2, and serine proteases) may contribute primarily to local effects (edema and pain) of the venom.

Pain

As indicated above, pain (sometimes excruciating) is the major local effect reported after *P. nigriventer* bites, occurring in ~90% of cases. Studies in rats suggest that hyperalgesia induced by PNV involves peripheral (tachykinin NK_1 and NK_2 and glutamate N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors), although none of these mechanisms is apparently involved in venom-induced rat hind-paw edema (Zanchet and Cury 2003). In addition, central (spinal) mechanisms involving neurokinins, excitatory amino acids, nitric oxide (NO) produced by inducible NO synthase (iNOS), pro-inflammatory cytokines (tumor necrosis factor α – TNFα – and interleukin-β1), and prostanoids have also been implicated in this pain (Zanchet et al. 2004; Costa et al. 2006). The activation of serotonin $5HT_4$ receptors in the vagus nerve may also be an important factor in venom-induced hyperalgesia (Costa et al. 2003). More recent work has confirmed the involvement of $5HT_4$ and vanilloid receptors in venom-induced pain (Gewehr et al. 2013). However, in contrast to rats, peripheral glutamate and histamine receptors apparently do not contribute to venom-induced pain in mice, whereas kinins released by tissue kallikrein-like activity of the venom and acting via bradykinin B2 receptors are involved; tetrodotoxin (TTX)-sensitive Na^+ channels,

Table 5 Principal toxin groups of *P. nigriventer* venom and their biological activities

Toxin group[a]	Isoforms	Principal target/ mode of action	Physiological manifestations during envenomation	References
PhTx1	Only PnTx1 – no isoforms (0.45% of venom)	**Voltage-gated Na^+ channels (Na_v)**: binds reversibly to outer mouth of the pore (site 1), with greatest affinity for $Na_v1.2$. Competitive blockade by μ-conotoxin GIIIB but not by tetrodotoxin (TTX). Reduces channel unitary current through partial blockade that is state dependent; no effect on voltage dependence. Human cardiac $Na_v1.5$ and arthropod Na_v channels insensitive to PnTx1	Excitation, salivation, tail elevation, spastic paralysis after i.c.v. injection in mice. No lachrymation, priapism, scratching, or contraction of guinea pig ileum. Ultrastructural alterations to axons but limited damage to neuromuscular junctions and muscle fibers (myotoxicity in 20–30% of fibers) in mouse phrenic nerve-diaphragm preparations in vitro; no change in MEPP frequency, membrane resting potential, or muscle contractility (1–5 μg/ml). Lethal (LD_{50}, 45 μg/kg and 61 μg/kg, i.c.v. for native and recombinant toxin, respectively; LD_{50} of venom in mice, 47–50 μg/kg, i.c.v.)	Diniz et al. (1990) Rezende Jr et al. (1991) Mattiello-Sverzut et al. (1998) Diniz et al. (2006) Martin-Moutot et al. (2006) Silva et al. (2012)
PhTx2	Mixture of at least nine related toxins (PnTx2-1 to PnTx2-9), the most lethal being PnTx2-1, PnTx2-5, PnTx2-6, and, to a lesser extent, PnTx2-9. The remaining toxins (PhTx2-2, PhTx2-3, PhTx2-4, PhTx2-7, and PhTx2-8) are much less active in	**Na_v channels**: delay (prolongation) of fast inactivation in Na_v by interacting with site 3 of these channels. Voltage-dependence of Na^+ conductance and inactivation shifted toward hyperpolarization (negative potentials). No effect on K^+ channels. PnTx2-6	Excitation: (hyper) salivation, lachrymation, pruritus, sweating, priapism, agitation, convulsions, spastic paralysis, respiratory distress. Slow contraction of guinea pig ileum mediated by acetylcholine and not reversed by washing. Stimulates neurotransmitter (acetylcholine,	Rezende Jr et al. (1991) Cordeiro et al. (1992) Araújo et al. (1993) Vital-Brazil and Fontana (1993) Mattiello-Sverzut and Cruz-Höfling (2000) Yonamine

(*continued*)

Table 5 (continued)

Toxin group[a]	Isoforms	Principal target/ mode of action	Physiological manifestations during envenomation	References
	rodents. PnTx2 – 3.5% of venom	shows greatest affinity for channels (six times greater than that of PnTx2-5) and is the most potent in slowing channel inactivation	glutamate) release. Membrane depolarization and increase in MEPP frequency in mouse phrenic nerve-diaphragm in vitro. Electrophysiological alterations blocked by TTX. Myonecrosis (swelling of SR, mitochondrial damage, sarcomere disorganization, myofiber hypercontraction) in skeletal muscle and damage to myelinated neurons (vacuolation, swollen mitochondria, nerve terminal vesicle depletion) in mouse phrenic nerve-diaphragm preparations in vitro. Lethality: LD_{50} for PhTx2 fraction in mice, 1.7 μg/kg, i.c.v. Lethal doses for PnTx2-1, PnTx2-5, and PnTx2-6: 1.62, 0.24, and 0.79, respectively. PnTx2-1, PnTx2-5, and PnTx2-6 cause scratching, lachrymation, hypersalivation, sweating, agitation, priapism, and spastic paralysis; PnTx2-9 causes tail erection, scratching, and reduced mobility at 1.40 μg/mouse	et al. (2004) Matavel et al. (2009) Nunes et al. (2012)

(*continued*)

Table 5 (continued)

Toxin group[a]	Isoforms	Principal target/ mode of action	Physiological manifestations during envenomation	References
PhTx3	Mixture of at least six related toxins (PnTx3-1 to PnTx3-6)	**Voltage-gated Ca^{2+} channels (Ca_V)**: blockade of L-type Ca^{2+} channels (PnTx3-2); blockade of Ca^{2+} channels: P/Q ≥ R > L > N (PnTx3-3). Blockade of glutamate release dependent on extracellular Ca^{2+} (PnTx3-3) **K^+ channels**: selective reversible blockade of transient outward (type A) K^+ channels (PnTx3-1, also known as PhKv); no effect on other K^+ channels (delayed-rectifying, inward-rectifying, large conductance Ca-sensitive, cardiac outward K^+ current – I_{to}) or T and L Ca^{2+} channels	Progressive flaccid paralysis that may last up to 24 h or more (PnTx3). For PnTx3-3 and PnTx3-4 (5 μg/mouse): flaccid paralysis and death in ≤30 min; reduction in neurotransmitter (acetylcholine, glutamate) release. Clockwise gyrations and flaccid paralysis after 6 h (PnTx3-2). PnTx3-1, PnTx3-5, and PnTx3-6: posterior limb paralysis, gradual decrease in movement and aggression. Lethality: LD_{50} of PnTx3 fraction in mice – 137 μg/kg, i.c.v. Paralysis of posterior limbs. Antiarrhythmic action in myocardial ischemia (reperfusion arrhythmias), mediated by an acetylcholine-dependent mechanism, i.e., nerve stimulation rather than a direct action on cardiac tissue. Enhances the frequency of spontaneous MEPPs (acetylcholine release) in rat phrenic nerve-diaphragm in vitro but no change in quantal size or content or in the kinetics of evoked end plate potentials	Rezende Jr et al. (1991) Cordeiro et al. (1993) Prado et al. (1996) Kushmerick et al. (1999) Almeida et al. (2011)

(*continued*)

Table 5 (continued)

Toxin group[a]	Isoforms	Principal target/ mode of action	Physiological manifestations during envenomation	References
PhTx4	Mixture of at least seven toxins (PnTx4 (1)-PnTx4(7)), with PnTx4-3, PnTx4 (5-5), and PnTx4 (6-1) being the best studied (especially the latter two). PnTx4(6-1) = 0.4% of venom	**Insect Na_v**: prolongs the Na current (delays channel inactivation) by binding to site 3 of Na_v. Prolongation of Na current results in greater depolarization and consequent opening of Ca_v, leading to exocytosis of neurotransmitter. Not active on mammalian Na_v. PnTx4(5-5) reversibly inhibits mammalian glutamate NMDA receptors; no effect on other glutamate (kainate, AMPA) or GABA receptors	Hyperactivity in insects: cramps, quivering, jerking, writhing, and trembling leading to muscle fatigue and paralysis; insecticidal. Little effect in vertebrates: PnTx4-3, PnTx4 (5-5), and PnTx4 (6-1) not toxic to mice at 30 μg i.c.v. (equivalent to 1.5 mg/kg) but inhibit glutamate uptake in rat brain synaptosomes. LD_{50} of PhTx4 fraction in mice = 480 μg/kg, i.c.v. PnTx4(6-2) ~75-fold less active in insects but causes clockwise gyrations in mice at 5 μg/ mouse	Figueiredo et al. (1995, 2001) De Lima et al. (2002) Oliveira et al. (2003)
PhM	Mixture of low molecular mass peptides, including tachykinin-like peptides	May modulate Ca^{2+} entry into nonvascular smooth muscle in a manner similar to that described for vascular smooth muscle. Interaction with specific receptors (in the case of tachykinin-like peptides)?	Nonlethal (0.1–0.3 mg injected i.c.v. showed no toxicity in mice). Contraction of guinea pig ileum in vitro	Resende Jr. et al. (1991) Pimenta et al. (2005)
Peptides	Mixture of low molecular mass peptides (3.5–4 kDa)	**3.5 kDa peptides** – similar in action to PnTx4 toxins **4.0 kDa peptides** – block L-type Ca^{2+} channels, in a manner similar to PnTx3 toxins	Lethal to insects but no effect in mice after i.c.v. injection Cause spastic paralysis and death in mice after i.c.v. injection	Richardson et al. (2006) Lúcio et al. (2008)

(*continued*)

Table 5 (continued)

Toxin group[a]	Isoforms	Principal target/ mode of action	Physiological manifestations during envenomation	References
Nigriventrine	Low molecular mass (422 Da), nonprotein neurotoxin	Stimulates c-Fos expression in various parts of the brain	Causes convulsions and tonic-clonic crises in mice when injected i.c.v. or i.v., with the latter route indicating the toxin can cross the blood-brain barrier	Gomes et al. (2011)
Proteins	Hyaluronidase, phospholipase A_2 (PLA_2), and proteases (serine proteases)	**Hyaluronidase** – facilitates venom/ toxin diffusion from site of inoculation by degrading hyaluronic acid and increasing tissue permeability **PLA_2** – may contribute to edema by stimulating formation of arachidonic acid metabolites **Proteases (serine proteases)** – may be involved in posttranslational modification of venom peptides. May also account for kallikrein-like activity of venom	The presence of these enzymes may enhance vascular permeability/edema formation and venom diffusion from the site of inoculation. Formation of kinins by kallikrein-like activity may contribute to pain. Proteolytic cleavage may contribute to the posttranslational modification and formation of additional peptides	Richardson et al. (2006) Okamoto et al. (2009) Gewehr et al. (2013)

[a]Toxin nomenclature based on De Lima et al. (2016) – see this review for nomenclatural variants of these toxins. i.c.v. – intracerebroventricular, MEPPs, miniature end plate potentials; SR, sarcoplasmic reticulum

acid-sensitive ion channels (ASIC), and transient receptor potential vanilloid 1 (TRPV1) channels (capsaicin receptor or vanilloid receptor 1) also appear to participate in the nociceptive response to venom in mice (Gewehr et al. 2013).

Prior to the introduction of local anesthetics, antivenom was widely used to control venom-induced pain. Although there has been no systematic clinical assessment of the efficacy of antivenom for treating this condition, experiments in mice indicate that pretreatment with antivenom markedly attenuates venom-induced pain (by 54%), whereas antivenom given 55 min after venom had no effect on the level of pain (Gewehr et al. 2013); the latter finding suggests that, at least in mice, once the sequence of events leading to pain is initiated, the presence of venom toxins may no longer be necessary, hence the lack of effect of antivenom. This issue has not been

addressed in clinical trials. The introduction of local anesthetics to control venom-induced pain in the 1950s (Fleury 1964) led to a subsequent marked decrease in antivenom use for this purpose. Local anesthetics function through their ability to block voltage-gated Na^+ channels activated by venom toxins, thereby preventing signal (action potential) transmission along sensory neurons. In mice, pretreatment with local anesthetic (lidocaine) markedly reduces (86%) venom-induced pain, whereas posttreatment (55 min after venom) has no effect. This finding is at variance with clinical observations in which the administration of local anesthetic at differing times after envenomation is effective in abolishing pain; this discrepancy may partly reflect interspecies differences, particularly with regard to the kinetics of circulating venom.

In addition to local anesthetics, standard analgesic treatment involving opioids (morphine, tramadol), dipyrone, and paracetamol (with or without codeine) has been used to treat venom-induced pain. In mice, dipyrone, morphine, and acetaminophen are generally more efficacious against this pain when given before venom than after, whereas for indomethacin the reverse is true (Gewehr et al. 2013). Pretreatment of rats with cyclooxygenase inhibitors (indomethacin, a nonselective inhibitor, and celecoxib – a type 2 cyclooxygenase inhibitor) also abolishes the venom-induced hyperalgesia in rats (Zanchet et al. 2004).

Although these two general approaches (local anesthetics and standard analgesics) have been used to treat pain caused by *P. nigriventer* bites, rigorous clinical studies are required to establish which treatment is best and what the ideal regimen should be.

Edema and Inflammation

Swelling, indicative of edema, is the second most common local manifestation associated with *Phoneutria* spp. bites, although it is only one-third as common as pain (Fig. 12). Although occurring in ~33% of *P. nigriventer* bites, the edema is generally mild, and its extent is frequently limited to the region bitten (e.g., finger) and, occasionally, the corresponding member (e.g., hand); extensive local and regional swelling such as seen with bites by *Bothrops* snake species is not observed in *P. nigriventer* bites. The generally mild edematogenic response is not usually a cause for clinical concern and frequently resolves without specific intervention. In agreement with the edema seen clinically, numerous experimental studies have shown that PNV causes edema in mice (Costa et al. 2006; Gewehr et al. 2013), rats (Antunes et al. 1992; Palframan et al. 1996; Costa et al. 1997, 2000, 2001), and rabbits (Antunes et al. 1992, 1993b; Marangoni et al. 1993a). Edema is also observed with venom from the related *Ctenus medius* (Okamoto et al. 2009). In rabbits, this edema is mediated essentially by activation of the tissue KKS, with bradykinin playing a major role in the responses (Marangoni et al. 1993a; Antunes et al. 1993b; Bento et al. 1995); histamine is apparently not involved in the venom-induced edema in rabbits (Antunes et al. 1992). The KKS probably also plays a role in

venom-induced edema in mice since venom-induced hyperalgesia in this species is mediated by kinins (Gewehr et al. 2013); in contrast, kinins do not contribute to venom-induced edema in rats (Palframan et al. 1996). In mice and rats, the venom-induced skin edema involves amines (Palframan et al. 1996; Costa et al. 2001) and neuropeptides (tachykinins/neurokinins) (Palframan et al. 1996; Costa et al. 1997, 2000), the latter released from sensory nerves after stimulation of prejunctional vallinoid receptors by the venom (Costa et al. 1997, 2000, 2001); in contrast, rat paw edema is reportedly not mediated by tachykinins and excitatory amino acids such as glutamate (Zanchet and Cury 2003). Part of this discrepancy may reflect differences in the doses of venom used in these studies.

Hemodynamic Alterations

Hypertension, tachycardia, bradycardia, hypotension, and shock occur to varying degrees in humans envenomed by *P. nigriventer*, as shown in Table 3, and may reflect the release of endogenous mediators such as catecholamines and acetylcholine (Bucaretchi et al. 2008). However, studies in animals have yielded divergent results regarding the role of these mediators in venom-induced hemodynamic alterations. In anesthetized rats, the intravenous injection of PNV (0.3 mg/kg) produces a biphasic response in arterial blood pressure characterized by short-lasting hypotension followed by sustained hypertension; this biphasic response is not seen with a lower dose of venom (0.1 mg/kg) that produces only transient hypotension (Costa et al. 1996). The hypotension partly involves the opening of adenosine 5′-triphosphate (ATP)-dependent K^+ channels, while the prolonged hypertension results from the direct activation of L-type Ca^{2+} channels, with no role for vasodilators such as acetylcholine, bradykinin, neuropeptides, and NO or vasoconstrictors such as angiotensin II, arachidonic acid metabolites, catecholamines, and endothelin (Costa et al. 1996). Similar hemodynamic alterations occur in rabbits, viz., only hypotension at a dose of 0.1 mg/kg, i.v. and a biphasic response (hypotension followed by hypertension) at a dose of 1 mg/kg (Estato et al. 2000). The route of venom administration influences the sensitivity to the responses observed since the intracerebroventricular (i.c.v.) injection of 0.03–0.1 mg of venom/kg reproduces the biphasic alterations observed with a venom dose of 1 mg/kg give i.v. Acetylcholine, bradykinin, angiotensin II, and excitatory amino acids are not involved in the responses to venom given i.c.v., whereas catecholamines acting via α_1-adrenergic receptors have an important role in the hypertensive phase by enhancing sympathetic outflow to the periphery (Estato et al. 2000). In addition to these general hemodynamic effects, PNV directly stimulates guinea pig isolated atria through adrenergic and cholinergic mechanisms (Vital-Brazil et al. 1988), whereas in rat isolated perfused hearts, the stimulation is essentially through adrenergic mechanisms (Costa et al. 1998). While part of this discrepancy may reflect differences in the innervation of these preparations (predominantly parasympathetic in guinea pigs compared to sympathetic in rats), it may also reflect the fact that the study in guinea pigs was done with undialyzed venom, while that in rats used dialyzed venom from

which some low molecular mass components (peptides and amines such as histamine and serotonin) were probably removed by dialysis. More recent work (Almeida et al. 2011) has identified a toxin PhKv (=PnTx3-1 of the PhTx3 group of toxins, Table 5) that attenuates reperfusion arrhythmias in a rat model of myocardial ischemia; this beneficial effect is blocked by atropine, indicating cholinergic muscarinic involvement in the protective action of PhKv. In agreement with this observation, PhKv causes bradycardia in vitro (rat isolated heart) and in vivo, with an increase in the RR, PR, and QT intervals. However, PhKv has no direct effect on cardiomyocyte action potentials or peak Ca^{2+} fluxes in cardiomyocytes in response to electrical stimulation, i.e., the toxin's effects are mediated indirectly, primarily via acetylcholine release. In this regard, PhKv has also been shown to release acetylcholine from presynaptic terminals in rat isolated phrenic nerve-diaphragm preparations (Almeida et al. 2011).

In agreement with the hemodynamic alterations indicated above, PNV directly contracts blood vessels (Antunes et al. 1993a), and peptides capable of contracting (Bento et al. 1993; Marangoni et al. 1993b) and relaxing (Weinberg et al. 2002) vascular smooth muscle have been purified from this venom. Blood vessel contraction in response to venom is dependent primarily on the entry of external calcium (Teixeira et al. 2004), and the relaxation is mediated by NO formation, with no involvement of acetylcholine, kinins, or vasodilatory arachidonic acid metabolites (Weinberg et al. 2002); the activation of ATP-dependent K^+ channels may also be involved (Costa et al. 1996).

Overall, these findings suggest that, at least in experimental animals, sympathetic and parasympathetic mechanisms have only a limited role in the hemodynamic responses to PNV. These mediators may, however, have important participation in other venom-induced manifestations such as sweating, salivation, lachrymation, diarrhea, and tremors. The extent to which adrenergic and cholinergic mechanisms are involved in the hemodynamic and cardiac responses to venom in humans is still unclear; the quantification of circulating catecholamine levels during envenomation could be useful in this regard (Bucaretchi et al. 2008).

Priapism

Priapism (long-lasting erections) caused by PNV was initially described in dogs (Schenberg and Pereira-Lima 1962, 1971), although the phenomenon also occurs with other arthropod venoms, particularly scorpions (Nunes et al. 2013). Priapism is not a consistent finding in human envenomations by *P. nigriventer*, but is most frequently observed in children with systemic envenomation (Cardoso et al. 2011). The mechanisms involved in this response include kinin formation via the tissue KKS and subsequent stimulation of NO release in rabbits (Lopes-Martins et al. 1994; Rego et al. 1996) and, in rodents, through NO formation stimulated primarily by two toxins, PhTx2-5 and PhTx2-6, that delay the fast inactivation of voltage-gated Na^+ channels (Yonamine et al. 2004; Nunes et al. 2012). Delayed inactivation of these channels results in greater depolarization that in turn leads to the opening of N-type

voltage-gated Ca^{2+} (Ca_v) channels in nitrergic nerves, with subsequent stimulation of iNOS and the release of NO [reviewed in Nunes et al. (2013) and De Lima et al. (2016)]. The action of these toxins appears to be primarily peripheral rather than via stimulation of the central nervous. The characterization of a potent erectogenic toxin from *P. nigriventer* has raised interest in the possibility of using this molecule as a starting point for the development of novel drugs for the treatment of erectile dysfunction in a variety of conditions such as aging and vascular dysfunction associated with diabetes (Silva et al. 2015; De Lima et al. 2016). The central role of NO in the venom-induced relaxation of corpus cavernosum smooth muscle is supported by an increase in NO levels detected in an adult patient bitten by *P. nigriventer* who showed systemic envenomation that included transient severe hypertension and priapism (Bucaretchi et al. 2008).

Neurological Manifestations

A variety of manifestations such as agitation, hypertonia, inability to stand up or walk due to spasticity, and prostration seen in humans are suggestive of interference with peripheral neurotransmission. In mice, the intramuscular and intraneural injection of PNV results in exacerbated electrical activity (marked increase in repetitive end plate potentials and miniature end plate potentials – MEPPs) and muscle fasciculation that lead to flaccid paralysis with subsequent recovery over the subsequent 12–14 h, although residual weakness may persist for 2–3 days; muscle membrane depolarization occurs in the acute phase of envenomation (1.5 h post-venom) but not at later stages ($\geq$3 h post-venom) (Cruz-Höfling et al. 1985). In rat isolated phrenic nerve-diaphragm preparations, PNV causes muscle membrane depolarization with no change in the duration of muscle action potentials and an increase in the rate of spontaneous acetylcholine release (= increased amplitude and frequency of MEPPs); these responses are mediated by the activation of sodium channels since they are abolished by TTX (Fontana and Vital-Brazil 1985). Subsequent investigation of the PhTx3 (=PF3) group of toxins demonstrated neuromuscular blockade through the inhibition of acetylcholine release in mouse diaphragm, without affecting muscle responses to direct stimulation, in contrast to PNV that affected the responses to both direct and indirect stimulation; PhTx3 reduced the frequency and amplitude of MEPPs and also reduced the quantal content in mouse diaphragm muscle, effects that were considered to reflect diminished entry of extracellular Ca^{2+} into the nerve terminals (Souccar et al. 1995). In contrast to these findings with the PhTx3 fraction, individual toxins from this group, e.g., PhKv (PnTx3-1), can stimulate acetylcholine release from nerve terminals in rat phrenic nerve-diaphragm preparations (Almeida et al. 2011). Together, these studies in vitro and in vivo indicate that PNV can cause reversible neuromuscular blockade that accounts for the venom-induced flaccid paralysis frequently seen in experimental animals. However, flaccid paralysis such as frequently described in experimental animals has not been observed in humans.

Central Nervous System

Numerous experimental studies in rodents have shown that PNV injected peripherally can affect the permeability of the blood-brain barrier and the expression of a variety of proteins involved in regulating barrier intactness (reviewed in Cruz-Höfling et al. 2016). However, it is unclear to what extent these alterations occur in humans, and their contribution to the clinical manifestations of envenomation is uncertain.

Other Manifestations

The cyanosis seen in some patients probably reflects a combination of respiratory disturbances, such as pulmonary edema resulting in poor oxygenation, cardiac failure/shock, and, more rarely, marked peripheral direct vasoconstriction. Vomiting and abdominal pain may indicate a stimulatory effect on the gastrointestinal tract, possibly through a combination involving the activation of autonomic pathways, the release of neuropeptides, direct activation of smooth muscle by toxins such as PhTx1 (Tx1) (Santos et al. 1999), and the low molecular mass fraction of the venom that contains tachykinin-like venom peptides (Pimenta et al. 2005). In rats, PNV delays gastric emptying partly through a catecholamine-mediated mechanism (Bucaretchi and Collares 1996). Pulmonary edema is a common feature in severe/fatal cases of envenoming (Table 3), and postmortem histopathological analysis in one fatal case revealed severe lung congestion (Fig. 17), but with no hemorrhage or inflammatory response. Toxins that have been shown to cause pulmonary edema and congestion include PnTx2-5 (Yonamine et al. 2004) and PnTx2-6 (Leite et al. 2012). The

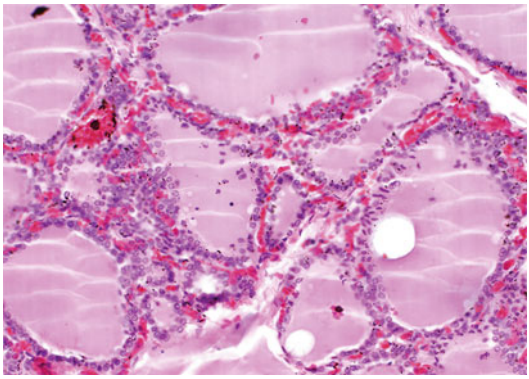

Fig. 17 Histological analysis of lung tissue in a fatal envenomation by *Phoneutria* spp. in a 3-year-old girl who developed cardiogenic pulmonary edema. Note the diffuse transudate filling the alveolar lumen (finely granular pink precipitate), capillary congestion, and lack of inflammatory cell infiltration (H&E, 100×). Death occurred 9 h post-bite. This case, which occurred in 1985, was briefly described in the case series reported by Bucaretchi et al. (2000), although no necropsy microscopic images were included in the original paper

intraperitoneal injection of PnTx2-5 in adult mice produces priapism, hypersalivation, and death by pulmonary edema and respiratory distress; these effects were abolished by pretreatment with 7-nitroindazole, a selective neuronal NOS inhibitor, indicating a possible role for NO in these responses (Yonamine et al. 2004).

Morphological Alterations

Although PNV and its toxins have been shown to cause muscle and nerve lesions in experimental animals (Cruz-Höfling et al. 1985; Love et al. 1986; Mattiello-Sverzut et al. 1998; Mattiello-Sverzut and Cruz-Höfling 2000), such damage has not been observed in human envenoming. Lethal cases in humans may be accompanied by pulmonary edema histologically (Fig. 17), but the effects in other organs have not been systematically examined. In experimental animals, PNV and its toxins induce pulmonary edema, vascular congestion, and hemorrhage (Yonamine et al. 2004; Leite et al. 2012).

Venom Kinetics

Little is known of the kinetics of PNV or its toxins in experimental animals or humans or of the relationship between circulating venom concentrations and the clinical manifestations. Circulating venom antigens have been detected by ELISA in envenomed mice (Chávez-Olórtegui et al. 2001) and humans (Chávez-Olórtegui et al. 2001; Bucaretchi et al. 2008), with concentrations in the latter ranging from 11 ng/ml to ~68 ng/ml. In mice injected subcutaneously with venom (0.25 mg/kg), the peak serum concentration was observed 15 min post-venom and remained detectable for up to 8 h post-venom (Chávez-Olórtegui et al. 2001). With regard to the tissue distribution of *P. nigriventer* toxins, Yonamine et al. (2005) used ^{125}I-labeled PnTx2-6 to examine the tissue distribution of this toxin in mice. In the circulation (blood), the toxin showed bicompartmental kinetics, with a rapid α phase and much slower β phase. Accumulation of the toxin was seen in various tissues, including the lungs (where this toxin causes pulmonary edema) and kidneys (probably indicative of renal excretion). In a subsequent study, Nunes et al. (2010) observed preferential accumulation of PnTx2-6 in the penis compared to testicles and brain after subcutaneous injection, a finding in agreement with this toxin's ability to cause priapism.

Fatal Cases

As mentioned previously (see "Epidemiology" in "Clinical Envenoming"), at least 15 deaths attributed to *Phoneutria* spp. have been reported in Brazil since 1903, with part of the available data summarized in Table 6. In several cases, clinical details are

unavailable, with the bites being reported by primary sources such as local medical doctors and others. These cases are described below in chronological order:

- Dr. J.L. Guimarães: reported the case of a 7-year-old boy, bitten on the ear by a "small spider" and who died 17 h post-bite. The case, which occurred in São Paulo City (1903), was cited by Brazil and Vellard (1925) and was described by Bücherl (1953b) as a possible fatal envenomation caused by *Phoneutria* spp. However, examination of the original detailed report (Guimarães 1903) revealed that the child developed local pain and local necrosis ("gangrene") at the bite site, evolving with fever (40.3 °C), intense prostration, jaundice, and dark urine, a clinical picture more compatible with severe local and systemic envenomation by a brown spider (*Loxosceles* spp.) evolving with intravascular hemolysis, or, perhaps, in view of the rapid evolution, a local necrotizing infection caused by gram-negative bacteria such as *Pseudomonas aeruginosa* that resulted in sepsis. The patient was treated with snake (antiophidic) antivenom antivenom (20 mL intramuscularly), the only antivenom available at the time.
- Dr. Novaes: described the case of a 45-year-old male bitten on the foot, who developed intense local pain and generalized hypertonia; death occurred ~3 h post-bite, in the city of Itanhaém, on the coast of São Paulo state; date not reported (prior to 1925; cited by Brazil and Vellard 1925).
- Dr. F. Gusmão: described the case of a 10-year-old boy bitten by a spider on the third right finger, with subsequent intense local pain, trismus, hypertonia, seizures, cyanosis, and respiratory palsy; death occurred ~30–40 min post-bite. This case may be classified as a very probable fatal envenomation caused by *Phoneutria* spp. The same doctor described three more cases cited by Vellard (1936), all of them involving males, including two slaves; no further clinical details were provided. All of the cases occurred in the city of Franca, in the state of São Paulo; no date reported (prior to 1936) (Vellard 1936).
- Fonseca (1949): described a fatal case among 415 patients bitten by *Phoneutria* spp. and followed up by the Instituto Butantan, from 1925 to 1945. According to Bücherl (1953b), this fatal case was treated with anti-*Ctenus* antivenom. No more published information is available for this case.
- Rosenfeld (1972): described four fatal cases among 3,830 patients bitten by *Phoneutria* spp. treated at the Hospital Vital Brazil, Instituto Butantan. These cases were cited in the list of fatalities among envenomed patients admitted to this hospital from 1954 to 1965. No more information has been published on these cases.
- Bücherl (1985): described receiving a bottle with alcohol containing one of the largest female *P. nigriventer* that he had ever seen, sent from a Police Station in the coastal city of São Sebastião, on the coast of São Paulo state. According to the policeman who brought the bottle, the spider was found under the bedsheet of a bed shared by two brothers (6 months old and 18 months old), with their father reporting to the local marshal that the two boys suddenly awoke during the night, crying and screaming, and died a few hours later. No more details were recorded; the date of the deaths was not reported (before 1972).

- Bucaretchi et al. (2000): described the case of a 3-year-old girl bitten by *Phoneutria* spp. on the third right finger, in the city of Araras, São Paulo state, and admitted to the University Teaching Hospital at UNICAMP in 1985, 3 h post-bite. The child developed intense local pain, agitation, diaphoresis, thoracic pain, vomiting (three episodes), tachycardia (HR = 120 bpm), and tachypnea (RR = 72 ipm) and was treated with local anesthesia and antiarachnid antivenom (Instituto Butantan; 5 vials, 25 mL). During the outcome, she developed diarrhea (5.5 h post-bite), crepitant rales in both lungs, cyanosis, and dyspnea, compatible with pulmonary edema (8 h post-bite). In view of the patient's worsening clinical condition, additional doses of antivenom (3 vials, 15 mL) were given ~8.5 h post-bite, but the patient died 9 h post-bite**.** Mechanical ventilation was not available at the time. As shown in Table 4, laboratory features revealed hypoxemia, leukocytosis, and hyperglycemia. Necropsy showed dilatation of the left cardiac ventricle and extensive pulmonary edema at microscopy (Fig. 17). The offending spider was brought for identification, with no description of the species; however, considering the geographical distribution, it was probably *P. nigriventer* (see Fig. 2).
- Santa Catarina PCC (2007): a postmortem blood sample from a 4-year-old boy was sent to the Laboratory of Toxicology of the Campinas PCC for quantification of the serum *Phoneutria* spp. venom concentration. The result was considered positive (ELISA, 67.8 ng/mL; cutoff = 17.1 ng/mL). According to the summary on the clinical chart sent by the Santa Catarina PCC, this boy was bitten on one of his feet by a spider while putting on his shoes (as witnessed by his father). The patient subsequently developed local pain and vomiting (one episode). At least 6 h post-bite, there was worsening of the clinical condition, with the child showing tachycardia, dyspnea, lung crepitant rales, and cyanosis. A chest radiography was compatible with "acute lung injury"; in sequence, the local medical staff started supportive life measures (intubation and mechanical ventilation), with an abundant frothy lung secretion being observed during intubation. Laboratory analysis revealed leukocytosis (Table 4). No antivenom was given. The child expired ~22 h post-bite. The bite occurred in Curitibanos, in the southern Brazilian state of Santa Catarina. This case was partially cited in the case report published by Bucaretchi et al. (2008), with comments on the ELISA postmortem result.

The cases described above indicate that of the 15 deaths reportedly caused by *P. nigriventer*, two deaths were probably caused by *Phoneutria* spp., another was very probably caused by this genus, and two only had sufficient details to confirm a causal nexus of fatal envenomation caused by *Phoneutria* spp. (Table 6).

Conclusions and Future Directions

The clinical findings described above indicate that patients bitten by *Phoneutria* spp. generally evolve with local manifestations, mainly pain, sometimes reported as distressing or excruciating. Severe life-threatening envenomation is very uncommon, occurring in <0.5% of the cases, mainly in children.

With regard to future directions, there is a need for a multicentric, prospective study to adequately assess the contribution of circulating venom concentrations, catecholamines, NO, and inflammatory mediators to the range and severity of clinical manifestations seen after envenoming and their relationship to other variables, such as the *Phoneutria* spp. involved, the age, sex and size of the spiders, the season of the bites, geographical distribution, and venomics. In addition, the new classification of bite severity proposed here should be validated, as should the efficacy of the doses of antivenom recently recommended by the (Brasil, Ministério da Saúde 2014) for the treatment of systemic envenomation. Finally, there is a need for detailed comparative experimental studies of the venoms from other non-Amazonian and Amazonian *Phoneutria* spp., including an assessment of their cross-reactivity and neutralization by Brazilian antiarachnid antivenom.

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North American Scorpion Envenomations

5

Anne-Michelle Ruha

Contents

Abstract

The Arizona bark scorpion, *Centruroides sculpturatus*, is the only scorpion endemic to the United States that produces a systemic envenomation in humans. Most stings do not result in serious symptoms however, and children are much more likely than adults to experience life-threatening effects. Serious envenomations are classified as Grade III and Grade IV. Findings include neuromuscular hyperactivity, with myoclonic muscle movements, fasciculations, thrashing and twisting of the torso, and restless agitation. Cranial nerve findings also occur and include opsoclonus, with dysconjugate and roving eye movements, as well as tongue fasciculations, hypersalivation, incoordination of pharyngeal muscles, and stridor. Local tissue findings at the sting site do not occur.

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Severe bark scorpion envenomation may be managed with supportive care and airway interventions as needed. Deaths occur from respiratory failure and hypoxia. An anti-*Centruroides* antivenom is available for treatment of serious bark scorpion stings. The antivenom reverses clinical toxicity within about an hour of administration. With or without antivenom treatment, most patients have full recovery from the envenomation in about 24 h.

Introduction

Scorpions are found throughout much of the world, with over 1700 species identified. A relatively small variety of species, numbering in the dozens, are found in North America, but only *Centruroides sculpturatus*, commonly known as the Arizona bark scorpion, is capable of producing envenomation in humans. *C. sculpturatus* belongs to the Buthidae family of scorpions and is one of eight *Centruroides* species that are dangerous to humans. These other *Centruroides* species are found in Mexico. In the United States, *C. sculpturatus* is found almost entirely within the state of Arizona. Its range may extend to areas that border Arizona within New Mexico, California, Utah, and Nevada, with envenomations reported in the Las Vegas area. The species range also extends to Mexico and Central America.

Several other scorpion species in the United States have been reported to produce local or allergic effects in humans, and poison centers receive calls regarding scorpion stings from across the country (Kang and Brooks 2017). Local and allergic reactions to stings are not commonly reported however, and have been best associated with stings by the common striped scorpion, *Centruroides vittatus*. One study documented development of IgE antibodies and subsequent allergic reactions in humans who had been stung by *C. vittatus* (More et al. 2004). A study of the clinical effects of stings by *Vaejovis spinigerus* and *Hadrurus hirsutus* in rats also demonstrated development of local edema (Stahnke 1938).

The majority of scorpion stings occur in the southern portion of the United States. Stings are reported year-round but are more common between March and October when the weather is warm (Kang and Brooks 2017). As expected, clinical findings associated with scorpion stings reported to poison centers outside of the southwestern United States typically describe local inflammatory effects such as edema or erythema. This differs significantly from findings reported in areas where *C. sculpturatus* is endemic (Kang and Brooks 2017).

The Arizona bark scorpion is a small yellowish-brown scorpion that is frequently distinguished from other native similar-appearing species by its ability to climb. It is often noted to be climbing up a wall or on a ceiling fixture inside residences. Its inability to climb glass surfaces is used as a preventive measure by protecting infants sleeping in cribs with the placement of glass jars around cribs legs. The bark scorpion can grow up to 7 cm. It is nocturnal and more stings are reported in the late evening and early morning, when both humans and scorpions are active (Kang and Brooks 2017).

Similar to other scorpion species, *C. sculpturatus* uses its venom to incapacitate prey and to defend itself. Two pincers located cephalid to the body are used to grasp prey, while the venomous sting is inflicted via a venom-containing telson located at the tip of a segmented tail (Isbister and Bawaskar 2014). Although scorpions generally avoid interaction with humans, *C. sculpturatus* is commonly found inside human dwellings, resulting in a large number of stings to humans. One poison center–based study revealed that 98% of stings occurred inside a residence (Kang and Brooks 2017). Over 10,000 stings are reported annually to Arizona poison centers (Kang and Brooks 2017). Many Arizonans have experienced numerous stings in their lifetime and do not consult a poison center, likely contributing to a great underestimation of the true number of stings that occur.

Venom

Biochemistry

Hundreds of biologically active components have been identified in the venom of scorpions. These include peptides which affect the function of ion channels; enzymes such as hyaluronidase, phospholipases, and metalloproteinases; peptides with antimicrobial and immunomodulatory activity; lipids; and other proteins (Osnaya-Romero et al. 2016).

The most abundant venom components are those that act at ion channels. These are divided into four groups depending on which channels are affected, and include sodium channel toxins, potassium channel toxins, chloride channel toxins, and calcium channel toxins. Ion channel toxins may act specifically in insects or in mammals (Santibanez-Lopez et al. 2015).

The toxins of primary relevance to human envenomation are those that act at sodium channels. Potassium channel toxins may also play a role in human envenomation, but this role is not as well defined as that of the sodium channel toxins (Santibanez-Lopez et al. 2015; Calderon-Aranda et al. 1999). Similarly, studies have demonstrated that *Centruroides* venom toxins are capable of producing a pro-inflammatory cytokine response in vitro and in animal models, but a clinical systemic inflammatory response has not been described in humans following envenomation (Corzo and Espino-Solis 2017).

More than 300 different sodium channel toxins have been identified in scorpion venoms. In some venoms, sodium channel toxins may comprise almost 10% of the protein content (Rendon-Anaya et al. 2012). These toxins are polypeptides weighing 6500–8500 Da and composed of 58–76 amino acid residues. They are bound by four disulfide bridges and are furthered classified as alpha or beta sodium channel toxins based on their action at the ion channel (Quintero-Hernandez et al. 2013).

Alpha sodium channel toxins were originally described in Old World scorpions found in Africa and Asia prior to being discovered in the venom of New World scorpions, including that of *C. sculpturatus* (Quintero-Hernandez et al. 2013). They

bind to receptor site 3 of the sodium channel while it is in its resting state and inhibit fast inactivation of the channel (Rendon-Anaya et al. 2012; Quintero-Hernandez et al. 2013; Campos et al. 2008). This results in prolonged depolarization of the neuron (Isbister and Bawaskar 2014).

Beta sodium channel toxins are also present in *Centruroides* venom and act at a different site on the sodium channel to enhance activation of the channel. They favor channel opening by binding to receptor site 4 and shifting the threshold of channel activation to a more negative membrane potential. This results in activation of the channel at a hyperpolarized membrane potential, up to as much as 20 mV, promoting repetitive firing of the neuron (Rendon-Anaya et al. 2012; Campos et al. 2007). The beta toxins are thought to bind to a voltage sensor in the inactivated state, prolonging the duration of inactivation at negative membrane potentials (Quintero-Hernandez et al. 2013).

Over 140 potassium channel toxins have been identified in scorpion venom. Toxins specific for potassium channels of the human ether-a-go-go (hERG) family have been identified in *C. sculpturatus* venom, but it does not appear that they contribute clinically to human envenomation (Quintero-Hernandez et al. 2013). The most concerning effect of blockade of hERG potassium channels would be prolongation of the QT interval and resultant cardiac arrhythmias. This is not known to occur following North American scorpion envenomation.

Pharmacokinetics

Numerous pharmacokinetic studies of scorpion venom in animal models have demonstrated that venom is absorbed rapidly following subcutaneous administration. Venom toxins have been detected in plasma within 2 min (Krifi et al. 2005; Devaux et al. 2004).

One study looking at the pharmacokinetics of subcutaneous administration of *Centruroides limpidus* venom in a rabbit model demonstrated a maximal blood venom concentration was achieved within 1 h, with a distribution half-life of 8 min, and a terminal half-life of 1.8 h. Most venom was eliminated in urine within 24 h. Intravenous injection of Fab2 antivenom 2 h after venom administration resulted in a redistribution of the venom from the extravascular to the vascular space. Venom concentrations increased in plasma 10 min after antivenom injection (Calderon-Aranda et al. 1999). In both animal models and humans, scorpion toxins may become undetectable after administration of appropriate antivenom (Krifi et al. 2005; Boyer et al. 2009, 2013).

Another study looked at pharmacokinetics of specific sodium channel toxins from the venom of *Androctonus australis*. When venom was administered subcutaneously to rabbits, it was rapidly distributed to tissues (Devaux et al. 2004). Plasma concentrations peaked within 30 min and then decreased rapidly, becoming undetectable by 24 h. These venom toxins were identified in urine within 2 h of administration and as the plasma concentration fell over 24 h, the urine concentration rose (Devaux et al. 2004).

Bark Scorpion Envenomation

Bark scorpion stings are extremely common in Arizona, with over 10,000 reported to the Arizona poison centers annually (Kang and Brooks 2017). This number almost certainly underrepresents the actual number of stings that occur, since many people do not consult a poison center following a sting.

The great majority of bark scorpion stings pose no threat to health and are not referred for medical treatment (Kang and Brooks 2017; Curry et al. 1983). However, even relatively minor stings are painful when they occur. Stings are felt immediately, although there may be some delay before onset of systemic symptoms when they occur. Local tissue effects do not occur following *C. sculpturatus* sting. In fact, the presence of erythema or edema following a scorpion sting in Arizona generally rules out diagnosis of bark scorpion envenomation. Occasionally, a tiny puncture mark is noted at the site of the sting but this is the exception, with the rule being no visible evidence of sting.

A grading system utilizing Grades I through IV is used in Arizona to describe the manifestations of bark scorpion envenomation along a spectrum (Curry et al. 1983). Stings that result only in pain localized to the sting site are described as Grade I. Grade I envenomations comprise most reports of stings to Arizona poison centers. In one study, 83% of patients were classified as Grade I (Curry et al. 1983). It is difficult to know how accurate this is however, since many patients with mild stings may not call the poison center, which results in underrepresentation of Grade I stings. Alternatively, patients may report a *C. scultpturatus* sting, when another species is actually responsible, overrepresenting the proportion of stings falling into the Grade I category. Pain localized to the site of the sting typically resolves quickly, almost always within 24 h.

Painful paresthesias that extend beyond the sting site, without other neurotoxic effects, characterize Grade II envenomations. These occurred in 9% of patients described in one poison center–based study (Curry et al. 1983). Tapping a distal extremity sting site can send shock-like vibratory sensations proximally up the extremity. Paresthesias and numbness typically resolve within 24 h but anecdotally may persist for up to a month. Curry et al. reported that 24% of patients with grade II envenomations experienced pain or numbness for greater than 24 h, although the patients were not followed longer to determine the duration of pain (Curry et al. 1983).

Grade III describes envenomations that result in either cranial nerve findings without presence of neuromuscular agitation or hyperactivity, or result in neuromuscular agitation or hyperactivity without cranial nerve findings. Cranial nerve findings include dysconjugate and roving eye movements (opsoclonus), tongue fasciculations, hypersalivation, dysarthria, sensation of throat swelling, and occasionally stridor. Neuromuscular agitation may be limited to subtle tremor or twitching of extremities, or may manifest as extreme myoclonic jerking with arching and twisting of the torso. Muscle fasciculations and tremor are common. Grade III envenomations are often also accompanied by diffuse paresthesias. Grade III envenomation is rarely severe, but the combined effects of painful lower

extremity paresthesias and numbness with dysconjugate gaze inhibiting vision can be debilitating while they last.

When clinical findings following bark scorpion sting include both cranial nerve findings and neuromuscular agitation, the envenomation is classified as Grade IV. There is a large spectrum of severity of illness within this Grade however (Coorg et al. 2017). A patient with a mild Grade IV envenomation may have findings limited to subtle tremors with occasional roving ocular movements (often described as nystagmus, although not true nystagmus). A patient with a severe life-threatening Grade IV envenomation often has hypersalivation, pronounced opsoclonus, tongue fasciculations, and exaggerated neuromuscular agitation with arching of the back, twisting of the torso, and myoclonic jerking motions of the extremities. Stridor and respiratory distress is present, likely due to a combination of inability to control excessive secretions, poor coordination of muscles of respiration, and loss of tongue and pharyngeal muscle control. Although pulmonary edema is occasionally reported on chest radiographs of patients with scorpion envenomation, this is not well described or confirmed, and an alternative diagnosis of aspiration may be a more likely explanation for the unusual finding (Gibly et al. 1999). Aspiration pneumonitis and rhabdomyolysis are potential complications of severe envenomation (O'Connor and Ruha 2012).

Patients with scorpion envenomation are awake and alert, although they may be in severe distress and appear to have altered mental status. They keep their eyes closed due to the distressing ocular effects of envenomation, which can give a false impression of encephalopathy.

Few patients overall with bark scorpion sting develop Grade IV findings. Such high-grade envenomation is much more likely to occur in young children than in older children or adults. In the poison center–based study by Curry et al., Grade IV findings were reported in only 3% of all cases. When looking at Grades of envenomation across different age groups, 17% of children under 5 years of age and 14% of children 6–10 years of age had Grade IV findings, while only 1% of adults were Grade IV (Curry et al. 1983).

The full spectrum and clinical course of illness is well described in a retrospective study of 88 children with Grade III or IV envenomation presenting to a pediatric tertiary care center (O'Connor and Ruha 2012). Ages ranged from 4 months to 12 years, with a mean age of 3.7 years. The mean time of onset of systemic neurotoxicity from the time of the sting was 20 min but was reported up to 130 min. Vomiting occurred in 38% of patients and was most often reported at the onset of systemic toxicity prior to presentation to a health care facility. Vomiting is typically limited to one or two episodes. Neuromuscular agitation occurred in all patients, and opsoclonus occurred in most. The next most common findings were tachycardia and hypersalivation. Hypertension occurred in half of patients, and fever in nearly a third. Respiratory distress was an important finding described in this study, occurring in a third of patients, with 24% of the study population experiencing respiratory failure necessitating endotracheal intubation and mechanical ventilation (O'Connor and Ruha 2012). Another retrospective study described 156 pediatric

patients with Grade III and IV envenomations. Respiratory distress was documented in 22% of patients (Coorg et al. 2017).

Adrenergic effects, such as tachycardia and hypertension, are characteristic features of *C. sculpturatus* envenomation. However, other cardiovascular effects are not expected or commonly described. A study looking at children in Mexico who experienced *Centruriodes* envenomation suggests that a mild degree of hypokalemia and prolongation of the QT interval on electrocardiogram may be associated with envenomation, but the clinical significance of this is unclear (Osnaya-Romero et al. 2016). This has not been described following *C. sculputuratus* envenomations in North America. However, patients with adrenergic findings from any toxin might be expected to experience hypokalemia and lengthening of QT, since potassium is driven into cells.

Adults may be at greater risk for adverse cardiovascular outcomes due to pre-existing medical conditions. Cerebrovascular or cardiovascular complications are anticipated in adult patients with risk factors due to the physiological stress of catecholamine excess that accompanies envenomation. Such effects are not common.

Adrenergic findings predominate in *C. sculpturatus* envenomation. The presence of severe agitation, tachycardia, and hypertension can appear clinically similar to methamphetamine or other sympathomimetic poisoning. In the southwestern United States, methamphetamine use and toxicity is common, and there are multiple reports of methamphetamine-poisoned children being misdiagnosed as having a scorpion envenomation (Kolecki 1998; Strommen and Shirazi 2015). Such misdiagnosis has also been reported in adults. When present, opsoclonus can best distinguish the two diagnoses, as this finding does not occur with methamphetamine poisoning.

Bark scorpion envenomation is also commonly misdiagnosed in children as seizure. For clinicians who are familiar with the appearance of scorpion envenomations, the distinction is clear. However, for clinicians who are new to the region without experience in the clinical manifestations of envenomation, seizure may be the most likely explanation for the violent "convulsions" noted in young patients who appear encephalopathic.

Death following *C. sculpturatus* envenomation is very rare. Nearly a century ago, bark scorpion envenomation was the leading cause of death resulting from a venomous bite or sting in Arizona (Stahnke 1950). Today, deaths are rare but do occur (Mowry et al. 2014). The mechanism of death is hypoxia due respiratory failure.

The duration of clinical effects in patients presenting with severe scorpion sting is variable and unpredictable. Although neurotoxic symptoms are generally reported to last under 24 h, some patients experience spontaneous resolution of Grade III and IV manifestations of envenomation in a much shorter time frame. In a retrospective pediatric study of bark scorpion envenomation, the mean length of stay in patients treated without antivenom was 28.7 h, but the range was 2–69 h (O'Connor and Ruha 2012). Despite presenting with Grade IV symptoms, some patients will have symptom resolution very quickly. Unfortunately this cannot be predicted.

Diagnosis

The diagnosis of bark scorpion envenomation is based on a history that supports the possibility of scorpion sting and identification of clinical findings consistent with envenomation. Although venom can be measured in serum and urine, there are no commercially available tests that would allow timely identification of toxins in order to affect diagnosis or treatment (Chase et al. 2009).

Scorpion sting is a diagnostic consideration throughout Arizona and surrounding regions of the southwest US where *C. sculpturatus* resides. Stings may occur indoors and outdoors, and are often reported in locations where scorpions have not previously been observed. In many cases the patient, or parent of a young child with apparent envenomation, never saw a scorpion prior to onset of symptoms. In such cases it is especially important to confirm that the onset of symptoms is consistent with a scorpion envenomation so as to not overlook other important etiologies of illness, such as infectious.

Sudden onset of crying and agitation in a previously well child is common. This may be followed a short time later by an episode of vomiting and then onset of opsoclonus, hypersalivation, and restless agitation. The child is inconsolable by the parent.

Adults are more likely to see and report a scorpion sting, however many stings occur without observation of a scorpion. Patients may awaken during the night with numbness and paresthesias in a single extremity and consider a stroke rather than a scorpion sting. They may also present with severe dysarthria due to tongue fasciculations, pharyngeal paresthesias, and loss of muscle control and appear to have experienced a stroke.

In adults or older children who are mainly experiencing paresthesias and are able to comply and assist with the exam, a "tap test" may be a useful aid in diagnosis. The examiner taps on the area where the sting is suspected to have occurred and this sends an electric shock-like vibration up the extremity. In patients who experience severe neuromuscular agitation this test is unlikely to be helpful or necessary.

In general, clinicians must keep scorpion envenomation in the differential diagnosis of patients presenting with sudden onset of neurological symptoms in regions where *C. sculpturatus* is endemic. The classic finding of opsoclonus can often "nail" the diagnosis when present.

Management

Supportive Care

Management of bark scorpion stings varies depending on the severity of envenomation. Most stings can be managed at home without referral to a health care facility. Grade I stings may be treated with over-the-counter analgesics such as acetaminophen or ibuprofen. Application of ice to the sting site has traditionally been recommended to treat pain, and there is some evidence to support its efficacy in

management of stings by other scorpion species (Aksel et al. 2015). Medications to treat allergic reactions, such as antihistamines, have not been shown to be helpful in the management of scorpion envenomation and are not recommended.

Grade II envenomations do not typically warrant presentation to a healthcare facility, however many patients do present for care as a result of diffuse painful paresthesias. These patients may also be managed with nonopioid analgesics such as acetaminophen or ibuprofen. Application of ice to the sting site may be helpful, as well as reassurance that symptoms will improve dramatically, if not resolve, within 24 h.

Grade III envenomations are not life-threatening but are likely to prompt presentation to a health care facility due to the physically distressing nature of the symptoms. Patients often have diffuse numbness and paresthesias, which can inhibit ambulation when the soles of the feet are involved. They also exhibit either cranial nerve or neuromuscular effects. When opsoclonus is present, patients report diplopia and nausea and keep the eyes closed to avoid these effects. Neuromuscular effects may be limited to mild muscle tremors or occasional myoclonic jerks, but in combination with numbness and paresthesias this also can inhibit ambulation. Supportive care management of Grade III symptoms is targeted at improving patient comfort with intravenous analgesics and benzodiazepines. Opioids are often used due to the discomfort patients experience from the combination of clinical effects. Patients typically require treatment in the emergency department or hospital until symptoms have improved enough for them to ambulate independently.

Grade IV envenomations require aggressive supportive care interventions. In addition to intravenous fluids, analgesics, and benzodiazepines, patients may require airway management. The major threat to life following a bark scorpion sting is respiratory failure resulting in hypoxia and death. Patients who present with stridor, respiratory distress, and/or inability to control their oral secretions should undergo endotracheal intubation if these findings are not quickly reversed with other less aggressive maneuvers. Some patients with severe clinical findings require large doses of benzodiazepines to control the neuromuscular hyperactivity associated with envenomation. In addition, opioids are administered to treat the painful paresthesias. These medications may increase the risk for developing respiratory failure.

All patients being treated for Grade IV envenomation should be placed on a cardiac monitor and pulse oximeter and be observed closely for signs of respiratory distress or respiratory depression. This may occur as a direct effect of venom toxicity or may be due to combined effects of venom and use of medications that affect respiratory drive. Suctioning of secretions and supplemental oxygen may be sufficient interventions for some patients but others will require endotracheal intubation. Many clinicians administer small doses of atropine to young patients with hypersalivation, but the efficacy of this treatment has not been studied. It's use has not been associated with harm (Suchard and Hilder 2001). This is in contrast to experience in other parts of the world where atropine use is contraindicated due to increased mortality.

Benzodiazepines are often considered first line in the treatment of Grade III and Grade IV scorpion envenomations. In patients with less severe neurotoxic effects,

benzodiazepines are often administered to provide relief from muscle fasciculations and myoclonic jerking movements. More commonly, benzodiazepines are given to counteract severe neuromuscular agitation and thrashing behavior in children. Gibley et al. reported use of continuous midazolam infusions in children admitted to the ICU with scorpion envenomation (Gibly et al. 1999). In another retrospective review of pediatric patients with Grades III and IV envenomations, benzodiazepines were the most commonly reported treatment, followed by opioids (O'Connor and Ruha 2012). Short-acting benzodiazepines, such as midazolam, are recommended for use in the emergency department setting. Duration of envenomation symptoms can be unpredictable and use of long-acting benzodiazepines may contribute to sedation outlasting venom effects. Short-acting medications are also ideal for use in patients who require immediate sedation while waiting for antivenom to take effect.

Opioids are recommended for the treatment of pain from severe scorpion envenomations. Verbal children and adults almost uniformly report pain following envenomation in addition to the other objectively observed venom effects. Pain likely contributes to the restlessness and inconsolability observed in very young children even in the absence of severe myoclonus. Fentanyl is an ideal opioid for use in the emergent setting not only because of its short duration of action but because it does not induce histamine release (O'Connor and Ruha 2012).

In patients who are admitted to the ICU for management of Grade IV envenomation, longer acting benzodiazepines, such as lorazepam, and opioids, such as morphine, are appropriate options. Another option for sedation of patients being mechanically ventilated is dexmedetomidine. This agent acts centrally to inhibit norepinephrine release from neurons, making it mechanistically ideal in combatting the hyperadrenergic effects of scorpion envenomation. Opioids and benzodiazepines are not indicated after 24 h from the sting event.

Antivenom

There is one antivenom approved by the United States Food and Drug Administration (FDA) for treatment of severe bark scorpion envenomation. Centruroides (Scorpion) Immune F(ab')2 (Equine) Injection (Anascorp®) (Fab2AV) is produced using the venom of four *Centruroides* species endemic to Mexico. These are *C. limpidus limpidus, C. l. tecomanus, C. noxius,* and *C. suffusus suffusus*. The venoms from these species are injected into horses, and the horse plasma then undergoes pepsin digestion to obtain the antibody fragments and remove the immunogenic Fc portion of the antibody. The product is purified and prepared as a lyophilized powder in vials (Therapeutics 2011). The antivenom has been shown to cross react with *C. sculpturatus* venom (Chase et al. 2009). A randomized, placebo-controlled, blinded trial also demonstrated its efficacy in treating *C. sculpturatus* envenomation (Boyer et al. 2009). In the trial, eight subjects received Fab2AV while seven subjects received placebo. Outcomes included clinical resolution of symptoms, mean diazepam doses, and detection of venom in plasma. All

subjects who received Fab2AV were asymptomatic at 4 h and had no detectable venom measured at 1 h. Six out of seven subjects in the placebo group continued to have symptoms at 4 h with detectable venom at 1 h. At discharge, the mean diazepam dose was significantly different between groups, with the Fab2AV group receiving 0.07 mg/kg versus 4.61 mg/kg in the placebo group (Boyer et al. 2009).

According to the FDA prescribing information, Centruroides (Scorpion) Immune F(ab')2 (Equine) Injection is indicated for the treatment of patients with clinically important signs of scorpion envenomation (Therapeutics 2011). This includes Grade III and Grade IV envenomations; however, the clinical importance of less severe Grade III envenomations can be argued and many clinicians would not consider a mild Grade III envenomation an indication for antivenom.

Anascorp® is provided as a sterile lyophilized powder in vials requiring reconstitution in 5 mL of normal saline at the time of use. The initial dose is three vials. Each vial is reconstituted and then combined and further diluted to a total volume of 50 mL. The total dose is infused intravenously over 10 min and the patient is observed for an additional 30–60 min for resolution of symptoms. If clinically important symptoms persist, another vial is administered and the patient is observed over the next 30–60 min (Therapeutics 2011). Additional single vials are administered until symptoms resolve; however, the clinical trials of Anascorp® performed in Arizona limited treatment dose to five vials and a postmarketing study demonstrated that patients rarely require the full five-vial dose (Boyer et al. 2013; Coorg et al. 2017).

Dosing of FabAV is controversial in Arizona. Since the initial FDA approval of the antivenom, the cost per vial charged to hospitals has been over $3000 (US), and hospitals charge patients significantly more per vial (Lutkin 2012). This has led some clinicians to use FabAV sparingly, providing a smaller initial dose than is recommended in the FDA prescribing information. One study retrospectively compared patients who had received sequential single vial doses of FabAV to patients treated as per the FDA-approved dosing recommendations, with an initial three-vial dose. It was found that the two groups were different at baseline. A larger proportion of patients received the alternative dosing strategy, however the group receiving the initial three-vial dose appeared to have a greater severity of illness at baseline. Those receiving an initial three-vial dose were younger and a greater proportion were documented to have respiratory distress. Despite this, none of the patients in the initial three-vial dose group were intubated, developed aspiration, or were hospitalized. In the sequential-vial dosing group, 8.5% of patients were hospitalized despite receiving FabAV, 2.4% were intubated, and 2.4% aspirated. These outcomes were not significantly different between groups, possibly due to the small number of patients in the initial three-vial dosing group (16 as compared to 82 in the alternative dosing group) (Coorg et al. 2017).

Antivenom should be not be given by the intramuscular route. Despite a report of apparent success in a 25-day-old patient administered IM Anascorp®, a pharmacokinetic study of Fab2AV given IM to human volunteers demonstrated slow release from muscle with a median time to peak plasma concentration of 45 h (Hiller et al. 2010; Vazquez et al. 2010). There is a case report of administration of Fab2AV by the

intraosseous route in a 16-month-old child with apparent effect (Hiller et al. 2010). Although not studied, this case report provides some evidence to support administration via an intraosseous line in an emergency situation.

All antivenom therapies have the potential to stimulate hypersensitivity reactions in humans. Anascorp® has been shown to be exceptionally safe in this regard compared to most antivenoms (Boyer et al. 2013). Results of multiple clinical trials comprising 1534 total patients who received Anascorp® reveal that only 0.2% of patients developed acute hypersensitivity reactions, while 0.5% developed late serum-sickness reactions. The acute reactions included urticarial rash and labored breathing, while the late reactions included rashes, myalgias, arthralgias, and fever (Boyer et al. 2013).

Conclusion and Future Directions

Envenomation by the bark scorpion, *Centruroides sculpturatus*, is a common event in the southwestern United States, but is only rarely life-threatening. Management should be targeted at protecting the patient's airway, since all deaths occur secondary to respiratory failure and hypoxia. Care may be entirely supportive or may involve administration of a $F(ab')_2$ antivenom derived from horses immunized with four *Centruroides* species endemic to Mexico. Antivenom reverses symptoms promptly and usually allows the patient to be discharged from the hospital, whereas supportive care for serious envenomations typically involves admission to an intensive care unit.

Future directions for management of *C. sculputuratus* envenomation may be aimed at development of an inexpensive and safe antivenom product that may be given to all patients experiencing pain as well as systemic toxicity due to envenomation.

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North American Coral Snake Envenomation

6

Mehruba Anwar and Jeffrey N. Bernstein

Contents

Abstract

North American coral snakes belong to the *elapidae* family. Medically significant North American coral snakes include *M. fulvius fulvius* (Eastern coral snake) and *M. fulvius tener* (Texas coral snake). They are famously known for their brightly banded black, red, and yellow pattern. Their bites are rare due to their docile nature and natural habitat. They may leave fang marks but their absence does not eliminate the possibility of envenomation. Neurotoxicity is the main feature of coral snake envenomations. It is mediated via blockade of presynaptic acetylcholine release as well as postsynaptic end-plate receptors. The major components of their venom are phospholipase A2 and 3 finger toxins. Symptoms of envenomation usually occur within 2 h and may involve slurred speech, cranial nerve palsies, bulbar paralysis, respiratory paralysis, and full flaccid paralysis. Prehospital treatment should focus on providing high-quality symptomatic and

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supportive care to the victim, focusing on airway, breathing, and circulation. Definitive treatment is North American Coral Snake Antivenin (NACSA) and should, optimally, be provided before the onset of symptoms. The dose is usually 3–5 vials. There is a small risk of allergic, anaphylactic, or anaphylactoid reactions. There have been no deaths reported when treatment is sought or sequelae of envenomation after recovery.

Introduction

North American coral snakes include the species *Micrurus* and *Micruroides.* They belong to the *elapidae* family which also includes cobras, mambas, and sea snakes. Although there are many subspecies of each, only *M. fulvius fulvius* (Eastern coral snake) and *M. fulvius tener* (Texas coral snake) envenomations are clinically relevant. Reported cases of envenomation by *M. fulvius tener* are rare. *Micruroides euryxanthus* (Arizona coral snake) is a relatively small. It is unable to deliver venom in sufficient quantities to cause clinically relevant envenomation due to its size (Roze 1996).

M. fulvius, is on average 18–39 inches, but has been known to grow up to 4 feet in length. They have a rounded head, flat snout, rounded black eyes, and smooth scales. They have a pair of fixed fangs in the front of the mouth. They do not have heat-sensing pits. The most well-known feature of *M. fulvius* is the brightly banded black, red, and yellow pattern, which gave rise to the mnemonic "red on yellow, kill a fellow; red on black, venom lack/friend of Jack." *M. fulvius* may be misidentified for nonvenomous *Lampropeltis triangulum elapsoides* (Scarlet kingsnakes) or *Cemophora coccinea* (scarlet snakes) due to a similar banded pattern. However, those snakes have red bands that are adjacent to black bands. *M. fulvius* is found in the southern Coastal Plain from North Carolina to Louisiana but is most prevalent in Florida (Jackson and Franz 1981). They most often reside in wooded, wet, and grassy habitats but sometimes encroach upon urban and suburban areas.

Epidemiology

Coral snakes are, in fact, docile snakes with an undeserved reputation of aggression. Most coral snake bites are provoked in some way. They are far less common than pit viper envenomations and account for less than 1% of venomous snakebites in North America. The first report of a coral snake bite was documented in 1883 (True 1883). In the last 10 years, the number of reported coral snake bites has remained steady with a range of 73–99 reported cases to poison centers with no clear trend (American Association of Poison Control Centers). In 2015, there were 77 reports of coral snake envenomations to poison centers. Of these, a majority had mild or moderate

outcomes and were treated at healthcare facilities with no reported deaths (Mowry et al. 2015). Of these, 42 occurred in Florida (Querybuilder). In 2009, the first case of death from coral snake envenomation in 40 years was reported in a man who did not seek medical care (Norris et al. 2009).

Pathophysiology

There is still much to be elucidated about *Micrurus* venom. The main feature of coral snake envenomation is neurotoxicity. However, studies have also shown varying degrees of cardiotoxicity, hematotoxicity, myotoxicity, and dermatotoxicity. Neurotoxic effects are mediated via blockade of presynaptic acetylcholine release as well as postsynaptic end-plate receptors by alpha neurotoxins resulting in a curare-like effect (Tanaka et al. 2010).

A recent sequencing of the venom gland transcriptome showed that 86% of the genome transcribes for the neurotoxic components Phospholipase A2 (PLA2) and 3 Finger Toxins (3FTx) (Margres et al. 2013). PLA2 is mainly responsible for both presynaptic and postsynaptic neurotoxicity. It also causes some myotoxicity, cardiotoxicity, and hematoxicity (Vergara et al. 2014). 3FTx postsynaptically inhibits acetylcholine at the nicotinic receptor or the neuromuscular end-plate. Other toxin classes identified within coral snake venom were long-chain neurotoxins, which are similar to 3FTx and have a similar mechanism of neurotoxicity and metalloproteinases which cause local and systemic hemorrhage in pit viper venom. The function of metalloproteinases in coral snake venom is unclear. Kunitz-type inhibitors were found to a lesser extent. They inhibit serine proteases, calcium, and potassium ion channels (Margres et al. 2013). Many other toxic components displaying enzymatic activity were also found within coral snake venom such as hyaluronidase, phosphodiesterase, natriuretic peptide, nucleotidase, nerve growth factor, vascular endothelial growth factor, leucine aminopeptidase, L-amino acid dehydrogenase, acetylcholinesterase, alkaline phosphomonoesterase, and L-amino acid oxidase (Tanaka et al. 2010; Margres et al. 2013). Other venom components include lipids, carbohydrates, riboflavin, zinc, calcium, magnesium, and potassium.

Coral snakes have a relatively simple venom delivery apparatus when compared to pit vipers. Their paired venom glands connect to fixed maxillary teeth or fangs via ducts. It also has a fairly inefficient system where the coral snake has to chew on its victim to deliver venom and may produce a velcro-like effect when the fangs are being pried off. Approximately 60% of coral snake bites are nonenvenoming. The average amount of venom available varies from species to species and is proportionate to the length of the snake (Auerbach 2012). A large coral snake can produce 20 mg of dried venom, which can be up to four or five lethal doses for a human adult (Fix 1980). The severity of a coral snake bite is related to the volume of venom injected and the size of the victim (Auerbach 2012).

The pharmacokinetics of *Micrurus* toxin is not well described. The average median lethal dose for an 18 g mouse is reported to be 9 μg. However, there is a

considerable difference in the LD50 between mammals and even among different species of mice and rats, therefore extrapolation to humans is not possible (Bolanos et al. 1978; SnakeDatabase). The venom is potent, and said to surpass that of the Mojave Rattlesnake (Auerbach 2012).

Diagnosis and Clinical Presentation

Coral snakes have been handled by the victim in most cases of envenomation, sometimes in an attempt to identify the snake. Misidentification of *Lampropeltis triangulum elapsoides* (Scarlet kingsnake) and *Cemophora coccinea* (scarlet snake) has also resulted in envenomation. Occasionally the value of a patient's herpetologic identification skills are in doubt, either because of intoxication, impaired visual acuity, or cover of darkness, and can only report that they have been bitten by an unknown or brightly colored snake. Pit vipers are known to strike swiftly from a small distance, if this history detail is given, the snake bite was most likely, not caused by a coral snake (Auerbach 2012).

Bites most often occur on the upper extremities. Fang marks are the most commonly reported clinical findings; however, lack of any local findings does not rule out a possible envenomation and should not lead healthcare personnel to underestimate the possibility of a true envenomation (Kitchens and Van Mierop 1987; Norris and Dart 1989). Fang marks can appear like abrasions or scratches and not typical fang marks associated with pit viper envenomation. Soft tissue swelling only occurs in approximately one-third of patients. Pain and paresthesia and muscle fasciculations at the site of a bite is commonly reported (Auerbach 2012; Kitchens and Van Mierop 1987). Injecting saline or lidocaine under bites does not necessarily express venom from wound or reveal breaks in the skin. Nausea and vomiting are also commonly reported symptoms, occurring in 25% of coral snake envenomation (Kitchens and Van Mierop 1987).

Symptom onset usually occurs within 2 h but have been delayed for up to 18 h (Fix 1980; Peterson 2006). Slurred speech and cranial nerve involvement might be the first neurological manifestation with bulbar paralysis and less commonly, full flaccid paralysis (Auerbach 2012). In one case, the victim's symptoms progressively worsened over 48 h and required ventilation. One patient described hearing "a million bees in [her] ears and pain that started in [her] throat and shot out of [her] vagina." She did not complain of difficulty swallowing but on exam had signs of inability to tolerate oral secretions. She remained intubated for 3 weeks. Aspiration pneumonia is a possible secondary complication. There are no long-term sequelae, neurological or otherwise, reported after coral snake envenomation. Even with severe envenomations requiring intubation, mentation is usually preserved and patients can usually communicate via writing (Kitchens and Van Mierop 1987).

For late presenters, a watch and wait approach with antivenom at the bedside has been advocated if no symptoms appear within 8 h. This should be done only with very reliable histories and with the patient's full understanding of the risks and ability to consent. Most patients who present to the hospital are asymptomatic.

Asymptomatic patients, if adequately treated with antivenom before the onset of symptoms, do not go on to develop toxicity. Therefore, a rapid risk-benefit assessment must be done with the help of a toxicologist to determine use of antivenom. Laboratory tests and imaging are usually not useful in the diagnosis of coral snake envenomations.

Treatment

For prehospital treatment, in general, tourniquet use is not recommended. However, a lymphatic compression technique that may be beneficial has been described in Australia. In an animal model, the localizing circumferential compression (LoCC) device delayed the onset of systemic toxicity and increased survival time after artificial truncal envenomation by *M. fulvius*. This was achieved by delaying lymphatic spread of venom (Hack et al. 2011). This is only recommended for a prolonged prehospital course and when there may be a delay to definitive treatment. Other treatments lack evidential support in a possible coral snake envenomation include ice/heat, electrical current, incision and drainage, fasciotomy, and/or suction. Immobilizing the affected limb and keeping at the level of the heart may delay venom entry into central circulation. Extraction devices (such as the Sawyer device) should not be used and have no proven clinical benefit for use with coral snake envenomations as possibly with crotaline envenomations (Alberts et al. 2004). Emergency Medical Services should apply standards of care and address supportive care as required. Prehospital times in Florida tend to be low and definitive care may not be far.

In-hospital treatment is based on whether the patient is symptomatic or asymptomatic. Asymptomatic patients with a good history of possible coral snake envenomation should be treated with North American Coral Snake Antivenin (NACSA). Because of the recent shortage of NACSA, there has been a wait and see approach advocated by some Poison Control Centers; however, this approach cannot be applied across the board as the risk of severe symptoms may not outweigh the benefits of withholding antivenin (Wood et al. 2013). It is not recommended to wait for end-organ toxicity as symptoms cannot be attenuated or reversed once they start. Theoretically and anecdotally, it may be possible to halt further progression of symptoms with antivenin. Asymptomatic patients who received five vials of coral snake antivenom have not been described to display subsequent signs of envenomation. There is no utility in prophylactically treating patients with corticosteroids or antibiotics. Tetanus vaccines/boosters and standard wound care methods should be applied. Patients should be observed for at least 24 h in an ICU setting (Peterson 2006; Wood et al. 2013).

For those patients who are already symptomatic, ABCs should be addressed first with high-quality symptomatic and supportive care given. Consideration should be given to early elective intubation (Kitchens and Van Mierop 1987). The average duration of ventilatory support is 2–3 weeks. There is a case in which respiratory support that was required for 6 weeks, but this was a definite outlier. Pain should be

treated with opioid pain medications as necessary. Isolated hypotension usually does not occur but has been described in victims who are already systemically ill from the envenomation. However, if the victim is hypotensive, standard pressors should be used. Patients who have severe envenomation and require respiratory support and/or vasopressors usually also require physical or occupational therapy. Patients should only be discharged after they have an asymptomatic period of 24 h (Corbett and Clark 2017). Neostigmine was used to improve symptoms in a single case report in Brazil of a patient with suspected *Micrurus* envenomation (Coelho et al. 1992). No experience with neostigmine is documented for treatment of North American coral snake envenomation, although it has been used for other elapids whose venoms exert postsynaptic neurotoxic effects on the neuromuscular junction (Gold 1996). The irreversibility of symptoms through the destruction of neurons by a presynaptic toxin would suggest that neostigmine may be at best a temporizing measure.

NACSA was developed in 1967 and is available for coral snake envenomations. It is an equine derived whole IgG to *M. fulvius* venom. All patients receiving antivenin should be asked about prior sensitization, as patients may have sensitization to horses or horse derived products. Pretreatment with corticosteroids or antihistamines is usually not necessary but a skin antigen test that is included with the antivenin packet could be utilized. Ten percent of patient may have false negative results. A positive test is still not a true contraindication in the case of an envenomation as the benefits of antivenin administration and avoidance of severe symptoms may outweigh the potential risk of an allergic reaction. However, possible adverse effects should be discussed carefully with the patient and use of concurrent antihistamines, corticosteroids, and/or epinephrine should be considered. The use of the skin antigen test and concurrent prophylactic treatments are at the discretion of the treating clinician (Corbett and Clark 2017).

(Wyeth Antivenin Micrurus Fulvius® is no longer in production as of 2003 but is periodically tested for stability by the Food and Drug Administration (FDA) and extended as needed. One lot (#L67530) is approved by the FDA for use. It was recently extended for use until Jan 31, 2018. This can be ordered from Pfizer by calling 1-800-666-7248 (Gold 1996). Prior to antivenom development, the mortality rate from coral snake envenomations was 10% (Corbett and Clark 2017). The usual dose is 3–5 vials given 4–8 h within time of the bite. A repeat course of 3–5 vials can be given, however, the indications for repeat treatment are left to the discretion of the practitioner. Up to 10 vials have been given (North American coral snake Antivenin). Each vial of antivenin neutralizes approximately 2 mg of venom (Kitchens and Van Mierop 1987).

Antivenom should be administered in an age/weight appropriate volume of fluid that can be delivered over an hour to an hour and a half. Prior to the onset of symptoms, the drip may be started slowly, giving the first 1–2 mL over 3–5 min while constantly observing for type I or immediate hypersensitivity reactions. If there is no adverse reaction, give a dose of the fluid with the antivenin that is appropriate for the age and weight of the victim. There is no specification as to what fluid should be used as a diluent (Toogood 1987). The Miami Poison Control Center protocol is to place five vials of antivenom in a weight-appropriate volume of diluent (250–500 mL

of either D5W or normal saline is appropriate for adults). An infusion is begun at a rate of about 3 mL/h. The rate may be doubled every 2 min as tolerated. The initial goal of therapy should be the administration of 3–5 vials over 1–1.5 h.

In one study, 18% of those who received antivenom had an adverse effect. However, most effects were minor and only 2% developed hypotension and 1% developed angioedema (Corbett and Clark 2017). The development of delayed serum sickness is a rare possibility with any antivenom administration. The risk of serum sickness is related to the number of vials of antivenom given (i.e., total foreign protein load). Serum sickness typically presents as a flu-like illness with arthralgias and rash, and responds well to antihistamines and a course of prednisone. Fear of serum sickness should not be used as an argument to avoid the use of antivenom. The concurrent use of beta blockers in victims has produced an exaggerated anaphylactic reaction (U.S. Food and Drug Administration). If an anaphylactoid, allergic or anaphylactic reaction develops, stop the infusion, and treat accordingly with H1/H2 blockers, corticosteroids, and epinephrine. Re-evaluate risk–benefit ratio for antivenom administration and possibly restart the drip at or below last tolerated rate. An epinephrine drip may be run concurrently. More dilute solutions may be less allergenic.

Coralmyn®, a coral snake polyclonal F(ab')2 antivenom is produced by the Mexican pharmaceutical company Instituto Bioclon. It has not undergone review by the FDA and has not been adapted for use in the USA. However, Coralmyn® may be effective in the neutralization of clinically important coral snake venom in the USA (Sánchez et al. 2008). Australian coral snake antivenom may also provide some level of neutralization of US coral snakes (Ramos et al. 2017).

Special Considerations

Children

Coral snake bites in children are rare. One case series identified 11 possible cases of coral snake envenomation in children. Children may be more at risk from envenomation since less venom is required to produce effects. The recommended treatment is the same as in adults since antivenom contains immunoglobulins, which have an affinity for venom. The amount of venom to be bound by antivenom, not the weight of the patient, is the determining factor for the effective dose of antivenom. However, reducing the total fluid volume may be reasonable to avoid fluid overload. All suspected coral snake bites in children should be admitted to the ICU (Sasaki et al. 2014).

Pregnant Women

There is extremely limited data on coral snake envenomation. There are no published case reports of coral snake bites in pregnant women. However, it may be possible to

extrapolate management techniques from reports of other elapids in pregnant women. There is a case report of successful antivenom use after a *Naja naja atra* bites in a 24 week pregnant woman (Lin et al. 2011). A literature review of snakebites during pregnancy showed a mortality rate of approximately 4% in envenomated pregnant women, and a fetal loss rate of approximately 20%. One study did not show that the administration of antivenom was an independent risk factor for adverse fetal outcome and was more related to the severity of envenomation in the mother (Langley 2010). Antivenom does not have a category with respect to its use in pregnant women. As the outcome in the infant relies so heavily on the health of the mother, pregnant women who have a good history of possible envenomation should be treated with antivenom (Corbett and Clark 2017; Seifert et al. 2009).

Snake Venom Sensitization

In rare cases, the victim of a coral snake bite may have prior sensitization to venom. This may be the case in herpetologists, snake handlers, or laboratory researchers who work with venom. One study shows an occurrence of anaphylaxis to snake venom in approximately 10% of cases (Isbister et al. 2012). In cases where there may be a suspicion for possible allergic or anaphylactic reaction to coral snake venom itself but antivenom is required for a strong suspicion of possible envenomation, antivenom should still be used, albeit, with precautions and concurrent use of an epinephrine infusion.

Conclusion

The North American Coral Snake, comprised of the genus *Micrurus* and *Micruroides*, is the only elapid indigenous to the United States. It is recognizable by its familiar red, yellow, and black pattern. While envenomation is relatively rare, victims may present with severe neurologic toxicity that includes respiratory paralysis. The presynaptic nature of the venom mandates that treatment with antivenom should precede the onset of symptoms. Antivenom shortages over the past decade have resulted in delay of treatment with neurologic involvement and the need for prolonged hospitalization.

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Part II

Clinical Toxinology in Australia, Europe, and Americas: Envenomation in Australia

Australian Snakebite and Treatment

7

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Contents

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Abstract

Life-supporting treatment may be required after envenomation by species of Australian snakes from terrestrial genera: *Pseudonaja* (Brown snakes), *Notechis* (Tiger snakes), *Oxyuranus* (Taipans), *Acanthophis* (Death Adders), *Pseudechis* (Black snakes), *Austrelaps* (Copperhead snakes), *Hoplocephalus* and by the species *Tropidechis carinatus* (Rough-scaled snake), *Paroplocephalus atriceps* (Lake Cronin snake), and most of the genera of sea snakes including *Hydrophis*, *Aipysurus*, *Laticauda*, and *Microcephalophis*. Envenomation causes paralysis, procoagulant coagulopathy or anticoagulant coagulopathy (both causing hemorrhage), and rhabdomyolysis with renal failure. Procoagulant coagulopathy may also cause acute cardiovascular collapse and microangiopathic hemolytic anemia. Lesser known species cause nonlife-threatening illness. Early administration of antivenom can neutralize toxins and halt but not reverse procoagulopathy and establish tissue damage such as destroyed nerve terminals and rhabdomyolysis, which mandate time and supportive medical therapy. The recommended antivenom dose for an envenomated snakebite victim is two vials but less or more may be required, preceded by low-dose subcutaneous adrenaline to prevent allergic reactions. The few toxins identified and purified include: presynaptic phospholipase A_2 neurotoxins taipoxin (Taipan), notexin (Tiger snake), textilotoxin (Brown snake); serine proteinase prothrombin activators in Taipan, Tiger snake, Brown snake, Rough-scaled snake, and Stephen's Banded snake venoms; blockers of cyclic nucleotide-gated ion channels (pseudechetoxin, pseudecin) in Black snake venom and a plasmin inhibitor in Brown snake venom.

Introduction

Australia is habitat to a large number of species of venomous terrestrial and marine snakes (Family Elapidae). The terrestrial elapid genera responsible for the majority of serious illness are Brown Snakes (*Pseudonaja*), Tiger Snakes (*Notechis*), Taipans (*Oxyuranus*), Black Snakes (*Pseudechis*), Death Adders (*Acanthophis*) and Copperhead snakes (*Austrelaps*), and several species including the Rough-scaled snake (*Tropidechis carinatus*) and Stephen's Banded snake (*Hoplocephalus stephensi*). The genera comprise several or many species. Most human deaths are due to Brown

and Tiger snake envenomation. Of numerous sea snakes, the most prominent is *Schistosa zwefeli* (formerly *Enhydrina shistosa*), the Beaked sea-snake.

Venoms contain numerous toxins, many of which remain unidentified. Toxins include phospholipase A_2 presynaptic neurotoxins, postsynaptic neurotoxins, serine proteinase prothrombin activators (causing procoagulant coagulopathy), and anticoagulants. This chapter discusses the known toxins, their effects and relates them to management of the envenomated victim. The mainstays of clinical management are antivenom and supportive care.

Snakebite

Epidemiology

The incidence of snakebite in Australia is not reliably known but the death rate is considerably less than in other countries despite the fact that the majority of Australia's snakes are extremely venomous. The low mortality is probably related to the infrequency of snakebite, an efficient healthcare system, and the effectiveness of antivenom treatment.

Approximately 3000 snakebites occur annually in Australia. The mean snake bite death rate in Australia over the last decade was 2.3/annum (Australian Bureau of Statistics, Welton et al. 2017), which yields an approximate death rate of 1/1000 bites. This mortality is a very small contribution to the annual World snakebite burden estimated by the World Health Organisation at 5 million bites with 2.5 million envenomations, at least 100,000 deaths (1 death/50 bites) and three times that the number of limb amputations or severe disability (http://www.who.int/mediacentre/factsheets/fs337/en/). In Europe where snakes are less venomous the death rate is 0.5/1000 bites (Chippaux 2012) but in Papua New Guinea where the majority of species are the same or are closely related to Australian snakes, the mortality is 120/1000 bites (McGain et al. 2004).

Death after snakebite in Australia usually occurs because of cardiac arrest out-of-hospital, snake bite in remote locations, envenomation unresponsive to treatment, or delayed or inadequate antivenom therapy. Approximately 500 victims per annum require antivenom treatment. Death and critical illness are due to sudden cardiovascular collapse (especially with Brown snake envenomation), progressive paralysis leading to respiratory failure, hemorrhage, and renal failure. Renal failure occurs as a consequence of rhabdomyolysis, microangiopathic haemolytic anemia, hypotension, hypoxemia, and to their combinations.

Snake bite is often "accidental" when a snake is trodden upon or suddenly disturbed. However, many bites occur when humans deliberately interfere with snakes or handle them. At special risk are herpetologists and snake collectors who sustain bites in the course of their work or hobby, but who also develop an allergy to venoms and to the antivenoms used in their treatment (Isbister and Brown 2012). Bites by exotic snakes have the additional problems of scarcity of antivenoms and a lack of expertise in treating envenomation.

Australian Snake Venoms

Overview

Venoms are complex mixtures of protein and polypeptide toxins, which immobilize and kill the snake's prey. Venoms contain hundreds of toxins which lead to death of prey and to effects on humans. Many toxins are phospholipase A_2 enzymes (PLA_2s), which have multiple effects including myotoxic, hemorraghic, hemolytic, hypotensive, platelet aggregating, convulsant, edema-forming, cardiotoxic, and pre- and postsynaptic neurotoxicity (Kini 2005). The procoagulant activity of venoms is caused by prothrombin activators which are similar in structure and function to activated human coagulation factor X (Xa). Other significant toxins in several genera include natriuretic peptides, which may cause natriuresis, diuresis, and vasorelaxation (Fry et al. 2005). In addition, the venoms of the *Pseudechis* genus contain toxins (pseudechetoxin, pseudecin), which interfere with membranes by binding to cyclic nucleotide-gated (CNG) ion channels (Brown et al. 2003). However, pseudechetoxin-like toxins are not confined to the *Pseudechis* genus and are also present in many other Australian snake venoms and target CNG ion channels, modulating the membrane potential and inhibiting the flow of membrane current in many tissues including brain, heart, and kidney.

The toxins, which threaten human life, cause paralysis, coagulopathy, and rhabdomyolysis. Paralysis is due presynaptic and postsynaptic neurotoxins (Table 1). Coagulopathy is due to either the procoagulant effect of prothrombin activators (factor Xa-like enzymes), with consumption of clotting factors or to a direct

Table 1 Main components of Australian snake venoms

Neurotoxins
Presynaptic and postsynaptic neuromuscular blockers present in all dangerous venomous snakes. Cause paralysis
Postsynaptic blockers readily reversed by antivenom
Presynaptic blockers are more difficult or impossible to reverse, particularly if treatment is delayed
Some presynaptic blockers are also rhabdomyolysins
Prothrombin activators
Present in most species
Cause procoagulant coagulopathy and possibly thrombotic microangiopathy with hemolysis and possible renal failure
Significant risk of spontaneous hemorrhage
Intrinsic fibrin(ogen)lysis generates fibrin(ogen) degradation products
Anticoagulants
Present in a relatively small number of dangerous species
Prevent blood clotting without consumption of clotting factors
Risk of hemorrhage
Rhabdomyolysins
Some presynaptic neurotoxins also cause lysis of skeletal and cardiac muscle
Apart from loss muscle of mass, may cause myoglobinuria and renal failure

anticoagulant effect. Coagulopathy exposes the victim to spontaneous hemorrhage while procoagulopathy also exposes the victim to the consequences of thromboembolism. When circulating venom has been neutralized by antivenom, it may be 4–6 h or longer before hepatic manufacture of clotting factors can restore normal coagulation. In general, toxins act rapidly but the possibility of local or lymphatic sequestration and delayed action exists.

Toxins in Australian Snake Venoms

Of the probable thousands of toxins to be found in snake venoms, only a relative few have been identified and characterized. Only a few percent of worldwide research into snake venoms concerns Australian snake venoms (Mirtschin et al. 2017). This article draws attention to the principal toxins and to the recent discoveries, which are directly relevant to the clinical management of an envenomated victim. Throughout, the names of snakes used by investigators are conserved even though they may have been subsequently revised. Additional detailed commentary on toxins discovered prior to 2000 may be found in *Australian Animal Toxins* (Sutherland and Tibballs 2001).

Research into Australian elapid venoms has mainly focused on the eight elapidae genera of greatest clinical significance: *Acanthophis*, *Austrelaps*, *Hoplocephalus*, *Notechis*, *Oxyuranus*, *Pseudechis*, *Pseudonaja*, and *Tropidechis* but recent studies have identified important toxins in many lesser species. The life-threatening toxins are phospholipases which disrupt neuromuscular function and coagulation. Neurotoxic toxins are divided into presynaptic phospholipases which often also have myotoxic effects and postsynaptic toxins. The latter are antagonists of the nicotinic receptor on the skeletal muscle and are rapidly acting. Depending on their sequence, postsynaptic toxins are subdivided into short- and long-chain toxins. These toxins display different binding kinetics and different affinity for subtypes of nicotinic receptors (Hodgson and Wickramaratna 2002).

Presynaptic Neurotoxic Phospholipases

The csecreted phospholipases of elapid (and viperid) venoms are a family of relatively low molecular weight extracellular phospholipases A_2 ($sPLA_2$) that require Ca^{++} for catalytic enzyme activity (Murakami et al. 2011). Those in the venom of elapid and viperid snakes, Group II $sPLA_2$s, inhibit neurotransmission at neuromuscular junctions in vertebrate skeletal muscle by attacking the motor neuron (presynaptic, beta-PLA_2). They have masses 13–19 kDa containing 5–8 disulfide bridges which confer a specific three-dimensional crystalline structure. They exist as monomers or complexes of 2–5 highly homologous subunits (oligomers) of which at least one is the active lipase.

Examples in Australian snake venoms are taipoxin (*Oxyuranus scutellatus*), notexin (*Notechis scutatus*), and textilotoxin (*Pseudonaja textilis*). The existence of oligomers greatly increases binding of the catalytic component to membrane receptors (Montecucco and Rossetto 2008). Although all neuronal tissue is vulnerable to attack by phospholipases, it is the events at the neuromuscular junctions which have been subject to most research using preparations of nerve muscle. The end clinical result of

PLA_2 action is flaccid paralysis but experimentally the actions are complex with a characteristic three-phase response (Sribar et al. 2014). When a toxin is added the initial response is a transient inhibition of muscle twitch which lasts several minutes. The second phase is a period of 10–20 min of increased muscle twitch and is related to indirect increased release of acetylcholine. In phase 3, the muscle twitching declines progressively and ceases at 1–2 h according to individual toxins but the resting membrane potential of the muscle is intact and it can still respond to neurotransmitter stimulation. These observations imply that the clinical onset of neurotoxicity is relatively slow – compared to coagulopathy which is quite rapid.

Inside the nerve terminal, numerous changes can be observed by electron microscopy: synaptic vesicles become depleted; Ω-shaped invaginations appear in the presynaptic membrane; large vesicles form in the cytosol; mitochondria are damaged; and the synaptic boutons detach from the postsynaptic membrane. These structures then degenerate and the neuronal cytoskeleton fragments. The final fragmentation of the nerve terminal is preceded by an influx of external Ca^{++} upon a change in conductance of certain plasma membrane calcium channels and increase in plasma permeability of storage sites of intracellular Ca^{++}. A common feature of the snake venom phospholipases is catalysis of hydrolysis of the ester bond at the sn-2 position of 1,2-diacyl-sn-3-phosphoglycerides, which are contained in anionic phospholipids: phosphatidylserine, phosphatidic acid, or phosphorylated phosphatidylinositols in the plasma membrane and in the cytosolic leaflets of subcellular organelles. Not all of the toxicity of the phospholipases is due to their lytic activity on the cell membrane or inside the cell. Apart from destruction of these phospholipids, the toxins must have, as yet ill-defined, receptors on the membranes. Several binding sites have been advanced of which one on rat brain synaptic membranes is a receptor for the beta subunit of taipoxin (*vide infra*) but its nature is unknown. The mechanism by which the toxins cross the plasma membrane to the internal cytoplasm is unknown. Once in the cytoplasm, toxins bind to cytosolic proteins including calmodulin and proteins from the lumen of the endoplasmic reticulum including crocalbin, a taipoxin-associated Ca^{++}-binding protein and protein disulfide isomerase, and a mitochondrial protein. Internalization of the toxins into nerve cells appears to be important for full manifestation of neurotoxicity.

Events inside the cell are speculative but the following (simplified) events have been proposed by Sribar et al. (2014) to explain observations and discoveries: (1) toxins bind to receptors on the axolemma of presysnaptic neuromuscular junction; (2) toxins hydrolyze phospholipids of synaptic vesicles associated with the axolemma causing their exocytosis and release of acetylcholine and change in conductance of certain Ca^{++} channels; (3) toxin rapidly internalizes into the nerve ending probably by multiple processes, one of which may include the recycling of synaptic vesicles; (4) toxin is translocated to the cytosol and to mitochondria; (5) toxin interacts with cytosolic proteins (calmodulin, 14-3-3p). Calmodulin stabilizes the toxin and increases its enzyme activity while 14-3-3p presents toxins to sites on the plasma membrane where synaptic vesicles endocytose; (6) by hydrolyzing the plasma membrane from the cytosolic side, the toxins prevent the function of amphiphysin, a protein which senses the curvature of membranes and required for

the formation and release (endocytosis) of synaptic vesicles from the presynaptic membrane; (7) toxins bind to receptors on mitochondria and induce opening of permeability transition pores. Mitochondrial oxygenation uncouples and production of ATP terminates. Subsequently, the mitochondria degenerates; (8) Ca^{++} homeostasis is lost due to extended phospholipolysis and consequently increased permeability of the cellular membranes. Calcium enters and triggers additional intracellular phospholipase and proteinase (calpains) activities which include dissipation of the F-actin cytoskeleton and extensive degeneration of the nerve ending.

Procoagulants

Many snake venoms worldwide contain prothrombinase complexes consisting of a serine proteinase factor Xa and cofactors Va, Ca^{++}, and phospholipids. These prothrombin activators are classified into four groups according to the requirement for cofactors Va, Ca^{++}, and phospholipids: group A and B prothrombin activators are metalloproteinases in viperid and crotalid venoms whereas group C and D are serine proteinases in elapid venoms.

Group C and D prothrombin activators are only found in Australian elapid snake venoms. Group C prothrombin activators resemble mammalian factor Xa-Va complex and only require Ca^{++} and negatively charged phospholipid but not FVa (Kini 2005). They have been purified and characterized from venom of Taipan (*Oxyuranus scutellatus*) venom (Walker et al. 1980; Speijer et al. 1986) and from Brown snake (*Pseudonaja textilis*) venom (Masci et al. 1988; Stocker et al. 1994; Rao and Kini 2002). Group D prothrombin activators are structurally and functionally similar to factor Xa (Kini 2005) and are strongly stimulated by addition of Ca^{++}, negatively charged phospholipid and FVa. They are present in venoms of Tiger snake (*Notechis scutatus*) venom (Tans et al. 1985), Rough-scaled snake (*Tropidechis carinatus*: trocarin) venom (Morrison et al. 1987; Marsh et al. 1997; Joseph et al. 1999; Rao et al. 2003), Stephen's banded snake (*Hoplocephalus stephensi*: hopsarin) venom (Rao et al. 2003), and the Peninsula Tiger snake (*Notechis ater niger*) venom (Williams and White 1989; Rao et al. 2003). These prothrombin activators usually constitute a considerable portion of the venoms (Joseph et al. 1999; Rao and Kini 2002; Rao et al. 2003) and in victims' blood would probably be at higher concentrations than their target coagulation factors (Kini 2005).

From a clinical perspective, the process of procoagulation caused by Australian snake venoms results in consumption of coagulation factors, including fibrinogen and the onset of intrinsic fibrinolysis. (Australian snake venoms do not appear to contain fibrinolytic agents.) The laboratory tests of prothrombin time and activated plasma thromboplastin time are both very prolonged, sometimes unmeasurable, and blood is incoagulable. Serum fibrinogen and other factors are very low or undetectable and fibrin(ogen) degradation products are generated. The platelet count is also often very low as a direct result of procoagulopathy. Infusions of purified Brown snake and Tiger snake prothrombin activators cause thrombocytopenia because of the end-product, thrombin, is a well-known aggregator of platelets. These effects are prevented by heparin (Tibballs 1998a; Tibballs et al. 1992). The platelet count is probably also depressed because venoms have a direct effect on platelets: in vitro venoms induce aggregation of washed platelets (Marshall and Herrmann 1989).

The procoagulant coagulopathy has been referred to variously as: "disseminated intravascular coagulation (DIC)" as may be caused by bacterial toxins; "defibrination coagulopathy"; and "venom-induced consumption coagulopathy (VICC)." The nature of the process remains insufficiently defined and hence these descriptive terms should not be regarded as necessarily explicative. Until the process is better understood, it is preferable to regard it simply as "procoagulant coagulopathy," which has three important clinical consequences as follows.

Spontaneous Hemorrhage

The risk of cerebral hemorrhage is not inconsiderable with procoagulant coagulopathy. For example, of 552 snakebite cases with procoagulopathy, 6 (1%) (who all had systemic hypertension) developed intracranial hemorrhages of whom 5 died despite antivenom therapy (Berling et al. 2015). Four of the victims had clinical evidence of hemorrhage within 8–12 h and 2 within 3 h. On this basis, it would be reasonable to administer exogenous coagulation factors but only after antivenom, particularly if the victim has hypertension, while waiting the endogenous hepatic reproduction of coagulation factors.

Acute Cardiovascular Collapse

This is also a consequence of procoagulant coagulopathy. The collapse may be transient or lead to death. It has been postulated that thromboembolic phenomena may also be the basis for acute cardiovascular collapse caused by venom and prothrombin activator from *Pseudonaja textilis* (Tibballs et al. 1989, 1992) and by venom and prothrombin activator from *Notechis scutatus* (Tibballs 1998a, b) – effects which are prevented by heparin. This is supported by the work of Chaisakul et al. (2013, 2015) who showed that the venom of *Pseudonaja textilis* and subunits of the prothrombin activator (Pseutarin C) isolated from its venom cause rapid cardiovascular collapse in animals. The mechanism of hypotension may be multiple. Chaisakul et al. (2012, 2014) also showed that cardiovascular collapse in animals was caused by a PLA_2 phospholipase in Papuan Taipan (*Oxyuranus scutellatus*) venom and by its subfractions via direct vasorelaxation and via the release of autacoids.

Microangiopathic Hemolytic Anemia (MAHA)

This is not infrequent and has been observed after Brown, Taipan, Tiger, and Stephen's banded snake envenomation (Isbister et al. 2007a; Ho et al. 2010; Johnston et al. 2017). Since no specific toxin has been identified in venoms of procoagulant snakes and it has not been reported after envenomation by an anticoagulant snake, it appears to be a consequence of procoagulant coagulopathy and not a separate condition. In this phenomenon, microthrombi lodge in small vessels and damage transiting red blood cells which appear on a blood film as schistocytes, fragmented or haemolyzed and accompanied by thrombocytopenia. A consequence of this microembolization is renal dysfunction and the victim may require renal support therapy such as hemofiltration or hemodialysis.

The clinical observations resemble those in hemolytic uremic syndrome and thrombotic thrombocytopenic purpura – conditions associated with deficiency of

or antibodies to ADAMTS13 (**a** **d**isintegrin **a**nd **m**etalloproteinase with a **t**hrombo**s**pondin type 1 motif, member 13) also known as von Willebrand factor-cleaving protease, a zinc-metalloprotease enzyme that cleaves von Willebrand factor (vWf) – a multimeric protein involved in blood coagulation. The absence of normal breakdown of vWf by ADAMTS13 leads to fibrin and platelet deposition of fibrin and platelets in small blood vessels, notably in the brain and kidney. Plasmapheresis is often undertaken to remove antibody to ADAMST13 and to replace deficiency. In a case of MAHA after presumed Tiger snake envenomation (Ho et al. 2010), plasmapheresis with fresh frozen plasma replacement was employed even though the ADAMTS13 activity prior to plasmapheresis was normal (85%). The role of plasmapheresis in addition to hemofiltration/dialysis however remains uncertain – renal failure was managed as well with or without plasmapheresis in addition to hemofiltration/dialysis in five of six patients with MAHA (Isbister et al. 2007b). For victims with renal failure requiring renal support (haemofiltration/dialysis) after coagulant procoagulopathy, a reasonable course would be measure ADAMST13 activity before embarking upon plasmapheresis.

Anticoagulants

Envenomation by Black snakes (*Pseudechis*) causes a state of anticoagulation. This is characterized by incoagulable blood with laboratory findings of prolongation of prothrombin time and activated plasma thromboplastin time but the preservation of serum fibrinogen level and the absence of fibrin degradation products. No specific anticoagulant toxins have been purified and sequenced but a PLA_2 which inhibits platelet aggregation in Copperhead (*Austrelaps*) venom has been isolated and characterized (Yuan et al. 1993).

Other Toxins

Natriuretic peptides have been identified in venom glands of numerous Australian elapids. Recombinant peptides from the Common brown snake (*Pseudonaja textilis*) and the Mulga snake (*Pseudechis australis*) were found to inhibit angiotensin converting enzyme activity (St Pierre et al. 2006). A natriuretic peptide is also present in venom of the Rough-scaled snake (*Tropidechis carinatus*) (Reeks et al. 2015). Their roles in envenomation are uncertain.

Effects of Generic Venoms and Identified Toxins

Pseudonaja spp. (Brown Snakes)

The Brown snake genus (*Pseudonaja*) embraces nine species: Eastern Brown (*P. textilis*), Dugite (*P. affinis*), Northern Tropical Brown (*P. nuchalis*), Peninsula Brown (*P. inframacular*), Five-ringed Brown snake (*P. modesta*), Ingram's Brown snake (*P. ingrami*), Spotted Brown snake (*P. guttata*), Western Brown snake (*P. mengdeni*), and Patch-nosed Brown snake (*P. aspidorhyncha*).

Envenomation by Brown snakes invariably results in procoagulant coagulopathy which is sometimes accompanied by myotoxicity (Johnston et al. 2017), but in massive envenomation or delayed/inadequate treatment envenomation by *P. textilis*, paralysis may also occur. The venom of *Pseudonaja textilis* is one of the most toxic of all snakes (Sutherland and Tibballs 2001). Brown snakes are responsible for most deaths from snake bite in Australia and most of these occur out-of-hospital (Johnston et al. 2017) probably as a result of hypotension.

The venom of *P. textilis* is known to contain a prothrombin activator complex, serine proteinase inhibitors, various phospholipase A_2s, and pre- and postsynaptic neurotoxins (Sutherland and Tibballs 2001) but new toxins are being discovered. Birrell et al. (2006) performed a proteonomic study of *P. textilis* venom: using two-dimensional gel electrophoresis, mass spectrometry, and *de novo* peptide sequencing; they identified most of the known venom proteins and discovered additional proteins previously not known to be present in the venom. In addition, using immunoblotting and posttranslational modification-specific enzyme stains and antibodies, a complexity and considerable regional diversification of venom between specimens was revealed. Modifications included phosphorylation, carboxylation, and glycosylation. The venom also has abundant glycoproteins with *N*-linked sugars that include glucose/mannose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and sialic acids. In addition, the venom has multiple isoforms of mammalian coagulation factors that comprise a significant proportion. Two of the identified proteins, a procoagulant and a plasmin inhibitor, are currently in development as human therapeutic agents.

Procoagulants

The venoms of *Pseudonaja* contain factor(s) which convert prothrombin to thrombin (prothrombin activators). Like other elapids, *Pseudonaja* venoms contain serine proteases whereas the prothrombin activator toxins in viperid and crotalid venoms are metalloproteases (Kini and Koh 2016). Several prothrombin activators (activated factor X-like) have been identified in *Pseudonaja* venom but the venom appears devoid of factors which directly activate factor X.

Masci et al. (1988) identified a prothrombin activator in *P. textilis* venom. It is a major component which has a MW of $\geq$200,000 composed of a number of subunits. It is related to the venom prothrombin activator of the taipan *Oxyuranus scutellatus*. The toxin coagulated citrated plasma, warfarin plasma, and Factor V- and Factor X-deficient plasmas to convert purified human prothrombin to thrombin and to hydrolyze the peptide p-nitroanilide substrate S-2222. Calcium ions and phospholipids had little if any effect on the rates of coagulation of citrated plasma or S-2222 hydrolysis catalyzed by this enzyme.

Rao and Kini (2002) purified a prothrombin activator, pseutarin C, from the venom of *Pseudonaja textilis*. It converts prothrombin to thrombin by cleaving both the peptide bonds Arg(274)-Thr(275) and Arg(323)-Ile(324), similar to the action of mammalian factor Xa. It is a protein complex (approximately 250 kD) consisting of an enzymatic and a nonenzymatic subunit. The enzymatic subunit has a high affinity for factor Va. The N-terminal sequence analysis of the catalytic subunit

of pseutarin C showed approximately 60% homology to mammalian factor Xa and approximately 78% homology to trocarin, a group D prothrombin activator from *Tropidechis carinatus* venom. The sequence of the nonenzymatic subunit of pseutarin C is similar to factor Va. Thus pseutarin C is structurally and functionally similar to the mammalian factor Xa-Va complex.

Reza et al. (2006) identified two molecular forms of factor X expressed in the liver of *P. textilis*. Both isoforms have molecular signatures and domain architecture of factor X. One isoform shows approximately 94% sequence identity with factor X from *Tropidechis carinatus*, whereas the other is much closer (90% identity) to the catalytic subunit of pseutarin C (but neither are expressed in the venom gland) suggesting that the latter is an intermediate in the evolution of venom prothrombin activator from blood coagulation factor X.

Other procoagulant toxins in *P. textilis* venom include textilinin-1, a 7 kDa Kunitz-type serine protease whose action by inhibiting plasmin (and trypsin) is an antifibrinolytic agent and is being developed as a systemic alternative to aprotinin as a hemostatic agent for surgery. Its crystalline structure has been determined. Other hemostatic toxins include a Factor Xa-like protein that displays potent procoagulant effects which are being developed as a topical hemostatic agent and a procoagulant cofactor that may have application as a systemic antibleeding agent in the treatment of internal bleeding and noncompressible hemorrhage (Millers et al. 2009; Earl et al. 2012).

The antivenom in current use (bioCSL Ltd Pty) is regarded as highly efficacious for neutralizing the effects of Brown snake venom but there may be components in venom not recognized in the immunization process and components once bound to receptors cannot be neutralized. Judge et al. (2006) identified similar proteins of mass 78–132 kDa, 32–45 kDa, and 6–15 kDa in venoms of *P. affinis*, *P. nuchalis*, and *P. textilis* which included a coagulant toxin (~42 kDa), a coagulant peptide (~6 kDa), and two PLA_2 (~14 kDa). Immunoblot recognition by the antivenom occurred predominantly for the higher molecular weight components with little recognition of 6–32 kDa MW species. The venoms caused presynaptic PLA_2 type nerve-muscle contraction which was not attenuated by antivenom treatment. Lack of antibody to some toxins could explain in part why victims of Brown snake envenomation sometimes succumb despite timely and adequate treatment with antivenom.

In 27 victims of Brown snake envenomation, antivenom at doses of 1–10 (median 3) vials cleared their blood of venom including in 9 victims given only 1 vial, while in vitro studies showed that antivenom prevented clotting and neutralized procoagulant activity of concentrations of venom observed in envenomated victims (Isbister et al. 2007b).

Presynaptic Neurotoxin

Textilotoxin, the major neurotoxic presynaptic PLA_2 of *Pseudonaja textilis* venom (Pearson et al. 1993), is a 623 amino acid five-subunit complex (subunit A, 118 residues; subunit B, 121 residues; subunit C, 118 residues; subunit D, two chains of 133 residues each). All subunits A, B, C, and D contain the putative phospholipase A_2 active site. Although only subunit A showed any mouse lethality on its own, all

subunits are necessary for maximum lethality. Unlike the other major presynaptic toxins in Tiger snake and Taipan venoms (notexin and taipoxin), it does not appear to have a significant rhabdomyolytic effect (Sutherland and Tibballs 2001) but myolysis does occur clinically in a small percentage of cases (Johnston et al. 2017). Compared with taipoxin in Taipan venom, textilotoxin is less potent and constitutes less of venom (5.7% vs. 20.4%) (Barber et al. 2012) explaining perhaps why neurotoxicity and rhabdomyolysis are uncommon in *Pseudonaja textilis* envenomation but common in *Oxyuranus scutellatus* envenomation.

Other Toxins

The venom of *Pseudonaja textilis* includes prothrombinase coagulation factors, neurotoxic textilotoxin phospholipase A2 (PLA2) subunits and "acidic PLA2," three-finger toxins (3FTx) and the Kunitz-type protease inhibitor textilinin, venom metalloproteinase, C-type lectins, cysteine-rich secretory proteins, calreticulin, dipeptidase 2, and a variant of coagulation factor 5a (Viala et al. 2015).

Notechis spp. (Tiger Snakes)

Historically, this genus included the Eastern or Mainland tiger snake (*N. scutatus*) and Black Tiger snakes (*N. ater*) with a number of subspecies of the latter including the Western Tiger snake (*N. ater occidentalis)*, Peninsula Tiger snake (*N. ater niger*), Krefft's Tiger snake (*N. ater ater*), King Island Tiger snake (*N. ater humphreysi*), and the Chappell Island Tiger snake (*N. ater serventyi*). Although they have evolved to exhibit significant variation in size, coloration, and its pattern, they are morphologically one species, *Notechis scutatus* (Keogh et al. 2005). Potent venom causes paralysis, rhabdomyolysis, and procoagulant coagulopathy.

Presynaptic Neurotoxins

Notexin is the main neurotoxic and myotoxic phospholipase A_2 protein in the venom of the Australian tiger snake, *Notechis scutatus*. The toxin comprises about 6% of venom, composed of 119 amino acid residues existing as a single chain and cross-linked by seven disulfide bridges with asparagine as the N-terminal. The molecular mass is 13,574 Da. Functionally, it initially disrupts the ability of neurons to release acetylcholine and in animal models kills by respiratory paralysis. In this process, calcium enters nerve terminals to cause massive release and exocytosis of synaptic vesicles perhaps in a manner similar to that of alpha-latrotoxin of *Latrodectus* spiders (Tedesco et al. 2009).

The effects of the toxin are not confined to disruption of acetylcholine release. Dixon and Harris (1996) showed by electron microscopy that notexin initially binds exclusively to the muscle sarcolemma creating small lesions. After degeneration of the sarcolemma, it enters muscle fibers which then also degenerate. Numerous case reports note rhabdomyolysis, myoglobinuria, and gross elevation of serum enzymes and isoenzymes, sometimes accompanied by renal failure. Experimentally, muscle enzymes are released into blood about 2 h after envenomation.

Numerous other presynaptic phospholipase neurotoxins, including those named notechis II-5, notexin II-5b, scutoxin A and B, and notechis Ns, are found in *Notechis scutatus* venom. They have similar mass, have high homology, and some are considered isoforms of notexin. The toxin has been used extensively in models of muscle repair and degeneration and cell apoptosis. A recombinant notexin has been produced, a three-dimensional structure proposed and its phospholipase activity and toxic properties determined to be highly dependent on its N-terminal asparagine (Simonato et al. 2014).

Postsynaptic Neurotoxins

At least three postsynaptic neurotoxins, including toxin 1 and toxin 2, are contained in *Notechis* venoms. They are rapidly acting, have 60–73 residues in a single chain with several disulfide bonds, have masses 6–8 kDa, and are easily reversed with antivenom in vitro (Sutherland and Tibballs 2001).

Hypotensive Proteins

Several proteins cause severe hypotension by an unknown mechanism. They are phospholipases, have 125 residues, and have molecular mass 18–21 kD (Sutherland and Tibballs 2001).

Procoagulant Toxins

Tans et al. (1985) isolated a prothrombin activator from *N. scutatus* venom. It has a molecular weight of 54 kD consisting of a heavy chain (32 kDa) and a light chain (23 kDa) linked by disulfide bridges. It has factor Xa-like activity which is greatly enhanced by the presence of phospholipids, calcium, and factor V. It causes a procoagulopathy resembling disseminated intravascular coagulation (DIC). Thromboembolism may be a cause of sudden clinical cardiovascular collapse: prothrombin activator infused intravenously into anesthetized mechanically ventilated dogs caused severe depression of systemic blood pressure, depression of cardiac output, and pulmonary hypertension. Systemic hypotension was postulated to be due to myocardial ischemia secondary to coronary thromboembolism. Pulmonary hypertension was also due to thromboembolism. Echocardiography showed the formation of thrombi within the chambers of the heart, right heart distention, and left ventricular hypocontractility (Tibballs 1998a, b).

Rao et al. (2003) purified group D prothrombin activators from *N. scutatus scutatus* (notecarin D), *N. ater niger* (notanarin D), and *Hoplocephalus stephensi* (hopsarin D) venoms (*vide infra*). All have a mass 46–47 kDa and highly homologous with each other and with mammalian proteinase complex Xa. Each is composed of a heavy and a light – a light chain of 18 kDa and a heavy chain of 30 kDa. A slight variation in the sequence of two toxins from *N. scutatus scutatus* (notecarin D1, D2) venom was attributed to variation in venom from snakes of different geographical locations. Williams and White (1989) also purified a prothrombin activator from Peninsula Tiger snake (*Notechis ater niger*) venom. It had an MW of approximately 58,000 in two chains and required factor V for full activity.

Oxyuranus spp. (Taipans)

Three distinct species of taipans (genus *Oxyuranus*) are recognized: Coastal taipan (*O. scutellatus*), Inland taipan (*O. microlepidotus*), and the Western Desert Taipan (*O. temporalis*). The Papua New Guinea Taipan (*O. s. canni*), which inhabits Saibai Island and probably Boiga Island in Torres Strait (Australian territory), is now also regarded as *O. scutellatus*. Their venoms cause paralysis, rhabdomyolysis, and procoagulant coagulopathy. The newly discovered species, *O. temporalis*, appears to have more potent postsynaptic neurotoxicity than the other generic species but has less or no presynaptic neurotoxic and less prothrombin activator activity (Barber et al. 2014).

Presynaptic Neurotoxins

The respective principal toxins in the respective venoms are the phospholipases taipoxin (*O. scutellatus*), paradoxin (*O. microlepidotus*), and cannitoxin (*O. s. canni*). These presynaptic neurotoxins also cause myonecrosis. Paradoxin is a very potent (beta-)presynaptic neurotoxin (Hodgson et al. 2007).

Taipoxin and paradoxin are trimeric molecules with a mass of 45.6 kDa and composed of alpha, two beta, and gamma subunits. The amino acid sequences of the subunits are known (Lind and Eaker 1982; Harrison and Aquilina 2016). In mice, the alpha subunit is most toxic, the gamma subunit is moderately toxic, and the beta-1 and beta-2 subunits are nontoxic (Lipps 2000) but enhance the activity of the other units (Cendron et al. 2012).

Taipan venom and taipoxin act very quickly at the nerve terminal. The neuromuscular inhibition in mouse phrenic nerve-diaphragm preparations caused by *O. scutellatus* venom and taipoxin was neutralized by F(ab′)2 and whole IgG antivenoms when preincubated, but if the antivenoms were added after the venom or taipoxin, neutralization was only achieved if antivenom was added within 10 min after venom (Herrera et al. 2016). Taipoxin (and notexin from *N. scutatus* venom) causes depletion of transmitter from the motor nerve terminals of rat soleus muscle within 1 h then followed by the degeneration of the motor nerve terminals and of the axonal cytoskeleton such that by 24 h most muscle fibers are completely denervated. Regeneration and functional reinnervation occur over 5 days, implying that recovery from established clinical neuromuscular paralysis caused by Taipan envenomation will be very slow. Treppmann et al. (2011) showed that taipoxin and paradoxin interrupt neurotransmission by disrupting synaptic vesicular excocytosis and by preventing synaptic filling as a result of dissociating the synaptophysin/synaptobrevin (Syp/Syb) complex on the synaptic vesicles.

Taipan equine-derived antivenom (bioCSL Ltd) is very expensive for Papua New Guinea where envenomation is common. Investigators have produced an alternative and tested it against taipan venom and taipoxin. An antivenom against taipan venom has been raised in chickens and harvested from egg yolk. Although considerably cheaper than production from horses, it had a lower ability to immunocapture the alpha subunit of taipoxin – the most important neurotoxin in the venom – and had lower ability to neutralize the coagulant and lethal activities of taipan venom. Moreover, it was more immunogenic in rabbits than equine-derived antivenom (Navarro et al. 2016).

Suramin, a P2Y receptor antagonist, approved for treatment of trypanosomiasis, prevented the in vitro neurotoxic effects of presynaptic neurotoxins in taipan venoms: taipoxin (*O. scutellatus*), paradoxin (*O. microlepidotus*), and cannitoxin (*O. s. canni*). Suramin completely blocked the taipoxin and cannitoxin-mediated inhibition of nerve-mediated twitches but only partially that caused by paradoxin and was ineffective against inhibition caused by textilotoxin, the major neurotoxin in common brown snake (*Pseudonaja textilis*) venom (Kuruppu et al. 2014).

Prothrombin Activator

Speijer et al. (1986) isolated a prothrombin activator from the venom of *Oxyuranus scutellatus.* It is a protein with an apparent molecular weight of 57,000 which rapidly activates prothrombin in the presence of Factor Va or in the presence of another venom protein component of molecular weight 220,000, suggesting that the prothrombin activator in the venom is a multimeric protein complex consisting of a Factor Xa-like enzyme and a Factor Va-like cofactor. Nakagaki et al. (1992) observed that the purified prothrombin activator was also an activator of human factor VII.

Cardiac Calcium Channel Blocker

Disorders of cardiac conduction are commonly observed after envenomation by the Papua New Guinea Taipan (Lalloo et al. 1997). The responsible toxin, Taicatoxin, is an L-type voltage-dependent cardiac calcium channel blocker (Fantini et al. 1996; Doorty et al. 1997).

Other Toxins

Numerous other toxins, including postsynaptic neurotoxins, are present in Taipan venoms. St Pierre et al. (2005) constructed a complementary DNA microarray of genes from the venom gland of the Coastal taipan (*Oxyuranus scutellatus*) identifying genes for neurotoxins, phospholipases A_2, a pseudechetoxin-like gene, a venom natriuretic peptide, and a nerve growth factor. These toxin transcripts were subsequently identified in venoms of seven other snake species (Inland taipan, *Oxyuranus microlepidotus*; Common brown snake, *Pseudonaja textilis*; Tiger snake, *Notechis scutatus*; Rough-scaled snake, *Tropidechis carinatus*; Stephen's banded snake, *Hoplocephalus stephensii*; Red-bellied black snake, *Pseudechis porphyriacus*; and Mulga snake, *Pseudechis australis*) all showing high homology and corresponding to known toxicities. For example, of the 3 PLA_2 isoforms identified in the microarray, the most abundant corresponded to the beta chain of taipoxin which by itself is not toxic but assists the long-known neurotoxic and myotoxic effects of the alpha chain of the toxin (Harris and Maltin 1982; Harris et al. 2000).

Acanthophis spp. (Death Adders)

Despite the collective name of Death Adders and adder-like appearance, the members of this genus are elapids which inhabit Australia, Papua New Guinea, and Indonesia. In Australia, the genus comprises *A. antarcticus* (Common Death Adder),

A. praelongus (Northern Death Adder, which some authors have divided into three species: *A. praelongus*, *A. hawkei*, and *A. rugosus*), *A. Pyrrhus* (Desert Death Adder), and *A. wellsi* (Pilbara Death Adder). Envenomation usually results only in paralysis but anticoagulant coagulopathy and rhabdomyolysis may also occur.

Long considered to contain only reversible rapidly acting postsynaptic toxins (Sutherland and Tibballs 2001), the venoms of the Common Death Adder and other species have also been found to contain presysnaptic toxins which could explain why victims occasionally present with delayed-onset neurotoxicity or respond poorly to antivenom (Johnston et al. 2012) or anticholinesterase treatment.

Blacklow et al. (2010b), using chick biventer cervicis nerve-muscle preparations, showed that in the group of Australo-Papuan Death Adders (*Acanthophis* spp.), potent presynaptic phospholipase A_2 neurotoxins were present in all geographic variations of *A. antarcticus* and in venom of *A. praelongus*, *A. rugosus*, *A. laevis* species but not in venoms of *A. wellsi* and *A. pyrrhus*. From the venom of *A. antarcticus*, Blacklow et al. (2010a) isolated a phospholipase A_2 toxin, P-elapotoxin-Aa1a (P-EPTX-Aa1), using liquid chromatography and by testing in a chick biventer-cervicis nerve-muscle preparation showed it binds irreversibly to motor neuron terminals to prevent acetylcholine release. It has a mass of 44,698 Da and is a heterotrimeric complex composed of alpha, beta, and gamma-subunits of which only the alpha-chain has significant phospholipase activity, similar to the alpha chain of taipoxin, a toxin from *Oxyuranus scutellatus* with which it shares significant homology. Preincubation of the toxin with Death Adder antivenom or suramin, or inhibition of the phospholipase activity by incubation with 4-bromophenacyl bromide, either prevented or delayed the onset of toxicity but, importantly from a clinical perspective, failed to reverse established neurotoxicity suggesting that antibodies can only neutralize unbound toxins.

Pseudechis spp. (Black Snakes)

This genus is comprised of six species: Mulga snake (*P. australis* which some authors divide into three species: *P. australis*, *P. pailsei*, and *P. weigeli*); Red-bellied black snake (*P. porphyriacus*); Butler's snake (*P. butleri*); Collett's snake (*P. colletti*); Spotted Black/Blue-bellied Black snake (*P. guttatus*); and Papuan Black snake (*Pseudechis papuanus*). The last named inhabits Saibai and Boigu Islands (Mirtschin et al. 2017), Australian territories in Torres Strait.

Mulga snakebite causes severe envenomation with severe rhabdomyolysis, paralysis, and anticoagulant coagulopathy (Johnston et al. 2013). The venom is used in experimental studies of rhabdomyolysis and muscle regeneration. Envenomation by other species of Black snake is less severe. Red-bellied Black snake (*P. porphyriacus*) venom causes mild experimental procoagulopathy (Sutherland and Tibballs 2001) but anticoagulant coagulopathy is usually observed clinically (Johnston et al. 2017). Collett's snake envenomation causes anticoagulation and skeletal and cardiac myotoxicity (Sutherland and Tibballs 2001; Johnston et al. 2017). Unlike envenomation by other Australian genera, local but minor tissue

damage at Black snake bite sites is usually observed while occasionally anosmia (loss of smell) with olfactory bulb atrophy (Sethi et al. 2016) and/or dysgeusia (alteration of taste) are residual effects.

The mechanism of occasional loss of smell after envenomation by these snakes is due to the presence of toxins named pseudechetoxin (PsTx) and pseudecin (Pdc) which block cyclic nucleotide-gated (CNG) ion channels. They probably occlude the pore entrance (Brown et al. 1999, 2003; Yamazaki et al. 2002; Suzuki et al. 2008). The channels play pivotal roles in sensory transduction by retinal photoreceptors and olfactory neurons. These toxins belong to a cysteine-rich secretory protein (CRiSP) family containing an N-terminal pathogenesis-related proteins of group 1 (PR-1) domain and a C-terminal cysteine-rich domain (CRD). The two toxins are highly homologous but PsTx is far more potent. PsTx and Pdc are 211 and 210 amino acids which are 96.7% identical, differing in only seven residues.

A proteome analysis showed that the venom of the Spotted Black snake (*P. guttatus*) contains l-amino-acid oxidases, phospholipases A_2, metalloproteases, nerve growth factors and ecto-5′-nucleotidases, cysteine-rich secretory proteins (CRiSPs) similar to pseudechetoxin, phospholipase B, and transferrin-like protein (Viala et al. 2014).

Antivenom is effective against effects of Black snake venoms. Antivenom did prevent myotoxicity more effectively, if given earlier, in rats injected with *Pseudechis australis* (Mulga snake) venom (Hart et al. 2014) and systemic envenoming in human victims of *Pseudechis australis* envenomation responds well to antivenom (Razavi et al. 2014).

Austrelaps spp. (Copperhead Snakes)

This genus comprises three species: Common Copperhead (*A. superbus*), Pygmy Copperhead (*A. labialis*), and the Highland Copperhead (*A. ramsayi*). These snakes are not regarded as highly venomous but clinical experience is limited. Envenomation causes paralysis with possible procoagulant coagulopathy and rhabdomyolysis.

These species have long been considered to have only postsynaptic neurotoxins but Marcon et al. (2013) identified several presynaptic neurotoxins from the venom of *Austrelaps superbus*. These included a multimeric presynaptic phospholipase A_2 neurotoxin, P-elapitoxin –As1a (P-EPTX-As1a), two novel monomeric presynaptic phospholipase A_2 neurotoxins, and a novel postsynaptic alpha-neurotoxin. In chick biventer cervicis nerve-muscle preparations, the presynaptic toxins inhibited nerve-evoked twitch contractions at the neuromuscular junction without inhibiting the contractile response to cholinergic agonists. High homology of P-EPTX-As1a with the alpha-chain of taipoxin (Taipan toxin) was noted. Additional other coagulopathic and myotoxic high mass proteins including a metalloproteinase, C-type lectin, acetylcholinesterase, and phospholipase B were also identified in venom. The same group of investigators (Marcon et al. 2012) investigated toxins in the venom of *Austrelaps labialis*. They isolated and characterized a postsynaptic neurotoxin, alpha-EPTX-Al2a composed of 75 amino acids with mass 8072.77 Da. This toxin has five disulfide bonds, has significant homology with classical long-chain alpha-

neurotoxins, and blocks responses to cholinergic agonists in chick biventer nerve-muscle preparations. Tiger snake antivenom prevented neurotoxicity if applied prior to toxin administration but was only able to partially reverse established neurotoxicity, an important implication for clinical management of envenomated human victim.

Tropidechis carinatus (Rough-Scaled Snake)

This species is confined to the coastal regions of southern Queensland and northern New South Wales. Envenomation causes procoagulant coagulopathy, neurotoxicty, and rhabdomyolysis (Gan et al. 2009) in a syndrome very similar to that of Tiger snake envenomation.

Several groups of investigators have isolated prothrombin activators from venom. Marsh et al. (1997) isolated two isoforms of a prothrombin-activating enzyme from venom which had a molecular weight of 64,500 under native conditions and 41,500 under reducing conditions. Prothrombinase activity was dependent on factor Va, phospholipid, and calcium ions. It did not have any phospholipase activity. Joseph et al. (1999) also isolated a prothrombin activator, Trocarin, and confirmed that its activity is enhanced by Ca^{++}, phospholipids, and factor Va, similar to that of factor Xa. They determined its amino acid sequence with a mass of 46,515 Da and that it was a glycoprotein highly homologous to factor Xa and sharing the same domain architecture. It consists of a light chain and a heavy serine protease chain which Venkateswarlu et al. (2002) determined by modeling had minor conformational and structural differences from factor Xa.

Reza et al. (2005) found that the eight introns of the gene for prothrombin activator toxin (Trocarin D) in *Tropidechis carinatus* venom gland cells and those of its gene for hepatic factor X (for its own haemostasis) are almost identical except for intron 1 and the promoter regions, and that they are similar to all mammalian factor X genes – a phenomenon explained by gene duplication. Han et al. (2016) showed that arginine-rich motifs in intron 1 regulate (suppress) expression of the gene in venom gland and mammalian cells.

Hoplocephalus spp.

This genus comprises three species: Stephen's banded snake (*H. stephensi*), Broad-headed snake (*H. bungaroides*), and the Pale-headed snake (*H. bitiorquatus*). Clinical experience with envenomation by this genus is very limited. Envenomation may cause procoagulant coagulopathy, paralysis, and possibly rhabdomyolysis.

In a case report, envenomation by Stephen's banded snake caused mild systemic symptoms and a defibrination coagulopathy which responded to treatment with Tiger snake antivenom (Hession 2007). In vitro using chick biventer cervicis nerve-muscle and mouse phrenic nerve diaphragm preparations, Hodgson et al. (2003) showed that the venom of this species had neurotoxic effects which were partially prevented and partially reversed by Tiger snake antivenom. The venom also had myotoxic effects. Tan et al.

(2006) showed that the venom of *H. Stephensi* contained a postsynaptic neurotoxin, hostoxin-1, which has a mass of 6660 Da whose action on chick biventer cervicis nerve-muscle preparation could be reversed with Tiger snake antivenom (bioCSL Pty Ltd).

Sea Snakes

Some sea snake venoms cause widespread damage to skeletal muscle with consequent myoglobinuria, neuromuscular paralysis especially by postsynaptic toxins or direct renal damage (Sutherland and Tibballs 2001). Most have not been researched. The principles of treatment are essentially the same as for envenomation by terrestrial snakes. The venoms of significant species are neutralized with antivenom to Beaked sea snake (*Hydrophis zwefeli*, formerly known as *Enhydrina schistosa*) (bioCSL Pty Ltd). The antivenom is raised with venom from a very similar Malaysian species now differentiated as *Hydrophis schistosa* but also known formerly as *Enhydrina schistosa* (Ukuwela et al. 2012). If Beaked sea snake antivenom is not available, Tiger Snake or polyvalent antivenom should be used. Sea snake bites are uncommon in Australia and no deaths have been recorded, but in Asian waters many deaths have been recorded.

Lesser Known Snakes

Whip snakes (genus *Demansia*) are not regarded as highly venomous to humans but the venom of the Black Whip snake (*D. vestigiata*) contains homologues of 13 distinct toxin families from other Australian elapids, such as factor X-like prothrombin activator, neurotoxins, phospholipases, cysteine-rich secretory proteins, textilin-like molecules, nerve growth factors, l-amino acid oxidases (which may retard putrefaction of ingested prey), vespryns, 5′nucleotidases, metalloproteinases, and C-type lectins as well as a novel dipeptidyl peptidase family (St Pierre et al. 2007).

The venom of the Brown-headed snake (*Glyphodon tristis*) has presynaptic neurotoxicity and myotoxicity both of which are prevented in vitro by bioCSL Pty Ltd polyvalent antivenom (Kuruppu et al. 2005).

The venoms of *Cryptophis boschmai*, *Denisonia devisi*, *Echiopsis curta*, *Hemiaspis signata*, and *Vermicella annulata* may all cause mild to moderate effects in humans. Venoms from all species, over the range of 4–40 kDa, contain three-finger toxins (6–8 kDa) and phospholipase A_2 (12–14 kDa). All venoms have in vitro neurotoxicity in chick biventer cervicis nerve-muscle preparation and all have PLA_2 activity. Polyvalent antivenom (bioCSL Pty Ltd) neutralized the inhibitory effects of *C. boschmai* venom but only delayed the inhibitory effect of the other venoms. The venoms of *C. boschmai*, *D. devisi*, and *H. signata* caused hypotension in vivo in an anesthetized rat preparation. *H. signata* has moderate procoagulant activity while the other venoms were weakly procoagulant (Pycroft et al. 2012).

In a case report of envenomation by a Lake Cronin snake (*Paroplocephalus atriceps*), procoagulant coagulopathy responded to treatment with polyvalent antivenom (Allen et al. 2013).

Snake Bite and Envenomation

Although a bite by a snake may be observed, envenomation may not occur because no venom or only a small amount of venom is injected, which occurs in about 50% of bites. Bites by Australian snakes may be relatively painless and may go unnoticed. This is in marked contrast to bites of many overseas crotalid and viperid snakes, where massive local reaction and necrosis are caused by proteolytic enzymes. In general, Australian snake venoms do not cause extensive damage to local tissues and are usually confined to localized mild swelling and bruising, and continued slight bleeding from the bite site. After Australian snake bite, paired fang marks are often visible but sometimes only scratches or single puncture wounds exist with or without local bruising.

Symptoms and Signs of Envenomation

Classical symptoms and signs and their onset are given in Table 2. Sometimes, not all possible symptoms and signs occur. In some cases, one symptom or sign may dominate the clinical picture, and in other cases, they may wax and wane. These phenomena may be explained by variations in toxin content of venoms of the same species in different geographical areas, and by variable absorption of different toxins.

The toxins or the mechanisms by which toxins cause many clinical effects of snake envenomation are not known. Common effects include general and

Table 2 Progressive onset of major systemic symptoms and signs of untreated envenomation[a]

<1 h after bite
Headache
Nausea, vomiting, abdominal pain
Transient hypotension associated with confusion or loss of consciousness
Coagulopathy (laboratory testing)
Regional lymphadenitis
1–3 h after bite
Paresis/paralysis of cranial nerves (e.g., ptosis, double vision, external ophthalmoplegia, dysphonia, dysphagia, myopathic facies)
Haemorrhage from mucosal surfaces and needle punctures
Tachycardia, hypotension
Tachypnea, shallow tidal volume
>3 h after bite
Paresis/paralysis of truncal and limb muscles
Paresis/paralysis of respiratory muscles (respiratory failure)
Peripheral circulatory failure (shock), hypoxemia, cyanosis
Rhabdomyolysis
Dark urine (due to myoglobinuria or hemoglobin)
Renal failure

[a]In massive envenomation or in a child, a critical illness may develop in minutes rather than hours

nonspecific symptoms such as headache, confusion, convulsions, nausea, vomiting, diarrhea, abdominal pain, pruritus, diaphoresis, and prostration. Although these are not life-threatening they do signify envenomation.

Transient and prolonged hypotension has been investigated and is probably related to procoagulation coagulopathy with myocardial ischemia and/or pulmonary hypertension culminating in transient systemic hypotension, as caused experimentally by Brown snake (Tibballs et al. 1989) and Tiger snake venom (Tibballs 1998b). Indeed, experimentally, thrombus formation within the heart is readily detectible by echocardiography and embolism to the pulmonary vasculature system evident histologically soon after envenomation of dogs with Tiger snake prothrombin activator and preventable with heparin (Tibballs 1998a). Chaisakul et al. (2013) likewise observed that rapid cardiovascular collapse caused by Brown snake venom in rats was preventable with antivenom or heparin. Prothrombin activators in venom gain access to the circulation within minutes after subcutaneous injection. Tachycardia and relatively minor ECG abnormalities are common. Other causes of hypotension such as direct cardiac toxicity are possible.

Tender or even painful regional lymph nodes are moderately common but are not per se an indication for antivenom therapy. Lymphadenitis also occurs with bites by mildly venomous snakes that do not cause serious systemic illness.

Occasionally intracranial hemorrhage occurs. In the case of untreated or massive envenomation, rhabdomyolysis may occur. This usually involves all skeletal musculature and sometimes cardiac muscle. The resultant myoglobinuria may cause renal failure.

A high intake of alcohol by adults before snake bite is common and may confound the cluster of symptoms and signs. Pre-existing treatment with anticoagulant (e.g., warfarin) or disease (e.g., gastrointestinal tract ulceration) may complicate management of coagulopathy.

Snake Bite in Children

Snake bite in young children presents additional problems. Envenomation is difficult to diagnose when a bite has not been observed or history unobtainable. The symptoms of early envenomation may pass unsuspected and the signs, particularly cranial nerve effects, are difficult to elicit. Bite marks may be difficult to distinguish from the effects of everyday minor trauma. Lastly, the onset of the syndrome of envenomation is likely to be more rapid and severe because of the relatively higher ratio of venom to body mass. Presentation may be cardiorespiratory failure.

Identification of the Snake

Identification of the snake is helpful but not essential for the clinician. However, if the snake cannot be identified, a specific monovalent antivenom, or a combination of monovalent antivenoms or polyvalent antivenom should be administered on a

geographical basis (Table 4). Nevertheless, identification guides the selection of the appropriate optimal antivenom and provides an insight into the expected syndrome. Although administration of the wrong antivenom may provide some neutralization of toxins (*vide infra*), a specific monovalent antivenom based on snake identify is far preferable.

Identification by Venom Detection Kit Test

The venom detection kit (VDK, Seqirus Australia) is an in vitro test for detection and identification of snake venom at the bite site, in urine, blood, or other tissue in cases of snake bite in Australia (and Papua New Guinea). It can be performed at the bedside or in the laboratory. It is an enzyme immunoassay using rabbit antibodies and chromogen and peroxide solutions. A positive result indicates only the type of antivenom to be administered if required. It detects venom from the five main genera including Tiger, Brown, Black, Death Adder, and Taipan. Individual species of snake within a genus cannot be identified by the test and several genera may yield a positive result in a specified well. The test is very sensitive, able to detect venom in concentrations as low as 10 ng/mL, and can yield a visual qualitative result in test wells in approximately 25 min but requires the absolute attention of the operator. The incidence of a false-positive test is very low (0–6%) and may be related to operator-error (Nimorakiotakis and Winkel 2016). On occasions, venom may be detected but the patient is asymptomatic and has no clinical signs of envenomation. A decision to administer antivenom should be made only on clinical grounds, not merely on the results of a VDK test. A very high concentration of venom in a sample may overwhelm the test and yield a spuriously negative result (Hook effect). If that possibility exists, a diluted sample should be retested.

Identification by Physical Characteristics

This can be misleading. Not all brown-colored snakes are Brown Snakes, not all black-colored snakes are Black Snakes and not all banded snakes are Tiger Snakes. Moreover, Brown Snakes, particularly juvenile snakes, may have bands and Tiger Snakes may lack characteristic bands. Juvenile snakes may not resemble the adult and can cause serious illness and death. Nonherpetologists should consult an identification guide with reference to scale patterns to identify a specimen correctly (e.g., Mirtschin et al. 2017), if antivenom therapy is to be based on morphological characteristics alone.

Identification by Clinical Effects

The appearance of a bite site cannot be used to reliably identify a snake. The constellation of symptoms and signs is useful but to a limited degree. For example, paralysis associated with procoagulant coagulopathy may be caused by a Tiger, Taipan, Brown, Rough-scaled Snake, or Stephen's banded snake but if rhabdomyolysis also occurs a bite by a Brown Snake is improbable. Paralysis associated with anticoagulant coagulopathy may be caused by a Black Snake or Death Adder, but if rhabdomyolysis also occurs a bite by a Death Adder is improbable. Paralysis with neither coagulopathy nor rhabdomyolysis may be caused by a Death Adder bite.

This information is of interest but of limited practical clinical importance. It is essential to administer antivenom at the first opportunity when indicated, rather than await manifestation of the full syndrome to select the appropriate antivenom.

Management of Snake Envenomation

The essentials actions in management are:

- Resuscitation – mechanical ventilation and restoration of blood pressure with intravenous fluids, inotropic and vasoactive agents as needed. Basic cardiopulmonary resuscitation at the scene may be live-saving: many deaths occur at the scene out-of-hospital (Johnston et al. 2017).
- Application of a pressure-immobilization first-aid bandage.
- Administration of antivenom.
- Performance of investigations.

From a practical point of view, one of the three clinical situations arises after snake bite. A plan of management for each of these is summarized in Fig. 1.

- Victim presents with a critical illness.
- Victim is envenomated but not critically ill.
- Victim is bitten but does not appear envenomated.

When the envenomated victim is not critically ill, time is available to identify the snake by investigations and to administer specific monovalent antivenom. A pressure-immobilization bandage should be applied if not already in place, and not removed until antivenom has been administered.

Period of Observation and Testing in Suspected Envenomation

When the victim has been bitten but not apparently envenomated, admission to hospital is advisable with observation and examination hourly for at least 12 h. The syndrome of envenomation may be very slow in onset over numerous hours with an initial period free of symptoms.

A test of coagulation should always be performed in suspected envenomation. In a study over 12 h of 206 victims who developed consumption coagulopathy, most victims (86%) had an elevated INR (>1.2) on admission to hospital and all had an elevated INR within 12 h of the bite. Of 33 victims who developed myotoxicity, a combination of CK $>$ 250 U/L and an abnormal aPTT identified all but two cases by 12 h. Nine victims who developed isolated neurotoxicity had a median onset of signs at 4 h after the bite (range 35 min to 12 h). The combination of INR, aPTT, and CK and repeated neurological examinations identified 238 of 240 (99%) cases by 12 h after bite (Ireland et al. 2010).

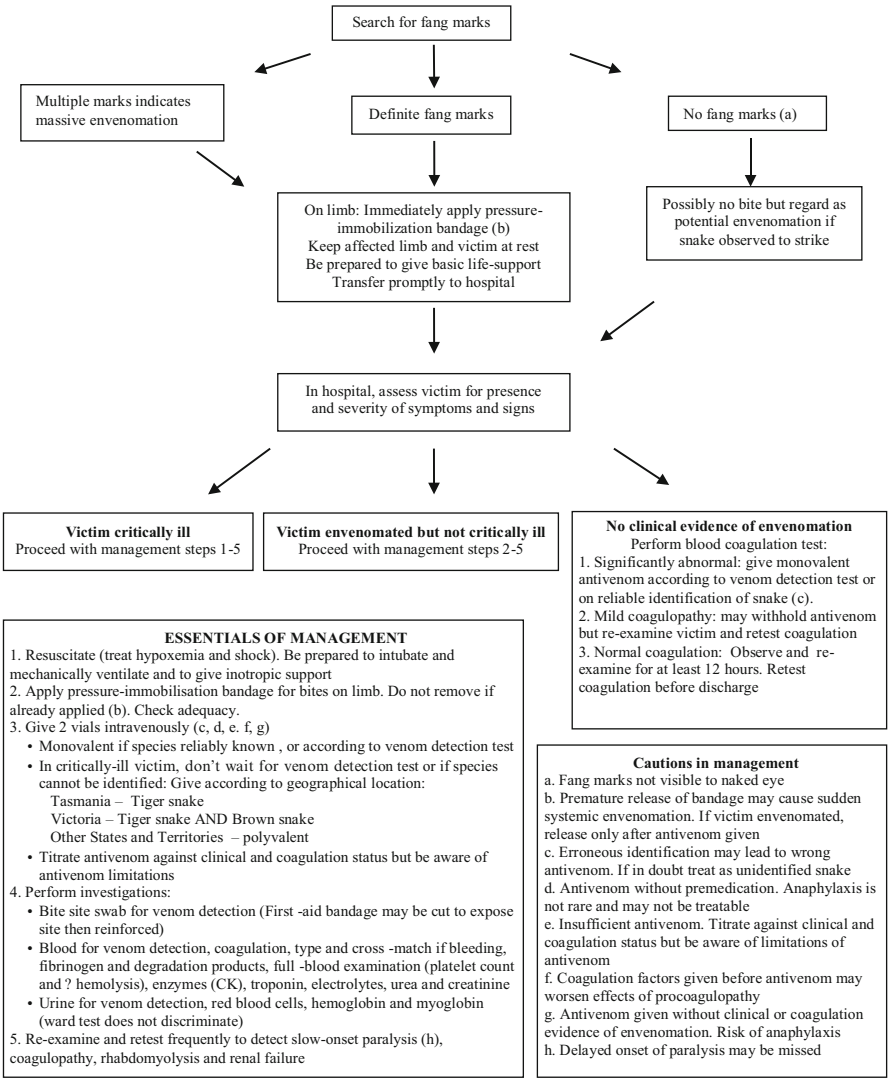

Fig. 1 Management of snake bite

Pressure-Immobilization Technique of First Aid

Since at least 95% of snake bites occur on the arms or legs, Sutherland's first-aid pressure-immobilization technique (Sutherland et al. 1979) is applicable in the majority of cases. With this technique, a crêpe (or crêpe-like) bandage, but preferably elasticised, is applied from the fingers or toes up the limb as far as possible, encompassing the bite site. It should be as firm as required for a sprained ankle. This provides some immobilization but additional immobilization is applied to the entire

limb by a rigid splint, with the aim of immobilizing the joints either side of the bite site preventing muscle action and hence limiting lymph flow. Both pressure and immobilization are essential for effective first-aid.

Venom is usually deposited subcutaneously. The systemic spread of venom is largely dependent on its absorption by way of the lymphatics (Howarth et al. 1994) or the small blood vessels. Application of a pressure less than arterial to the bitten area when combined with immobilization of the limb effectively delays the movement of venom to the central circulation. Although it is a first-aid technique designed for use in the field, it should be part of initial management in hospital of an envenomated victim since it halts further absorption of venom.

Removal of the Pressure-Immobilization Bandage

Removal in the case of envenomation may precipitate a sudden elevation in blood concentration of venom and collapse of the victim. On the other hand, first aid has not been proven to allow inactivation of snake venom in humans. Its removal, therefore, should be dictated by the circumstance. When an asymptomatic snakebite victim reaches hospital with the recommended first-aid measures in place, these should not be disturbed until antivenom, appropriate staff and equipment have been assembled. If the victim is symptomatic and antivenom is indicated, the first-aid measures should not be removed until after antivenom has been administered and reapplied if the victim's condition deteriorates. A swab of the bite site may be obtained by removing the splint temporarily and then cutting a window in the bandage. Thereafter the bandage should be made good and the splint reapplied.

Antivenom

BioCSL Pty Ltd (Parkville, Australia) produces highly purified equine monovalent antivenoms, F(ab′)2 fragments of IgG against the venoms of the five main terrestrial snakes, including Tiger Snake, Brown Snake, Black Snake, Death Adder, and Taipan. A polyvalent antivenom – a mixture of all of these is also available. A sea-snake antivenom is also produced using Beaked Sea-Snake (*Enhydrina schistosa*/*Hydrophis zwefeli*) venom. Although marketed as "monovalent antivenoms," such preparations are actually polyvalent containing considerable quantities of antibodies against toxins of other species due to use of the same horses in the immunization process (O'Leary et al. 2007; O'Leary and Isbister 2009). However, that is neither a constant nor guaranteed property.

The role of antivenom in snake envenomation is of utmost importance and life-saving even when administered late but it has limitations. Antivenom antibodies bind to free venom toxins and thus prevents their actions but they cannot be expected to reverse all established harmful effects and they cannot halt processes such as the coagulation cascade which once started cannot be halted by neutralization of the initiating agent. A theoretical model of venom-induced consumptive coagulopathy suggests that antivenom has a negligible effect on reducing the recovery time of coagulation unless the antivenom is administered almost immediately after

Table 3 Antivenom and initial dosages when snake identified

Snake	Antivenom	Units dose (vials)
Brown snakes	Brown snake	2000 (2)
Chappell island tiger snake	Tiger snake	12,000 (4)
Copperheads	Tiger snake	3000–6000 (1,2)
Death adders	Death adder	6000 (1)
Mulga (King Brown) snake	Black snake	18,000 (1)
Papuan black snake	Black snake	18,000 (1)
Red-bellied black snake	Tiger snake *or* Black snake[a]	3000 (1) 18,000 (1)
Rough-scaled (Clarence River) snake	Tiger snake	6000 (2)
Sea snakes	Sea snake *or* Tiger snake	1000 (1) 3000 (1)
Taipans	Taipan	12,000–36,000 (1–3)
Tiger snakes	Tiger snake	6000 (2)

[a]Smaller protein mass Tiger snake antivenom preferable. Antivenom units per vial: Brown snake 1000; Tiger snake 3000; Black snake 18,000; Taipan 12,000; Death adder 6000; polyvalent 40,000. Note: (1) If the victim on presentation is critically ill, 2–3 times these amounts should be given initially; (2) Additional antivenom may be required in the course of management since absorption of venom toxins may be delayed

Table 4 Antivenom and initial dosages when identity of snake uncertain

State	Antivenom	Unit dose (vials)
Tasmania	Tiger snake	6000 (2)
Victoria	Tiger snake *and* Brown snake	6000 (2) 2000 (2)
New South Wales and ACT; Queensland; South Australia; Western Australia; Northern Territory	Polyvalent	40,000 (2)
Papua New Guinea	Polyvalent	40,000 (2)

Antivenom units per vial: Brown snake 1000; Tiger snake 3000; Black snake 18,000; Taipan 12,000; Death Adder 6000; polyvalent 40,000. Note: (1) If the victim on presentation is critically ill, 2–3 times these amounts should be given initially; (2) Additional antivenom may be required in the course of management since absorption of venom toxins may be delayed

envenomation (Tanos et al. 2008) – an impossible clinical aim. Moreover, it is simply unknown which precise toxins are neutralized by antivenom and to what extent. Some toxins do not generate antibodies in the immunization process (Judge et al. 2006).

Antivenom should be administered according to the identity of the snake or if unknown or doubtful, according to the result of a carefully performed VDK test (Table 3). If neither of these criteria can be fulfilled, and if the situation warrants immediate antivenom therapy, the geographical location (Table 4) may be used as a guide, since the distribution of many species is known (Shea 1999). Polyvalent

antivenom should not be used when a monovalent antivenom could be used appropriately. For bites by uncommon snakes, when antivenom is indicated, polyvalent antivenom or a monovalent antivenom as indicated by a VDK test should be chosen. Although specific monovalent antivenoms have some true but limited cross-reactivity with venoms from other species presumably due to homologous toxins, this is also due to their polyvalency. Such cross-reactivity is variable and may not be useful clinically for the treatment of envenomation by the major genera (Brown, Tiger, Taipan, and Death Adder) but is evidently beneficial in some circumstances; for example, Tiger snake antivenom is effective in treatment of victims of envenomation by Copperhead snakes, Rough-scaled snake, some Black snakes (e.g., Red-bellied Black), Stephen's Banded snake, and by lesser known species.

Premedication

Antivenom should be preceded by premedication with low-dose subcutaneous epinephrine (adrenaline), approximately 0.25 mg for an adult and 0.005–0.01 mg/kg for a child, at least 5–10 min before the commencement of infusion. In the moribund or critically ill victim when it is essential to administer antivenom quickly, the epinephrine may be given intramuscularly or even intravenously in smaller doses. However, in general, epinephrine is not recommended by either of those routes because of the risk of intracerebral hemorrhage due to the combination of possible epinephrine-induced hypertension and venom-induced coagulopathy. Although intracerebral hemorrhage has been recorded in the past in association with premedication, all such cases occurred after intravenous epinephrine, and none with subcutaneous epinephrine which does not cause significant changes in heart rate or blood pressure (Dassanayake et al. 2002).

The incidence of all immediate-type reactions and anaphylaxis is about 25% and 10%, respectively (Isbister et al. 2008) and the occasional death due to antivenom (Williams et al. 2007) are sufficient to warrant premedication with epinephrine, which is the only medication proven effective in reducing the incidence of snake antivenom-induced reactions and their severity (Premawardhena et al. 1999; de Silva et al. 2011).

It is not reasonable to forgo premedication on the assumption that if anaphylaxis occurs it will be treatable. Iatrogenic anaphylaxis has a high mortality despite vigorous and expert resuscitation (Pumphrey 2000). If an adverse reaction to the first vial of antivenom has not occurred, subsequent vials do not need to be preceded by epinephrine. The reaction rate to polyvalent antivenom is higher than to monovalent antivenoms and should not be used when a monovalent antivenom or combinations will suffice.

The antihistamine promethazine is ineffective in this setting (Fan et al. 1999) and contraindicated because it may cause obtundation and hypotension, both of which may exacerbate and confound a state of envenomation. Other drugs such as steroids and aminophylline are also not useful in preventing anaphylaxis because their actions, apart from being unproven, are too slow in onset, but steroids are useful for preventing serum sickness.

Dose

The dose of snake antivenom has always been uncertain and controversial. There has been a tendency for clinicians to administer increasing larger doses of antivenom but recent studies of case series of victims in the Australian Snakebite Project (ASP) have prompted a call to restrict the dose to one vial in all cases of envenomation.

On the basis that a median dose of one vial of antivenom clears the blood of circulating procoagulant toxins, the authors of the Australian Snakebite Project (ASP) study of 715 envenomated victims (2005–2015) have proposed that one vial of the relevant antivenom is a sufficient dose for all victims of snakebite (Johnston et al. 2017; Isbister et al. 2013a). However, rigid adherence to this "one vial policy" has a number of problems including the lack of knowledge of the effects of antivenom on multiple venom toxins other than clearance of procoagulants from blood and is meaningless for neurotoxic and myotoxic symptoms. The kinetics of the neurotoxins and myotoxins are unlikely to be those for procoagulants.

The "one vial policy" has been criticized by some clinicians as a "precariously narrow clinical strategy" (Ou et al. 2015) but without detailing well the reasons for their opinion.

Arguably, the neutralization dose of antivenom should be regarded as the maximum dose beyond which no further improvement is gained but that is difficult to derive theoretically and difficult to derive during the practical clinical management of an individual victim because the effects of antivenom are not instantaneous. This applies to all effects of envenomation, including coagulopathy due to procoagulopathic toxins which cause consumption of coagulation factors. Such coagulopathy will not resolve for approximately 6 h while coagulation factors are remanufactured, provided adequate antivenom has been administered.

The neutralization dose for a particular victim by a particular snake is uncertain for numerous reasons. Above all is the fact that the neutralization doses for all effects of venom toxins in humans are unknown. Antivenom is tested in small animals and does determined to prevent death. However the neutralizing dose in vivo in humans cannot be extrapolated accurately from such data. Units of antivenoms are standardized per weight of venom: 1000 units neutralizes 10 mg of venom.

The yield of venom from "milking" is very variable. Mirtschin et al. (2006) recorded that the average dry weight yields of venom from South Australian Tiger snakes (*Notechis scutatus*) is 34 mg (SD 26 mg, maximum 336 mg) and from Victoria is 33 mg (SD 25 mg, maximum 224 mg). Similarly, the average dry weight yield from South Australian Common Brown snakes (*Pseudonaja textilis*) is 8 mg (SD 5 mg, maximum 51 mg), while the Queensland specimens yielded an average of 26 mg (SD 20 mg, maximum 155 mg). Taipans (*Oxyuranus scutellatus*) yielded an average of 146 mg (SD 102 mg, maximum 882 mg). Since one vial of Tiger snake antivenom (3000 units) theoretically neutralizes 30 mg of venom, one vial of Brown snake antivenom (1000 units) theoretically neutralizes 10 mg of venom and one vial of Taipan antivenom (12,000 units) neutralizes 120 mg, the likelihood of undertreatment with one vial for envenomation by these species, if based on "milking" results, is obviously sizeable.

The amount of venom delivered during an actual bite is always unknown and experimentally it is quite variable (Morrison et al. 1982, 1983). Moreover, snakes may bite multiple times and absorption of toxins from the bite site or sites of sequestration is probably a continual process, implying that initial several vials and serial administration of antivenom may be required. Morrison et al. (1982, 1983) proffered freshly killed mice to snakes and then determined the amount of venom injected. The median amount injected by the first bite of Common Brown snakes was 3.8 mg but the range was 0.05–9.5 mg. Similarly, the median amount injected by Tiger snakes was 3.4 mg with a range of 0.003–36.7 mg and the median by Taipans was 19.8 mg with a range of 0.2–119 mg. On this basis the contents of one vial, each antivenom would be expected theoretically to neutralize several times over the median amount of venom injected experimentally at a first bite by respective species but the maximum dose injected in all cases is very close to the maximum neutralization capability, provided that neutralization doses are the same for humans as they are for small animals. There is no margin for error in massive envenomation as occurs in multiples bites, bites by large specimens of snake, bites in which the snake must be forcibly removed or when the victim is unusually susceptible to envenomation.

The "one vial policy," derived from the ASP study, is based on the median dose derived from all degrees of envenomation by all snakes. This is intrinsically problematic and unsafe: it should rather be based on the upper dose range or maximum effective dose. According to the study, although less antivenom was used in 2014/2015 compared with 2005 with no differences in mortality, the upper quartile ranges of antivenom doses used in 2014/2015 were actually considerably above the median dose used. In 2014/2015, the upper quartile dose for envenomation by Brown snakes was approximately 1.5 vials, for Tiger snakes 2.0 vials, and for Taipans 1.5 vials (together comprising 61% of envenomations) (Johnston et al. 2017). These observations alone suggest that the neutralizing doses of antivenom for bites by these snakes are more than one vial. Only in bites by Death Adders, Mulga snakes and Red-bellied Black snakes (comprising 23% of envenomations) did the upper quartile range equate to the median dose – which is not surprising for Black snake bites since bites by these cause anticoagulant coagulopathy – an effect which is readily reversed with antivenom and measurable.

Determination of the neutralization dose in humans is also confounded by a lack of knowledge of which toxins and to what degree they are neutralized by antivenom antibodies. The pharmacokinetics of tissue-bound toxins including the possible release of toxins, penetration of antivenom to tissues, possible sequestration of toxins, and the effect of the timing of antivenom on bound toxins in tissues are simply unknown and require research. Victims may present late after envenomation when some toxins have already become bound to target tissues and cannot be easily neutralized or tissues are already damaged. Some victims in such circumstances have required mechanical ventilation for many weeks despite large amounts of appropriate antivenom.

Moreover, it is difficult to reconcile recommendations of authors of the ASP study with the clinical outcomes of the victims in the study. For example, although one vial

of antivenom "cleared the blood of venom," the effects on victims with procoagulopathy after Brown snake envenomation suggest a more cautious approach than simply recommending one vial for every patient for all degrees of envenomation by all snakes. Of 136 victims with procoagulant coagulopathy, 6 (4%) died, 7 (5%) had a cardiac arrest, 5 (4%) had major hemorrhage, 15 (11%) had thrombotic microangiopathy, and 37 (27%) had hypotension (Allen et al. 2012). It is impossible to discern if this morbidity and mortality was due to procoagulant coagulopathy alone or caused by other untreated effects of multiple toxins in venom. The neutralizing doses for all effects of venom are simply unknown. In any case, it is difficult to draw valid conclusions from uncontrolled case series which although of high quality in the instance of ASP study is actually very low or low on any scientific scale of evidence, such as with the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) system.

Against a "one vial policy," the manufacturers of the antivenoms (bioCSL Pty Ltd) do not support dosing with one vial and recommend higher doses – but based only on the consensus opinion of an expert committee (White 2013). It should be taken into account that in a study of monovalent antivenom batches (admittedly some outdated), the potencies of antivenoms were quite variable with some considerably below and others little above their specified neutralization content: Brown snake 0.6–1.3, Tiger snake 0.6–1.2, Taipan 0.9–1.0, Death Adder 0.3–1.0, and Black snake 0.8–1.0 (O'Leary and Isbister 2009).

Whatever is adopted as a dosing policy, it cannot relate to serum sickness which occurs whatever the dose (Ryan et al. 2016) nor can it relate to adverse reactions which almost invariably occur with the first dose of antivenom.

In desperate circumstances of life-threatening envenomation, seemingly unresponsive to antivenom, it is difficult for a clinician to differentiate organ dysfunction which could be reversed with antivenom and dysfunction which cannot lead to over-treatment. At present, clinicians managing a human victim of envenomation simply cannot know whether or not additional doses of antivenom will improve or reverse tissue effects.

Contrary to the recommendations derived from the ASP study, hospitals should stock at least two vials of appropriate antivenoms and clinicians should be prepared to administer two vials at the outset of treatment and administer additional vials if clinically indicated. As a general rule, two vials of antivenom would probably suffice but less or more may be indicated according to the snake involved (species, size, location), number of bites, and the effects on the victim. The preparedness should always be to treat life-threatening envenomation with some margin of safety. Undertreatment may be fatal while overtreatment with more than one vial, although expensive, is otherwise probably not harmful and may be attributable to a lack of understanding of the incapability of antivenom to restore established organ dysfunction and lack of knowledge of the time required for the hepatic manufacture of consumed coagulation factors.

Too many uncertainties render a "one vial policy" unsafe and unreasonable. The initial appropriate dose of antivenom for envenomation should be two vials of an appropriate monovalent or the polyvalent antivenom. Less antivenom may be

sufficient for minor envenomation but clinicians should aim to administer two vials at the outset of treatment of envenomation and to administer more antivenom if clinically indicated.

Administration

The decision to administer antivenom must be based on clinical criteria of envenomation, and not restricted to the result of a VDK test. A positive VDK test of a biological sample confirms a diagnosis of envenomation and assists the choice of antivenom if required, but does not imply that antivenom should or should not be given. Clinically, VDK tests have a false positive rate and may lead to antivenom being administered to a nonenvenomated victim with a risk of anaphylaxis (Johnston et al. 2017).

If the victim is significantly envenomated, antivenom must be administered as there is no other effective treatment. Snake antivenoms must be given by the intravenous route or in dire circumstances if a vein cannot be cannulated, by the intraosseous route. The large volume of fluid and slow absorption render the intramuscular route useless in emergencies.

A test dose of antivenom to determine allergy should not be done. It is unreliable and a waste of precious time.

The antivenom may be injected or infused slowly into a running intravenous line or diluted in Hartmann's or other crystalloid solution in approximately 1 in 10 volumes in a burette and administered over 15–30 min if the situation is not critical. This method may reduce the risk of an anaphylactoid reaction resulting from its binding with complement. For small children, if multiple vials are required, the dilution may be less to prevent excessive fluid administration. In emergencies, the antivenom may be infused quickly in high concentration.

Adverse Reactions

Antivenom infusion should be administered in a location equipped and staffed by personnel capable of managing anaphylaxis. Intramuscular epinephrine is the key treatment in a dose of approximately 0.25–1.00 mg for adults and 10 μg/kg for children. Antivenom therapy should be discontinued temporarily and restarted when the victim's condition is stable.

Lesser degrees of immediate adverse reaction restricted to headache, chest discomfort, fine rash, arthralgia, myalgia, nausea, abdominal pain, vomiting, and pyrexia may be managed by temporary cessation of infusion and administration of steroids and antihistamine before recommencement.

A delayed hypersensitivity reaction, serum sickness, should be anticipated and patients warned of the symptoms and signs, which usually appear several days to 2 weeks after antivenom administration. Severity may range from a faint rash and pyrexia to serious multisystem disease including lymphadenitis, polyarthralgia, urticaria, nephritis, neuropathy, and vasculitis. The incidence of serum sickness is not dependent on the dose of antivenom administered and has an incidence of about 30% (Ryan et al. 2016). It is, therefore, reasonable to administer prophylactic treatment, a course of steroids (e.g., prednisolone 1 mg/kg per day for 5 days), to every patient treated with any dose of antivenom.

Withholding Antivenom

If a coagulopathy or systemic symptoms or signs of envenomation are present, specific monovalent antivenom should be administered after identification of the species or as indicated by a VDK test. If the victim has had no symptoms or signs of envenomation and only a mild coagulopathy is present, as for example achieved with warfarin therapy, it may be acceptable to withhold antivenom in the anticipation of spontaneous resolution, but coagulation should be checked at intervals and the victim maintained under surveillance until coagulation is normal. Antivenom may be withheld if envenomation is so mild that spontaneous recovery may occur or the consequences of antivenom administration are likely to outweigh the benefit to be gained (e.g., in a herpetologist with minor effects and known to have an allergy to antivenom).

Investigations and Monitoring

Tests should be performed regularly, interpreted quickly, and treated promptly to counter venom effects and its complications. Serial coagulation tests and tests of renal function are especially important. Absorption of venom from the bite site is a continuing process that should be anticipated. Apart from regular monitoring of vital signs and oxygenation, the following are specifically needed.

Bite Site

A swab for venom testing should be done. It has the highest likelihood of detecting venom provided the site has not been washed. The bite site may be squeezed to yield venom if it has been washed. A positive result identifies venom but does not prove envenomation.

Urine

Test the urine for venom that may be present when venom in blood has been bound by antivenom and is therefore undetectable. Urine should also be tested for blood and protein. If the urine is pigmented, a distinction should be made between hemoglobinuria and myoglobinuria, which is impossible with simple ward tests. Hourly urine output should be monitored.

Blood

- Coagulation tests should include prothrombin time, activated partial thromboplastin time, serum fibrinogen, and fibrin degradation products. Point-of-care INR testing machines yield unreliable results in snakebite coagulopathy (O'Rourke et al. 2013) and should not be used.
- A full-blood examination and blood film for hemoglobin level, evidence of hemolysis, and platelet count. A mild elevation in white cell count is expected.
- Electrolytes, urea, creatinine, and creatine phosphokinase (isoenzymes and troponin are useful) to monitor rhabdomyolysis and possible renal compromise.

Electrocardiogram

Sinus tachycardia, ventricular ectopy, and ST segment and T-wave changes are not uncommon. These effects may be the direct result of venom toxins or from electrolyte disturbances caused by rhabdomyolysis or renal failure.

Secondary Management

Coagulation Factor and Blood Transfusion

Although coagulopathy often resolves after antivenom it does not restore coagulation per se – it permits newly manufactured coagulation factors to act unopposed by venom. This is not failure of antivenom therapy. If hemorrhage is occurring, or if coagulation is not restored after several doses of antivenom over several hours, it is prudent to administer fresh frozen plasma (FFP) and to remeasure coagulation at intervals. FFP administered 6 h after antivenom restored clotting function in most victims (Isbister et al. 2013b). Because regeneration of coagulation factors takes many hours, the treatment of isolated coagulopathy entirely with antivenom while waiting for their regeneration exposes the patient to serious haemorrhage. Administration of coagulation factors, such as in the form of fresh frozen plasma, should be preceded by antivenom to neutralize venom prothrombin activator as otherwise consumption coagulopathy may worsen. Platelets may be required but whole blood is rarely needed.

Intravenous Fluids, Rhabdomyolysis, and Renal Protection

After acute resuscitation, administer intravenous fluids in sufficient volume to maintain urine output at about 40 mL/kg per day in an adult and 1–2 mL/kg per hour in a child to prevent tubular necrosis as a consequence of rhabdomyolysis. Life-threatening hyperkalemia and hypocalcemia may develop with rhabdomyolysis. Renal failure may also be secondary to thrombotic microangiopathy. Hemofiltration or dialysis may be required.

Heparin

Although this anticoagulant has prevented the action of prothrombin activators in animal models of envenomation, it does not improve established consumption coagulopathy. It is not recommended. Emphasis instead should be on treating the cause by neutralizing venom with antivenom.

Analgesia and Sedation

Australian snake bite may cause pain and although not usually severe, may require analgesia. However, sedation is always required for the mechanically ventilated venom-paralyzed victim and analgesia for rhabdomyolysis.

Care of the Bite Site

Usually, no specific care is required. Occasionally the site may blister, bruise, ulcerate, or necrose, particularly when a first-aid bandage has been in place for a considerable time or when the bite was by a member of the Black Snake genus, such as a Mulga Snake or Red-bellied Black Snake.

Other Drugs

Antibiotics are not routinely required but should be considered as for any potentially contaminated wound. Sea-snake bites may cause Gram-negative infections. Tetanus prophylaxis should be reviewed.

Bites by Exotic Snakes and Uncommon Australian Snakes

Zoo personnel, herpetologists, and amateur collectors who catch, maintain, or breed endogenous snakes or who import or breed exotic (overseas) snakes are at risk, as are personnel in the Australian Quarantine and Inspection Service (AQIS) who encounter exotic species. Specific antivenoms to the venoms of uncommon Australian snakes do not exist, but neutralization is provided by polyvalent antivenom or by monovalent antivenom, as indicated by the VDK.

Exotic snake antivenoms are maintained by some institutions including Royal Melbourne Hospital (tel: +61 3 9342 7000); Royal Adelaide Hospital (tel: +61 8 8222 4000); Ballarat Hospital (tel: +61 3 5320 4316); Venom Supplies Ltd, Tanunda, South Australia (tel: +61 8 8563 0001); Australian Reptile Park (Tel: +61 2 4340 1022); and Taronga Zoo (Mosman, tel: +61 2 9978 4757). Usually, administration of exotic snake antivenom to a victim is delayed compared to endogenous snakebite. Protocols to ensure timely management should be in place at zoos and hospitals (Othong et al. 2012).

Long-Term Effects of Snakebite

After appropriate timely treatment, recovery is expected but it may be slow, taking many weeks or months, particularly from a critical illness or after delayed presentation involving neurotoxicity and rhabdomyolysis. Isolated neurological or ophthalmic signs may persist. Long-term loss of taste or smell occurs occasionally. The psychological effects of snakebite, including depression and posttraumatic stress disorder, may necessitate expert assistance.

Conclusions and Future Directions

Australian snake venoms are complex mixtures of numerous toxins. Only a relative few toxins have been researched and even those incompletely. Presysnaptic phospholipase A_2s destroy the motor nerve terminal and cause rhabdomyolysis. Procoagulant serine proteinases consume coagulation factors exposing the victim to spontaneous hemorrhage and causing thromboembolism which probably results in acute cardiovascular collapse and renal failure. More work is needed on identifying toxins in venoms and understanding their modes of action. The pharmacokinetics and pharmacodynamics of snake venom toxins on organ systems, particularly on

their excitable membranes is known or understood only at a basic level. Australia is fortunate to have efficacious and effective snake antivenoms but all aspects of antivenoms: their production, their efficacy against individual toxins and effects, and their use are in need of further study. Lastly, potential alternative or additional forms of treatment such as Suramin, anticholinesterases for postsynaptic neurotoxins and synthetic polymer nanoparticles which can neutralize and sequester PLA_2 from snakes and bees (O'Brien et al. 2016) require investigation and development.

Cross-references

- ► Exotic Envenomation in the United States
- ► Injury and Envenomation by Exotic Snakes and Other Venomous Pets in Europe
- ► North American Coral Snake Envenomation

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Spider Envenomation in Australia

8

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Contents

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Abstract

Known species of spiders number tens of thousands worldwide, grouped into over a hundred families and nearly 4000 genera. Millions of toxins are estimated to exist in their venoms but only a very small fraction, several hundred, has been identified and characterized. Protein toxins of lethal potential to humans are present in venoms of Australian genera in the family Hexathelidae (*Atrax* and *Hadronyche,* Funnel-web spiders), in the family Actinopodidae (*Missulena,* Mouse spiders), and the one species of the family Theridiidae (*Latrodectus hasselti,* Red-back spider). The principal toxins in *Atrax* and *Hadronyche* venoms and probably in *Missulena* venom are δ-hexatoxins (42-residue peptides) which slow the inactivation of voltage-gated sodium channels, cause repetitive action potentials in neural tissue, and subsequent uncontrolled release of neurotransmitters with characteristic clinical syndromes of envenomation. An effective antivenom is available. These toxins have a characteristic internal cystine knot motif conferred by disulfide bonds. Other small peptides in funnel-web spider venoms, ω-hexatoxins, target insect voltage-gated calcium channels while κ-hexatoxins target insect calcium-activated potassium channels. The principal toxin in *Latrodectus* venom is a 1180-residue protein (α-latrotoxin), which forms a tetramer on nerve ending membranes creating pores for the ingress of calcium and subsequent exocytosis of synaptic vesicles and release of transmitters. Internal calcium stores are also released. Envenomation causes a universally recognized constellation of symptoms and signs, "latrodectism" which is effectively treated with antivenom. The syndrome also results from envenomation by *Steatoda carpensis* (a Cupboard spider), another of the Theridiidea family.

Introduction

Spiders are arthropods in the Class Arachnida and Order Araneae. The number of species is not accurately known but is immense. In 2014, nearly 45,000 species of spiders in 3935 genera and 114 families were known to exist worldwide (Platnick 2014). The estimated number of species is approximately 170,000 (Coddington and Levi 1991). In Australia, approximately 2000 species comprise 22 families. With the exception of one herbivorous species, all spiders are predators and kill their prey, mainly insects, with venom and then liquefy it with digestive enzymes before consummation.

Spider Toxins

Scientific clinical investigation of the venoms and their toxins of spiders has mainly concerned about 100 species in 7 genera. These are *Photoneutria* (Brazilian armed or wandering spider), *Atrax,* and *Hadronyche* (Australian funnel-web spiders, family Hexathelidae), *Loxosceles* (American brown spiders), *Chilobrachys* (Chinese tarantulas), *Latrodectus* (Widow and red-back spiders, family Theridiidae), and *Agelenopsis* (American funnel-web spider).

Research into spider toxins is driven by numerous aims: to derive treatments for cases of human envenomation; to understand the structure and function of ion channels in biomembranes; to understand the process of exocytosis and release of neurotransmitters; to derive new therapeutic drugs; and to derive effective antisecticides for agricultural industries and for control of insects transmitting human diseases.

The number of spider bioactive peptides in venom is estimated conservatively at over 10 million with only a small fraction thus far characterized. At present, the spider toxin database, Arachnoserver (www.arachnoserver.org) lists 1544 toxins from 100 spider species of which 117 toxins originate from the Hexathelidae family and 469 toxins from the Theraphosidae family.

Although this chapter focuses on dangerous Australian spiders, it refers to non-Australian species and occasionally to scorpions to illustrate various properties of spider venoms. In order to discuss Hexathelidae spider toxins and human envenomation, it is necessary to briefly review ion channels which are targeted by toxins and the nomenclature of toxins.

The Targets of Toxins: Ion Channels

Excitable biomembranes have protein channels embedded in their phospholipid structure for the passage of ions. The channels open and close in response to changes in membrane voltage initiated by interaction of peptide toxins with the protein of the

ion channels and lead to the perturbations in action potentials. At least 9 subtypes of voltage gated sodium channels have been identified ($Na_v1.1$–$Na_v1.9$) for conductance of sodium ions. Their location in mammalian tissues varies. Subtypes $Na_v1.1$–1.3 are located in the central nervous system, $Na_v1.4$ and $Na_v1.5$ are located in skeletal and cardiac muscle, respectively, and $Na_v1.6$–$Na_v1.9$ are located in the peripheral nervous system. In contrast, insects, the main prey of spiders, have only one subtype, the *para*-Na_v channel which has about 50–60% identity with the various mammalian subtypes. (The naming of the insect channel refers to discovery of a mutant insect channel causing reversible temperature-sensitive *paralysis* in *Drosophila melanogaster,* fruit fly.) The channel subtypes have similar architecture with 4 homologous domains each containing 6 transmembrane segments of which 4 segments form the voltage sensor and 2 determine the selective pore of the channel. Several sodium channel subtypes ($Na_v1.5$, $Na_v1.8$, and $Na_v1.9$) are resistant to blockade by tetrodotoxin with the remainder sensitive. Different spider toxins target different sites of specific sodium channels and have different effects on the action potential.

The potassium channels of biomembranes also consist of 4 identical domains each having a voltage sensing domain and a region which forms the pore-forming domain.

Whereas vertebrates have a large range of subtypes of protein calcium channels (for example, L-type Ca_v1, N-type $Ca_v2.2$), those of insects are limited in subtype to just 3 (Ca_v1–3). While the identities of insect channels in different insect species are quite similar, their identities with various human subtypes are less. For example, human $Ca_v3.1$, the closest human ortholog, has only 66% identity of *Drosophilia melanogaster* Ca_v3 (King et al. 2008). It would be expected that spider toxins targeting insect calcium channels would have limited effect on human calcium channels.

Animal venom peptides primarily modulate sodium channels by either physically blocking the pore or by binding to an allosteric site which induces a conformational change which in turn alters the equilibrium between the open, closed, or inactive state (Nicholson 2007). Spider venom toxins are all allosteric modulators but different spider venoms modulate in different ways. Based on their primary structure and cystine scaffolds, spider venom toxins comprise at least 12 families of Na_v channel toxins and estimated to comprise more than 10 million bioactive peptides of which only a minute proportion have been characterized (Klint et al. 2012).

Although most spider toxins, from diverse families, primarily target sodium channels they do have some activity on other ion channels, that is, potassium and calcium channels. The sodium channel toxins are peptides generally of 30–40 amino acids with the core of the molecule stabilized by 3–4 disulfide bridges creating the so-called inhibitory cystine knot (ICK) motif (Bosmans and Swartz 2010). The toxins probably interact with the voltage sensors of the membrane ion channels after partitioning into the lipid membrane and binding to the domains at the protein-lipid interface.

Other spider toxins, “vanillotoxins,” also target the “Vanilloid receptor 1” or “Capsaicin receptor” (Transient receptor potential cation channel subfamily V

member 1, TRPV1), an excitatory channel expressed by sensory neurons in pain pathways. First identified in venom of the West Indian "Trinidad Chevron" tarantula, *Psalmopoeus cambridgei* (Siemens et al. 2006) and in that of the Chinese bird spider, *Ornithoctonus huwena* (Bohlen et al. 2010), these toxins are also of 30–40 amino acids with a typical inhibitory cystine knot motif and have some inhibitory activity on voltage-gated potassium channels.

Nomenclature of Spider Toxins

The naming of animal toxins (snakes, spiders, scorpions, sea anemones) is bewildering and likely to become incomprehensible considering that venoms, including spider venoms, contain very numerous toxins of which only a small fraction have been identified. Consequently, King et al. (2008) proposed a new system based on the main target of the toxin and its taxonomic origin including the family, genus, and species of the animal. In essence, the family abbreviation of the animal is preceded by a Greek letter (upper or lower case) indicating biological function (e.g., α = targets alpha receptor; ω = inhibits voltage-gated calcium channel (Ca_v); δ = delays inactivation of voltage-gated sodium channel (Na_v); κ = inhibits voltage-activated potassium channel (K_v); λ = inhibits calcium-activated potassium channel (K_{Ca})) followed by the first letters of its genus and species name of the animal. Where applicable this system is used in this chapter. Thus, for example, the toxin δ-HXTX-Ar1a from *Atrax robustus* indicates that this toxin (TX) slows sodium channel inactivation (δ) and it originates from the Hexathelidae family of spiders whose genus is *Atrax* (A) and whose species is *robustus* (r). The terminal numeral (1) and lower case letter (a) distinguishes it from other toxins from the same animal.

Therapeutic Uses of Spider Toxins

Although about 10 therapeutic drugs in regular use are derived from animal toxins, the earliest being captopril, an angiotensin converting enzyme inhibitor derived from a pit-viper venom, none are as yet derived from spider venom toxins. However, numerous additional animal toxin-derived drugs are in preclinical studies including one from the Trinidad chevron Tarantula spider which targets acid sensing ion channel (ASIC)1a and is being considered for the treatment of chronic pain (King 2011). Other spider toxins hold promise such as the toxin μ-TRTX-Tp1a from a Peruvian tarantula, *Thrixopelma pruriens*, that inhibits human $Na_v1.7$, a channel responsible for pain sensation (Cardoso et al. 2015). Another is a family of 4 peptides each of 75–77 residues (Hi1a-d) in *Hadronyche infensa* venom which have marked similarity with PcTx1, the prototypical ASIC inhibitor, PcTx1, from the unrelated tarantula spider *Psalmopoeus cambridgei*. Hi1a comprises two tandem PcTX1-like sequences in two homologous inhibitor cystine knot motifs. It protects the rat brain from neuronal injury after stroke (Chassagnon et al. 2017).

Spider toxins may become used as insecticides incorporated into the food of insects. For example, the cloned toxin Magi 6 from *Macrothele gigas* (Japanese funnel-web spider) incorporated into the tobacco genome by using tobacco mosaic virus is an effective defence against herbivorous insects (Hernandez-Campuzano et al. 2009).

Australian Funnel-Web Spiders, Genera *Atrax, Hadronyche,* and *Illawarra*

These spiders of the family Hexathelidae number 35 classified species which are distributed along the eastern coast of Australia including Queensland, New South Wales, Victoria, and Tasmania and the peninsula Gulf areas of South Australia (Gray 2010). Others remain to be classified. Not all classified species are known to be venomous to humans but serious illness or death has resulted from bites by *A. robustus* (Sydney funnel-web), *H. cerberea* (Southern tree funnel-web), *H. formidabilis* (Northern tree funnel-web), *H. infensa* (Darling Downs funnel-web), *H. macquariensis* (Port Macquarie funnel-web), and *H. versuta* (Blue Mountains funnel-web). These spiders are essentially ground-dwellers, occupying burrowed retreats in sheltered microhabitats such as beneath logs and rocks and inside decayed logs and tree stumps and natural soil crevices. Several species of *Hadronyche* are associated with standing trees, but they are not confined to this habitat. These spiders construct loosely structured funnel-shaped or tube-shaped webs with lateral "trip-wires," When alerted, the spider rushes out to capture and kill prey, usually beetles and other insects but sometimes small reptiles. They are aggressive, tending to rear up on their rear legs in a threatening display with venom visibly accumulating on the tips of large heavy fangs.

The funnel-web spiders have an evil reputation and are probably the most venomous spiders worldwide. However, most of the recognized species are not known to have caused significant human illness. This is particularly true of the smaller species, which are represented in parts of Victoria, Tasmania, and South Australia. To date, life-threatening envenomations by these spiders have been restricted to areas of New South Wales and southern Queensland. Thirteen deaths have been recorded and have been limited to New South Wales (Sutherland and Tibballs 2001). None have occurred since a funnel-web spider antivenom became available in 1980. This does not mean that further deaths will not occur, especially in infants, since the onset of the syndrome of envenomation can be very rapid and it may not be possible to administer antivenom in time. The importance of first aid in funnel-web spider bites cannot be overstressed.

Identification of these spiders is difficult since there are a number of other genera of large dark-colored (black/dark brown) spiders inhabiting the same territory as funnel-webs. In the three species of *Atrax* (*A. robustus*, *A. yorkmainorum*, A. *sutherlandi*) the second leg of the male spider has a prominent tibial spur (conical apophysis), but only *A. robustus* is regarded as highly venomous to humans. Several of the male *Hadronyche* species (*H. cerberea, H. versuta, H. emmanalizae,*

H. formidabilis, H. alpina) also have such a spur but it is less prominent than in the *Atrax* species. Some of the *Hadronyche* species are also highly venomous. The purpose of the spur is to lock the female's second leg in a raised position to enable mating and in so doing prevents descent of the female's fangs. The observation of the presence of a spur is of some use from a clinical point of view. If a spur is prominent, the spider is quite possibly a dangerous male *Atrax robustus.* However, if the spur is small or absent it does not guarantee that the spider does not belong to another highly dangerous species. An excellent guide to funnel-web spider identification is by Gray (2010).

Toxins

Early efforts to identify funnel-web toxins were carried out on the venom of *Atrax robustus*, particularly the venom of the male spider. However, these efforts were hampered by the relative resistance of lower order laboratory animals to *A. robustus* venom, its tendency to adhere to laboratory glassware, the unusual fact that the venom of female spiders was about 10 times less toxic than that of male spiders and the variation in toxicity from specimens of the same species collected from different geographical areas. Curiously, new-born mice and monkeys are very susceptible to venom and have been successfully used in research.

Effects in Monkeys

The syndrome of envenomation in monkeys is very similar to that in humans who have a particular vulnerability to *A. robustus* venom. Sutherland (Sutherland and Tibballs 2001) found that *Cynomolgus* monkeys invariably died within 4 h of receiving 3 mg/kg of female venom intravenously and not infrequently died within 8 h after the same dose injected subcutaneously. A dose of male venom 0.5 mg/kg given intravenously or subcutaneously almost invariably resulted in death. Male venom at a dose of 0.2 mg/kg either intravenously or subcutaneously frequently resulted in death. Unless the monkey was intubated and the pharynx kept free of secretions, the animals sometimes died during the first 10 min from asphyxia due to laryngospasm and/or inhalation of secretions.

In closely monitored monkeys, Duncan et al. (1980) determined the principle actions of the venom with the principle aim to determine what intensive treatment in humans would be beneficial since at the time it was not considered feasible to produce an antivenom and clinical treatment to date had been somewhat empirical. The gross features of the syndrome were similar to those described by other workers. Lacrimation, salivation, and fasciculation occurred within a few minutes of the intravenous injection and within 20 to 25 mins of the subcutaneous injection. After the subcutaneous injection, the fasciculation occurred locally at the site of the injection almost immediately, then gradually spread to involve the whole limb before becoming generalized. Facial and tongue fasciculation were the first signs of

intravenous envenomation. An alteration in conscious state occurred within minutes of the intravenous injection. Some monkeys developed pinpoint pupils, while the pupils of others became dilated, eccentric, and nonreactive. Piloerection was a feature in all cases. All the six monkeys which received venom intravenously died.

The cardiorespiratory effects of venom were dramatic. Initially, hypotension occurred within 30 to 60 sec of intravenous injection and at 5 to 10 min after subcutaneous injection. Thereafter, the blood pressure steadily increased to a hypertensive levels at 14–17 min after intravenous injection and at 60 min after subcutaneous injection. The hypertension was maintained for several hours but abated to normal in survivors and fell to hypotensive levels in nonsurvivors. Disturbances of cardiac rhythm were observed: usually sinus tachycardia accompanied by ventricular ectopic beats or bigeminy and transient second degree atrioventricular block. Overt pulmonary edema occurred in some monkeys during the acute hypertensive phase but in the absence of cardiomegaly. The pulmonary edema fluid had a raised total protein content. An acute metabolic acidosis occurred whether the venom was administered subcutaneously or intravenously. The mean base deficit was maximal approximately 1 h after the intravenous envenomation and 90 min after the subcutaneous. Spontaneous resolution of this imbalance occurred and was largely complete, by 4 h after envenomation. A variable degree of respiratory acidosis occurred. A short period of apnoea followed intravenous envenomation, lasting less than 10 min followed by hypoventilation which necessitated mechanical ventilation for several hours. Intracranial hypertension was observed and in the nonsurvivors a low cerebral perfusion pressure culminated in brain death. Hyperthermia, fluid losses, and hemoconcentration were noted but urine output remained satisfactory and tissue edema was absent. Massive rises in creatine kinase levels were recorded along with massive excretion of catecholamines in urine. Although the syndrome could be prevented or largely abolished by treatment with atropine, phenoxybenzanime, and propranolol, the urinary excretion of catecholamines was not affected by this therapy. It was considered that the syndrome was largely caused by release of acetylcholine at autonomic and neuromuscular junctions and by release of catecholamines from autonomic nerves. Although these experiments clarified many aspects of the clinical presentation, some aspects remained unclear. Although a link was made between intracranial hypertension and pulmonary edema, the effect of catecholamines causing heart failure was not at that time contemplated but rather a direct cardiac effect of toxin(s) was considered. In addition, the causes of cerebral depression and intracranial hypertension were unclear. There was no obvious evidence that the venom caused an alteration in capillary permeability. High levels of serum endogenous catecholamines have subsequently been measured in 2 victims of severe envenomation (Isbister et al. 2015).

Identification of Lethal Toxins

Although considerable detail of funnel-web toxins is known, the knowledge probably concerns a mere fraction of the peptides in venom. Palagi et al. (2013), using

mass spectrometry, identified approximately 800 peptides in each venom of female *Hadronyche cerberea* and *Hadronyche infensa* and approximately 400 peptides in venoms of male *Illawarra wisharti* and *Hadronyche cerberea*. Identification of the toxins has been difficult.

δ-Hexatoxins

Struan Sutherland observed that the components of crude venom most toxic to mice and producing a syndrome in monkeys similar to crude venom had molecular weight in range of 15–25 kD when isolated by ultrafiltration membranes. The active component in this fraction was originally designated "atraxotoxin" but subsequently found to have a much smaller molecular weight when derived by Sephadex column fractionation (Sutherland and Tibballs 2001). Subsequently, "atraxotoxin" was renamed "robustoxin" by Sheumack et al. (1985) which in turn was referred to by Little et al. (1998) as δ-atracotoxin-Arl but which is now known as δ-HXTX-Ar1a (King et al. 2008). The common name is "δ-hexatoxin."

Sheumack et al. (1985) isolated "robustoxin" from male *A. robustus* venom and determined the sequence of the 42-residue peptide (MW 5.8 kDa). They found none in female venom. The subcutaneous LD_{50} of the toxin in newborn mice was 0.16 mg/kg and it accounted for 10% by weight of the crude male venom and was responsible for the major effects of the unfractionated venom. It was the first neurotoxin described having disulfide-bridged cystine residues at both the amino- and carboxytermini. It was unusual to find cystine residues in a group of three (at residues (14–16). Pallaghy et al. (1997), using NMR, determined its three-dimensional structure. The four disulfide bonds, exist at residues 1–15, 8–20, 14–31 and 16–42, confer a structure consisting of a small three-stranded, anti-parallel β-sheet and a series of interlocking γ-turns at the C-terminus. It also contains a cystine knot, three distinct charged patches on the surface, and an extended loop with aromatic and nonpolar residues. These are features possessed by phylogenetically diverse venoms and toxins which bind to voltage-gated ion channels (Norton and Pallaghy 1998; Nicholson 2007).

"Robustoxin" (δ-HXTX-Ar1a) has considerable primary sequence homology with "versutotoxin" (δ-HXTX-Hv1a) from *H. versutus* (Nicholson et al. 1996) which is also a 42-residue peptide (Brown et al. 1988). Significant homology has also been found between these two toxins and another 42-residue toxin from *H. versuta* (δ-HXTX-Hv1b) and a 42-residue peptide toxin δ-ACTX-Hs20.1a (δ-HXTX-Iw1a) of *Hadronyche* sp. 20 (*Illawarra wisharti*) (Nicholson et al. 2004). Moreover, these toxins also have structural homologies with the 42-residue neurotoxin δ-mussulenatoxin-Mb1a (δ-MSTX-Mb1a, δ-AOTX-Mb1a) from the Australian eastern mouse spider *Missulena bradleyi* (*vide infra*).

Several decades of research was necessary to understand the mode of action of these toxins which is to change the electrical field in nerve membranes. This produces repetitive spontaneous action potentials in nerves, which in turn leads to muscle fasciculation. These toxins slow sodium channel inactivation to maintain

sodium currents during depolarization (Nicholson et al. 1994, 1996, 1998; Little et al. 1998). The overall effect of this modulation of sodium channel function is to prolong the action potential and initiate spontaneous repetitive firing, leading to transmitter release and paralysis. This is consistent with the muscle fasciculations and autonomic effects, such as lacrimation and salivation noted in envenomated monkeys and humans. The δ-hexatoxin from *Hadronyche versuta* venom is produced by an intronless gene that encodes a prepropeptide that is posttranslationally processed to yield the mature toxin (Pineda et al. 2012). Another δ-hexatoxin from *H. versuta* venom, δ-ACTX-Hv1b (now δ-HXTX-Hv1b), has the same action but is less potent than other δ-hexatoxins and has vertebrate but no insecticidal activity (Szeto et al. 2000).

The family of δ-hexatoxins from Australian funnel-web spiders all comprise 42–44 residue peptides with 4 disulfide bonds of which 3 are arranged in an ICK motif. This motif is structurally similar but not amino acid sequentially similar across a variety of other spider and marine snail toxins (Nicholson 2007; Yamaji et al. 2009). All such toxins from the Australian funnel-web spiders bind to neurotoxin receptor site 3 and exert their effect by slowing inactivation of vertebrate and insect tetrodotoxin-sensitive sodium channels, similar to certain scorpion α-toxins, Magi 1 and Magi 4 toxins from *Macrothele gigas* (Japanese funnel-web spider), Jinghaotoxin-1 toxin from *Chilobrachys jingzhao* (Chinese tarantula), PhTX1–4 from *Phoneutria nigriventer* (Brazilian armed spider), and some sea anemone toxins (Nicholson and Little 2005; Nicholson 2007).

ω-Hexatoxins

The venom of *Hadronyche versuta* contains a family of 37-residue peptides which targets the insect voltage-gated calcium channels but not mammalian calcium channels (Fletcher et al. 1997; Wang et al. 2001; Tedford et al. 2004). Thee toxins cause slow onset of irreversible flaccid paralysis in insects (Fletcher et al. 1997). One of these toxins, ω-ACTX-HV1 (Fletcher et al. 1997), is now known under the nomenclature of King et al. (2008) as ω-HXTX-Hv1a which primarily targets the insect calcium channel Ca_v1 and to a lesser extent insect calcium channel Ca_v2 but has no effect on vertebrate calcium channels (King et al. 2008). The structure of ω-HXTX-Hv1a is that of a β-hairpin protruding from a disulfide-bonded globular core comprising 4 β-turns with the 3 intramolecular disulfide bonds forming an inhibitor cystine knot (ICK) motif, similar to that in other neurotoxic animal peptides. It has very high resistance to heat, organic solvents, and proteinase which is due to its inhibitory cystine knot motif (Herzig and King 2015) while its insecticidal activity is due to its β-hairpin (Tedford et al. 2001).

A similar toxin from *A. robustus*, ω-ACTX-Ar1a (ω-HXTX-Ar1a), a homolog of ω-ACTX-Hv1a (ω-HXTX-Hv1a) from *H. versuta,* while causing a reversible pore blockage of both mid-low- (M-LVA) and high-voltage-activated (HVA) insect calcium channels, also causes a modest block of insect voltage-gated sodium channel but had no activity on voltage-gated potassium channels (Chong et al. 2007).

These "ω-hexatoxins," which target insect voltage-gated calcium channels (subtype Ca_v1), have been identified as multiples orthologs of ω-HXTX-Hv1a in the venoms of other Australian funnel-web spiders including *A. robustus*, *H. infensa* (Toowoomba, Darling Downs, or Port Macquarie funnel-web spider), and *H. venenata* (Tasmanian funnel-web spider). In addition, they are present in the venom-gland transcriptome of *H. modesta* (Victorian funnel-web spider) (Pineda et al. 2014). Orthologs of these toxins also exist in the venom of the Australian eastern mouse spider, *Missulena bradleyi,* but not in any other venomous animal (King 2007).

Another family of ω-hexatoxins exist in Australian funnel-web spider venoms. It is a unique family of 42–45 insecticidal peptides of which the prototype toxin ω-ACTX-Hv2a causes instantaneous but reversible paralysis of insects by blockade of as yet an undetermined subtype of voltage-gated calcium channels (Wang et al. 2001) but likely to be subtype Ca_v2 (King 2007) and it may target vertebrate calcium channel $Ca_v2.1$ (King et al. 2008). These toxins have the classic inhibitory cystine knot motif but have not been identified in any other venomous animal.

Κ-Hexatoxins

Another family of toxins, the "Janus-faced atracotoxins," so-called because of the asymmetrical orientation of their charged residues which recalls Janus, the two-faced Roman god, are specific blockers of invertebrate Ca(2+)-activated potassium channels (K(Ca)). These are 36- or 37-residue peptides with 4 disulfide bridges one of which is vicinal (adjacent) to the other 3 disulfide bridges which endow the molecule with its classical cystine knot motif. The vicinal disulfide bridge is essential for insecticidal activity (Wang et al. 2000; Gunning et al. 2008), initially identified in *H. versuta* venom. These toxins are not homologous with either δ-hexatoxins or ω-hexatoxins. The prototypic member of the toxin family J-ACTX-Hv1c would be named λ-HXTX-Hv1c under the nomenclature system of King et al. (2008) but is referred to as κ-HXTX-Hv1c by Pineda et al. (2014). Orthologs of κ-HXTX-Hv1c were identified in *H. modesta* venom-gland transcriptome but not in those of *H. infensa*, *A. robustus,* or *H. venenata* (Pineda et al. 2014). Like the ω-hexatoxins, the κ-hexatoxins are members of a spider toxin superfamily, consisting of small disulfide-rich peptides, which have evolved from a single ancestral gene, but whereas the ω-hexatoxins are under a positive Darwinian selection, the κ-hexatoxins are under a negative influence (Pineda et al. 2014).

Other Toxins

A 68-residue peptide toxin, ACTX-Hvf17 (HXTX-Hvf17), was isolated form *Hadronyche versuta* venom by Szeto et al. (2000). Its function is unknown since it has no activity on insect or vertebrate ion channels but by its homology to a mamba snake toxin, it may assist in digestion of prey. Another 38-residue peptide with

atypical ICK motif has been isolated from *H. infensa* venom but whose function is unknown (Nicholson et al. 2006).

Genus *Atrax*

The three members of this genus (*robustus, yorkmainorum, sutherlandi*) are confined to the southeastern coast and adjacent highlands. The northern boundary of the genus is the Hunter River in New South Wales and its southern limit extends into the north of eastern Victoria (Gray 2010). They are far more similar in form than is seen among the *Hadronyche* species. For example, all male *Atrax* spiders have a characteristic large conical tibial spur on their second legs (Gray 2010) but of the three, only *A. robustus* is considered extremely venomous.

A. Robustus, the Sydney Funnel-Web Spider

In contrast to other species, the male is more dangerous to humans than the female. Although its range is limited to a radius of some 160 km from the center of the city of Sydney, it has potential contact with a densely populated metropolis. The spider is found as far west as Lithgow in the Blue Mountains. Its northern and southern limits are the Hunter River and the area of the Shoalhaven River, respectively. It is a large black spider and the sexes are distinct in mature specimens. The male is more delicate in build. The spinnerets are long, with the terminal segment characteristically the longest. The massive chelicerae (fangs) are paraxial and, when the spider strikes, the fangs thrust downward inflicting a wound not unlike a snake bite.

Juvenile and mature female spiders build lairs in crevices of rock ledges or in soil around the foundations of houses, but they prefer to enlarge natural cavities like rotting root lines. The white silken web is usually a roughly woven divided tube rather than a funnel and, in fact, “tube-web” would be a more accurate name for this spider. The retreat has radiating irregular trip lines, and contact with which by prey will cause the speedy emergence of the spider. Many spiders may constitute a colony. The diet is mainly beetles and other large insects, usually in proximity of the lair. Hunting occurs at night. The spider strikes viciously at anything moving within range. Winged prey, such as a fly or moth, is knocked down by the front legs of the spider rearing high on its hind legs, but crawling insects are seized by a quick lunge, often after being stalked.

The paired venom glands are curved and flask-shaped, and measure about 5 mm in length. The venom duct is about 4 mm in length and opens at a small orifice dorsally above the tip of the very tough fangs which can penetrate the skulls of small animals.

Both sexes are quite aggressive and, when disturbed, stand on their rear four legs with front legs raised high and chelicerae erect. Visible drops of clear venom appear on the tips of their large fangs.

Genera *Hadronyche* and *Illawarra*

The *Hadronyche*, numbering 31 classified species, are distributed along eastern Australian coast and adjacent hinterland from northeast Queensland to the Gulf Ranges region in South Australia, including Victoria and Tasmania. Like Atrax species, they are mostly ground dwellers but several species are associated with standing trees (*H. formidabilis*, Northern tree funnel-web) and *H. cerberea* (Southern tree funnel-web). They are a diverse group but differentiated from *Atrax* and *Illawarra* genera by subtle anatomical features (Gray 2010) and mass profiles of venom peptides (Wilson and Alewood 2006). Identification and differentiation of one specimen from other species of funnel-web spiders is a very difficult task for nonarachnologists.

Human Envenomation

The outcome of a bite by a male or female *A. robustus* is quite uncertain. Fortunately, no constitutional symptoms develop in the majority of human victims, particularly if they have been bitten by female spiders. However, the bite of a funnel-web spider may be rapidly fatal. The recorded fatalities were probably due to male *A. robustus* (Sutherland and Tibballs 2001) but bites by numerous *Hadronyche* species may also be life-threatening (Dieckmann et al. 1989; Harrington et al. 1999; Miller et al. 2000; Isbister et al. 2005). The dangerous species of *Hadronyche* include: *cerberea*, *formidabilis*, *infensa*, *macquariensis* (previously sp.14), *nimoola* (previously sp. 7), and *versuta*. Most bites occur in the early summer months when the males tend to roam into houses and camps and the spider has sought refuge in clothing or shoes. Multiple bites to the hands may occur in children and infants, when they accidentally pick up an object that the spider is grasping.

Signs and Symptoms

Local Effects

Invariably the bite site of either a male or female spider is extremely painful for hours and even days due to trauma inflicted by the large strong fangs and toxins in the venom. The large size of this spider and its evil reputation may produce fear and variable degree of panic in both the victim and bystanders. Difficulty is sometimes experienced in removing the spider, particularly if the fangs are deeply embedded. No local necrosis of tissues occurs and postmortem studies have not revealed any significant pathology. Local fasciculation could be expected to develop within minutes of the bite, but spread of this could be limited by prompt application of effective first aid.

General Effects

Symptoms of systemic envenomation by either *Atrax* species (Sutherland 1972; Sutherland and Tibballs 2001) or *Hadronyche* species (Harrington et al. 1999; Miller et al. 2000; Isbister et al. 2005) can develop within 10 min if effective first aid measures have not been applied. The symptoms and signs are consistent with the release of neurotransmitters at neuromuscular junctions and from the sympathetic and parasympathetic autonomic nervous system as responses to the effects of toxins on ion channels.

The first systemic symptom is usually numbness around the mouth and spasms and/or fasciculation of the tongue. Ensuing features of the early part of the classical syndrome in humans are nausea and vomiting, abdominal pain, piloerection, profuse sweating, brisk salivation, bronchorrhoea, lacrimation, and severe dyspnea. The victim's mental state may rapidly progress from confusion to being quite irrational or even comatose. Pupillary dilation may be present. Blood pressure initially may be markedly elevated with peripheral signs of severe vasoconstriction evident. Tachycardia and ectopy may be present. Hypotension may occur later in the syndrome. Gross pulmonary edema occurs in most severe cases and was considered by clinicians, e.g., Fisher et al. (1980), to be noncardiogenic in origin. However, high levels of catecholamines may be responsible for development of catecholamine-induced cardiac failure (Takotsubo cardiomyopathy) as reported by Isbister et al. (2015) and observed in other envenomations by scorpions and certain jellyfish (see ▶ Chap. 11, "Clinical Management of Envenomation by Australian Carybdeid Cubozoan, Hydrozoan, and Scyphozoan Jellyfish") by same author in this publication. However, direct effects of toxins on the heart are also possible considering the known effects of toxins on cardiac sodium channels, the increases in creatine kinase in experimental envenomation (Duncan et al. 1980), and the observations of raised levels of creatine kinase and troponin levels in cases of severe human envenomation (Isbister et al. 2015). The precise actions of venom on cardiac function remain to be determined.

Paralysis does not occur, but in serious cases muscle fasciculation causes hypoventilation. Muscle twitching may be prolonged and violent, making the management of the semiconscious patient quite difficult. Usually, induction of therapeutic paralysis with muscle relaxants is required to overcome spasm of the jaw so that an airway can be inserted not only to relieve obstruction of the airway caused by secretions, muscle spasm, and obtunded consciousness, but also to facilitate mechanical ventilation. Profound coma may last for hours and appears unaffected by adequate oxygenation and the maintenance of blood pressure at a normotensive level. The demonstration by Duncan et al. (1980) that envenomated monkeys developed brain death, related to raised intracranial pressure and systemic hypotension, suggests that a similar mechanism operating in humans could in part explain the coma and the development of irreversible brain damage.

Later in the syndrome, the generalized muscle fasciculatory activity continues, but the production of secretions subsides. Marked gastric dilation may be present. Death is due to hypoxemia caused by a combination of hypoventilation, airway obstruction, pulmonary edema, and obtundation of the conscious state. Some victims have died with a progressive hypotension preceding an irreversible cardiac arrest,

despite adequate ventilation. Rarely, a relatively symptomless period may occur in the midst of the syndrome and uneventful recovery may be incorrectly predicted. In small children where the dose of venom per body weight may be much higher, the whole syndrome can progress much faster to death from 15 to 90 min after a bite. Adults who have received an effective bite may die after 30 h or later. No cases of even temporary confusion or coma have been recorded without the victim having some of the characteristic early symptoms of envenomation.The development of a consumptive coagulopathy cannot be attributed directly to the venom but is probably due to prolonged peripheral circulatory failure.

Clinical Management of Envenomation

A seriously envenomated victim of a funnel-web spider bite may survive providing the following are available:

1. Effective first aid
2. Access to skilled medical care with facilities for endotracheal intubation, mechanical ventilation, infusion of potent vasodilators and inotropic agents, and access to extracorporeal life support (these aspects not discussed further)
3. Antivenom

First Aid

Whenever a victim is bitten on a limb by a large dark spider in the known distribution of funnel-web spiders, the pressure-immobilization technique of first aid (Sutherland et al. 1979) should be applied immediately. This technique is known to be effective in preventing onset or progression of the syndrome from experimental studies (Sutherland and Duncan 1980) and from human case reports (Browne 1997; Miller et al. 2000, 2016; Sutherland and Tibballs 2001; Isbister et al. 2005). First aid should not be removed until an intravenous line is in place, antivenom is available, resuscitation equipment ready, and monitoring apparatus connected – all this preferably in an intensive care unit or emergency department. Sudden movement of the venom as occurs when first-aid is removed may precipitate a critical illness. The timing of removal of first-aid is given in Table 1.

Spider Bites Other Than on the Limbs

These are uncommon even though two fatal cases resulted from bites to the trunk: one to the breast and the other to the buttocks (Sutherland and Tibballs 2001). Both cases were adults, who reached hospital in good condition. Even if pressure can safely be applied to the bitten area, it is not certain that it alone, without immobilization, is of much value.

Table 1 Emergency initial management of funnel-web spider bites

Type of first aid given before arrival	Action if asymptomatic	Action if symptomatic[b]
None	Observe for 4 h	Apply pressure-immobilization bandage Resuscitate and treat symptoms Source and administer antivenom urgently
Pressure-immobilization bandage	Source antivenom Release bandage Observe 4 h Treat symptoms if they develop	Leave bandage in position Resuscitate and treat symptoms Source and administer antivenom urgently
Arterial tourniquet[a]	Source antivenom Release tourniquet Observe 4 h Treat symptoms if they develop	Maintain tourniquet if not harmful, otherwise Apply pressure-immobilization first aid Resuscitate and treat symptoms Source and administer antivenom urgently

[a]Not recommended for first aid, but still often employed
[b]Admit all symptomatic victims to intensive care if available

Antivenom and Administration

Indications for Antivenom

There is no doubt that an antivenom manufactured by Commonwealth Serum Laboratories (CSL) Pty Ltd. is effective. It was developed by the late Dr. Struan Sutherland and made available in late 1980 and quickly proved effective in treating envenomation (Fisher et al. 1981). Since introduction of antivenom, no deaths have been recorded from funnel-web spider envenomation, and the clinical response to its administration is both rapid and complete (Isbister et al. 2005).

Urgent administration of antivenom is required if there is evidence of envenomation by a funnel-web spider. The presence of *any* of the following signs or symptoms indicates that significant envenomation has occurred:

1. Muscle fasciculation in the limb involved or remote from the bite, usually first seen in tongue or lips with systemic spread of venom
2. Marked salivation or lacrimation
3. Piloerection
4. Significant tachycardia
5. Hypertension in a previously normotensive patient (late in the syndrome the victim may become hypotensive)
6. Hypoxemia, hypoventilation, dyspnea, pulmonary edema
7. Disorientation, confusion, or a depressed level of consciousness

In most cases of bites by funnel-web spiders, little or no venom is injected and no illness occurs. As a generalization, the victim either develops no illness or the full severe syndrome.

Premedication

Despite a small incidence of adverse reactions, no premedication is indicated. Pretreatment with a steroid (hydrocortisone sodium succinate 100 mg or equivalent) may be given but has not been proven to prevent adverse reactions to any medication. Likewise, antihistamines do not prevent adverse reactions and indeed may be harmful by their sedative and hypotensive actions. Premedication with adrenaline, the only medication proven to prevent and ameliorate reactions to other (snake) antivenom (Premawardhena et al. 1999), is not indicated in this situation in which the spider venom causes the release of endogenous catecholamines. Skin testing is not recommended with any antivenom.

Dosage and Effectiveness

The antivenom is purified IgG antibody derived from immunization of rabbits with male *Atrax robustus* venom. The appropriate dosing to treat envenomation is not certain. A vial, containing 125 units of antivenom, neutralizes 1.2 mg of such venom in vitro. The average yield of venom obtained by "milking" spiders (aspirating venom from fang tips) is approximately 0.14–0.18 mg but the average amount obtained from dissected glands is 0.81 mg (Sutherland and Tibballs 2001).

Conceding that the amount of venom injected is always unknown and the aforementioned data, the minimum initial dose of antivenom for a mild case is 2 ampoules; repeated in 15 min if there is no clinical improvement. Double the dose for a severe case, and thereafter according to the clinical status of the victim. The antivenom should be given intravenously, slowly initially. It is uncommon to require more than 6 ampoules of antivenom. In 75 victims requiring antivenom, the median dose administered was 3 ampoules, but the range was 1–17 (Isbister et al. 2005). Although venom can be measured in blood and may be useful in assisting in determining dosing (Miller et al. 2016), the prudent course is to administer antivenom until no further clinical improvement is evident after which spontaneous recovery will be determined by the supportive and secondary treatment (*vide infra*).

Fortunately, when administered soon after envenomation, antivenom usually rapidly restores a degree of normality and preventing the worsening of an extremely difficult clinical problem. However, funnel-web spider antivenom, like all animal antivenoms, cannot alone restore normal function of established tissue damage. For example, antivenom did not restore low cardiac output considered to be due to catecholamine-induced cardiomyopathy in 2 victims of envenomation, one of whom

had high levels of endogenous catecholamines and both had high serum levels of troponin and creatine kinase and required infusions of inotropic agents to maintain cardiac output before spontaneous recovery (Isbister et al. 2015).

Although the antivenom is raised against male *Atrax robustus* venom, it appears to be effective for treatment of envenomation by all other male and female members of the Atrax genus and for members of the *Hadronyche* genus (Knight and Sutton 1982; Harrington et al. 1999; Miller et al. 2000; Isbister et al. 2005). However, the venom of female *A. robustus* and venoms of male and female members of the genera of funnel-webs are not identical. Graudins et al. (2002) showed that the antivenom does not bind to all protein bands in *Atrax robustus* venom subjected to electrophoresis and Western blotting but, importantly, it did bind to male and female spider proteins (from a variety of species) of size below 6500 Ka and above 40,000 Da and to δ-ACTX-Hv1a (δ-HXTX-Hv1a) (4856 Da), the lethal toxin of *H. versuta*. Similarly, the antivenom reversed and neutralized the muscle contracture and fasciculation caused by *Atrax robustus* venom and a variety of other male and female funnel-web venoms in a chick *biventer cervicis* nerve-muscle preparation. Interestingly, muscle contracture and fasciculation in this preparation was caused by the female venom of several species including *A. robustus*, *A. illawarra*, *H. cerberea*, *H. formidabilis*, and *H. versuta*) but not by female venom of *H. infensa*, *H. lamington* (*H. lamingtonensis*), H. sp. 20 (*H. wisharti*), and *H. valida.* Despite these differences, there does not appear to be a current need to manufacture species-specific or gender-specific antivenom.

Duration of Hospitalization

The use of antivenom has markedly reduced the time in hospital. Survivors of envenomation before availability of antivenom were usually hospitalized for several weeks, but this has been reduced to several days by use of antivenom (Sutherland and Tibballs 2001). Nowadays, it is unusual for the hospital stay to extend beyond 2 days. Indeed, the average duration of hospitalization of envenomated victims treated with antivenom over the period 1981–2004 was 1.8 days compared with an average of 3.5 days for those before antivenom was available (Isbister et al. 2005).

Acute Allergic Reactions and Delayed Serum Sickness

Acute allergic reactions are uncommon, occurring in 2 of 75 patients receiving antivenom of whom one required treatment with adrenaline (Isbister et al. 2005). Apart from immediate reactions, the possibility of delayed serum sickness (Type 3 immune complex disease) should be borne in mind and short-term steroid therapy commenced should the slightest suggestion of its occurrence be detected. However, the only adverse report has been a solitary case of mild delayed serum sickness (Miller et al. 1999).

Intensive Management

In addition to measures above, atropine may be required to control excessive secretions. Although an initial dose for an adult is 0.6–1.2 mg and 0.02 mg/kg for a child, larger and repeated doses may be required to control excessive secretions and contamination of the airway. Endotracheal intubation and mechanical ventilation are required in severe cases of envenomation to overcome upper airway obstruction and to achieve adequate oxygenation and carbon dioxide clearance in the face of airway obstruction, pulmonary edema, acute heart failure, and obtundation.

In the initial phase of the syndrome, sympathetic blockade may be beneficial for hypertension and severe tachycardia. However, as hypotension occurs after an initial transient phase of hypertension, it may be necessary to infuse an inotropic agent. Since dobutamine appears to be the preferred drug in the treatment of hypotension caused by scorpion envenomation in which a hyperadrenergic state also occurs, this is probably also the most appropriate drug in severe funnel-web spider envenomation.

If hypotension and low cardiac output are refractory to inotropic support, veno-arterial (rather than veno-veno) extracorporeal membrane oxygenation may be required. Repeated echocardiographic examination may assist in determining the rapidly changing state of cardiac contractility. To date, all published cases of severe cardiorespiratory failure have been successfully managed without the need for extracorporeal support (Fisher et al. 1980; Isbister et al. 2005, 2015) but it was considered in a case refractory to antivenom and requiring high dose inotropic support (Isbister et al. 2015).

Genus *Missulena*, (Family Actinopodidae), Mouse Spiders

Sixteen species of this genus are found in Australia. A characteristic of this squat, heavily built spider is that in a few species the male has bright red fangs and fang bases but in most species these are all black. The males are also known as red-headed trap-door spiders, although they do not live in burrows and often wander in broad daylight. The slightly larger female is usually dark brown or black. Most species are slow moving, but aggressive, and venom drops are often seen at the tips of their very large fangs as they rear backwards. The fangs open widely and venom may easily be "milked" from the fangs with a pipette.

Venom from female *Missulena occatoria* was tested in new-born mice and found to have a subcutaneous LD_{50} of 0.2 μg and caused muscle fasciculation in rat phrenic nerve diaphragm preparations (Sutherland 1979). Rash et al. (2000) presented in vitro evidence that the venom of the male eastern mouse spider (*Missulena bradleyi*), but not the venom from the female spider, contains a potent neurotoxin which causes release of endogenous neurotransmitters. The venom caused rapid fasciculations and augmented indirect twitches in the mouse phrenic nerve diaphragm preparation and contraction of chick biventer muscle preparations. Muscle contraction was inhibited

by d-tubocurarine, a neuromuscular blocker and by tetrodotoxin, a sodium channel blocker suggesting that the venom acts via sodium channels similar to the δ-hexatoxins in funnel-web spider venom. Indeed, Gunning et al. (2003) isolated a 42-residue peptide with characteristic 8 cystine residues, δ-MSTX-Mb1a, which was highly homologous to δ-hexatoxins of funnel-web spiders and which had the same sodium channel-gating effects. In contrast, the venoms of the female spider and that of both male and female northern mouse spiders (*M. pruinosa*) did not cause contraction nor fasciculation in chick *biventer cervicis* nerve-muscle preparations (Herzig et al. 2008). Contraction in vitro is inhibited by prior exposure to *Atrax robustus* antivenom (Rash et al. 2000) and reversed by *Atrax robustus* antivenom (Herzig et al. 2008) thus supporting the use of this antivenom for treatment, if necessary, of human envenomation by mouse spiders.

Human Envenomation

Usually, bites by these spiders cause only minor or moderate effects (Isbister and Gray 2002) but a 19-month-old child developed a life-threatening illness, similar to that caused by funnel-web spider envenomation, after a bite by a male eastern mouse spider, *Missulena bradleyi* (Rendle-Short 1985). The victim vomited and then rapidly became seriously ill with loss of consciousness, hypertension, sweating, muscle spasm, and stridor. She received red-back spider antivenom with no improvement and subsequently given funnel-web spider antivenom some 12 h after the bite. By 90 min her blood pressure returned to normal and her conscious state rapidly improved. She was found also to have a thrombocytopenia and neutropenia which lasted 3 days but recovered well.

Genus *Latrodectus*

"Latrodectism," is the long-known syndrome caused by the bite of species of spiders from the genus *Latrodectus* (Family Theridiidae, comb-footed spiders). The syndrome includes agonizing pains, cramps, spasms, motor unrest, board-like abdomen, "facies latrodectismica," sweating, oliguria, hypertension, anxiety, mental excitation, and an extended convalescence. Clinical manifestations of the venom from different species are similar but not identical.

Members of the genus *Latrodectus* are found in most tropical to temperate parts of the world. The present global classification comprises 31 species and numerous subspecies (Platnick 2014). The Australian species, *L. hasselti,* the red-back spider, was previously been regarded as a subspecies, *L. mactans hasselti.* This spider is the commonest cause of potentially serious envenomation in Australia and, as a consequence, red-back spider antivenom is the most frequently used of all antivenoms. Only the female of the species is dangerous to man and animals.

L. hasselti has established itself in New Zealand and the males readily mate with the resident *L. katipo* females. New Zealand also has a second species, *L. atritus.*

Several victims are treated annually in New Zealand with CSL Ltd's red-back spider antivenom. *L. hasselti* has also become established in Osaka, Japan, after probable importation from Australia.

Globally, the best known species is *L. mactans,* the American black widow spider. In Europe, epidemics of latrodectism due to *L. tredecimguttatus* have occurred from time to time. Australia probably has the highest rate of latrodectism per head of population in the world. As far as can be determined the properties of the venom of all species and subspecies and the clinical syndromes produced by them are similar. There are, however, differences in the venoms of the species.

Latrodectus Hasselti, the Red-Back Spider

L. hasselti is widely distributed over Australia, but it is less common in the high humidity of tropical and subtropical regions. It can be found in metropolitan regions and plentiful in most areas of bushland.

Description and Habits

A mature female *L. hasselti* has a characteristic appearance. The body length is about 10 mm and the cephalothorax is very small compared with the large round abdomen, which usually bears a bright red or orange dorsal stripe. The shape of the red stripe is variable and it may be fragmented; very rarely it is pink or light gray. On the abdominal underside the stripe is hourglass-shaped or two triangular shapes. The hourglass-shape is characteristic of the genus throughout the world but is reduced in some species. The body and slender legs are black and the first pair is longer than the others. The male spider is not dangerous – it has a much smaller body length of only some 3 mm and an overall color of light brown rather than black and has a whitish abdomen with posterior red stripe and several oblique dark markings on each side.

The mature female spins an irregular web often on suburban property near houses and in outhouses, gardens, or refuse. The web consists of a snare, trap threads, and a retreat. The snare is a central tangled mass of web from which very sticky "trap" threads radiate. Insects that fly into these "trap" threads or beetles that stumble into them find that they contract rapidly toward the central snare, making escape very difficult. The third part of the web is a tubular retreat, generally extending into some suitable crevice. After mating, the spider spins five or six spherical silk egg sacs, some of which may contain several hundred eggs. The pale yellow egg sacs are about 10 mm in diameter and are usually suspended in the spider's retreat, where they are closely guarded against wasps seeking to lay eggs in them. The spiderlings hatch after about 3 weeks and several moultings occur over some months before adulthood is reached. The young spiderlings are highly cannibalistic but enough reach maturity to maintain a significant threat to public health. Unlike *Atrax robustus, L. hasselti* is not aggressive and if molested will usually curl up and feign death.

Venom and Toxins

The major active component in *Latrodectus* venom which affects vertebrates is a protein named α-latrotoxin, which has a molecular mass of approximately 130 kDa. As a tetramer or dimer it acts at nerve endings of vertebrate and invertebrate neurons to release transmitters. The toxin causes exocytosis of synaptic vesicles. The result of the action of the toxin is the development of a patchy paralysis at the neuromuscular junction and the widespread release of catecholamines.

The principal toxins in *Latrodectus* venoms are vertebrate specific toxins, α-latrotoxins, which are responsible for the clinical syndromes of envenomation. These toxins are similar between species but not identical. That of *L. hasselti*, α-LTX-Lh1a comprises 1180 residues (~ 132 kDa) with 93% sequence identity with α-LTX-Lt1a from *L. tredecimguttatus* (Graudins et al. 2012). Alpha-latrotoxin has an N-terminal sequence and a C-terminal which includes multiple ankyrin-like repeats each of approximately 30–34 amino acids which facilitate its polymerization. A monoclonal antibody raised against α-LTX-Lt1a failed to neutralize the neurotoxicty of other *Latrodectus* venoms in a chick *biventer cervicis* nerve-muscle bioassay. Five insect-specific latrotoxins (LIT), α, β, γ, δ, and ε of molecular mass 110–140 kDa and a crustacean-specific latroxin (α-LCT) have been identified in *L. tredecimguttatus* venom as well as low molecular weight proteins (latrodectins) which augment the toxicity of the latrotoxins (Rohou et al. 2007; Graudins et al. 2012).

Recent transcriptome analyses of venom and of venom glands of various species of *Latrodectus* have revealed numerous toxins. From *L. hesperus* (Western black widow) venom, Haney et al. (2014) identified a trove of diverse toxins in venom including transcripts for over 20 paralogs for latrotoxins, inhibitor cystine knot toxins, CRISPS, hyaluronidases, chitinase, and proteases. From *L. tredecimguttatus* venom gland, 146 toxin-like proteins were identified which formed 12 distinct families (He et al. 2013). Six previously identified toxins from *L. tredecimguttatus,* including α-LTX-Lt1a and its analogues, and homologues to 16 known toxins from other different spider species and scorpions were identified. Broadly classified, these protein toxins included neurotoxins and their assistants which interfere with transmission of neural signals, those with trypsin-like activity to assist in prey digestion and protease inhibitors which prevent toxin autodegradation. Interestingly, no ion channel toxins were present in *L. tredecimguttatus* venom. However, the transcriptome of *L. mactans* venom contains 14 cystine-rich peptides of which 5 are toxins with inhibitor cystine knot structures known for actions on ion channels (Oldrati et al. 2017).

Mode of Action of α-Latrotoxin

Alpha-latrotoxin targets binds to presynaptic receptors (latrophilin 1, neurexin 1α) and leads to exocytosis of transmitter vesicles either by entry of extracellular calcium or by release of intracellular calcium. On the cell membrane α-LTX forms tetramers which insert into the membrane creating pores permeable to calcium. Inflow of

extracellular calcium causes exocytosis of vesicles which in turn results in massive release of neurotransmitters into synaptic clefts and postsynaptic receptor activation. Additionally, dimers or tetramers of α-LTX complex with latrophilin 1 (a G protein-coupled receptor, GPCR) which activates membrane-associated phosphatidylinositide-specific phospholipase C (PLC) which in turn leads to an increase in presynaptic inositol triphosphate and diacyl glycerol and subsequent release of calcium from intracellular stores (Ushkaryov et al. 2004; Luch 2010) and synaptic vesicular exocytosis.

Human Envenomation

Diagnosis of envenomation is usually straightforward. The spider is usually seen but if not a bite by *L. hasselti* should be considered in the differential diagnosis of acute illness or distress in an infant or young child, particularly if they have a painful skin lesion.

Prior to the availability of antivenom in Australia, a bite by a red-back spider could be fatal from respiratory failure. Indeed, approximately 15 deaths had been recorded (Sutherland and Tibballs 2001). The onset of serious illness is slow. In the largest survey, of 2144 bites (Sutherland and Trinca 1978), the majority of bites occurred on upper or lower extremities when the spider was disturbed in man-made objects. The main symptoms and signs are listed in Table 2. Most victims required antivenom.

The bite is usually a pin-pricking pain, but it may be unnoticed. The site becomes hot and erythema and edema develop rapidly. Localized sweating often occurs. The swelling is generally limited to an area of several cm in radius from the bite site; occasionally it is extreme. Approximately 5 min after the bite, intense local pain commences and increases in severity and distribution. In most cases, pain is the predominant symptom; the patient is sometimes distraught and even hysterical because of its intensity. Movement of the affected limb often significantly increases the pain. About 30 min after the bite, pain and swelling are often experienced in the regional lymph nodes. If abdominal pain occurs, it is worse when the lower extremities or genitals were bitten, probably due to lymph node involvement. Sometimes severe pain develops in parts remote from the bite site, for example, in an opposite limb or the opposite side of the trunk. About 1 h after the bite, headache, nausea, and vomiting often occur. Sweating is a common manifestation and profuse in some cases. Mild pyrexia may occur.

When treatment is delayed, malaise, restlessness, muscle weakness, and incoordination are likely to occur. Movement of the affected limb increases the pain significantly. Severe tremors may be experienced and are particularly distressing. Tachycardia is very common and severe hypertension may occur presumably due to catecholamine release. A transient, but unpleasant, arthralgia may occur, and those with pre-existing arthritis may suffer an exacerbation of their condition. Albuminuria may occur. A fine rash, often described as being like "prickly heat," is seen in some cases, in some a generalized pruritic rash, and in a few generalized urticaria. Ten

Table 2 The main signs and symptoms in 2144 cases* of *L. hasselti* bite (Sutherland and Trinca 1978)

Local effects	*Percent*
Pain	76
Erythema	33
Edema	24
Heat	19
Pruritis	4
Regional effects	
Pain and swelling of regional lymph nodes	19
General effects	
Pain other than at bite site	39
Nausea or vomiting	20
Sweating	15
Malaise	10
Paresthesiae	10
Pyrexia	8
Insomnia	8
Dizziness	8
Headache	4
Rash	4
Hypertension	3

*20 per cent of these cases were inadequately reported. Of the 2144 cases, at least 95.7 per cent received antivenom and, thus, the syndrome of latrodectism would be modified to some extent

patients in the series of 2144 required assisted ventilation (Sutherland and Trinca 1978).

Uncommon, even bizarre, signs and symptoms developed in some cases. These were tetanic spasms, tingling in the teeth, swelling of the tongue, bite site infection, convulsions, excessive thirst, severe diarrhea, anaphylactic reaction to the venom, blotchy rash on face, hemoptysis, dyspnoea, dysuria, severe trismus, persistent anorexia, periorbital edema, and/or conjunctivitis. Patchy areas of what was described as "bizarre sweating" were not uncommon.

Treatment with Antivenom

The purpose of antivenom is to treat pain and importantly to avert delayed but serious neurological illness. If untreated, effects of envenomation may persist for months but delayed antivenom is effective. Envenomation does not always cause serious illness. Definite and distressing evidence of envenomation will almost invariably be obvious within an hour or two. If no signs or symptoms have developed within 24 h other than at the bite site, antivenom could be withheld and managed with analgesics alone. In other circumstances, antivenom should be administered.

Red-back spider antivenom has been available since late 1956. It is a purified horse immunoglobulin. An ampoule contains 500 units, which will neutralize in vitro 5 mg of venom, and consists of about 1 ml of a 6% (w/v) protein solution. This is a very small volume and quantity of protein compared with other antivenoms. Administration of several ampoules of antivenom is commonly required at intervals of approximately 2 h, allowing evaluation of a preceding dose. Acute allergic reactions to the preparation are possible but very uncommon (approximately 0.5%).

Premedication is not necessary but as always with such products, clinical staff should be ready to treat anaphylaxis. The only medication known to ameliorate the severity and reduce the rate of anaphylaxis to an antivenom has been adrenaline (*vide supra*), given subcutaneously. In a case of known allergy to horse protein, premedication with adrenaline (0.25 mg adult, 0.005 mg/kg for a child) before red-back antivenom is warranted, but such therapy should not be given either intramuscularly or intravenously lest it exacerbates hypertension caused by envenomation. In such case also, a parenteral steroid (e.g., methylprednisolone 1–2 mg/kg) may be helpful but is of unproven efficacy, as are antihistamines.

Since the onset of effects of envenomation is slow and the antivenom is a small volume, it may be given either intramuscularly or intravenously. In cases of late presentation and/or with severe envenomation, the intravenous route is preferable which can be given via a diluted solution.

First Aid

No special first aid is recommended. No attempt should be made to restrict venom movement, because of the relatively slow action of the venom and because holding the venom at the bite site may increase the severity of the local pain. The bite site should not be incised or excised. Applications of ice and water mixed in a plastic bag may give some relief. The best course is to seek medical aid at once, bringing the offending spider for identification in a suitable container.

Other Spiders

Of the numerous other species of spiders in Australia, the bites of only a relative few have caused pain and inflammatory signs at the bite site and minor (non-life-threatening) systemic effects (Isbister and Gray 2002). These included members of the Families Araneidae (Orb-weavers), Clubionidae (Sac spiders), Corinnidae (Darting spiders), Desidae (Lace web spiders, including *Badumna insignis*, Black house spider), Lamponidae (White-tail spiders), Lycosidae (Wolf spiders), Salticidae (Jumping spiders), Sparassidae (Huntsman spiders), Zodariidae (Spotted or ant spiders), and other Mygalomorphae (Trapdoor, mouse, and tarantula spiders). With few exceptions, venoms and toxins of these have not been investigated. The venom of *Selenotholus foelschei* (Family Theraphosidae) causes minor effects in humans but is lethal to dogs, probably by blocking vertebrate voltage-activated Na^+-channels

(Herzig and Hodgson 2008). Hardy et al. (2013) isolated an orally active insecticidal toxin from the Australian tarantula, *Selenotypus plumipes*. Another member of the Theridiidea family, *Steatoda capensis* (a Cupboard spider), is very similar in morphology to *Latrodectus hasselti* but without a dorsal stripe and its bite may cause an envenomation syndrome very similar to that of *Latrodectus hasselti*. It is treated effectively with red-back antivenom.

Conclusions and Future Directions

Although intensive research of the venom of selected species of Australian funnel-web and related spiders (genera *Atrax* and *Hadronyche*) has identified and characterized selected toxins which cause human illness, the vast majority of their toxins and the toxins of numerous other Australian spiders remain unidentified. Nonetheless, study of these toxins has contributed enormously to our understanding of the functioning of biomembrane ion channels and to the clinical management of envenomated victims. Similarly, the identification of the principal toxin in the venom of *Latrodectus hasselti*, red-back spider, has contributed to our understanding of neuronal vesicle exocytosis and to the management of envenomated victims of this and related spiders. Fortunately, effective antivenoms for the management of victims of funnel-web and red-back spiders are available. In contrast, the twin aims of deriving therapeutic drugs and insecticides from spider toxins has not so far resulted in productive outcomes. In these endeavors, research is at an investigational stage.

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Envenomation by Australian Hymenoptera: Ants, Bees, and Wasps

9

James Tibballs

Contents

Abstract

The venoms of the order Hymenoptera, comprising ants, bees, and wasps, contain numerous toxic substances including a vast array of peptides, which serve to cause cell lysis and disrupt intracellular processes. Australia has numerous indigenous species of all Hymenoptera, but the imported European honeybee (*Apis mellifera*) and the European wasp (*Vespula germanica*) have added

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significantly to the burden of allergic reactions expected principally from stings by members of the indigenous ant genus *Myrmecia*. Although Hymenoptera toxins from multiple stings may damage organs and tissues especially muscle, hepatic, and renal and disrupt coagulation, a large number of toxins are allergenic and share significant homology between species and between bees and wasps. The overwhelming clinical effects of humans are allergic reactions varying from minor local inflammation to life-threatening IgE-mediated anaphylaxis which tends to progress with repeated venom exposures. However a state of immune tolerance may be achieved by regimens of repeated exposure to small quantities of venoms or recombinant allergens (venom immunotherapy). The diagnosis, monitoring, and prediction of the immunoreactivity of individual allergic victims are major difficulties in clinical management but facilitated with wider adoption of serum tryptase measurement and new techniques of in vitro basophil activation testing.

Introduction

Stings by families of Hymenoptera (Formicidae, ants; Apoidea, bees; Vespoidea, wasps) may envenomate and cause marked local reactions and/or cause dangerous allergies including life-threatening anaphylaxis.

Hymenoptera stings in Australia are considerable health hazards but trending downward or static and less than in other world regions. In Australia during the period 1979–1996, bee and wasp stings caused 42 deaths – equal to the number of snakebite deaths in the same period and at an annual incidence of 0.15 per million population. Bee and wasp stings caused 942 admissions to hospital in a 1-year period 1995–1996, second to spider bites (1,281 admissions) and more than snakebites (549 admissions) (Winkel et al. 1999). Over a 3-year period mid-2002 to mid-2005, stings by bees and wasps caused 3,557 hospitalizations (bees caused 77% and wasps 22%), while ant stings caused 347 admissions yielding a net annual average of 1,300 hospitalizations due to Hymenoptera stings (Bradley 2008). In the 14-year period 2000–2013, ant, bee, and wasp stings caused 17,000 admissions at an annual average of 1,200 (Welton et al. 2017). The 32 arthropod deaths in this period, 0.11 per million population per year, were all due to Hymenoptera stings by bees (25), ants (5), and wasps (2), while no deaths were due to spider bite. In Europe, the annual incidence of fatalities from Hymenoptera stings is between 0.1 and 0.5 per million with over 100 deaths per year, while in the United States, the annual incidence is approximately 0.2 per million population with 60 deaths per annum.

Ants

Worldwide, the number of species and subspecies in the family Formicidae (ants) is estimated at over 15,300 in 17 subfamilies and which constitute the most abundant of all venomous animals on Earth (Aili et al. 2014). In Australia, 1,559 known

species comprise 109 genera in 12 subfamilies and include 17 species, which have been introduced (http://www.antwiki.org/wiki/Australia). However, Anderson (2007) estimates that about 6,500 species could exist in Australia.

Only a few genera may cause serious illness in humans. Rarely, death may be attributed to massive tissue damage following prolonged attack. Cleland (1931) described three such cases: one involved an infant abandoned by its mother and the other two were drunken men who fell and lay upon ant mounds. Although many species have powerful mandibles and can deliver a painful bite, allergy to ant venom is the most serious clinical problem.

Venom is manufactured in bilateral cylindrical or convoluted glands and stored in a venom reservoir. The venom is delivered by injection into the victim by a stinger, a modified ovipositor situated at the distal base of the abdomen (gaster). Although most of the 13 subfamilies of ants deliver venom by sting, those of the Formicinae subfamily deliver venom by spraying through a special opening, the acidopore, a round orifice also located at the base of the gaster. The principal toxin of non-stinging ants is formic acid, although acetic acid may also be sprayed.

The types and roles of toxins of stinging ant venoms are highly variable, depending on the genus, but are generally used for subduing prey, defense against other predators and competitors, defense against microbial pathogens, and social communication. Ant venoms are composed of a complex mixture of chemicals, which include proteins, peptides (<100 amino acids), enzymes, biogenic amines, hydrocarbons, alkaloids, and formic acid.

In some subfamilies, the venom is joined in the venom duct on delivery by the contents of Dufour's gland. The excreted content of this gland, found in all Hymenoptera, is used principally by ants for communicative roles such as trail-marking, territory-marking, signaling alarm-defense, slave-making, and sex pheromones. The chemicals delivered are saturated and unsaturated hydrocarbons, terpenoids, aliphatic alcohols, fatty acids, sugars, quinones, and aromatic compounds (Mitra 2013).

Toxins

The predominant toxins in ant venom are peptides, but only 75 from 11 ant species (in 6 subfamilies) have been fully sequenced, representing less than 0.1% of the estimated number of all ant venom peptides (Touchard et al. 2016; Aili et al. 2014). It is expected that over one million peptides will be eventually identified in ant venoms, representing a rich source of novel drugs, including insecticides and antibiotics. Structurally, these peptides are linear, dimeric, and inhibitor cysteine knot (ICK)-like peptides which have cytolytic and neurotoxic properties. Small linear peptides, usually less than 35 amino acid residues and devoid of disulfide bonds, prevail in ant venoms. These toxins probably disrupt cell membranes giving rapid insecticidal, hemolytic, and antimicrobial properties, but only a few have thus far been characterized. For example, a linear peptide in the venom of the non-Australian bullet ant (*Paraponera clavata*) exhibits paralytic vertebrate and

non-vertebrate neurotoxic effects by modulating voltage-gated sodium (Na_v) channels, while a dimeric peptide in the venom of the non-Australian ant (*Ectatomma tuberculatum*) blocks voltage-gated calcium (Ca_v) channels and also has a cell membrane pore-forming cytolytic effect (Touchard et al. 2016).

Australian Ant Species

The common causes of painful ant sting and allergy in Australia are the numerous members of the genus *Myrmecia* and the species *Rhytidoponera metallica*. The venoms of many species of the genus *Myrmecia* generate IgE antibodies in humans although common peptides in each venom may be responsible. Australia has 90 known species of *Myrmecia*, and more are expected to be discovered. *Myrmecia* ants are all highly aggressive stinging species. They have large eyes enabling use of visual cues to navigate and a prominent powerful pair of mandibles. When the skin of a human victim is firmly grasped (bitten), the ant curls up its body and thrusts in a long sharp barbless sting. Multiple stings and envenomations may be made in quick succession. Dangerous imported ants include *Solenopsis invicta* (red imported fire ant).

Jumper, Jack-Jumper, or Jumping-Jack Ants

Numerous species comprise members of *Myrmecia* with these common name terms. They are aggressive and ferocious. The common names derive from their behavior, displaying characteristic frenetic movement and jumps made especially when agitated or disturbed. Species of *Myrmecia* are especially common in southeastern Australia and Tasmania, where populations may become very dense in the higher mountains. The most well-known and abundant member, *M. pilosula*, has a black/dark brown body 10–12 mm in length and has characteristic yellow or orange mandibles and yellowish leg tips. It ranges from north of Brisbane west to the vicinity of the small coastal town of Denmark on the western side of the Great Australian Bight in Western Australia. It is present on Kangaroo Island and ranges north in South Australia to the Flinders Ranges. It is normally a diurnal forager. The name *M. pilosula*, as currently used, almost certainly refers to a complex of closely similar "sibling" species with different chromosome counts. Several of these "sibling" species are known to cause severe reactions in stung humans (Brown et al. 2011). The venom chemistry of jumper ants is probably similar but more allergenic than the venom of the larger bull ants.

Bull Ants, Bulldog Ants, Giant Bull Ants, and Inchman Ant

Many different species comprise this group of *Myrmecia*, so described by their common names because of their large body size (typically 20 mm, but up to 25 mm), large mandibles, and tenacious grip. One species, *Myrmecia pyriformis*, is very common in open sclerophyll woodland in southeastern Australia, ranging west to Adelaide, inland in New South Wales to the Canberra area and north to

southern Queensland, but not Tasmania where another bull ant, *M. forficata*, exists. It builds conspicuous mounds and is normally a night forager. *M. pyriformis* shares its range in part with several other species of large bulldog ant, with which it can be very easily confused.

Toxins of *Myrmecia* Species

Study of the toxins of *Myrmecia* venoms has been almost entirely from the venom of one species, *Myrmecia pilosula.* Wanandy et al. (2015) reviewed the known structure and functions of peptide toxins from *M. pilosula* and have proposed nomenclature from the viewpoint of allergenicity, but in the light of the expectation of discovery of many thousands of toxins to be yet identified in ant venoms, such nomenclature may become redundant. Nonetheless, the 15 or 16 identified toxins may be grouped into 5 families (pilosulins 1–5) existing as monomeric or dimeric structures linked by disulfide bonds shared by cysteine amino acid residues. They have cytotoxic, hypotensive, histamine-releasing, and antimicrobial activities. Several are either major or minor allergens. Antimicrobial activities against Gram-negative and Gram-positive fungi are vital to ensure the ant colony is protected against invading species and against species brought in by hunting and foraging.

Touchard et al. (2016) has recently proposed a new system of nomenclature for ant venom toxins based on biological activity of the toxin; ant subfamily, genus, and species; and the molecular scaffold and amino acid sequence of the toxin. For some known toxins, this proposition represents an additional revision of nomenclature. Consequently, the same toxin has been cited in several different ways. For example, the same linear allergenic peptide from *Myrmecia pilosula* has been variously named as Myr p 1, pilosulin 1, and Myr p 1.0101 but is henceforth proposed as M-myrmeciitoxin-Mp1a-f (M-MIITX-Mp1a-f). The following commentary on identification and characterization of toxins over half a century necessarily utilizes superseded nomenclature. The toxins of *Myrmecia* spp., generally known by their trivial names as "pilosulins," have been studied extensively, but many are still uncharacterized.

De La Lande et al. (1965) found extracts made from *M. pyriformis* venom sacs to be rich in histamine. They were also found to contain a smooth muscle stimulant and to have histamine-releasing and hemolytic activities as well as a hyaluronidase (Lewis and De La Lande 1967). The intraperitoneal LD_{50} of these extracts in mice of 20–30 g weight was between 2 and 10 mg/kg. Lewis et al. (1968) identified phospholipase A activity in *M. pyriformis* venom. Wanstall and De La Lande (1974) demonstrated the presence of seven protein components of *M. pyriformis* venom sac extracts and by chromatography obtained a fraction that resembled the bee toxin melittin and which contained most of the biological activity. Venom collected by electrical stimulation of the venom sacs was similar in activity to whole sac extracts. Cavill et al. (1964) collected venom from the venom reservoir of *M. gulosa* (a bull ant), which upon analysis proved similar to *M. pyriformis* venom.

Matuszek et al. (1992) found that the venom of *M. pilosula* also contained histamine and a heat-sensitive hemolytic factor and may cause release of cyclooxygenase products. Matuszek et al. (1994) identified phospholipase A_2, phospholipase B, hyaluronidase, and phosphatase activities in the venoms of both *M. pilosula* and *M. pyriformis* but greater activities in the latter.

The major allergens in *M. pilosula* venom, Myr p1 and Myr p2, were identified by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) and by blotting and have been cloned (Donovan et al. 1993, 1996; Street et al. 1996). The expressed clones appeared to account for most, but not all, of the venom-specific IgE antibody binding observed on SDS-PAGE blots of native venom (Donovan et al. 1996). In the venom gland, Myr p1 is a 112-residue polypeptide but undergoes extensive posttranslational processing and appeared in the venom as a family of C-terminal IgE-binding homologous polypeptides of 56, 48, 45, 42, and 27 residues. Myr p2 is expressed as a 75-residue polypeptide and undergoes posttranslational processing to appear in the venom sac as a C-terminal 27-residue polypeptide (3,208 Da) (Donovan et al. 1996) which is bound to a 23-residue polypeptide as a heterodimer. The 56-residue component of Myr p1 (~ 6,052 Da) and the 27 C-terminal residue of Myr p2 (~ 3,212 Da) bind substantial IgE in direct binding assays. These were subsequently named, respectively, "pilosulin 1" (Donovan and Baldo 1997; Wu et al. 1998) and "pilosulin 2" (Donovan and Baldo 1997). The 56-residue peptide of Myr p1 and the Myr p2 heterodimer are both membranolytic cytotoxins, with hemolytic activity, that appear to have a similar mode of action to the 26-residue peptide melittin from the venom of the honeybee *Apis mellifera* (Wu et al. 1998; King et al. 1998). The membranolytic properties might assist tissue penetration of the venom. Evidence from studies using synthetic peptides as inhibitors in the venom-specific IgE antibody radioimmunoassay indicates that 20 C-terminal residues of Myr p1 are responsible for its IgE binding, whereas the 23-residue polypeptide associated with Myr p2 does not bind IgE but is important for its cytotoxic membranolytic properties (Donovan et al. 1994).

Davies et al. (2004), using mass spectrometry, identified more than 50 peptides in venom all in the range 4–9 kDa. They identified the previously reported pilosulin 1 derived from Myr p1 but found that it existed mainly as a variant in which isoleucine replaced valine at residue 5 ([Ile^5] pilosulin 1). They also identified Myr p2 as the bis-disulfide-linked heterodimer consisting of the previously reported pilosulin 2 (~3,155 Da) and another peptide (~2,400 Da). The dimer was later named pilosulin 3 whose components were named pilosulin 3a and pilosulin 3b (Wiese et al. 2007). In that study of Western blot tests and IgE radioallergosorbent tests of venom components with the sera of 54 patients allergic to ant venom, 13 separate IgE-binding bands were observable in reactions with whole venom. However, the number of bands recognized by individual serum was 1–6 with a median of 3. On the basis of these reactions, pilosulin 3 (Myr p 2) (allergen name Myr p 2.0101) was classified as the only major allergen identified, while [Ile^5] pilosulin 1 (Myr p 1) (allergen name Myr p 1.0102) and a previously unknown peptide of mass 8,198 Da (pilosulin 4.1) (allergen name Myr p 3.0101) were

classified as minor allergens, but another five antigens are yet to be characterized and named. Inagaki et al. (2004) using the RNA from *Myrmecia banksi*, a member of the *M. pilosula* complex, identified cDNA clones which encoded four pilosulins: pilosulin 1 and 2 as previously known and two novel pilosulins, named pilosulins 3 and 4, of 74 amino acids 84 amino acids, respectively, which both have antimicrobial activity. (Inagaki's pilosulin 3 is a variant of pilosulin 3b, already described by Davies et al. (2004).) Proteomic analysis of *M. pilosula* venom (Wiese et al. 2006) confirmed the presence of many peptides of molecular weight <10 kDa most of which contained disulfide bridges. A dimer of 8,546 Da named pilosulin 5 was identified, but pilosulin 4, previously identified by Inagaki et al. (2004), was not identified. Instead, a variant named pilosulin 4.1a of 8,198 Da was identified. Subsequently, Inagaki et al. (2008) confirmed that pilosulin 5 is encoded by RNA of *M. banksi* – it is a dimer peptide connected by a disulfide bridge and has histamine-releasing property. A 20 N-terminal segment of pilosulin 1 has broad antibacterial and anti-*Candida* activity (Zelezetsky et al. 2005).

Green-Head Ant, Metallic Pony Ant, and *Rhytidoponera metallica*

At least 76 species of *Rhytidoponera* are known to exist in Australia. Like *M. pilosula*, this species as presently conceived includes a large number of closely similar sibling species. *R. metallica*, sensu lato, is one of the most abundant and easily collected variables of Australian insects. It is found throughout Victoria, New South Wales, Australian Capital Territory, South Australia (including Kangaroo Island), most areas of Western Australia (not the Kimberley), southern region of the Northern Territory, eastern Queensland, and Tasmania. It mainly inhabits open and moderately wooded areas including metropolitan parks and gardens. The worker ant is 5–7 mm with variation in a metallic color from green to purple to red violet. It is a diurnal predator of insects, killing by envenomation, and forager of seeds and honeydew.

Nothing is known about the toxins of the venom, but there is little doubt that the species causes allergic reactions in humans. It was among ants collected from geographical regions in Australia, mainly northern coastal New South Wales and South East Queensland, where patients had experienced allergic reactions to ant stings and from whom sera samples of IgE reacted with green-head ant venom (Brown et al. 2011).

Imported Ants

Red Imported Fire Ant, *Solenopsis invicta*, and Tropical Fire Ant, *Solenopsis geminata*

Although eight native species of the genus *Solenopsis* exist in Australia, the two introduced invasive species, *S. invicta* and *S. geminata*, are dangerous to humans and negatively impact the country's biodiversity, costing an estimated $1.5 billion annually. These species, along with four other imported species (*Wasmannia auropunctata*, little fire/electric ant; *Pheidole megacephala*, African big-headed

ant; *Anoplolepis gracilipes*, yellow crazy ant; *Linepithema humile*, Argentine ant), have been subject to expensive but partially successful eradication programs. *S. invicta*, a South American species, was first detected in Brisbane in 2001 and had infested some 36,000 ha in South East Queensland before reduction to 561 ha by 2011. Infested areas are inhospitable for humans and livestock. Several hundreds of millions of hectares are infested in Southern United States. The ants are omnivorous, destroying plants and animals, crops and plastic components of irrigation, and electrical systems. They reproduce and spread rapidly, form super colonies, and aggressively attack any invader of their territory.

Toxins in *S. invicta* Venom

Alkaloids comprise more than 90% of the contents of venom with proteins comprising the remainder. The alkaloids are mainly cis and trans stereoisomers of numerous 2,6-dialkylpiperidines (Chen and Fadamiro 2009). Two such alkaloids (solenopsin A and isosolenopsin A) caused hypotension and reduced left ventricular contractility in ventilated rats and seizures, respiratory arrest, and death in spontaneously breathing rats (Howell et al. 2005). Such actions may explain the occasional non-allergic death of a human victim after multiple stings.

Dos Santos Pinto et al. (2012), using gel electrophoresis and mass spectrometry, sequenced 46 proteins in total of which 21 are toxins, another 5 are related to communication, and others are considered to be contaminants from tissues surrounding venom glands. The numerous functions of these small toxin proteins (peptides) were deduced from databanks of proteins from other species of venomous animals. Broadly, the toxins comprise five groups which cause allergic reactions, neurotoxicity, venom diffusion, tissue damage, and inflammation and disruption of homeostasis. Four well-known allergens from fire ant venoms (Sol i 1, Sol i 2, Sol i 3, and Pac c 3) were identified, but another (Sol i 4) was not. Sol i 1 is a phospholipase A_1 which like a phospholipase A_2, also identified in the venom, disrupts biological membranes leading to pore formation, cell lysis, inflammation, and tissue damage. Sol i 2 is a transporter/capturer of pheromones (Borer et al. 2012) but is a major allergen in human victims of fire ant sting, causing IgE-mediated anaphylaxis. Sol i 3 and Pac c 3 are members of the cysteine-rich secretory protein (CRISP) family which have strong inflammatory properties. Myotoxin 2-like proteins, similar to the PLA_2 of crotalid snake venoms, were identified. These proteins cause skeletal muscle necrosis around the site of venom injection and increase local microvascular permeability and cytolysis. The venom contains an enzyme – a disintegrin and metalloproteinase – similar to snake venom metalloproteinase, which causes myonecrosis, skin and tissue damage, hemorrhage, edema, and coagulopathy. Another protein cytolytic venom component is similar to the hemolytic toxin PSTx-60 from sea anemone. Three different neurotoxins, for use in prey capture, were identified. One is similar to U5-ctenotoxin Pk from the spider *Phoneutria keyserlingi*, one is similar to *Scolopendra* toxin from the centipede

Scolopendra angulata, and another is similar to alpha-toxin Tc48a from the scorpion *Tityus cambridgei*. Three ponericin-like peptides with broad-spectrum activity against Gram-positive and Gram-negative bacteria were detected. These are similar to some of the antibacterial and insecticidal peptides from a neotropical species of ant, *Pachycondyla goeldii*. A transferrin in the venom, similar to that from wasp and bee venom, may also have an antibacterial role. The venom included a group of proteins (thioredoxin peroxidase, glutathione S-transferase, and cytochrome c oxidase) which are similar to those in honeybees and wasp venoms, protecting their other venom components against oxidation. Another protein toxin is a vascular endothelial growth factor, also in honeybee and wasp venoms, which increases vascular permeability and venom diffusion. A PLA_2 inhibitor is also present which probably prevents self-hydrolysis of the venom gland cells. The last toxic venom component is atrial natriuretic peptide, which causes hypotension. The venom also contains a group of five peptides related to chemical communication. They act as odorant-binding proteins, chemosensory proteins, and pheromone-binding proteins. These are in addition to different types of pheromones originating in Dufour's gland.

Although the protein allergens comprise a mere 0.01% of the venom of *S. invicta* (Hoffman et al. 1988), they are potent and have been extensively studied. Sol i 1, the most abundant protein in the venom, is an enzymatic serine hydrolase lipoprotein lipase (phospholipase) composed of 346 amino acids and carbohydrate (probably mannosylated glycan). It has homology with other phospholipases in vespid wasp venom. Cross-reactivity of sera from patients allergic to honeybee venom is due to its carbohydrate component, whereas cross-reactivity to yellow jacket wasp venom is due to both its lipoprotein lipase and carbohydrate component (Hoffman et al. 2005). Sol i 2 is a potent protein allergen able to evoke IgE-mediated anaphylaxis in humans. The crystal structure of Sol i 2 has been determined to be a globular homodimeric protein with a disulfide bridge linking the monomers and with three disulfide bridges linking the 5 α-helices of each monomer. Its role is uncertain, but by its close homology with an odorant- and pheromone-binding protein of the fruit fly *Drosophila melanogaster*, its function is probably similar (Borer et al. 2012). Sol i 3 is a protein of 212 amino acids (mass 23,968 kDa). The crystal structure is 7 α-helices and 6 β-strands arranged as three stacked layers giving rise to an α-β-α sandwich. Its function is unknown. Although this protein has strong homology to the wasp venom allergen Ves v 5, immunological cross-reactivity is not observed in patient sera probably because the conserved surface patches of the two molecules are too small to serve as common epitopes (Padavattan et al. 2008). Sol i 4, one of the four original allergens described by Hoffman et al. (1988), has not been as well characterized. It is a 117 amino acid protein of mass 13.3 kDa. Its function is unknown. It is strongly antigenic to humans and along with Sol i 2 is the major allergen in the queen ant venom which lacks Sol i 1 and Sol i 3 found in worker ant venom (Lockwood et al. 2012). Queen ant venom is less antigenic than worker ant venom not only due to its different content of protein allergens but also due a lesser content of alkaloids.

Human Envenomation

Myrmecia Species

The sting of these ants causes immediate pain which may be severe for 5 or more minutes. Usually some swelling and inflammation occur, and the area may be aching and itchy for a few days. The most important medical aspect of ant sting, however, is the development of an allergy. A generalized reaction or anaphylaxis may occur after an initial sting or after subsequent stings.

The overall prevalence of a sting by any ant among the population of Victoria was estimated at 2.9%. The combined systemic reaction rate to either jumper or bull ants or both was 2.4%. Of victims stung by *Myrmecia* spp., (88%), only 3% had no reaction, 85% had local reactions, and 10% had systemic reactions (Douglas et al. 1998). Among the population of Tasmania, the prevalence of stings by *M. pilosula* was estimated at 2.7%, and 70% of these had sustained anaphylaxis (Brown et al. 2003a). Of Australian victims of ant sting sustaining anaphylaxis, 89% were caused by *Myrmecia* spp. and 11% by *Rhytidoponera metallica* (green-head ant). The *Myrmecia* species responsible were those of the *pilosula* complex (66%), other jumper ants (*M. nigrocincta*, *M. ludlow*, *M. chasei*, *M. swalei*), and 11 species of bulldog ants (Brown et al. 2011). Allergic deaths have occurred from stings of *M. pilosula* and *M. pyriformis* (Brown et al. 2001; McGain and Winkel 2002). Most deaths occurred in victims known to be allergic, had comorbidities, and had not used adrenaline.

Solenopsis Species

"Fire ants" are so-called because their sting causes a burning pain. From a toxicological viewpoint, in Australia, this species appears to have been contained but not eliminated. Only one case of anaphylaxis from *S. invicta* sting has been reported in Australia (Solley et al. 2002), and over the period 2002–2005, no hospitalizations were attributed to its stings (Bradley 2008). In contrast, in Southeastern United States, numerous cases of hypersensitivity reactions are recorded annually, mainly due to *S. invicta* and less due to *S. richteri* (black imported fire ant) stings. Stings from *S. invicta* are occasionally lethal, about 1–2 per annum (Prahlow and Barnard 1998). Most human inhabitants of the Southeastern United States have been stung by imported fire ants, amounting to many millions of stings per annum and an approximate allergic status of the population of a few percent. About half the population are stung each year. Typically, a victim is stung by numerous ants near simultaneously because they attack en masse, but a single sting can evoke an allergic response.

At the site of a fire ant sting, a classic wheal-and-flare reaction develops within 30 min associated with intense inflammatory signs but then a pathognomonic vesicle develops within 24 h and becomes a sterile pustule that remains for several days before crusting. Classic signs and symptoms of anaphylaxis may also develop, rapidly. Whereas acute allergic reactions are mainly due to the protein allergens, the sting site lesions are due to the alkaloids in the venom.

Bees

Australian Bees

Australia has over 1,500 species of native bees of which a few are stingless but the majority can sting, multiple times. Since most native species are solitary and do not defend their nests, stings are unlikely to cause serious envenomation unless a victim is allergic. For example, a 27-year-old farmer, known to be allergic to native bees, died in circumstances suggesting an anaphylactic reaction to a sting by an unspecified native bee (Harvey et al. 1984). Native social bees such as of the genus *Tetragonula* (formerly *Trigona*) and *Austroplebeia* are found only in northern and eastern Australia and do not have stings. In contrast, there are many native wasps that do sting man, and some of these resemble bees. The study of bee venom and human envenomation has been almost exclusively confined to *Apis mellifera* and related species.

The Common or European Honeybee: *Apis mellifera*

A. mellifera has been husbanded by Europeans for thousands of years and has been introduced to each country to which they have settled. Because of our fondness for honey, very few people go through life without suffering at least one bee sting. The sting of the honeybee, unlike those of ants, wasps, and native bees, is barbed, and so, once the sting has been driven into the victim, it is generally not possible for the bee to remove it. Usually, the bee swivels around the sting, which is anchored in the skin, and it and its associated venom gland are torn out and left behind. The bee is then doomed to die because of the damage to its abdomen, but the muscles controlling the movement of the lancets inside the sting continue to pump venom and cause further penetration of the sting shaft into the tissue of the victim.

Allergy to the venom of *A. mellifera* is a worldwide problem. In the United States, more people die from anaphylactic reactions to bee sting than from all other venomous creatures combined. A similar situation exists in Australia. Many thousands of Australians are known to be highly allergic to bee venom, and they may suffer severe local or general reactions after a sting. One or two of them die each year, about the same number of deaths from snakebite (Harvey et al. 1984; Winkel et al. 1999; Welton et al. 2017).

Death can occur in non-allergic victims who receive vast numbers of stings, but this is extremely rare. Several such deaths have occurred overseas (Franca et al. 1994) after massive envenomation by "killer" bees (Africanized honeybees, *Apis mellifera scutellata*) which are more hostile but have less venom than European *A. mellifera* and whose lethality does not differ substantially from European *A. mellifera.* Clinical features of envenomation included hemolysis, rhabdomyolysis, adult respiratory distress syndrome, hepatorenal dysfunction, coma, shock, coagulopathy, and myocardial damage. There is no antivenom. The toxic reaction to massive bee or wasp envenomation may be delayed up to 18 h after the stings. It would be prudent to admit to hospital such patients not only for analgesia but also for

early detection of multiple organ failure. Victims have survived massive stinging. For example, Pursley (1973) reported the case of a 35-year-old male who received 2,243 stings over a 4.5-h period and recovered. Sometimes death may occur following a sting to the pharynx, when a bee (or a wasp) has entered a can of soft drink and has then been accidentally swallowed. The cause of death in such cases is probably respiratory obstruction due to local edema and may not be an allergic response. Such deaths are reported from time to time, and, obviously, it is best to use a straw when drinking from a can on days when bees are active.

Venom and Toxins

No bee venom has been as extensively researched as that of *A. mellifera.* Several groups of investigators have published the venom proteome (Van Vaerenbergh et al. 2014; Matysiak et al. 2014). Over 100 peptides and proteins are identifiable of which over 33 are known or postulated to be toxic. Many toxins have partial homologies with those of other species of bees, wasps, and snakes.

Clinically, the most important components of bee venom are large protein enzymes including phospholipase A_2 (PLA_2), hyaluronidase, and acid phosphatase which are all highly allergenic (Table 1). Twelve peptides or proteins have thus far been designated as allergens. While hyaluronidase facilitates the penetration of venom, PLA_2 disrupts cell membranes. Bee venom also contains many small peptides of which melittin, apamin, and mast cell degranulating (MCD) peptide are the most significant.

Melittin is a 26 amino acid sequence (GIGAVLKVLTTGLPALISWIKRKRGG) structure inactive as a tetramer stored in the venom sac but strongly cell membrane lytic when it dissociates into monomers when diluted on injection. Melittin and PLA_2 are synergistic, causing lysis of cell membranes, e.g.; of muscle cells, fibroblasts, and hepatocytes; and of intracellular membranes, e.g., mitochondrial membranes. Potential clinical uses for melittin include its use as an antimicrobial agent, as a coating on medical devices, as an antiviral agent, as an adjuvant for vaccines, as an anti-inflammatory agent, as an anti-atherosclerotic agent, and as an anticancer agent. Lastly, the endosomolytic property of melittin or derivatives may successfully deliver small interference RNA into the cellular cytoplasm, as gene therapy (Moreno and Girault 2015).

Apamin is an 18 amino acid sequence neurotoxic peptide (CNCKAPETALCARRCGGH) which allosterically blocks Ca^{++}-dependent K^+ channels. It facilitates learning and restores deficits in neurological function and may prove useful in degenerative brain diseases, e.g., Parkinson's disease and related disorders. It preserves stored red blood cells and crosses the blood-brain barrier for which it may see use as a drug shuttle (Moreno and Girault 2015). MCD is a 22 amino acid sequence peptide causing release of histamine and which is contained in the venom in large amounts. Other physiological amines in bee venom include dopamine, noradrenaline, and serotonin. Also found in the venom are sugars, phospholipids, α-amino acids, and numerous volatile pheromones which control many social activities of

Table 1 The toxins of *Apis mellifera* venom (After Van Vaerenbergh et al. 2014; Matysiak et al. 2014; Blank et al. 2013)

Name	Allergen name
Peptides	
Melittin	Api m 4
Apamin	
Secapin	
Mast cell degranulating peptide	
Tertiapin	
Esterases	
Phospholipase A_2–1	Api m 1
Phospholipase A_2–2	
Group XV phospholipase A_2	
Acid phosphatases 1, 2, and 3	
5′ – Nucleotidase	
Carboxylesterase 6	Api m 8
Proteases and peptidases	
CLIP serine protease	
CUB serine protease 1	Api m 7
CUB serine protease 2	
Putative trypsin	
Snake serine protease	
Dipeptidyl peptidase IV	Api m 5
Serine carboxypeptidase	Api m 9
Prolylcarboxypeptidase	
Metalloprotease	
Protease inhibitors	
Api m 6 (serine protease inhibitor)	Api m 6
Serpins 1, 2, and 3	
Carbohydrate metabolism	
Hyaluronidase	Api m 2
N-sulfoglucosamine sulfohydrolase	
Endochitinase	
Growth factors	
Platelet-derived growth factor	
Imaginal disc growth factor 4	
Major royal jelly proteins/precursors	
MRJP5, MRJP8, and MRJP9	Api m 11
Other toxins	
C-type lectin	
Icarapin	Api m 10
Vitellogenins/precursors	Api m 12

bees including attraction to an already stung victim. Table 1 summarizes the main toxin constituents of bee venom.

Royal Jelly

Severe allergic reactions, including fatal asthma and anaphylaxis, have been reported after ingestion of royal jelly – a hypopharyngeal gland secretion of honeybees used as a health tonic. Sensitization to royal jelly ingestion is high, raising IgE antibodies to the allergens Api m 11 which are not directly related to the allergens in venom.

Human Envenomation

The annual Australian mortality from bee sting over the period 2000–2013 was 0.086 per million (Welton et al. 2017) which was the same as that over the period 1960–1981 (Harvey et al. 1984).

Although systemic reactions to bee stings are not uncommon, occurring in about 1 in every 200 people worldwide, fatalities occur in only approximately one in five million people worldwide per year, less often in children. In Australia approximately 3% of the population have systemic reactions to bee stings, and another approximate 3% have local reactions. About 15% of populations have IgE antibody to bee venom. The extent of the allergy varies from case to case and over a period of time and may even fade out over a number of years if no further exposure occurs. The rate of loss of sensitization is about 12% per year.

The families of beekeepers have a higher rate of bee venom allergy than the general population due to their greater exposure to bees. The partners of beekeepers are particularly prone to bee allergy, possibly due to inhalation of allergenic particles adhering to work clothing. When "immune" beekeepers have had a break of weeks or months from contact with bees, they sometimes respond in an allergic fashion when stung upon their return to the hives. This may reflect a fall in the blocking antibodies due to the lack of stimulating bee stings. Hobby beekeepers, whose families have bee venom allergies, should be encouraged to abandon beekeeping. The professional apiarist usually maintains the hives far away from home, safeguarding the family.

Wasps

Hundreds of species of indigenous wasps exist in Australia; most are solitary and do not pose high risk to humans unless nests are concentrated in one area. Other wasp species are social, that is, they live in colonies, posing more risk of stings to humans. Some wasps are quite large, and their stings can be very painful, but a victim is unlikely to receive a series of stings by solitary wasps over a period, and hence hypersensitivity to their venom must be very rare. Moreover, unlike introduced European and English wasps, native wasps are not attracted to food consumed by humans, and so encounters with them are unlikely unless their nests are disturbed.

Some wasps possess an ovipositor, an organ to deposit eggs, but in some wasps (aculeates), the ovipositor has become modified to a sting. Like bees only female

wasps can sting. The unbarbed hollow sting is used to deliver venom to subdue prey or used as a defense or for communication.

Many solitary wasps are parasitoidal, that is, they lay their eggs onto prey (ectoparasitoid) or inject them into prey (endoparasitoid) which becomes food for their larvae. The wasp injects neurotoxins (Moreau and Asgari 2015) into the cerebral ganglia of the prey which immobilizes but doesn't kill it, along with a symbiotic polydnavirus or viruslike particles incorporated into the wasp genome. The virus disables the prey's immune system preventing it from attacking the wasp egg, while other wasp toxins slow the metabolism or arrest the development of the prey thus guaranteeing a lasting food source. The venoms of parasitoidal wasps are thus not used as defensive weapons and lack the major allergenic properties of eusocial wasps.

Genus *Polistes*: Social or Paper-Nest Wasps

Native and introduced species of social or paper-nest wasps exist in Australia. An introduced species of the genus *Polistes* was known to exist in Western Australia in 1950. *P. dominula*, the European paper wasp, inhabits the southwest of Western Australia, and *P. chinensis*, the Asian paper wasp, has been identified in New South Wales. Members of this genus have a slender body and tiny waist and are dark brown with yellow bands across the abdomen. They are distinguishable from the genus *Vespula*, as described below. These wasps often build a nest of wood fiber mixed with saliva under eaves. It is shaped roughly like an inverted cone and suspended by a narrow stem. From below, it can be seen to contain dozens of honeycomb-shaped brood cells. Any disturbance near the nest may provoke an attack by numerous wasps. Both native species, *P. variabilis* and *P. humilis*, can give a very painful sting, and successive stings over a period may lead to hypersensitivity. Other native paper wasps, e.g., *Ropalidia* spp., may also cause adverse reactions, which are not identified by skin testing with *Polistes* antigen.

Vespula germanica (European Wasp) and *Vespula vulgaris* (English Wasp)

These two *Vespula* species are very similar – both have alternating transverse black and yellow abdominal bands (stripes), but the European wasp is distinguished by black dots within the bands of yellow. These and other wasps are called "yellow jackets" in the United States. Some paper wasps also have black and yellow abdominal stripes (e.g., paper wasp, genus *Vespidae*; European paper wasp, *Polistes dominula*), but the waist is more conspicuous in the *Vespula* species than in genus *Polistes* wasps because the abdomen is squarely cut off rather than tapered as in *Polistes*.

Vespula species have large communal nests built with masticated wood fiber, usually underground in cavities or at ground level in concealed situations, such as in wall cavities, and often near or in compost heaps. Unfortunately, because of the warmer climate, they do not abandon their nests in winter as they usually do in the

northern hemisphere. When disturbed, they are likely to attack either singly or in large numbers. Although wasps are carnivorous, *Vespula* are attracted by sweet-smelling foods.

V. vulgaris was first discovered in Australia in 1958 and *V. germanica* in 1959, and both have since spread widely. The prospect of eradication is now very slim since there are neither parasites, nor predators, nor pathogens present in their range. Moreover, there is no competition (except between the two species) and no predation by resident *Vespula* or *Vespa* species. Introduction of a parasitic wasp (*Sphecophaga vesparum*) has been unsuccessful.

Multiple stings may cause initial hypotension or hypertension followed by delayed hemolysis, myolysis, and renal failure. One case of multiple stings in Australia was reported by Levick and Braitberg (1996) in which a child received approximately 120 stings. In addition to severe pain and edema around the stings, the victim experienced a fluctuating conscious state, hypertension, and tachycardia for approximately 36 h but no hemolysis, myolysis, or hepatorenal dysfunction and recovered well.

Elsewhere, particularly in Southeast Asia but not known in Australia, species of the genus *Vespa* (hornets), e.g., *Vespa affinis* (lesser banded hornet), are dangerous. The mode of injury or death is principally via hemolysis, rhabdomyolysis, and hepatic and renal toxicity (Xuan et al. 2010). The "Australian hornet" is actually a species of paper wasp, *Abispa ephippium*, the "Potter wasp."

Wasp Venoms and Toxins

The major Australian health problem with wasps is the development of allergy after repeated stings, not only by the same species but also by different wasp species and by bees. Most of the wasp species of the genus *Polistes* (social wasps) in Australia are natives, but almost nothing is known about their venoms. The most troublesome are introduced species of genus *Polistes* and also species of the genus *Vespula* (*germanica*, European; *vulgaris*, English).

The venom of *Polistes* and *Vespula* wasps contains many proteins, some which are very similar to allergenic toxins in bee venom and include hyaluronidase (Ves v 2), phospholipase A_1 (Ves v 1, Pol d 1) antigen 5 (Ves v 5, Pol d 5), acid phosphatase (small amounts), dipeptidylpeptidase (Ves v 3), vitellogenin (Ves v 6), and mast cell degranulating substance. Venom extracts and recombinant antigens from both genera are available for skin testing and immunotherapy. Wasp and bee venoms have similar enzyme composition such that both *Vespula* and *Apis* (bee) venoms bind to both specific IgE antibodies suggesting that multiple sensitivities are due to cross-reactivity between antigens. Numerous venom nontoxic and toxic components remain to be identified.

Although wasp venom toxins have similarities with bee venom toxins, the predominant toxins in wasp venoms are mastoparans and bradykinins. Mastoparan is a tetradecapeptide (INLKALAALAKKIL), which has a wide range of biological effects. It is a component of many wasp genera, including *Polistes*. It inserts into

phospholipid biomembranes. At the cell surface, it causes lysis, or it interacts with G proteins at the cytoplasmic interface to disrupt transmembrane signaling and to stimulate phospholipases. Inside the cell, it permeabilizes mitochondrial membranes and sarcoplasmic reticulum causing mobilization of Ca++, stimulating apoptosis, and/or culminating in cell death. Hemolysis and histamine release from mast cells are observable clinical effects, but it has potential as an antibiotic and tumor cell toxin (Moreno and Girault 2015).

Human Envenomation

Mortality due to wasp stings in Australia is measurable but lower than that from bee stings. McGain et al. (2000) identified seven deaths from wasp stings over the 20-year period 1979–1998. All these occurred in rural settings in males who had a history of wasp and/or bee allergy, but none had received venom immunotherapy or carried injectable adrenaline. The species were suspected of being a *Polistes* or *Ropalidia* species, but none were identified. Walton et al. (2017) recorded two deaths from wasp stings over the 14-year period 2000–2013 compared with 25 from bee stings.

Clinical Management of Hymenoptera Stings

First Aid

Most victims stung by an ant, bee, or wasp merely suffer a brief period of discomfort, consisting of a sharp sting, local swelling, and pruritus. Stinging into loose tissue, such as around the eyelids or genitals, may produce marked edema. All these effects are direct pharmacological consequences of the components of bee venom.

On being stung by a bee**,** the priority for the victim is to remove the sting and associated venom sac as quickly as possible without over concern for the method of removal since the degree of envenomation is directly related to the duration it is embedded in the skin. If removal is delayed beyond 2 s, there is no difference in the degree of envenomation however long removal of the sting requires (Visscher et al. 1996). The quickest technique is to simply scrape off the sting with a fingernail.

For all Hymenoptera stings, the application of an ice pack (e.g., ice and water mixed in a plastic bag) gives pain relief (Balit et al. 2003), but analgesics and antihistamines may be indicated to reduce the swelling, but they appear to have little effect when there is gross swelling of the stung area. Steroids may give some relief from the marked local pruritus and distressing urticaria, which occur in the more serious cases.

If the victim is known to be allergic, the employment of pressure-immobilization technique is untested but may be useful.

Adults and children who have suffered from a previous general reaction should have ready access at all times to an injectable form of adrenaline. Both the patient

and those around them inside and outside hospital should be instructed how to give this drug promptly. Although several proprietary preparations of self-injectable pre-filled syringes of adrenaline are available worldwide, the only preparations in Australia are "Anapen" (0.3 mg), "Anapen Junior" (0.15 mg), "EpiPen" (0.3 mg), and "EpiPen JR" (0.15 mg) autoinjectors. Two autoinjectors should be accessible. Although the median dose to treat anaphylaxis is 0.3 mg, the range is 0.1–0.8 mg (Korenblat et al. 1999).

Clinical Management of Systemic Reactions

Administration of Adrenaline

The initial treatment, sine qua non, is prompt administration of intramuscular adrenaline. If there is any evidence of general allergic reaction, then it is recommended that adrenaline be given. For an adult, the initial intramuscular dose of adrenaline should be 0.25–0.40 ml of a 1 in 1,000 dilution. The initial dose for a child is 10 μg per kilogram intramuscularly. The intramuscular route is preferable – with this route, the peak plasma levels are reached in a mean of 8 min versus 34 min with the subcutaneous route (Simons et al. 1998). This should be repeated in 10 min if no improvement has occurred. Adrenaline by inhalation should be given if there is any evidence of upper airway obstruction. An inhalation of 5 ml of 0.1% (1:1,000) solution or 0.05 ml/kg of 1% L-isomer or 2.25% racemic mixture should be given by ambulance crew or hospital staff. Repeated doses of adrenaline may be required intramuscularly or as an infusion of 0.05–1.0 μg/kg/min (0.3 mg/kg diluted to 50 ml infused at 0.5–10 ml/h).

Supportive Treatment

Should upper airway obstruction or respiratory failure develop due to bronchospasm and pulmonary edema, then endotracheal intubation (or cricothyrotomy or tracheostomy) and mechanical ventilation with oxygen may be required. Systemic hypotension, which may occur due to vasodilation and leakage of fluid from capillaries, should be treated with intravenous fluids. Antihistamines and steroids are not the best initial treatment, nor are they useful in preventing biphasic and protracted anaphylaxis. The management of anaphylaxis is essentially the application of the basic principles of management of airway, breathing, and circulation with the specific use of adrenaline, colloid solutions, aggressive therapy for bronchospasm, and possibly corticosteroids. It must be stressed that adrenaline is the mainstay of treatment both outside and inside the hospital. Unfortunately, bee sting anaphylaxis is frequently mismanaged in hospitals.

It is important to realize that persons receiving β-blocking drugs or angiotensin-converting enzyme (ACE) inhibitors have a potentiated risk of anaphylaxis. Resuscitation is difficult since hypotension is likely to be refractive to adrenaline. In such cases, glucagon (therapy for β-blocker toxicity) may be required in addition to adrenaline and dopamine.

All persons who have suffered a large local reaction or systemic reaction to a sting should be reviewed by an allergist or by a physician with some experience in the immunotherapy of insect allergy.

Diagnosing Insect Allergy

Diagnosing insect venom allergy is traditionally made by a combination of the clinical features of stings, skin testing (prick/intradermal), and detection of venom-specific IgE antibodies that bind to the surface of mast cells and basophils. Further exposure to venom in these individuals may trigger the release of histamine, slow reacting substances of anaphylaxis, and the eosinophil chemotactic factor, all of which may combine to produce the devastating clinical syndrome of anaphylaxis.

However the presence of venom-specific IgE is associated with only a modest chance (10–15%) of prediction of a severe reaction in stings victims who have no previous reaction and a widely variable chance, 5–75%, of those with a previous allergic reaction. That is, although skin venom tests and serum IgE tests are very good at confirming a diagnosis, they have imperfect sensitivity and limited positive predictive value (Golden 2014). Severity of reaction is correlated with frequency but not severity of previous reactions. Basophil activation tests offer greater diagnostic and predictive accuracy but are not yet in wide use. While the measurement of histamine is not practical, other mediators released by basophils are now measurable. These tests are the secretion of mediators of allergic inflammation, such as increase in serum levels of the phospholipid leukotriene C4 (LTC4) and the cytokine interleukin 4 (IL-4), and the in vitro detection of expression of proteins (e.g., CD63, CD203c) on the basophil plasma membrane, which can then be detected with specific antibodies by means of flow cytometry (MacGlashan 2013). Raised basal serum tryptase also predicts allergic reaction. These in vitro tests, which avoid the risk of reactions, can assist in determining which insect sting the individual may be allergic, especially in the case of possible "double sensitization," and therefore which allergen to employ in venom desensitization therapy.

Allergic Responses

Clinical manifestations of bee venom allergy may be classified into four grades and are relevant to other allergenic insect venoms. They serve as a guide to anticipate subsequent reactions. With each further exposure to venom, the individual may proceed to a more severe degree of reaction.

Grade 1: Local swelling which is more severe than a "normal bee sting." It may involve a large area of a limb and take days to subside. Pruritus may be very distressing.

Grade 2: Mild generalized reaction with urticaria and/or edema of eyelids, lips, or other areas remote from the sting.

Grade 3: Marked generalized reaction with bronchospasm, dyspnea, and extreme distress.

Grade 4: Severe reaction with collapse, hypotension, and possibly death.

In the majority of Grade 3 or 4 cases, there has been a clear worsening of the reaction to subsequent bee stings and hence opportunity to seek medical advice.

This scale may be replaced by a simple classification of reactions to:

Mild: pruritus, urticarial, erythema, mild angio-edema, rhinitis, and conjunctivitis
Moderate: mild asthma, moderate angio-edema, abdominal pain, vomiting, diarrhea, and minor and transient hypotension
Severe: respiratory difficulty (asthma/laryngeal edema), hypotension, collapse or loss of consciousness, seizures, and incontinence

Laboratory Investigations

In most cases of bee venom allergy, the diagnosis is clear-cut, as the offending bee is seen and/or its sting and venom sac are left behind on the victim's skin. Estimations of plasma levels of specific IgE and IgG and serum tryptase should be carried out before commencing immunotherapy and again near the end of the course. A decline in specific IgE and tryptase and an elevation of specific IgG indicate a satisfactory response to the injections of bee venom.

Immunotherapy

For the hypersensitive individual, immunotherapy with venom (VIT) or selected recombinant antigen (e.g., rApi m 3, rApi m 10) is the only intervention known to reduce morbidity and possibly mortality. Whole bee/body extracts for this purpose are redundant, and venom obtained by electrical stimulation is preferred because it contains less nontoxic proteins and more toxic proteins than venom obtained from dissected venom glands (Li et al. 2013). Imported preparation of both *Polistes* spp. and *Vespula* sp. venoms is available for use by allergists for desensitization immunotherapy. Before commencing VIT, it is important to clarify whether the culprit insect is a bee or a wasp, notwithstanding that there is commonly overlapping allergenicity of antigens in both venoms.

The mechanism of the efficacy VIT is not entirely clear. In some individuals, exposure to bee venom stimulates the formation of antibodies directed against any of the main allergenic proteins listed above. However, beekeepers who receive regular batteries of stings may develop "immune tolerance." Beekeepers and subjects undergoing VIT develop IgG (mainly IgG_4) antibodies ("anti-idiotypic antibody," "blocking antibodies") against venom-specific IgE antibody and a decrease in venom-specific IgE and undergo a decrease in T-cell proliferation and in Th2 cytokine (IL-4, IL-5, IL-3) and Th1 cytokine secretion. These changes are mediated by the anti-inflammatory cytokine IL-10 from monocytes, type 2 T helper cells, mast cells, and subsets of T and B cells. However, the protective effect in beekeepers is not observed unless several hundred stings per year are sustained, and in contrast the risk of allergic reaction is heightened if stings number <10/year (Müller 2005).

VIT is very effective but not completely. In a meta-analysis (Boyle et al. 2012) of 6 randomized trials and 1 quasi-randomized trial of immunotherapy in

392 victims who had had systemic or large local reactions to ant, bee, or wasp stings, the risk of systemic reactions to subsequent stings was 2.7% compared with 39.8% in victims receiving no immunotherapy. The risk ratio for preventing system allergic reaction was 0.10 with 95% confidence interval 0.03–0.28, and the risk ratio for preventing large local reaction was 0.41 with 95% confidence intervals of 0.24–0.69. The effect on mortality was immeasurable because of the rarity of this outcome. There was a significant risk of systemic reaction to VIT: of 150 individuals treated with VIT, 14 (9.3%) sustained a systemic reaction. Anaphylaxis occurring during VIT may be ameliorated with omalizumab, a humanized monoclonal IgE antibody.

Immunotherapy is restricted to individuals who have experienced a systemic reaction to a sting. Systemic reactions refer to Grade 3 or 4 reactions and include bronchospasm, upper airway obstruction (not due to a local sting), or hypotension. Local reactions, which predict a risk of a systemic reaction as $<10\%$, are not regarded as indications for immunotherapy. A generalized cutaneous reaction alone (Grade 2) is an indication for immunotherapy if a test for specific IgE antibodies is positive and subject to review if a test is negative.

Numerous guidelines for immunotherapy exist (e.g., Krishna et al. 2011). Indications for venom immunotherapy are a systemic allergic reaction (Grade 3 or 4 or severe reaction) and a subsequent positive venom skin test or venom IgE radioallergosorbent (RAST) assay. These tests do not predict anaphylaxis with a subsequent sting with absolute reliability. A regimen of immunotherapy aims to administer increasing amounts of venom protein and to establish the maintenance dose of within times varying from 0.5 day to 3.5 months. Accelerated schedules are known as "Rush" immunotherapy. The efficacy of therapy may be tested by a provoked sting. The maintenance dose is administered at intervals of 4–6 weeks. Immunotherapy in adults may be discontinued after 3–5 years but with an approximate 17% residual risk of a systemic reaction (Golden 2001). VIT should be conducted with caution when the victim is taking beta-blocker or ACE inhibitor medication or has mastocytosis (mast cell proliferation).

Little has been published of the Australian experience in using bee venom for immunotherapy. Hobday (1998) stated that none of 20 children experienced an adverse reaction when subjected to a bee sting 6–8 weeks after completing a 2-year course of monthly injections. However, a clinically important number of children do not "outgrow" allergic reactions to insect stings, but VIT leads to a lower risk of subsequent reaction compared to adults (Golden et al. 2004).

Immunotherapy should only be conducted by trained personnel in locations equipped to manage all aspects of anaphylaxis.

Government-funded efficacious desensitization immunotherapy is available for victims allergic to ant *Myrmecia* species (Brown et al. 2003b) in Tasmania (Royal Hobart Hospital), Victoria (Monash Medical Centre), and South Australia (Royal Adelaide Hospital). The antigen used is a standardized content of the major allergen Myr p 2 and two minor allergens Myr p 2 and Myr p 3 (Wiese et al. 2008).

Conclusions and Future Directions

Hymenoptera venoms contain numerous toxins most of which remain uncharacterized and whose functions are unknown. Many toxins are able to disrupt biomembrane integrity and function. However, envenomation rarely causes organ damage except after multiple stings. The principal toxicological outcome from Hymenoptera stings is allergic reactions due to the numerous venom protein allergens which may be common or have significant homology between species of a family or between families, particularly between bees and wasps creating double sensitization. The immediate use of adrenaline on site and in hospital is necessary to prevent fatal outcomes. Individual victims of stings experiencing a large local reaction or anaphylaxis must consult a specialist with the aim of achieving immune tolerance by means of venom immunotherapy. The many thousands of bioactive components present in Hymenoptera venoms offer opportunities to derive new therapies for a range of diseases, especially the development of antibiotics arising from the need of social Hymenoptera to prevent infection in their colonies. The peptides melittin and apamin from bee venoms and mastoparan from wasp venoms are being investigated for numerous therapeutic applications.

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Tick Paralysis of Animals in Australia

10

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Abstract

This review encompasses all aspects of tick paralysis relevant to animals in Australia. Tick paralysis is a major animal health problem along the east coast of Australia. Each year an estimated 10,000 dogs and cats are presented to veterinarians for treatment. Many other animal species have been documented to be affected with tick paralysis including horses, cattle, sheep, bats, and other native animals. *Ixodes holocyclus* and, to a lesser extent in southern Australia, *Ixodes cornuatus* are the causative tick species. Tick attachment to the host is usually followed by a latent period of 3–6 days during which time the tick engorges and salivary glands enlarge producing a neurotoxin. Paralysis signs are generally caused by the engorgement of one tick but less commonly multiple ticks can be found and occasionally no tick is found. Typical early clinical signs include hind limb ataxia which generally progresses to quadriplegia and death from respiratory failure. Atypical cases may present without generalized paralysis but with only facial paralysis, anisocoria, or gastrointestinal signs such as vomiting and regurgitation may occur making diagnosis difficult. Unexplained actions of the tick toxin include cardiac effects which may contribute to pulmonary edema and ultimately respiratory compromise. The historically elusive molecular nature of the salivary gland secreted 5 kD protein neurotoxin has recently been described and the gene sequence determined. The principle therapeutic agent used in the treatment of affected animals is hyperimmune canine serum. Supportive care of severely affected animals appears important to improve survival outcomes. Response to treatment of early mildly paralyzed animals is good with over 90% of treated reported animals surviving. Animals presenting with severe respiratory compromise and quadriplegia have a poor survival prognosis despite intensive treatment. A range of preventative acaricides are currently available including new generation long-acting oral medications, topical liquids, and washes.

Abbreviations

CSIRO	Commonwealth Scientific and Industrial Research Organisation
g	Gram
HT-1	Holocyclotoxin 1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
im	Intramuscular
ip	Intraperitoneal

iv	Intravenous
kD	Kilodalton
kg	Kilogram
LD50	Lethal dose 50%
mg	Milligram
mL	Milliliter
NSW	New South Wales
Qld	Queensland

Introduction

Tick paralysis caused by *Ixodes holocyclus* is a major veterinary problem in Australia, and it is estimated at least 10,000 domestic animals are presented to veterinarians for treatment each year (Stone and Aylward 1987). A wide range of domestic animal species are affected including dogs, cats, horses, cattle, sheep, and poultry. Tick paralysis is most commonly reported to occur in domestic dogs and less commonly cats and other farm animal species. While the life cycle of the tick has been understood for many decades, the elusive molecular nature of the toxin causing the neurological signs has been inadequately described until recently (Thurn et al. 1992). *Ixodes holocyclus* has long been recognized as the cause of tick paralysis in Australia although a morphologically similar and closely related species *Ixodes cornuatus* is also capable of causing paralysis.

This review has focused on the issues associated with tick paralysis in animals in Australia. While there are other ticks found outside of Australia (Malik and Farrow 1991) that can cause clinically significant paralysis, they have not been included in this review. The goal of this review was to encompass all the relevant and current information available on tick paralysis in animals in Australia. Although humans can be affected with all stages of the life cycle of the paralysis tick and human deaths have been reported (Pearn 1977), this material has not been covered in this review. The Australian veterinarian Sir Ian Clunies Ross has made a major contribution to understanding the biology of the paralysis tick and developed the first canine tick antiserum; much of his early work is recognized in this review (Ross 1935).

General

History

Tick paralysis has long been a problem on the east coast of Australia and was first documented in 1884 but the problem had been recognized since the early settlement of the continent (Dodd 1921). Experimental studies to better understand the biology of the tick and its ensuring disease process were first begun in the 1920s (Dodd 1921; Ross 1926, 1927a). Sir Ian Clunies Ross experimentally engorged ticks on dogs and

was able to demonstrate that paralysis did not occur until 3–5 days after attachment and sometimes up to 13 days. Ross also found that the toxin was likely to be produced from the tick salivary glands because injection of homogenized glands into normal dogs produced similar clinical signs (Ross 1926).

Immunity was observed in dogs that had recovered from tick paralysis and Ross proposed that a hyperimmune serum made in dogs might be the only effective treatment but that its production "*. . .would be difficult and costly. . .*" (Ross 1927b). Ross subsequently produced a hyperimmune serum and documented its therapeutic use in over 100 clinical cases of naturally occurring tick paralysis in dogs (Ross 1935). Ross reported a 75% recovery rate with the serum and commented that veterinary practitioners at that time generally had a 10–60% recovery rate without the serum (Ross 1935). Ross used a simple mouse protection bioassay to measure serum neutralizing potency and demonstrated that his canine tick antiserum could protect a mouse from 20–40 lethal doses of engorged tick extract (Ross 1935). While the tick serum proved useful, some veterinary practitioners experienced deaths leading to the conclusion that the serum was most useful only in the early stages of the disease process following tick removal (Hindmarsh and Pursell 1935). Commercial production of canine tick antiserum first began in the 1930s at the Commonwealth Serum Laboratories using the methods developed by Ross (Oxer 1948; Ross 1935).

Life Cycle of *Ixodes holocyclus*

Knowledge of the life cycle of *Ixodes holocyclus* has been progressively obtained using both laboratory colonies of ticks and field data from various infested animal species and the environment (Ross 1924). *Ixodes holocyclus* has a life cycle similar to other members of the *Ixodes* tick family. It is a three-host tick, gorging on a blood meal and then dropping off its warm blooded host between each successive stage of the life cycle. The life cycle is comprised of your stages: egg, larva, nymph, and adult. The engorgement period for each stage can last up to 14 days and is dependent upon environmental temperature and humidity. Large numbers of larvae, nymphs, and adults (females) may be found simultaneously on the same individual. Larvae and nymphs drop off the host when engorged and undergo a molt on the ground into the next stage of the lifecycle. When conditions are favorable the ticks "quest" and are then actively seeking a host, attracted by carbon dioxide, heat, and movement (Hall-Mendelin et al. 2011a). Engorged and sexually mature adult females detach and lay 2000–3000 eggs into moist litter. Males also seek warm blooded hosts but rarely take a blood meal from the host; instead they mate on the host and feed on the hemolymph of engorging female ticks by piercing their cuticle on their ventral side (Ross 1924). The development from egg to adult takes around 1 year. However, cold conditions lengthen the developmental period. Dryness affects all stages adversely and this limits ticks to a habitat with a high relative humidity (>85%). The eggs hatch in 40–60 days during the summer months in moist leaf litter. The emerging

Table 1 Key morphological features used to differentiate between *Ixodes holocyclus* and *Ixodes cornuatus* ticks (* indicates most significant features) (Adapted from Barker et al. 2014)

Character	State	*I.cornuatus*	*I.holocyclus*
*Scutum proportion	Longer than wide	+	
	Wider than long		+
Punctations on scutum or conscutum; distribution	Sparse	+	
	Dense		+
Cervical groove length	Short	+	
	Long		+
Trochanter spurs (males)	Indistinct		+
	Distinct	+	
*Leg color pattern	None	+	
	Legs 1 and 4 darker than legs 2 and 3		+
Tarsi terminal profile	Gradually stepped		+
	Steeply stepped	+	
*Cornua on dorsal basis capituli	Absent	+	
	Present		+

larva can survive for weeks without attaching to a host. They appear mainly in summer and autumn (February to May). Nymphs can also survive without a host for long periods. They appear predominantly in autumn and winter (March to September) followed by adults with numbers peaking in late spring to early summer (September through to December) (Doube 1975).

The bandicoot is an important intermediate host in Australia for *Ixodes holocyclus*, and bandicoots have been found with more than 60 adult ticks attached without clinical signs of tick paralysis (Jones 1991). There has been conjecture as to whether the bandicoot has innate natural immunity or that it acquires protective immunity through chronic exposure; development of immunity through exposure appears the favorable hypothesis (Stone and Wright 1981). The two significant bandicoot species are the short nose bandicoot found in eastern Queensland and north east NSW, and the long nose bandicoot found in eastern Queensland, eastern NSW and Victoria (Doube 1975; Ross 1924). Many other animal species can also function as host and provide the necessary blood meal, including the domestic animals such as dogs, cats, cows, horses, sheep, and chickens (Ilkiw 1983).

Distribution of *I. holocyclus* and *I. cornuatus* in Australia

Tick paralysis in Australia in animals is primarily caused by *Ixodes holocyclus* although a morphologically similar species – *Ixodes cornuatus* – is responsible for a lesser proportion of cases. Distinction between these two Ixodid species has been

possible by careful morphological examination and more recently the key anatomical differences have been better defined aiding their morphological differentiation (Barker and Walker 2014; Table 1). *Ixodes cornuatu*s has been associated with clinical signs of paralysis in animals (Beveridge and Coleman 2004), and the tick is found in south eastern Australia, including Tasmania, where *Ixodes holocyclus* is not currently found (Jackson et al. 2007). The geographical distribution of *Ixodes holocyclus* is considered to be from east of Lakes Entrance in Victoria, along the east coast of Australia, up to the northern tip of the east coast to Cape York Peninsula extending variable distances inland but mostly less than 50 km from the coast (Fitzgerald 1998).

Toxins in *Ixodes holocyclus*

Isolation and Characterization of the Salivary Origin Neurotoxin

The quest to understand the molecular nature of the paralyzing neurotoxin produced by *Ixodes holocyclus* has been long and tortuous. A salivary-gland origin toxin was first hypothesized by Ross to be the paralyzing agent (Ross 1926). Ross correctly concluded this because: (i) he was unable to transfer the toxicity from a paralyzed animal through injection of whole blood or body fluids into another dog or experimental animal (although the concentration of toxin in serum is probably extremely low) (Ross 1926); (ii) injection of salivary-gland extracts from engorged ticks were shown to reproduce the disease (Ross 1935). Multiple reports since the early work of Ross continued to demonstrate the toxin was produced in the salivary glands following tick engorgement, but the molecular nature of the toxin remained elusive (Kaire 1966; Stone and Wright 1981). The production of toxin in the salivary glands reaches peak levels after 3–5 days, and this coincides with onset of progressive paralysis in the host (Goodrich and Murray 1978; Murray and Koch 1969). It was not until the application of chromatographic methods to the purification process that the causative neurotoxin was found to be a 5–6 kD protein molecule (Thurn et al. 1992). Thurn's purification process yielded a toxin with 313-fold greater toxicity in neonatal mice by mass than a crude tick extract. Commercial canine tick antiserum also blocked binding of the 5–6 kD toxin to rat synaptosomes (Thurn et al. 1992). The authors maintained that the previous work (Stone et al. 1979; Stone and Aylward 1987; Stone and Wright 1980) on descriptions of a 60 kD causative toxin was probably compromised by the binding of the toxin to serum albumin. A number of publications reported the molecular weight as 60–80 KD but were probably describing albumin (Thurn et al. 1992). The name holocyclotoxin (HT-1) was proposed to describe the neurotoxin from *Ixodes holocyclus*. Chemical synthesis of HT-1 has revealed a molecule with four disulphide bonds and a folded three-dimensional structure but no in vivo toxicity data was reported for this synthetic molecule (Vink et al. 2014). HT-1 appears to have structural similarity to scorpion neurotoxins and contains eight cysteine residues leading to a "cysteine knot" structure (Vink et al. 2014).

Mechanism of Action of the Neurotoxin

The physiological action of the neurotoxin produced by *Ixodes holocyclus* has been studied experimentally. However, much of the knowledge of the proposed mechanism of action is largely based on unrepeated work published by a single author (Cooper and Spence 1976).

Tick toxin was found to result in a temperature dependent reduction in neurotransmission at the neuromuscular junction, caused by an inhibition in the amount of acetylcholine released (Cooper and Spence 1976). To demonstrate this effect preparations of phrenic-nerve diaphragm muscle obtained from mice paralyzed with ten *Ixodes holocyclus* nymph for 3.5–4.5 days were incubated in an electrolyte solution at various temperatures. The action of the tick toxin was studied by electrical stimulation of the isolated nerve preparations and measurement of the resulting twitch in the connected diaphragm muscle (Cooper and Spence 1976). There was a delayed effect of 6–7 h between incubation of mouse nerve-muscle tissue in buffer containing the tick toxin extract upon inhibition of the neuromuscular junction (Fitzgerald 1998). The delay between exposure of the neuromuscular junction to tick toxin and paralytic effect is consistent with clinical signs typically observed in affected animals when treatment is begun and when toxin is injected (Cooper et al. 1976). Cooper postulated that this delay could be due to either chemical modification of the toxin in vivo or simply a delay in reaching the site of action. Due to the temperature dependence and absence of biding of the neurotoxin at low temperatures implementation of "controlled hypothermia" was advocated as a treatment of clinical paralysis. When considering clinical adoption of this recommendation Fitzgerald (1998) was careful to advise that to avoid compounding the generalized illness and depression associated with tick paralysis, core temperature should not be allowed to fall below 34–36 °C (Fitzgerald 1998). In a literature review of the available knowledge on controlled hypothermia for treating tick paralysis it was concluded that until further research is conducted that animals not be exposed to excessively high or low temperatures (Fearnley 2002).

Studies of isolated ulnar and tibial nerve preparations obtained from dogs with experimentally induced tick paralysis did not reveal any delay in nerve conduction velocities or pulse amplitudes in nerve trunks. Isolated muscle preparations from these dogs similarly did not reveal any change in action potential shape but a reduction in amplitude of the muscle contraction. This experimental information further supported the argument that the site of action of the tick toxin in at or near the neuromuscular junction (Cooper et al. 1976).

Non-Neurological Effects Associated with Engorged Adult *Ixodes holocyclus*

An anticoagulant, found in extracts of *Ixodes holocyclus*, has been described (Anastopoulos et al. 1991). The activity of the anticoagulant was found to be greatest

when extracted from the gut of the tick, although limited activity was also found in salivary-gland secretions. The high levels in the gut may be due to reingestion by the tick during feeding of the salivary-gland origin anticoagulant (Anastopoulos et al. 1991). It is postulated that the anticoagulant assists in maintaining blood flow while the tick is feeding. The anticoagulant appears to have no clinical or pathological significance in animals.

The presence of cardiac acting toxins in the salivary secretions of *Ixodes holocyclus* was demonstrated by its action on rat cardiac function (Campbell et al. 2004). Prior incubation of tick toxin extracts with canine tick antiserum prevented the cardiovascular effects in live rats and excised rat cardiac tissues (Campbell et al. 2004). A positive inotropic response was observed upon the in vitro cardiac tissues from rats, and at higher doses arrhythmic responses in the right atria were also recorded in live rats. The responses of rat cardiac tissues to tick toxin were consistent with the mechanism of action being a blockade of potassium channels (Campbell et al. 2004). No histological or ultrastructural changes were identified in rats in which tick paralysis was experimentally induced by multiple tick attachment and engorgement. It is unclear whether the cardiac effects are due to different toxins to those that cause generalized paresis.

Toxic Effects from Injection of Unfed Ticks

There has been argument over the toxicity of unfed *Ixodes holocyclus* ticks when injected into mice. The toxicity of salivary-gland extracts produced from unfed ticks was found in one study to be relatively high and declined during the first 24 h after initiation of feeding only to resume toxicity at the end of feeding (Stone et al. 1979). However, work performed subsequently by a different group examining the toxicity of unfed ticks was in complete contrast to the work of Stone. No toxicity was observed from injection of unfed ticks into mice (Davey et al. 1988). Up to 20 unfed ticks were injected into each mouse and without toxicity. The absence of toxicity in mice injected with homogenates of unfed adult ticks was also demonstrated in two other studies (Goodrich and Murray 1978; Kaire 1966). Davey questioned how such a large difference could be observed between their studies and that of Stone who reported toxicity in unfed ticks.

Cuticle Toxin

Extracts of the cuticle of unfed ticks were reported to be toxic (Doube et al. 1995). Toxicity was described as being associated with the cuticle washings of fully engorged larvae, nymphs, and adults. A respiratory paralysis was reported to occur in mice 9–10 h after intraperitoneal injection (Jones 1991). The substance was described as being a deep red water soluble material that appears from the time the tick drops off the host until ecdysis or oviposition (Jones 1991). No further descriptions of this cuticle associated toxin have been described.

Larvae Toxicity

The newly hatched larvae can cause an intense skin irritation in humans and animals exposed to large numbers (Ross 1935). Neurotoxicity resulting from the attachment of 500 larvae to 500 g guinea pigs has been reported but it appears toxicity per larvae is much less than in adult ticks as large numbers of larvae are required (Oxer and Ricardo 1942). Clinical neurotoxicity due to larvae infestation is possible but appears to be a rare clinical event in domestic animals. One clinical case of tick paralysis was described in a 4.5 kg domestic cat with an estimated 200–300 *Ixodes holocyclus* larvae attached (Fitzgerald 2007). The clinical signs described were mild and the cat recovered within 48 h following administration of canine tick antiserum and removal of the larvae.

Nymph Toxicity

Toxicity from attachment of nymphal stages of *Ixodes holocyclus* has been described in a dog (Ross 1932) and experimental guinea pigs (Oxer and Ricardo 1942). A 6-month-old cocker spaniel was found to have "not less than 100" engorging nymph attached around its eyes and face; the dog developed lethargy, mild hind limb paresis, but recovered without antiserum and physical removal of all nymph (Ross 1932). Five days later the same dog again had over 70 engorged nymph attached, exhibited mild hind limb paresis, and once more spontaneously recovered after 7 days (Ross 1932). Guinea pigs weighing 400–500 g in which 25 nymphs were attached died of paralysis within 7 days while 1–12 nymphs were unable to cause paralysis (Oxer and Ricardo 1942). Nymph may attach and engorge on dogs but scratching by the dog may dislodge many of the attached nymphs (Oxer and Ricardo 1942). Nymphs have only minor significance in causing clinical paralysis in domestic animals although skin irritation may be more relevant.

Egg Toxicity

Homogenates of eggs obtained from 17 different species of Ixodid ticks were lethal when injected into guinea pigs at a dose rate of approximately 300 mg/kg (Riek 1957). Death was not associated with paralysis but appeared to due be a fatal generalized toxemia, different to the paralysis syndrome seen with adult ticks. Egg toxicity is of no clinical relevance to domestic animals.

Clinical Signs

Dogs and Cats

In a study designed to document the clinical signs of tick paralysis in eight Beagle dogs with 3–4 ticks attached, the interval from attachment to first clinical signs was 5.5–7.0 days (Ilkiw et al. 1987). Seven of eight dogs developed clinical disease,

consisting of ascending flaccid motor paralysis, and died within 18–32 h of onset of signs (Ilkiw et al. 1987). One dog with three ticks attached did not die nor show any clinical signs of paralysis which the authors considered was due either to immunity of lack of toxin production by the ticks. The authors noted a tendency for dogs which developed clinical signs early to more rapidly progress to fatal paralysis (Ilkiw et al. 1987). The first noticeable clinical signs were for those dogs that barked, a mild hoarse or husky tone to the bark, which was then followed by hind limb ataxia. The dogs appeared healthy, ate and drank normally, and did not vomit. As the paresis progressed to also involve the front legs, 20% of dogs were observed to vomit and retching noted in some dogs if food was placed in front of them (Ilkiw et al. 1987). As paralysis progressed, the dogs were unable to right themselves and adopted lateral recumbency, respiration was associated with grunting noises, and swallowing reflexes were affected (Ilkiw et al. 1987). In the terminal stages the mucous membrane color was gray, pupils dilated, and death occurred within 2 h of loss of all withdrawal reflexes (Ilkiw et al. 1987). Clinical signs of tick paralysis in cats are similar to that described for dogs (Schull et al. 2007).

Focal neurological defects associated with or without generalized paralysis have been described in animals with tick paralysis (Holland 2008). Unilateral facial paralysis and anisocoria were described in 27 clinical cases occurring in dogs and cats (Holland 2008). When facial paralysis and anisocoria was present the tick attachment site was always located on the head or neck and always ipsilateral to the side of facial paralysis. Asymmetrical focal neurological deficits were a consistent finding in a 9.4% (27/286) of clinical cases in dogs and cats presented with naturally occurring tick paralysis (Holland 2008). The focal neurological lesions were described to persist for many days and sometimes weeks after resolution of generalized signs of tick paralysis (Holland 2008).

Cattle and Sheep

Tick paralysis caused by *Ixodes holocyclus* has long been recognized as a production limiting problem in some farming regions of Australia. Tick paralysis is primarily a problem in young calves, although large calves and adult cattle can occasionally be affected (Doube 1975; Doube and Kemp 1975). Young calves born in the spring are particularly at risk of developing fatal paralysis in endemic tick areas. In an experimental infestation of cattle, two ticks were insufficient to cause paralysis in 2-week-old calves but that ten ticks were required to induce paralysis (Doube 1975). Older calves of 80–160 kg bodyweight were paralyzed by the attachment of 25 ticks per animal resulting in 3–10 ticks fully engorging (Doube and Kemp 1975). Management strategy to avoid calving cattle in the spring on susceptible pastures has been described as effective in reducing the mortality in calves (Doube and Kemp 1975). Further work (Doube et al. 1977) clarified doubts from earlier work that one tick could paralyze a calf and supported the hypothesis that the fatal dose was between four and ten ticks per calf. Cutaneous cellular immune responses and in particular a basophil response to tick feeding appears to be part of the mechanism whereby cattle (and guinea pigs) may develop acquired resistance to tick infestation

(Askenase et al. 1982). Acute nonfatal side effects including collapse, tachycardia, and mucous membrane pallor associated with intravenous administration of canine tick antiserum to calves have been described, and these may be a factor limiting clinical treatment, along with cost, in use of this product in bovines (Schull 2007).

In sheep, a 42% mortality rate was described in one outbreak in 1968 affecting a sheep flock in Victoria (Sloan 1968). The sheep were reported to be covered by large numbers of ticks particularly on the face, and death was due to recumbency and paralysis (Sloan 1968).

Horses

Horses are susceptible to tick paralysis with cases described in foals, adult horses, and a miniature pony (Ruppin et al. 2012; Tee and Feary 2012). The death of five yearling foals, each with up to 40 engorged ticks present, has been described (Bootes 1962). In another series of 103 cases of tick paralysis in horses of varying ages and bodyweights, an overall survival rate of 74% was reported (Ruppin et al. 2012). Clinical signs of tick paralysis in horses appear similar to that described for dogs with a progressive flaccid paralysis leading to recumbency and respiratory paralysis. At least 43/103 horses were described as recumbent at the time of presentation for veterinary treatment, and prolonged recumbency was associated with a higher risk of death (Ruppin et al. 2012). Paralysis ticks were generally located on the head, neck, axilla, torso, and inguinal areas of affected horses with no ticks located on the legs (Ruppin et al. 2012). The mortality rate in horses is higher than that for small animals, and this was considered due to the greater complications associated with recumbency and respiratory compromise (Ruppin et al. 2012).

Other Animals

Tick paralysis has been described in a llama in Australia (Jonsson and Rozmanec 1997). The llama presented recumbent, unable to rise and pronounced unilateral facial paralysis but was euthanized after 3 days due to complications from hepatic lipidosis (Jonsson and Rozmanec 1997). Flying foxes have been reported as dying in large numbers from tick paralysis (Campbell et al. 2003). The bats fall from the trees due to paralysis and succumb to predation or paralysis complications and populations have reportedly fallen by tens of thousands.

Diagnosis

Typical Presentations

Diagnosis of "typical cases" in geographical areas where *Ixodes holocyclus* is endemic and seasonal, usually pose little difficulty. The easily recognizable signs of ascending flaccid lower motor neuron paralysis often accompanied or preceded by dysphonia and/or vomiting and/or regurgitation and/or gagging and retching are

arguably pathognomonic (Westwood et al. 2013). A typical history involves residence in a tick habitat or a visit to in the previous 3–6 days. The speed of onset of these signs and the rate of progression appears variable.

Locating an engorged *Ixodes holocyclus* (or *cornuatus*) will support the diagnosis but differential diagnoses such as myasthenia gravis, acute polyradiculoneuritis, snakebite (especially brown snake, *Pseudonaja textilis*), spinal pathology, and botulism may need to be excluded.

A retrospective examination of 325 cases of tick paralysis in dogs and cats presented to six veterinary practices in Sydney, NSW, revealed that 10% of dogs and 4% of cats presented with no tick visible; 77% of dogs and 81% of cats had one tick; 8% of dogs and 15% of cats had two or more ticks; while 4% of dogs had more than three ticks (Westwood et al. 2013). Season was important with 92% of cases presented in the spring and summer. The most common presentation in dogs (46%) and cats (47%) was for a mild gait abnormality. The median hospitalization time for dogs and cats was 2 days (Westwood et al. 2013). The authors reported that 9% of dogs and 11% of cats had been treated on at least one previous occasion for tick paralysis. Despite the detailed clinical data there was no overall death rate reported in the study (Westwood et al. 2013).

There are no specific diagnostic tests for diagnosing tick paralysis. A rapid patient-side test would greatly facilitate diagnosis of tick paralysis in suspect patients where no tick can be found. Until such time veterinarians must rely heavily on suspicion of tick paralysis and exclusion of other more readily diagnosed differential diagnostic conditions.

Atypical Presentations

The diagnosis of tick paralysis can be extremely challenging with atypical cases. These include "out-of-season" presentations, those on which a tick cannot be found, those outside of a known geographical area (Kelers et al. 2012), or those with limited or unusual signs.

Tick paralysis is most commonly reported to occur in the spring and summer but cases can occur all year round. Cases that occur outside of the typical season for tick paralysis are possible and veterinarians need to be vigilant and maintain a high suspicion index to ensure diagnosis (Fitzgerald 1998).

Cases of tick paralysis may occur outside of a known endemic geographical area for various reasons (Beveridge 1991). These may be caused by previous exposure to a tick infested area, transportation of tick infested materials with ensuring fomite transmission, or changing geographical distribution of the causal ticks. Two separate cases of tick paralysis caused by *Ixodes holocyclus* in dogs that had never left the Melbourne area were described as occurring in the outer east of Melbourne, which is not a known tick area (Beveridge 1991). Straw bedding material originating from Queensland was suspected to have carried questing *Ixodes holocyclus* ticks which subsequently caused tick paralysis in a dog in Melbourne (Beveridge 1991). A dog was imported into New Zealand from Sydney, Australia, that developed clinical

signs of tick paralysis and died within days of arriving in New Zealand; presumably the dog was transported with a tick already attached (Hutton 1974).

Presentation of dogs to veterinarian with suspicious clinical signs of tick paralysis such as lower motor neuron paralysis and recent exposure to a geographical tick region – but in which no tick can be found – are very challenging for veterinarians to manage and clients to understand. Repeated searching of dogs is essential and total body clipping of all hair may facilitate the location of engorged ticks (Fitzgerald 1998). More than 70% of ticks attach to the head and neck region in experimentally and naturally infested dogs, while only 10% of ticks attached to the ventral body (Atwell et al. 2000). Dogs with long hair coats were found to be more likely to have more ticks attached than shorter hair coat dogs (Atwell et al. 2001).

Dogs may present with an atypical history of vomiting or regurgitation and no apparent neuromuscular weakness (Campbell and Atwell 2001) (Malik et al. 1988). Careful veterinary diagnostic evaluation to exclude other causes of vomiting is essential along with regular and repeated whole body searches for ticks. Evidence of megaesophagus, defined as esophageal dilation on radiographs, was found in 70% (28/40) of dogs diagnosed with tick paralysis (Campbell and Atwell 2001). The development of megaesophagus was not significantly associated with the tick location being on the neck or throat region. Megaesophagus was more likely to be detected in older dogs with tick paralysis and recovery of esophageal function lagged behind that of generalized tick paralysis (Campbell and Atwell 2001). Vomiting and regurgitation without classical signs of paralysis has been described and a series of three such case presentations has been described (Malik et al. 1988). The extreme difficulty in obtaining a diagnosis was highlighted in this report. Multiple days of hospitalization elapsed before diagnosis of tick paralysis, death of two of the dogs, and exploratory laparotomy surgery performed in one case in an attempt to diagnose the cause of the gastrointestinal upset which later died (Malik et al. 1988). None of the three dogs were reported to have displayed signs of generalized paralysis demonstrating the difficulty veterinarians face with atypical tick paralysis cases.

The high frequency of megaesophagus in dogs with tick paralysis and the radiographic visibility on plain views of this condition would suggest that this may be a useful diagnostic screening test in suspect cases. Thoracic radiographs could be used to support administration of tick antiserum in cases where no tick can be found.

Treatment

Tick Antiserum

The principle treatment in cases of tick paralysis for decades has been the use of canine tick antiserum harvested from hyperimmune donor dogs. Despite the widespread use there is only anecdotal data to support its effectiveness. Almost all clinical case studies have not included untreated tick infected control animals. Early administration of tick antiserum is generally advocated to obtain the best patient survival and minimize

complications (Fitzgerald 1998). Ross first described the use of canine tick antiserum for the treatment of tick paralysis in dogs and commented that over 75% of cases survived with antiserum, but no detailed case data was published (Ross 1935).

Canine tick antiserum is prepared based on the original methods developed by Ross (Oxer 1948). Large breed dogs are progressively exposed to increasing numbers of ticks until they develop sufficient immunity to withstand engorgement of 30 or more ticks simultaneously (Oxer 1948). Serum is then harvested from the dogs at regular intervals following boosting of immunity by applying additional ticks to each dog. Early tests of serum neutralizing potency were performed using a death-as-endpoint adult mouse protection assay (Kaire 1965; Oxer 1948; Ross 1927b, 1935) and reported varying protective potencies of 3–50 mouse lethal dose 50% per milliliter of serum. A neonatal mouse protection assay that used a 24-h paralysis index was described and adopted for commercial serum batch release testing (Stone et al. 1982). Neonatal mice appear to differ in their response to tick extracts compared to adult mice, with paralytic signs being more readily observed, whereas adult mice tend to die quickly with minimal paralytic signs (Stone et al. 1982). More recently an enzyme immunoassay has been used to determine specific IgG antibody levels (Hall-Mendelin et al. 2011b) to tick proteins, and this has been adopted by regulatory authorities as a potency test (Westwood et al. 2013). However, there is conjecture over whether it is IgG or IgM that is neutralizing the tick toxins. The rapid decline in immunity of dogs following tick exposure suggests that IgM may be the predominant neutralizing class of immunoglobulins although serum specific IgG levels appear to be measurable (Hall-Mendelin et al. 2011b).

All commercial tick antiserum products in Australia contain either phenol or cresol as a preservative to limit microbial growth. Administration of large volumes of antiserum to low bodyweight animals may lead to high serum levels of phenol, which may create a theoretical risk of toxicity, although this has not yet been described.

The dose of tick antiserum to administer to animals with tick paralysis has long been the subject of discussion by veterinarians. However, almost no consideration is given to the variable potency of the relatively crude tick antiserum, which is a recognized issue (Westwood et al. 2013). Veterinarians adopt one of two regimes for determining the dose of canine tick antiserum to administer. Some practitioners have advocated a dose proportionate to bodyweight in the range of 0.5–1.0 mL/kg, while others have recommended a fixed minimum volume of 10–20 mL per animal or per tick (Fitzgerald 1998; Webster 2014a). Treatment with a single large dose of tick antiserum as early as possible in the disease process is recommended rather than attempting to titrate the dose to clinical effect (Fitzgerald 1998). Intravenous administration is essential due to the slow absorption of large molecular weight serum immunoglobulin proteins from subcutaneous or intramuscular sites. Intraperitoneal administration of canine tick antiserum is used by some veterinarians to minimize the risk of anaphylactic reactions to the canine proteins, particularly in cats (Schull et al. 2007). In a comparison of iv and ip administration of antiserum to cats, no reactions were recorded with ip route but a 5% reaction rate with iv route; both groups had the same survival outcome (Schull et al. 2007).

Only one published study could be found that compared outcomes of untreated control dogs with experimentally induced tick paralysis and dogs treated with tick antiserum (Ilkiw and Turner 1988). Six *Ixodes holocyclus* ticks were each applied to 40 dogs but only 20 dogs developed signs of paralysis and four dogs were then allocated to five treatment groups including an untreated control. It was not stated if the ticks were removed from the dogs prior to applying the treatment and the stage of paralysis is not clear. Dogs that received no tick antiserum all (4/4) died within 3.3 h. Dogs that received tick only tick antiserum and no other drugs 75% (3/4) died within 38.3 h. Tick antiserum prolonged the time to death. The surviving dog took 57.8 h to walk again and a further 4 days to be fully recovered. Four dogs were treated with tick antiserum plus dexamethasone (0.5 mg/kg) every 12 h 75% (3/4) of the dogs survived. Treatment with tick antiserum plus promethazine resulted in 50% (2/4) of dogs surviving, while treatment with tick antiserum plus phenoxybenzamine (1 mg/kg) resulted in 100% (4/4) dogs surviving. The overall results of this study are difficult to interpret as the numbers are small, some details are missing, and not all dogs became affected with tick paralysis. The authors reported that based on the phenoxybenzamine results veterinary practitioners had adopted that as a treatment alongside tick antiserum (Ilkiw 1983). Despite the encouraging preliminary clinical report on the use of phenoxybenzamine, it appears to have not been widely accepted and its use is not included in more recent treatment recommendation reviews (Fitzgerald 1998; Jones 1991).

The time period from administration of tick antiserum to visible clinical improvement has been reported as a minimum of 12 h (Fitzgerald 1998; Ilkiw and Turner 1988). A worsening of clinical signs during this time period commonly occurs until improvement is noted.

Administration of canine tick antiserum is reported to have occasional adverse effects in dogs, and these events are more likely to occur in cats (Schull 2007). A survey of veterinarians reported that 3% of dogs treated intravenously with tick antiserum experienced an adverse reaction. Acute death of five cats following intravenous injection of canine tick antiserum was reported by a veterinary practitioner despite pretreatment of each cat with antihistamine, steroids, and adrenaline (Fitzgerald 1998). In 82% of adverse reactions in one study bradycardia, mucous membrane pallor, hypotension, weakness, and depression were described (Atwell and Campbell 2001). The physiological nature of this reaction was attributed to activation of the Bezold-Jarisch reflex which is triggered by activation of intra-cardiac receptors (Zucker and Cornish 1981). The remaining 18% of reactions were associated with tachycardia, cutaneous reactions, vomiting, diarrhea, and restlessness and were thought to be anaphylactic-type reactions (Atwell and Campbell 2001). The administration of atropine prior to tick antiserum was reported to decrease the incidence of adverse reactions from 2.7% to 0.5% (Atwell and Campbell 2001). Cats were more likely to demonstrate an anaphylactic reaction (2.6%) than dogs, which was attributed to the administration of foreign canine protein (Atwell and Campbell 2001). Warming to tick antiserum to body temperature prior to administration is also recommended to minimize reactions.

Supportive Care

General supportive veterinary care of tick paralysis animals is recognized as an important part of treatment. Patient survival has been improved from 75% as reported in the early years (Ross 1935) of tick paralysis treatment where tick antiserum was heavily relied upon to the more recent era where 95% survival is expected (Atwell et al. 2001). Improvements in hospitalization and supportive therapies have likely led to these better outcomes. The generalized paralysis caused by toxins produced by *Ixodes holocyclus* results in a range of pathophysiological disturbances throughout the body, and these require considered management to optimize patient outcomes.

Location, removal, and identification the tick are important and necessary preliminary treatment procedures. Removal of the tick should be done as soon as possible to limit further absorption of toxin by the patient. The most common site for tick attachment is around the head and neck but occasionally ticks are reported as located inside the roof of the mouth or even inside the anal sphincter (Jones 1991). Identification of the tick should be performed to ensure that it is *Ixodes holocyclus* or *cornuatus*, which are the only two species known to cause tick paralysis; use of a dissecting microscope and anatomical key are essential for accurate identification (Barker and Walker 2014). Many other nontoxic *Ixodes sp*. and non-*Ixodes* ticks may also be found on dogs and cats throughout Australia.

The use of acaricidal washes, dips, or application of topical insecticides to diagnosed or suspect cases of tick paralysis in dogs has been recommended (Jones 1991). Complete immersion of a sedated animal in synthetic pyrethroid and organophosphate solutions typically used for cattle is adopted by some veterinarians with or without prior clipping of the hair coat (Jones 1991). This practice may be of benefit in cases where no tick can be found and the clinical signs and suspicion index are consistent with tick paralysis. However, there is no published data to support the efficacy of this acaricidal treatment in preventing the onset of tick paralysis. Other insecticidal preparations such as fipronil, afoxolaner, and fluralaner appear effective in killing already attached engorging ticks, but the process may take up to 48–72 h and does not necessarily guarantee that paralysis will not develop in nonimmune dogs (Fisara and Webster 2015).

Intravenous fluid therapy using a balanced electrolyte such as Hartmann's solution administered at maintenance rates is indicated for patients that are unlikely to consume oral fluids within the following 24 h (Webster 2014b). Fluid therapy must be monitored to avoid fluid overload which may exasperate or lead to development of clinical pulmonary edema (Webster 2014b).

Bladder paralysis and the inability to pass urine may require management through either manual expression or catheterization. Facial paralysis may lead to dry eyes and ulceration; the use of ocular lubricants may be necessary. Mild sedation may be useful to reduce anxiety and facilitate clipping and radiography; acepromazine (0.05 mg/kg) has been reported as adequate and useful (Jones 1991).

Respiratory support may be required in some patients depending upon case severity; support may include intranasal or tracheal oxygen or mechanical ventilation

(Webster et al. 2013b). The use of mechanical ventilation has been shown to improve survival rates but is costly and labor intensive to manage. In a study of dogs (54) and cats (7) that were ventilated due to tick paralysis, the mean duration of ventilation was 23 h but ranged from 3 to 144 h (Webster et al. 2013b). When cases euthanized on cost basis were excluded, 75% of ventilated animals survived (Webster et al. 2013b). Improved survival was noted for animals with hypoventilation but appeared less effective for animals when hypoxemia was present (Trigg et al. 2014) with only 50% survival with hypoxemia and 90% for hypoventilation (Webster et al. 2013b).

Dogs with tick paralysis have been shown to have varying degrees of left-sided heart failure (Campbell and Atwell 2003). Dogs with tick paralysis had normal systolic blood pressure, radiographic evidence of pulmonary venous congestion and peribronchial fluid infiltration, and a reduction in echocardiographically derived functional indices (Campbell and Atwell 2003). Despite the recognition that heart failure may be occurring, no specific cardiac treatments or medications are currently recommended.

Antibiotics would appear to be useful for management of existing aspiration pneumonia in paralyzed animals. Evidence for this was found in the postmortem examination of 25 dogs that had severe respiratory failure in which all dogs had histological lung changes and 15/25 had bronchopneumonia (Webster et al. 2013a).

Pulmonary edema and congestion were noted in 9/25 dogs euthanized for tick paralysis, and this probably contributes to the development the hypoxemia observed in some patients (Webster et al. 2013a). Pulmonary edema is a consistent finding in animals affected with tick paralysis. It is unclear what the pathophysiology of this process is but it is an ongoing area of research interest (Webster 2014a). Treatment of pulmonary edema with diuretics may appear logical but diuretics raise the potential for other fluid therapy complications and has not be recommended for management of pulmonary edema from tick paralysis (Webster 2014b).

Esophageal dysfunction and megaesophagus appear to be relatively common in dogs with tick paralysis resulting in vomiting and regurgitation (Campbell and Atwell 2001; Malik et al. 1988). Secondary esophagitis may develop with megaesophagus and medications such as cimetidine or omeprazole to reduce acidity of gastric secretions may be indicated (Campbell and Atwell 2001). When introducing oral fluids and food during recovery feeding from an elevated position is thought to provide gravity assisted esophageal movement. Antivomiting medications such as metoclopramide and maropitant are often given to tick affected animals, but their efficacy for preventing aspiration pneumonia remains unproven (Webster 2014b). It is recommended that tick paralysis affected animals should not be fed fluids or solids by mouth until such time that neuromuscular function has been sufficiently restored that aspiration pneumonia will be avoided (Webster 2014b).

Despite some animals showing apparent recovery from generalized tick paralysis, occasional cases of unexplained sudden death have been reported to occur within a few weeks of recovery. Stressful stimuli, however mild, may precipitate sudden death (Fitzgerald 1998; Ilkiw 1983). A convalescent period of 2 weeks posttreatment with only nonstressful activity has been recommended to minimize sudden unexpected deaths (Ilkiw 1983). The mechanism triggering sudden death appears to be

cardiac related. Electrocardiographic abnormalities have been recorded in dogs recovering from tick paralysis (Campbell and Atwell 2002). A prolonged QT interval was observed in dogs at the time of presentation for tick paralysis, at 24 h post treatment, and at discharge from veterinary care when each animal results were compared to QT interval measured 12 months after recovery (Campbell and Atwell 2002). T wave morphology was also altered in dogs at the time of presentation. Resolution of ECG changes lagged behind that of apparent clinical recovery. The authors concluded that prolongation of the QT interval seen on ECG likely predisposes dogs to sudden death from ventricular tachycardia, similar to long QT syndrome diagnosed in humans (Campbell and Atwell 2002). A similar prolongation of the QT interval was also measured in the spectacled flying fox, a native bat species which is commonly affected with tick paralysis (Campbell et al. 2003). Dogs with nonsevere clinical tick paralysis may still be predisposed to sudden death because of the delayed recovery from ECG abnormalities, and that the tick antiserum does not appear to reverse this ECG pathology (Campbell and Atwell 2002). The incidence of sudden unexplained death in dogs recovering from tick paralysis is not known but a prospective survey of 500 cases of tick paralysis in dogs found nearly half of the 33 cases that ultimately died were reported by veterinarians as having been "unexpected" (Atwell et al. 2001). Further studies are needed to better understand the nature of the cardiac events that may lead to sudden death in treated animals.

Prevention

Acaricides

The development of effective acaricidal preparations that are economical and can be conveniently applied or fed to dogs and cats has been a big improvement since the 1990s for prevention of tick paralysis. The current next generation of Australian Pesticide Veterinary Medicine Authority (www.apvma.gov.au) licensed acaricides available in 2016 within Australia for the prevention of tick paralysis include the orally administered fluralaner (Bravecto, MSD, Australia) and afoxolaner (Nexgard, Merial, Australia). Orally administered fluralaner has been reported to be effective against *Ixodes holocyclus* for at least 115 days and out to 143 days with 95% efficacy (Fisara and Webster 2015). Fluralaner has also been shown to kill adult ticks within 72 h of product administration, although the partially engorged dead ticks may still remain on the dog (Fisara and Webster 2015). Afoxolaner has a minimum duration of action of 30 days and is currently licensed in Australia as for control of *Ixodes holocyclus* infestation in dogs. Fipronil is a topical acaricidal agent that is licensed for control of *Ixodes holocyclus* on dogs and cats for up to 2–3 weeks depending upon the formulation used. Fipronil must be applied externally and the manufacturer recommends that dogs not be washed or swim within 48 h of application.

A number of other products have been licensed for control of *Ixodes holocyclus* in dogs and cats in Australia. Topical skin application of a combination of imidacloprid and permethrin (Advantix, Bayer, Australia) is licensed for control (kills and repels)

of paralysis ticks for up to 2 weeks. An insecticidal wash containing pyrethrins, piperonyl butoxide, and *N*-octyl bicycloheptene dicarboximide (Fido's Free-Itch Rinse Concentrate, MavLab, Australia) is licensed for prevention of paralysis tick attachment for up to 3 days following a single wash. A collar worn by dogs containing flumethrin and propoxur (Kiltix Collar for Dogs, Bayer, Australia) is licensed for control of paralysis tick for 6 weeks. Skin irritation may be a problem for some dogs with prolonged wearing of the collar. The collar manufacturer advises that the collar may lose effectiveness if there is prolonged contact with water. Recently a collar containing imidacloprid and flumethrin has been licensed for use in dogs and cats as a flea and tick preventative (Seresto, Bayer, Australia). The product has a registered claim of 4 months prevention of *Ixodes holocyclus* infestation. An external skin wash product containing permethrin (25:75 cis:trans) (Permoxin, Dermcare-Vet, Australia) is licensed for control of paralysis tick for 7 days. A chemical impregnated collar containing deltamethrin (Scalibor, Intervet, Australia) is licensed for control of paralysis tick in dogs for up to 14 weeks. The manufacturer advises removal of the collar if the dog is swimming in waterways as the active ingredient is toxic to fish and other aquatic life forms. Accidental ingestion of the collar by dogs may result in toxicity with ataxia, drooling, and vomiting.

Paralysis tick preventative options for cats are primarily limited to the use of the active ingredient fipronil formulated in either spray or topical products. All products are registered as an aid in the control of tick paralysis and do not guarantee complete prevention.

All acaricidal products require regular application and lapses with re-treatment of animals may be a primary reason for development of clinical paralysis.

Development of a Toxin Vaccine

The long-term goal of the work undertaken at Commonwealth Scientific and Industrial Research Organisation by Dr Bernie Stone was to develop a vaccine for domestic animals in Australia against the tick paralysis toxin (Stone 1990; Stone et al. 1979). Stone sought to have a one, two, or three-shot vaccine that would protect dogs and livestock from the paralyzing toxin (Stone 1990). Stone adopted the approach of using toxin extracted from ticks engorged on rats and was able to demonstrate protective immunity in the serum of both immunized rabbits (Stone 1983) and dogs (Stone et al. 1983). Stone produced a more refined extract from the crude engorged tick preparations and cross linked this with glutaraldehyde and demonstrated that this toxoided immunogen also resulted in serum that was protective against whole tick toxin extracts (Stone and Neish 1984). Despite this experimental success the large scale production of a commercial tick vaccine eluded Stone up until the time of his death (Scott 2005). Arguably the key factor limiting progress to commercialization would have been producing large enough quantities of toxin from engorged ticks and use of a more animal friendly adjuvant other than Freund's.

Following on from the preliminary work of Stone, a group at the University of Technology in Sydney applied recombinant protein production techniques to the

problem of making a tick toxin vaccine (Masina and Broady 1999). Broady and colleagues produced a recombinant form of holocyclotoxin expressed in *E. coli* using the DNA sequence defined in their previous work (Thurn et al. 1992). A report was published in 1999 that described production of a recombinant form of the toxin HT-1 and that "*. . .antibodies produced against the recombinant fusion protein show a significant level of protection against native toxins indicating a vaccine is possible. . .*" (Masina and Broady 1999; Nicholson et al. 2006). Significant homology to scorpion neurotoxins was noted with the DNA sequence for HT-1. No further publications by Broady on this method of vaccine production have been produced. Chemical synthesis of HT-1 was described more recently along with structural analysis, but no data on the biological activity or immunogenicity was published (Vink et al. 2014).

A vaccine would have theoretical advantages – should it work – in protecting dogs and livestock against the paralyzing toxin. However, vaccine technology is now competing against marked improvements in long-acting acaricidal products for companion animals and while in the 1970s immunological control may have appeared a very attractive strategy, this may not be so in the current era.

Other Prevention Methods

Unarguably the best method of preventing tick paralysis is to avoid exposure of susceptible animals by not taking them into endemic tick areas. For a large part of the animal population in Australia, tick paralysis will never be an issue because they live permanently outside of these tick areas. For holidaymakers who travel from non-endemic areas into endemic areas, the risk of tick paralysis must be managed.

Regular daily tick checks of animals have often been prescribed as a method of early detection of paralysis tick attachment (Ross 1934). Ross recommended a daily routine of feeling for attached ticks in a dogs coat, rather than relying only on combing and visual searching, was a highly effective preventative method in his experience (Ross 1934). Inexperienced animal owners who attempt this may be at risk of inadvertently not locating a tick and the animal developing paralytic signs. Researchers who work with ticks and track where they attach can still have difficulty locating all applied ticks, combined with variable locations across the animal's body this method is probably of low sensitivity for early detection of paralysis ticks.

Conclusions

Tick paralysis continues to present challenges to domestic animals, animal owners, and veterinary practitioners along the east coast of Australia. The enigmatic nature of the neurotoxin has been revealed through a long and tortuous

pathway of discovery. There has been a gradual increase in the understanding of the toxin and its mechanism of action. The relative paucity of basic experimental studies of the pathogenesis of the toxin implies that care must be taken in extrapolating the published data to a clinical setting. Despite the 90 years that have elapsed between the publication of Sir Ian Clunies Ross's foundation studies on the tick biology and actions in the dog, there is still much that is unknown about tick paralysis. Effective preventative treatments are now widely available but pet owner compliance continues to be a reason to develop ever better clinical treatments and preventatives. Despite the significant amount of research work into a tick toxin vaccine, there is currently no commercial product available. The next century of discovery should surely reveal more of the secrets of this much feared little parasite (Figs. 1, 2, 3 and 4).

Fig. 1 Photograph of the dorsal surface of the nonengorged adult Australian paralysis tick *Ixodes holocyclus* showing darker colored legs 1 and 4 (Photo credit: A. Padula)

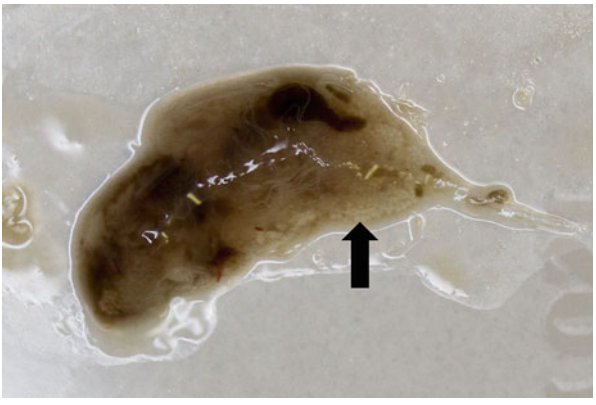

Fig. 2 Anterior body extract of a fully fed *Ixodes holocyclus* showing the enlarged salivary gland tissue and acini (*arrow*) (Photo credit: A. Padula)

Fig. 3 Adult unengorged *Ixodes holocyclus* crawling over skin of rat prior to attachment (Photo credit: A. Padula)

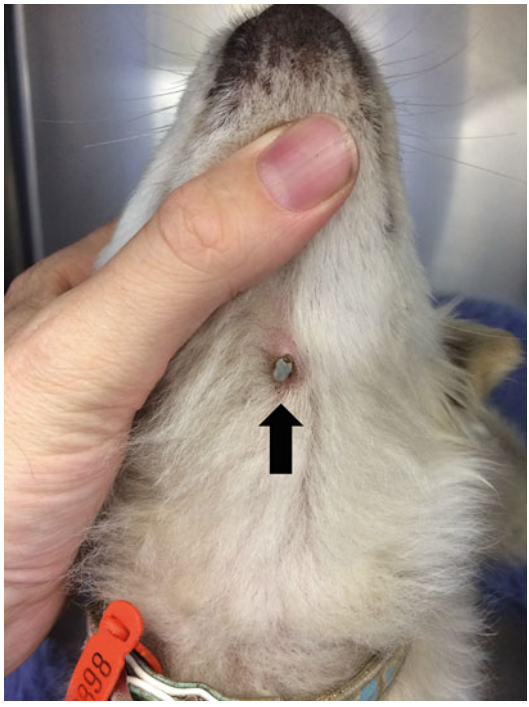

Fig. 4 Engorged adult *Ixodes holocyclus* tick attached to ventral neck of dog resulting in paralysis (Photo credit: A. Padula)

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Clinical Management of Envenomation by Australian Carybdeid Cubozoan, Hydrozoan, and Scyphozoan Jellyfish

11

James Tibballs

Contents

Abstract

The waters surrounding the continent harbor numerous species of jellyfish classified as carybdeids, hydrozoans, and scyphozoans. Some of the carybdeids, small jellyfish with single tentacles arising from each of the four corners of a cuboid bell, have only relatively recently been identified. The stings of carybdeids cause a clinical syndrome known as "Irukandji syndrome" – the delayed onset of severe pain in association with hypertension and sometimes progressing to acute cardiac failure, resembling Takotsubo cardiomyopathy and necessitating

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mechanical ventilation and inotropic support. The severe syndrome is consistent with endogenous release of catecholamines by toxins acting on sodium channels and by cytolytic toxins causing pore formation of cardiac cells. Although initially recognized in Australia and typified by the sting of *Carukia barnesi*, it is being increasingly recognized in victims of stings by similar jellyfish in other oceanic regions. Some protein toxins of *Carukia barnesi* and *Malo kingi* have a molecular mass of 43–46 kDa and have some homologies with cytolytic pore-forming toxins of chirodropids *Chiropsalmus quadrigatus* and *Chironex fleckeri* and homologies with ion channel neurotoxins of sea anemones. A hydrozoan jellyfish, *Physalia physalis*, found in Australian waters but distributed widely throughout the world, is the most frequent cause of mild jellyfish stings. Its protein toxins are known to be cytolytic, acting by cell membrane poration and allowing cellular entry of calcium. Numerous other jellyfish of the class Scyphozoa in Australian waters include *Pelagia noctiluca*, *Cyanea capillata*, and *Aurelia aurita*. At present, no specific therapy exists for stings by any of these jellyfish and toxins of all remain to be comprehensively identified and studied.

Introduction

This chapter is an overview of Australian carybdeid Cubozoan, hydrozoan, and scyphozoan jellyfish, their venoms and toxins and relevance to the clinical management of the envenomated victim. The animals are briefly described. Some species inhabit seas and oceans elsewhere in the world. Envenomation by chirodropid (multitentacled) Cubozoan jellyfish is considered in ▶ Chap. 12, "Australian Chirodropid Cubozoan Jellyfish Envenomation" where the classification of these jellyfish and their common mechanism of envenomation are described.

Carybdeids (Jellyfish Causing "Irukandji Syndrome")

Carybdeids (order Carybdeidae) like chirodropid jellyfish are also "box" jellyfish, having a cubic medusal bell but otherwise differ substantially. From each corner of a carybdeid bell arises an unforked arm (pedalium) bearing a single tentacle in contrast to those of chirodropids which bear many tentacles. Two of the families of Carybdeidae are distinguished which cause Irukandji syndrome: Carybdeidae and Alatinidae. Although the capture of culprit stinging jellyfish and their subsequent identification are often problematic due to their small size and translucency, approximately 25 species of jellyfish have been associated directly or indirectly with Irukandji syndrome (Gershwin et al. 2013). Carybdeids of medical importance in Australia include species members of at least six genera: *Alatina*, *Carukia*, *Carybdea*, *Gerongia*, *Malo*, and *Morbakka*.

Although originally recognized in tropical Australia, Irukandji syndrome has been increasingly recognized as a clinical entity worldwide, occurring from jellyfish stings in oceans between latitude 53°N and 38°S (Gershwin et al. 2013). In Australia, Irukandji stings have principally occurred in the vicinities of the Australian Great Barrier Reef and the North West Shelf but have also been recorded along the coasts of New South Wales and Victoria. Other prominent regions include Southeast Asia, the Caribbean, and the Islands of the Pacific (Papua New Guinea, Fiji, Vanuatu, Tahiti, Samoa, and New Caledonia). Sporadic reports also come from China, Japan, South Africa, the Middle East, and Europe.

Just as the sting of the Australian *Chironex fleckeri* is the archetypical envenomation of chirodropids, the sting of the Australian *Carukia barnesi* is that which causes a clinical syndrome known as "Irukandji syndrome."

Carukia barnesi

For many years people bathing near Cairns in Queensland suffered an unusual type of marine sting, which Dr Hugo Flecker, in 1952, called "Irukandji syndrome," but the jellyfish responsible was not discovered until 1961 by Dr Jack Barnes. The jellyfish was described fully by Dr Ronald Southcott who compounded the generic name *Carukia* from *Carybdea* and Irukandji. The latter derives from the name of an Aboriginal tribe, the Yirrganydji, who formerly inhabited the area.

The sting differed from others in that, although only moderately painful and local effects not serious, a severe general illness often occurred about 30 min later. Although the Irukandji syndrome is synonymous with *Carukia barnesi*, the syndrome is now known not to be confined to a sting by this species alone.

This creature is minute compared with other dangerous jellyfish. Its squarish bell is barely 12 mm wide, and its four tentacles vary in length from a few centimeters to 35 cm. The body and tentacles are almost completely transparent in water. Clumps of nematocysts (mammillations) appear as tiny red dots over the bell as well as in ring formations on the tentacles. Very little is known about the habits of *C. barnesi.* It appears in shallow coastal and in deep water outside the Great Barrier Reef in the summer months and may come close to the shore when seas are quite rough.

Envenomation (the "Irukandji Syndrome")

Local Effects

The victim rarely sees the jellyfish but is often, but not always, aware of a slight sting to the upper body while swimming in deep water. Sometimes the sting is unnoticed, and it is the onset of symptoms which forces the victim to leave the water. There is no banding or puncture mark, just an oval area of barely perceptible erythema measuring 5 cm by 7 cm, which is much larger than the area of contact with the bell. Irregularly spaced papules ("goose pimples") up to 2 mm in diameter develop within 20 min of the sting and then fade, but the erythema may last several days.

General Effects

From 5 min to as late as 2 h after the sting, but usually after about 30 min or so, marked general symptoms may develop. Severe low back pain, cramping muscle pains, nausea, vomiting, profuse sweating, headache, restlessness, and agitation almost invariably occur, sometimes with hypertension. Abdominal pain is associated with spasm of the muscles of the abdominal wall, and cramps occur in the muscles of the limbs. Full recovery occurs after 1 or 2 days of nausea, pain, and prostration. In 1964, Dr Jack Barnes stated:

> Of the 60 documented cases of Irukandji stinging, 55 patients presented with abdominal pain, 35 complained of limb pains, 34 were reported to have cough, nausea or vomiting, and 24 had severe backache. Neuralgias and joint pains were troublesome symptoms in all four patients observed in hospital overnight, and doubtless the incidence would have been higher had more patients remained under observation. (Irukandji victims are usually treated as outpatients, and discharged when presenting symptoms abate. Unnecessary suffering and anxiety result from this practice).
>
> Tingling, shivering, weakness, cramps, headache, dry mouth and itch were recorded infrequently. Here again the true incidence may well have been higher, for these are second-rank symptoms, likely to be disclosed only in response to specific inquiry. The one effect usually ignored by victims, but figuring prominently in medical records, was profuse sweating.

Barnes also described in detail the effects when the first captured specimens of *C. barnesi* were applied to the arms of himself, his son, and a surf lifesaver. The illnesses they suffered incorporated all the described features.

Several subsequent studies have added detail to the symptomatology. In a study of 62 victims presenting in Cairns during 1996 (Little and Mulcahy 1998), the symptoms and signs were abdominal cramps (40%), hypertension (50%), back pain (39%), nausea and vomiting (39%), limb cramps (34%), chest tightness (26%), and marked distress (24%). In a series of 88 patients with Irukandji syndrome reporting to Broome Hospital after stings, mainly at Cable Beach between 2001 and 2003, 80% of victims had cutaneous signs of a sting, 50% were hypertensive, 90% required opiate analgesia, and 17% required hospital admission (Macrokanis et al. 2004). The most recent series reported from Darwin (Nickson et al. 2009) included 87 victims of whom 65% had severe pain of rapid onset (<30 min) and 63% had visible sting lesions.

Cardiovascular Effects and Acute Cardiac Failure

Cardiogenic pulmonary edema was originally described in several individual case reports and in case series. In a retrospective review of 12 serious cases, pulmonary edema was a prominent feature (Little et al. 2003). The initial phase of envenomation was mild skin pain followed at 30 min by considerable muscle pain and cramps with associated tachycardia and hypertension. After a mean of 14 h post sting (range 1.5–18 h), pulmonary edema was evident radiologically and in some cases was associated with hypokinetic cardiac function, reduced cardiac output, and raised cardiac enzymes. In several cases, the necessary successful treatment was oxygen

therapy, diuretics, vasodilators, inotropic support, and mechanical ventilation. Of note is the fact that in none of the "mild" cases was a positive identification made of a creature causing the envenomation although in one case, a macerated tentacle appeared to belong to an unnamed carybdeid jellyfish.

In the series of cases reported from Darwin (Nickson et al. 2009), cardiovascular features included hypertension and ECG abnormalities among which were atrial and ventricular ectopy, atrioventricular conduction defects, ST segment elevation, and T-wave abnormalities, in one case of ventricular tachycardia associated with cardiomyopathy, acute pulmonary edema, and raised troponin level. Other serious involvement included renal failure (two cases) and one case of pancreatitis and ileus. No patient needed mechanical ventilation or sustained intracerebral hemorrhage. Nematocysts of variable morphology were present on the skin in seven patients, suggesting that different species may have been responsible.

Indeed, identification of the jellyfish is persistently problematic. In a retrospective study (Huynh et al. 2003) of 116 patients presenting to Cairns Base hospital with Irukandji syndrome over a 12-month period 2001–2002, 39 of 50 who had skin scrapings performed had *Carukia barnesi* nematocysts identified from skin scrapings. One patient who died had an unidentified cnidome in skin scraping, later identified as that of another carybdeid: *Malo maxima* (Gershwin 2005a). In a prospective study of victims of jellyfish stings presenting to the Royal Darwin Hospital (O'Reilly et al. 2001), 4 of 40 patients had Irukandji syndrome, but characteristic nematocysts could not be identified in sticky-tape sampling of sting site in any of them.

Thus, although it is now certain that *Carukia barnesi* can cause Irukandji syndrome, other species may also be responsible for the most severe form of the illness which includes cardiac failure.

The mechanism of cardiac failure is unlikely to be secondary only to hypertension (Takotsubo cardiomyopathy). In some cases the hypertension has been both brief and mediocre before the onset of cardiac failure. There is considerable scientific investigational evidence that jellyfish venoms cause pores in cell membranes ("poration") which of course may disrupt cell function and allow the ingress of other lethal toxins. The series of human envenomations by carybdeids reporting cardiac failure and raised cardiac enzymes or elevated serum troponin levels (Little et al. 2003, 2006; Nickson et al. 2009) strongly suggests that cardiac cell membrane poration, in addition to hypertension, is a factor resulting in cardiac failure.

Fenner et al. (1986a) had speculated that the mechanism of hypertension may be caused by catecholamine release, which itself is probably caused by sodium channel perturbations. Although hypertension due to catecholamine release has been demonstrated in animal models of *Carukia barnesi* envenomation (Tibballs et al. 2000; Winkel et al. 2005; Ramasamy et al. 2005), it has yet to be confirmed in human cases. Antihypertensive therapy may be required in the initial phase of management. Infusions of the alpha-adrenergic blocker phentolamine have been used successively for this purpose, but a more "titratable" short-acting nitrate would be

preferred because of imminent cardiac failure and hypotension caused by envenomation. Sublingual nitrates have been proposed as pre-hospital treatment of hypertension.

Differential Diagnosis

In 1964, Barnes observed that stinging capsules had not been recovered from *C. barnesi* stings and suggested that negative skin scrapings indirectly assist differentiation from other jellyfish stings and that the diagnosis is usually clear-cut on clinical grounds. Greater success in nematocyst identification has been achieved in more recent times (Huynh et al. 2003). Importantly, the condition can be simulated by gastric poisoning and by various surgical emergencies such as peptic ulcer, ruptured spleen, ruptured ectopic pregnancy, acute appendicitis, actual myocardial infarction, and decompression sickness.

Barnes predicted correctly that the "Irukandji" syndrome would prove to be caused by a variety of species of jellyfish. In so far as the effects of muscle pain are concerned, this has been observed for species of *Tamoya*, for *Physalia*, and for *Carybdea* and other genera both inside and outside Australian waters (Yoshimoto and Yanagihara 2002; Tibballs et al. 2012; Gershwin et al. 2013). Although several detailed case reports are available (see Tibballs et al. 2012), on only one occasion was the victim able to offer a description of an offending jellyfish. This was a 28-year-old diver who experienced a typical Irukandji syndrome after contact with a small box-shaped jellyfish with four tentacles with an overall size of a thumb. His sketch of the animal closely resembled *C. barnesi* (Hadok 1997).

First Aid

Although domestic vinegar has been shown to inactivate undischarged nematocysts of *Chironex fleckeri*, *Carybdea rastonii*, and *Tamoya* sp., this has not been decisively demonstrated for *C. barnesi*.

Clinical Management and Prognosis of Envenomation

Pain relief is the most important feature of management in mild-moderate cases. The majority of victims in a large series of case reports have required parenteral analgesia (Macrokanis et al. 2004; Nickson et al. 2009).

Most cases of stings in the area of Cairns are mild-moderate (Mulcahy and Little 1997; Little and Mulcahy 1998; Huynh et al. 2003). However, two fatalities have been attributed to unseen jellyfish causing the Irukandji syndrome further south and on the Great Barrier Reef (Fenner and Hadok 2002; Huynh et al. 2003). In these fatalities, intracerebral hemorrhage was associated with severe hypertension. One of these occurred off Hamilton Island in the Whitsunday Islands (20°20′S, 148°56′E) and the other some 1,300 km north on the Great Barrier Reef off Port Douglas.

In severe cases in which pulmonary edema and cardiac failure are present, admission to an intensive care unit is needed. Mechanical ventilation or continuous positive airway pressure (CPAP) and "titratable" inotropic and vasodilator therapy may be required.

Interestingly, although *Chironex fleckeri* antivenom binds to this crude venom preparation in vitro (Wiltshire et al. 2000), it is clinically ineffective in treatment of the Irukandji syndrome (Fenner et al. 1986b), and it did not prevent tachycardia and vessel contraction in vitro caused by *Carukia barnesi* (Winkel et al. 2005), *Alatina mordens* (Winter et al. 2008), or *Malo maxima* (Li et al. 2011).

Corkeron used intravenous magnesium sulfate (10 mmol loading dose plus an infusion of 5 mmol/h for 20 h) to immediately ameliorate pain and hyperadrenergic features (hypertension, agitation, diaphoresis, piloerection, and dyspnea) of Irukandji syndrome in a previously well 26-year-old man who had been stung by an unknown jellyfish. The basis for use of magnesium was its ability to decrease vascular resistance in hyperadrenergic states and its suppression of catecholamine release. The analgesic effect however remains unexplained. Of additional interest was a high troponin I level (6.4 mcg/L) associated with only a moderate level of hypertension (170/100 mmHg) and normal echocardiographic function, which suggests an action of venom in addition to its hypertensive effect. In a further series of ten victims with Irukandji syndrome, seven from Queensland and three from Western Australia, intravenous magnesium salts provided pain relief, as assessed by serial pain scores before and after treatment, and a reduction in blood pressure (Corkeron et al. 2004). However, in two other cases from Western Australia, magnesium failed to provide analgesia and to prevent cardiac failure, with a raised troponin level in one case, and pulmonary edema requiring mechanical ventilation (Little 2005). The mechanism of action of magnesium remains unknown.

The clinical use of sodium channel blockers in the treatment of Irukandji syndrome has not been addressed at all, yet some agents such as procainamide (class 1a antiarrhythmic agent) and lidocaine and phenytoin (class 1b antiarrhythmic agents) are in common use for other conditions.

Venom of *Carukia barnesi*

The venom appears to contain a potent neuronal sodium channel modulator as indicated by in vitro experiments using whole-animal extracts. Winkel et al. (2000) observed that it caused tachycardia in rat and guinea pig isolated atria, an effect which was prevented by pretreatment with tetrodotoxin (Na^+ channel blocker). It caused contraction of rat mesenteric artery not affected by tetrodotoxin, prazosin, or conotoxin GVIA. None of these effects of the extract were influenced by Commonwealth Serum Laboratories (CSL) Ltd. *Chironex fleckeri* antivenom. In human atrial muscle strips, the extract caused an initial fall and then a sustained increase in contractile force which was decreased by propranolol. In anesthetized piglets, the extract caused sustained tachycardia and systemic and pulmonary hypertension. Blood samples of these piglets contained marked elevations of noradrenaline and adrenaline (Tibballs et al. 2000; Winkel et al. 2005). This hyperadrenergic state was advanced to explain at least in part the clinical features of the Irukandji syndrome. Similarly, in anesthetized rats, venom derived from nematocysts and from tentacle extracts (devoid of nematocysts) also induced hypertension, which could be attenuated by prazosin suggesting a catecholamine-mediated action (Ramasamy et al. 2005).

Another investigator has suggested the venom has cell membrane pore-forming activity. Ávila-Soria (2009) developed a cDNA library of *Carukia barnesi* which predicted a venom content to include proteins, named CbTX-1 and CbTX-2, which are similar to known pore-forming toxins and ion channel (Na+, K+) neurotoxins in sea anemones and in the jellyfish *Aurelia aurita*. Indeed, injection of CbTX recombinant proteins caused lethal paralysis in cockroaches. The toxins have a mass of 43–45 kDa, homologous with toxins that other investigators had identified including Mk-332-1 and Mk-332-2 from *Malo kingi*, with CqTX-A from *Chiropsalmus quadrigatus* and CfTX-1 and CfTX-2 from *Chironex fleckeri*, and are antigenic to CSL *Chironex fleckeri* antivenom.

Other Jellyfish Causing Irukandji Syndrome

Carybdea xaymacana

This small carybdeid has been found along the coasts of Western Australian coast and Queensland and is structurally distinct from *Carybdea rastonii*, which is also present (vide infra). Specimens captured near Cairns are reported to have caused back pain, nausea, sweating, and hypertension among five children (Little et al. 2006), but the identity may have been mistaken for *Carukia barnesi* (Gershwin 2006). Experimentally, venom derived from nematocysts of this species caused elevation of cytosolic Ca++ in rat ventricular myocytes which was blocked by lanthanum, a nonspecific channel and pore blocker, but not by verapamil, an L-type Ca++ channel antagonist (Bailey et al. 2005). Although it also caused hemolysis of sheep and human erythrocytes, this effect was not correlated with its lethality for *Artemia* sp. prawns. Like other jellyfish venoms (*Chironex fleckeri*, *Chiropsalmus* sp.; see ▶ Chap. 21, "Animal Venoms and Nephrotoxic Effects") examined by these investigators, lethality was opined to be related to a pore-forming action.

Malo maxima

This species inhabits the waters in tropical Western Australia and is abundant offshore along the 80 mile beach. Identified in 2005, it superficially resembles the related species *M. kingi* except that the bell is somewhat taller – up to 5 cm. This jellyfish is probably the species recognized by members of the pearl diving industry in Broome as the cause of an Irukandji-like syndrome, except that the sting may be sharply painful before the onset of the full syndrome (Gershwin 2005b).

Biochemical and pharmacological evidence suggests that the cardiovascular effects of venom of *M. maxima* are similar to that of *Carukia barnesi* as responsible for Irukandji syndrome (Li et al. 2011). Analysis of crude venom extracts (CVE) of *M. maxima* revealed a protein content and sizes similar to those of *Carukia barnesi*. The CVE increased contractility of atrial tissue, but this was diminished by pretreatment with propranolol or tetrodotoxin (sodium channel antagonist) suggesting an effect mediated by catecholamines released by sodium channel activation. Pretreatment with magnesium also decreased atrial contractility but not mesenteric artery contraction leading the investigators to suggest that any clinical

antihypertensive effect of magnesium was via a cardiac and not a peripheral vascular effect. Interestingly, pretreatment with neither the alpha-blocker prazosin nor the N-type calcium channel antagonist ω-CTX GVIA diminished mesenteric artery contraction by SVE which suggests that neither alpha-blockade nor calcium channel antagonists would be helpful in clinical management of hypertension. Pretreatment with the calcitonin gene-related peptide 8–37 ($CGRP_{8-37}$), that is, a CGRP receptor antagonist, decreased SVE-induced atrial contraction and increased the sensitivity of mesenteric artery contraction suggesting that the venom causes release of the sensory neurotransmitter CGRP and consequent pain. Since CGRP release is known to be dependent on N-type calcium channels, the investigators speculated that the mechanism of the apparent clinical analgesic effect of magnesium for Irukandji syndrome is via competitive antagonism of calcium ions decreasing calcium influx causing reduction in release of CGRP and consequently of pain.

Malo kingi

This species, in North Queensland Pacific waters, was identified and named by Gershwin (2007). It has a bell about 3 cm tall, half as wide, and narrower at the apex. It has mammillations (collections of stinging cells) on the apex and on the walls of the bell. The four tentacles, one at each pedalium (corner), are segmented by halolike thin sheet-rings perpendicular to the tentacle axis. Tentacular nematocysts are found only on these halo rings. The characteristic nematocysts match those found on the skin of a man, Robert W King, after whom the species is named, who died from toxin-induced hypertension and intracranial hemorrhage (Huynh et al. 2003).

Ávila-Soria (2009) developed cDNA libraries for *Malo kingi* and *Carukia barnesi* from which venom toxins were predicted. *M. kingi* venom would have two putative cytolytic proteins, MkTX-A and MkTX-B, but only the full structure of the former was determined with 434 amino acids and a mass of 48.55 kDa. The mass of the toxins is similar to other cytolytic pore-forming jellyfish toxin family already identified in *Chironex fleckeri* (CfTX-1 43 kDa, CfTX-2 45 kDa), *Chiropsalmus quadrigatus* (CqTX-A 44 kDa), *Carybdea alata* (CaTX-A 43 kDa), and *Carybdea rastonii* (CrTXs 43–45 kDa). In addition, the venom of *M. Kingi* would include proteins (Mk-332-1 and Mk-332-2) with homology to known ion channel (Na^+, K^+) neurotoxins in sea anemones. Other components of venom would include peptidases (serine, metallo, cysteine, threonine) and peptidase inhibitors.

Carukia shinju

This carybdeid species has also been identified in waters offshore from the 80 mile beach, Broome, where it has long been recognized as a source of stings sustained by pearl fishermen (Gershwin 2005a). The species nomenclature "shinju" is a reference to the Japanese word for "pearl." Its bell is a rounded pyramid approximately 16 mm tall with warts (collections of nematocysts) scattered over the apex. The four tentacles, one arising from each corner, have aggregations of nematocysts forming distinct circular bands with an inferior margin greater in circumference than the superior margin giving an appearance described as "neckerchief-like tails." It is postulated that this species, on the basis of its morphological and genetic similarities,

to be the cause of a syndrome similar to that produced by the Queensland *Carukia barnesi*.

Alatina mordens

This species was identified by Gershwin (2005b) with a causal linkage to Irukandji syndrome. Two cases were recorded from Osprey Reef near Cairns in 2002 (Little et al. 2006). Evidence that the release of endogenous catecholamines is the cause at least in part of the Irukandji syndrome evoked by this species was provided by an examination of the in vivo and in vitro cardiovascular effects of *Alatina nr mordens* (Winter et al. 2008). In that study, venom derived from nematocysts caused a long-lasting pressor response in anesthetized rats accompanied by large increases in plasma concentrations of adrenaline and noradrenaline. Also an evidence of a key role of release of endogenous catecholamines was the observation that the venom contracted rat vas deferens tissue and that this was reduced by pretreatment with guanethidine (which blocks release of noradrenaline) or by reserpine (which blocks transport of noradrenaline into synaptic vesicles enabling it to be degraded by monoamine oxidase). Whereas pretreatment of rats with the α-adrenoreceptor antagonist prazosin inhibited the pressor response to venom, pretreatment with a β-adrenoreceptor antagonist did not, thus suggesting that β2-adrenoreceptor stimulation does not contribute to the clinical hypertensive effect of the venom. Although CSL Ltd. *Chironex fleckeri* antivenom failed to inhibit effects of *Alatina mordens* venom, SDS-PAGE analysis showed that the venom is comprised of multiple proteins of mass from 10 to 200 kDa of which some were antigenic for the antivenom suggesting these two genera may have at least in part homologous toxins.

A Hawaiian member of the genus, *Alatina moseri* (formerly *Carybdea alata*), sometimes causes Irukandji syndrome (Yoshimoto and Yanagihara 2002). Vinegar or a commercial preparation (Sting No More™) containing copper gluconate, urea, and magnesium sulfate was found to be the best first-aid treatments to prevent tentacle discharge (Yanagihara et al. 2016), while hot-water immersion was superior to application of ice packs for analgesia (Wilcox and Yanagihara 2016).

Gerongia rifkinae

This species, inhabiting the waters in Northern Territory and Queensland, was known previously as the "Darwin carybdeid," responsible for a mild form of Irukandji syndrome (O'Reilly et al. 2001). It was described and named in by Gershwin and Alderslade (2005). The bell is a robust cuboid, approximately 6 cm in height with a rounded apex. The four tentacles, one at each corner, are round in cross-section with an expansion ("flaring") at their origin from the bell.

Morbakka fenneri ("Morbakka")

This species, known as the "Moreton Bay stinger" and the "fire jelly," is a relatively large carybdeid Cubozoan found from Cape York in north Queensland to Moreton Bay in the south. Although it closely resembles *Tamoya virulenta*, it has been recognized as a new species of a new genus *Morbakka fenneri* (Gershwin 2008). In 1985, Dr Ronald Southcott had proposed the name "Morbakka" – derived from

"Moreton Bay carybdeid medusa." Characteristically, the transparent bell is deeper than it is wide with measurements of a large specimen 18 cm and 12 cm, respectively. The four tapered tentacles are mauve in color and extend to 60 cm.

A sting raises a white weal approximately 10 mm wide and surrounded by a red flare resembling a superficial burn. It is associated with severe burning pain of almost immediate onset and lasting 24 h. The appearance of the lesion and the sensation give rise to the common name "fire jelly." There may also be associated respiratory distress and throbbing lumbar pain as in the "Irukandji syndrome." The lesions may show a characteristic tapering corresponding to the tentacle and may show "ladder" markings similar to a chirodropid sting. The lesions may vesiculate and become pruritic. Skin necrosis may occur. The surface of the bell has nematocysts arranged in clusters (warts) which may cause multiple punctate lesions within an area of erythema. Typical undischarged and discharged nematocysts may be obtained from skin scrapings. Undischarged nematocysts are inactivated by vinegar (Fenner et al. 1985).

This species may cause a severe illness. A stung diver experienced back and body pain accompanied by sweating, nausea, and hypertension. The victim required hospitalization for 2.5 days and had ECG abnormalities with an elevated troponin l level (5.4 mcg/L) (Little et al. 2006), suggesting actions of catecholamines and cardiac membrane pore formation.

Carybdea rastonii ("the Jimble")

Carybdea rastonii is a small four-tentacled carybdeid jellyfish with a translucent body about 2 cm across. The maximum size is 3 cm in width and 5 cm in length. The tentacles stretch out some 5 cm to perhaps 30 cm. When contracted, the tentacles are denser and hence easier to see than the bell which is almost invisible. It swarms and usually rises to the warmer surface of the sea in the early morning and evening.

Carybdea rastonii was first discovered in St Vincent Gulf, South Australia, but now known to inhabit the southern waters of Australia including the Indian Ocean east of Cape Leeuwin and into the Pacific Ocean as far north as Newcastle, including Tasmania and Victoria. The same named jellyfish in Japan is a different species sometimes alternatively called *Carybdea mora* or *Carybdea brevipedalia*. Other species also named *Carybdea rastonii* exist in Hawaiian and Californian waters and are also species different to the Australian *Carybdea rastonii* (Dr Lisa Gershwin: Personal communication October 2016). In the Atlantic Ocean and Mediterranean Sea, a similar jellyfish, *Carybdea marsupialis*, occurs.

Envenomation

The tentacles bear ovoid nematocysts which average 28 by 16 μm. Stings by *C. rastonii* are usually but not always immediately painful with lesions almost invariably linear and frequently four in number, ranging from 10 to 20 cm in length. Southcott subjected himself to a number of stingings by severed tentacles and described in detail the local effects (Cleland and Southcott 1965). In most cases, the pain is of moderate severity and lasts up to 2 h. Weals 3–12 mm in width develop and are surrounded by a flare some 4 cm in radius. Some stings produce small

blisters of varying sizes. The swelling resolves over several days, but usually some pigmentary changes are evident for at least 2 weeks after the stinging. Southcott could not recover nematocysts by scraping the region of self-inflicted stings, so examination of a piece of tentacle may be necessary to confirm the diagnosis. He considered the stings would not effectively penetrate the thick skin of an adult's palm.

Cleland and Southcott (1965) relate a number of minor stings caused by *C. rastonii*, but there have been no reports of significant systemic effects following stings by reliable identification of this jellyfish. A case of Irukandji syndrome occurred at Queenscliff in Victoria where *C. rastonii* may be found, but a jellyfish was not seen (Cheng et al. 1999).

Tentacles, cut from living jellyfish, caused severe pain lasting from 10 min to 8 h when placed on forearms of 25 volunteers (Ohtaki et al. 1990). Erythema and weal appeared within minutes and subsided within 24 h to 3 days. In 15 of the volunteers, at 7–13 days after application of the tentacles, linear erythema and papulovesicular lesions with pruritus occurred, lasted a week, and left slight pigmentation. The histological findings of these flare-up lesions resembled those of allergic contact dermatitis.

First Aid and Clinical Management

As with other box jellyfish stings, domestic vinegar should be poured over the adhering tentacles (Fenner and Williamson 1987). Alcohol should not be used for this purpose. Application of local anesthetic agent such as lignocaine in an ointment or spray may be warranted when lesions are extensive.

Venom

Winkel et al. (2002a) demonstrated in vitro that a crude venom extract from the Australian *Carybdea rastonii* contracted blood vessels, an action that was absent in Ca^{++}-free media and which was reduced but not abolished by felodipine, nicardipine, and by T-type and L-type voltage-operated calcium channel antagonists (mibefradil, verapamil). When infused in vivo, the venom extract caused increase in heart rate and blood pressure which were abolished by verapamil. However, the tachycardia and hypertension, in contrast to similar effects of *Carukia barnesi* venom (Tibballs et al. 2000), were not associated with catecholamine release leading the investigators to speculate that if an Irukandji-like syndrome is caused by this jellyfish, it would be secondary to direct Ca^{++}-dependent actions on vascular tissues.

A partially purified toxin (pCrTX) from the tentacles of the Japanese *Carybdea rastonii* contracted aortic strips which were attributed to the release of endogenous catecholamines and to an influx of Ca^{++} in smooth muscle (Azuma et al. 1986a). A portion of such contraction was attributed to release of prostaglandins and a direct effect on smooth muscle not dependent on Ca^{++} influx (Ozaki et al. 1986). The toxin also contracted intestinal smooth muscle which was attributed to release of prostaglandins (Nagase et al. 1987). The partially purified toxin and purified proteins (CrTX-1, CrTX-II, CrTX-III) obtained from the tentacles aggregate platelets

by increasing cation permeability permitting an influx of calcium (Azuma et al. 1986b, c).

Nagai et al. (2000a) isolated protein toxins CrTX-A and CrTX-B with respective molecular masses of 43 and 46 kDa from the nematocysts and from tentacles of Japanese *Carybdea rastonii.* The 450 amino acid sequence of CrTX-A, localized primarily in nematocysts, was derived from cDNA which encoded both toxins. This toxin was fatally toxic to mice at 20 μg/kg (i.v.) and to crayfish at 5 μg/kg (i.p.) and caused inflammation when injected subcutaneously. The toxins have some sequence similarity to toxins derived from *Chiropsalmus quadrigatus* (Nagai et al., 2002) and to nematocyst hemolytic toxin CaTX-A (43 kDa, 463 amino acid sequence deduced from cDNA encoding) and to hemolytic CaTX-B (45 kDa) from *Carybdea alata* (Hawaiian box jellyfish, now *Alatina moseri*) (Nagai et al., 2000b) leading this group of investigators to suggest (correctly) that these toxins represented a novel class of bioactive proteins. Importantly, these observations contributed to the concept that cytolytic and pore-forming toxins of Cubozoan jellyfish have common evolutionary precursors (Brinkman et al. 2014; Jouiaei et al. 2015).

Hydrozoan Jellyfish

Physalia ("Bluebottle," "Portuguese Man-of-War")

This is also called the Pacific man-o'-war. It is a colony of siphonophores (class Hydrozoa) of which one possesses the stinging capsules, nematocyst, which are normally used for defense and capture of prey. It is the most frequent cause of significant jellyfish stings in Australia. Currently, it is uncertain whether *Physalia* exists as two or more separate species. Fenner et al. (1993) argued for differentiation into two species on the basis that one has several main tentacles (*P. physalis*), while the other has only one main tentacle (*P. utriculus*). The multitentacled species exists either as a small morph ("Pacific man-o'-war") or as a larger morph ("Portuguese or Atlantic man-o'-war"). *Physalia* is found in hot and temperate waters of the world. Both the single-tentacled and the small multitentacled specimens may be found in Australian waters.

Each *Physalia* consists of a number of individual animals, which have developed specific roles. One is a gas-filled float into which excreted carbon monoxide becomes diluted with atmospheric oxygen and nitrogen. The float measures from 2 to 13 cm in length and keeps the group "sailing" on the water surface. Because of its float, the jellyfish remains permanently on the surface of the sea. *Physalia* consists of two mirror images which rely on movement by sailing either to the left or to the right at 45° to the wind. Other members of the group are involved in reproduction, while a third group has developed polyps with associated stinging tentacles responsible for the collection of food.

The tentacles, which consist of a bunch of short frilled tentacles and a trailing long tentacle(s), serve effectively as a sea anchor. Parts of the float and all the tentacles are bright blue. The fishing tentacle which may be 13 m long and is

responsible for most of the stings. Being a surface creature, *Physalia* presents a significant hazard to swimmers particularly when the creatures gather in large numbers, for example on European and South American Atlantic coasts and on Mediterranean coasts. Beached *Physalia* may still deliver a sting, even though dehydrated.

Human Envenomation

When the main tentacle contracts, nematocysts become arranged in "stinging buttons," and contact may produce weals consisting of a corresponding line of "beans or buttons" composed of a blanched center surrounded by erythema. In contrast, uncontracted tentacles may give fine linear stings. Sharp pain is instantaneous and weals and/or red lines developing rapidly. The severity and appearance of local effects are in proportion to the size of the tentacle and its state of contraction. In severe cases vesicles may develop, but in most cases, signs of the injury have faded within 24 h. Local pain may last some 2 h and may spread throughout the whole limb or around the trunk when body stings have occurred. Movement of the injured limb may increase the severity of the pain.

Although no definite fatalities have been attributed to *Physalia* in Australia, it can cause severe local pain and occasionally serious illness. For example, Gollan (1968) described the collapse of a victim with apnea and pulselessness some 15 min after a sting attributed to *Physalia*. Fortunately, the victim responded dramatically to an intravenous injection of an antihistamine and soon recovered suggesting there may have been allergic basis to this severe reaction.

Several deaths from acute cardiorespiratory arrest (Burnett and Gable 1989; Stein et al. 1989) and near-fatal outcome due to unknown mechanisms on contact with the larger Atlantic *P. physalis* have occurred on the southeast coast of the United States. The death of a victim off the island of Sardinia was attributed to anaphylaxis (Prieto et al. 2015). In Chinese seas, *Physalia physalis* is among a group of several jellyfish suspected of hospitalizing several thousand victims and killing 13 in the period 1994–2007 (Dong et al. 2010).

Significant general signs are uncommon, but headache, vomiting, abdominal pain, and collapse may occur. Eye injuries have also been attributed to *Physalia* in Australia. Burnett et al. (1986a, 1988) accumulated clinical and laboratory evidence of the development of hypersensitivity to the *Physalia* as well as to other jellyfish. Jellyfish toxins and embedded nematocyst structures and contents are potent causes of immunological reactions to jellyfish stings (Tibballs et al. 2011).

Differential Diagnosis

Physalia is well known, and the offending creature is usually clearly visible. The characteristic injury produced usually allows distinction from other jellyfish stings. Skin scrapings may demonstrate characteristic spherical nematocysts.

Management of Envenomation

First Aid

Vinegar was observed to cause discharge of nematocysts at a grade of 2 on a scale of 5, while methylated spirits caused discharge to grade 5, in contrast to water which caused no discharge (Exton 1988). Vinegar caused discharge of up to 30% of nematocysts of *Physalia utriculus* (Fenner et al., 1993) and discharge of *Physalia physalis* nematocysts in suspensions of tentacles (Birsa et al. 2010). Thus vinegar is not recommended as a first-aid treatment, and methylated spirits should not be used. Adherent tentacles can be picked off the skin.

Pain Relief

Loten et al. (2006) showed in a controlled trial that immersion in hot water was superior to application of cold packs or ice. Indeed, reviews of that study and of others support the use of hot-water immersion over ice packs for pain relief of cnidarian envenomation (Li et al. 2013; Wilcox and Yanagihara 2016). The Australian and New Zealand Resuscitation Council (www.resus.org.au) recommends immersion in hot water for pain relief (Guideline 9.4.5). Persistent pain may respond to the application of a local anesthetic ointment, such as lignocaine 5%. Most stings are quite minor in nature, but they do pose a significant public health problem from time to time.

Venom and Toxins

Lane (1960) extracted crude venom from homogenized *P. physalis* nematocysts and found it to be a complex mixture of labile proteins. Mice injected with venom showed initial hyperactivity and tremors but then developed a progressive flaccid paralysis and died of respiratory failure within 1–48 h, depending upon the dose administered. Peptides derived from venom retained toxicity (Lane et al. 1961).

Burnett and Calton (1974) isolated nine mouse lethal factors from *P. physalis* nematocyst suspension and determined their molecular weights to be approximately 150 kDa. Tamkun and Hessinger (1981) purified a toxin, physalitoxin, from isolated *P. physalis* nematocysts, which had hemolytic and lethal effects with lethal dose similar to crude venom. Physalitoxin is a glycoprotein containing 10.6% carbohydrate with an estimated molecular weight of 240 kDa. A high molecular mass toxin (P3) from *P. physalis* was observed to reversibly block glutamate receptors (Mas et al. 1989).

In vitro studies showed that *P. physalis* venom caused exocytosis of mast cell granules and their eventual cell lysis which suggested the release of histamine (Cormier 1984). The venom caused vasodilation in skeletal muscle vascular beds of anesthetized dogs (Loredo et al. 1985) and in isolated rabbit arterial ring segments (Loredo et al. 1986). Venom obtained from the smaller of two nematocyst organelle types, separated by flow cytometry, was lethal to chick embryonic cardiocytes (Burnett et al. 1986b).

The toxins of the venom probably interfere with voltage-gated ion channels and cause poration of cell membranes. Lane (1967) showed the toxin produced marked

conduction disturbances in the hearts of rats and a potent stimulator of smooth muscle. The cardiovascular systems of the rat and dog were affected adversely by venom (Larson and Lane 1966; Hastings et al. 1967). The mechanism of action was suggested to be depolarization by inhibition of the Na^+/K^+-ATPase activity (Larson and Lane 1966). Positive inotropy in isolated rabbit atria proportional to the extracellular calcium level was observed by Bonlie et al. (1988). Burnett et al. (1985) observed that the calcium channel blocker, verapamil, delayed death in envenomated animals and suggested it may be a therapy for envenomation. However, although venom increases calcium influx into chick heart cells, the action is unaffected by a range of L-type calcium channel blockers and by a T-type calcium channel blocker but is inhibited by certain trivalent and divalent transitional metals (Edwards et al. 2000) which are known to block calcium channels. These investigators also observed that sodium influx, also increased by venom, was not blocked by flecainide, a sodium channel blocker, and suggested that the venom may act by activating or forming nonselective channels or pores ("poration") in membranes (Edwards et al. 2000). In a cnidocyst cDNA library, Bouchard et al. (2006) identified a variety of voltage-gated ion channel proteins including those for a Ca++ channel and for a Shaker-like K+ channel.

Support for the concept of "poration" or an action of permeabilizing the plasma membranes of cells was supported by later studies (Edwards and Hessinger 2000) which showed that the venom caused release of cytoplasmic lactate dehydrogenase (LDH) at the same dose which caused influx of calcium. These effects were observed not only with embryonic chick heart cells but also with rat pituitary cells and fetal rat lung cells and fibroblasts. The effects were not blocked by ouabain (which blocks Na+/K+-ATPases) or by vanadate (an ATPase inhibitor) but were blocked by Zn++. The transitional metal lanthanum blocked Ca++ influx but not the cytolytic activity of the venom. Further studies using electron microscopy (Edwards et al. 2002) revealed that cultured cells exposed to venom acquired 10–80 nm diameter lesions in membranes and that the extent of LDH release was related to the number and diameter of the lesions and could be suppressed by osmotic agents. These results implied that irrespective of a possible effect on ion channels, *Physalia* venom produces pore-like structures in cell membranes which lead to colloidal osmotic swelling with subsequent release of intracellular proteins and cell lysis.

Scyphozoan Jellyfish

These jellyfish inhabit temperate waters worldwide. Generally, they have a flat bowl, mushroom-shaped, or umbrellalike semitransparent bell which is scalloped at the edges to form a variable number of lappets from which arise a variable number of tentacles. From the underside of the center of the bell arise four stout mouthparts or arms which mingle with the more delicate tentacles. The jellyfish of medical importance in Australia include species of the genera *Pelagia*, *Cyanea*, and *Aurelia*.

Pelagia noctiluca ("the Little Mauve Stinger")

Pelagia noctiluca as its name implies is an open-water (pelagic) species which has a somewhat nocturnal phosphorescent bell measuring from 3 to 12 cm in diameter in mature specimens. The edge of the bell is scalloped into sixteen lappets. Eight tentacles arise from the bell edge. It is generally colored pink, mauve, or light brown. The upper surface of the bell and the four frilled mouthparts are studded with collections (warts) of nematocysts. *P. noctiluca* has a wide distribution in the oceans of the world, being found in tropical zones as well as colder areas, such as the north Atlantic and north Pacific. It is abundant in the Mediterranean Sea where envenomations are common (Queruel et al. 2000) and where it is regarded as the most dangerous jellyfish.

Human Envenomation

This species has not caused fatalities in Australia but has proved to be a nuisance when it has appeared in the sea during major surfing championships. Its tentacles or bell can cause immediate local pain and irregularly shaped weals resembling urticaria. Dyspnea may occur after massive stingings. Recurrent skin eruptions may occur without repeated contact with the jellyfish (Mansson et al. 1985).

Epidemics of stings due to *P. noctiluca* have occurred in the Adriatic Sea. Along the coasts of Yugoslavia in 1978, an estimated 250,000 people were stung. No serious injuries were reported, but in some cases the initial intense pruritus did not subside for a week. Trawlers and fishing boats had problems during this epidemic as the jellyfish fouled their screws and fishing gear, as well as filling their nets.

Although stings are usually not considered to endanger life, one victim had a cardiorespiratory arrest soon after a sting in the Mediterranean Sea (Bianchi et al. 2011). After prolonged successful resuscitation on the beach, ECG showed signs of myocardial infarction, while echocardiography revealed apical akinesis and severe left ventricular dysfunction with an ejection fraction of 30%. Coronary angiography showed normal coronary arteries but mid-left ventricular ballooning which the authors ascribed to catecholamine sting-induced Takotsubo cardiomyopathy.

First Aid

The utility or otherwise of the application of vinegar and heat or cold temperature is unknown.

Venom

Little is known about the venom, but it is antigenic as are many jellyfish venoms (Tibballs et al. 2011). A case of anaphylaxis after possible contact with *P. noctiluca* has been recorded (Togias et al. 1985). Reactivity with *Chrysaora quinquecirrha* and *Physalia physalis* monoclonal antibodies has been observed (Olson et al. 1985). Calcium-dependent activation of nematocysts is blocked by treatment with gadolinium (Salleo et al. 1994). Crude venom derived from nematocysts is cytotoxic to cultured fibroblasts (Mariottini et al. 2002).

Cyanea capillata ("the Hair Jelly")

This jellyfish has many other common names including "sea blubber," "hairy stinger," "sea nettle," "lion's mane," and "snottie." A number of other species in the genus *Cyanea* are distributed widely throughout all oceans. *C. capillata* is found in all Australian waters. This jellyfish has a flattened platelike (saucer-shaped) bell, the edge of which is turned inward and shaped into 16 lappets. It may be more than 30 cm across in Australian waters, but in the colder waters its size may be very much greater. It is a semitransparent white, light yellow, or brown. From beneath the central part of the bell arise the four mouth arms which secrete thick mucus – hence the common name "snottie." These mingle with a mass of numerous tentacles, more than a thousand, which arise more peripherally as eight V-shaped clusters. The tentacles, delicate and hairy, may trail many meters and detach easily but which are still capable of inflicting a painful sting.

Human Envenomation

No deaths have been attributed to this species in Australia, but in Chinese seas, it is among other scyphozoans which hospitalized approximately 2,000 victims and killed 13 in the period 1983–2007 (Dong et al. 2010). Stings by this species are associated with prominent weals. Contact with the tentacles may produce a burning feeling which develops into a severe pain and formation of prominent weals which have a characteristic sawtooth pattern. The pain and swelling subside after 15 min or so, leaving a bright red streak which may persist for several days, a valuable diagnostic aid. Shortly after the stinging, nausea and abdominal pains may develop. At times, profuse sweating, muscle cramp, and respiratory distress are seen. As with other jellyfish, injuries to the eye have occurred after contact with the tentacles or nematocysts shed by members of this genus.

First Aid

Vinegar should not be poured over the lesion or any adhering tentacles since it causes discharge of nematocysts (Fenner and Fitzpatrick 1986). Cold packs relieve the pain (Exton et al. 1989).

Venom

Rice and Powell (1972) isolated a single protein, toxic to mice, from nematocysts taken from specimens of *Cyanea capillata* collected in Chesapeake Bay in the United States. Walker et al. (1977) extended earlier findings concerning the lethal cardiotoxic effects of the toxin in experimental animals by tissue culture studies. The main toxin was found to directly disturb enzymes involved in ion transport and was a basic protein with mass 70 kDa. Xiao et al. (2010a) showed that the mechanism of hypotension in rats caused by a tentacle extract and nematocyst-derived venom, observed previously, was cardiotoxicity with acute heart failure with increases in serum lactate dehydrogenase, creatine kinase, and MB enzymes, while histological examination showed congestion of the lung and liver and electron microscopy revealed disruption of cardiac sarcomeric filaments. Using an isolated rat perfused

heart model, Beilei et al. (2012) observed that the recovery of cardiotoxicity induced by tentacle-only extract could be improved by pretreatment with calcium channel blockers nifedipine and verapamil but only partly with propranolol or phentolamine and not at all by atropine or neostigmine, leading these investigators to suggest that the mechanism of action of venom might be overactivation of L-type Ca++ channel receptors. Zhang et al. (2014) observed that a tentacle extract depressed rat heart function in vivo and also in isolated rat perfused hearts: effects which inhibited by L-type Ca++ blockade. In cultured myocytes the extract caused an increase in cytoplasmic Ca++. Extracts from tentacles contracted aortic tissue in vitro (Wang et al. 2013a). Interestingly, although tentacle extracts readily caused lysis of a range of mammalian red blood cells in suspensions, they were inactive in whole blood leading investigators to suggest that a factor in blood plasma, possibly albumin, has a protective role (Xiao et al. 2010b; Wang et al. 2012) which may explain why hemolysis is not a prominent feature of human jellyfish envenomation.

Lassen et al. (2011) isolated a human hepatocytoxic protein named CcTX-1 of mass 31.17 kDa from fishing tentacle venom of *C. capillata*. The amino acid sequence of CcTX-1 was derived for 85% of the protein and partially matched the known sequences of hemolytic (pore-forming) proteins from the Cubozoan jellyfish *Carybdea alata* (CaTX-1) and *Carybdea rastonii* (CrTX-1). Additional work by this group (Lassen et al. 2012), using a fishing tentacle extract, identified a neurotoxic polypeptide of mass 8.22 kDa with Na(v) channel blocking activity as determined in a mouse neuroblastoma cell line. In addition to pore formation, lipid peroxidation (of cell membranes) was identified as another potential mechanism underlying hemolysis caused by tentacle extract (Wang et al. 2013b).

Aurelia aurita ("Saucer or Moon Jellyfish")

Aurelia species have a worldwide distribution and have been observed in Australian waters. *A. aurita* is characterized by a smooth flattened saucerlike bell of diameter up to 30 cm and whose edge is slightly scalloped into eight lappets. It is colored blue-white. The numerous tentacles are very short and appear as a fringe from the edge of the bell. The four oral arms are thick and prominent.

Although it has been regarded as relatively harmless (Cleland and Southcott 1965), Burnett et al. (1988) described a case of significant envenomation occurring in the Gulf of Mexico. A swimmer sustained large stings of an arm and knee. Instant pain was followed within a few minutes by urticaria, then by ulceration which encrusted 3–9 days later. The initial pain disappeared within 30 min but was followed by regional pain after 4.5 days which lasted for 24 h. Post-inflammatory hyperpigmentation was still visible at the sites after 2 weeks. Immunospecific antibodies against *Aurelia* antigens were detected which also cross-reacted with antigen derived from *Chrysaora quinquecirrha* (North American sea nettle) another jellyfish of the order Semaeostomeae. *Aurelia aurita* is one of the several scyphozoan jellyfish which collectively have caused numerous hospitalizations and deaths of a number of victims in China (Dong et al. 2010).

Venom

In comparison to other jellyfish, the toxicity of its venom has been little researched. Tentacle extract has a fibrinogenlytic activity (Rastogi et al. 2012).

Other Jellyfish

There are many other species of jellyfish which can cause painful stings to humans in Australian waters, but nothing or very little is known about their venoms. These include *Catostylus mosaicus* ("blubber jellyfish") which can cause hemolysis, edema, and hemorrhage, *Pseudorhiza haeckeli*, *Olindias singularis*, *Chrysaora* sp., and *Cassiopea* sp. ("upside-down jellyfish").

Conclusions and Future Directions

Envenomation by numerous Australian carybdeid jellyfish cause the Irukandji syndrome which is probably the result of protein toxins disrupting biomembrane sodium channel conductance and resulting in excessive release of catecholamines with resultant hypertension and cardiac failure. Toxins also cause pore formation in cardiac myocytes contributing in the severe case of envenomation to cardiac failure. The toxins remain to be fully identified and characterized. No specific treatment exists for the envenomated victim. However, pain relief and standard clinical cardiorespiratory support have been successful in severely envenomated victims. Numerous other jellyfish, classed as hydrozoan and scyphozoans, also inhabit Australian waters. These can cause painful stings but do not present serious threats to life. Immersion of a stung limb in hot water appears to be an effective analgesic first-aid technique for *Physalia* stings. The investigation of toxins of all these jellyfish is still at an early stage. Their modes of action, structure, and character remain to be discovered and verified.

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Australian Chirodropid Cubozoan Jellyfish Envenomation

12

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Contents

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Abstract

The Australian box jellyfish, *Chironex fleckeri*, is a large dangerous jellyfish inhabiting the waters of northern Australia and nearby Southeast Asia. Despite intensive research over half a century, limited knowledge exists about its venom and toxins and of a related but much less potent chirodropid species *Chiropsella bronzei*. The jellyfish kills prey rapidly and may severely injure or kill humans with an array of potent toxins. The clinical treatment of human victims is supportive with limited assistance from an existing antivenom. The principal toxins are proteins of around 40–45 kDa which probably perforate cell membranes leading to immediate lysis and subsequent loss of function of vital organs and tissues including cardiac function. In addition, smaller proteins probably damage the function of excitable tissues by perturbations of function of voltage-gated ion channels. These mechanisms occur across the phylum Cnidaria whose members have homologous toxins. No specific treatment for the human victim exists, but effective treatments might be developed from observations that suggest that the poration and ion-channel effects of the toxins are susceptible to blockade with metallic compounds.

Introduction

This chapter is an overview of Australian *chirodropid cubozoan* jellyfish and their venoms and toxins related to the clinical management of the envenomated victim. Approximately 50 species of jellyfish have been assigned to the biological class Cubozoa – all are characterized by a square or box-shaped medusal bell. Chirodropida (multiple tentacles arising from the medusal corners – "Chirodropids") and Carybdeida (single tentacles arising from the medusal corners – "Carybdeids") constitute its two orders. Chirodropida is divided into two families: Chirodropidae and Chiropsalmidae. This chapter considers Australian chirodropid jellyfish. The Carybdeids and other jellyfish are discussed in a companion chapter.

Jellyfish belong to the phylum Cnidaria, which also includes corals, sea anemones, sea pens, and hydroids. They number about 10,000 species, mainly inhabit salt-water environments, and are characterized by cnidocysts – specialized cellular structures. One type of cnidocyst, possessed by all Cnidaria, are penetrant nematocysts. These "stinging cells" explosively discharge eversible tubules, which deposit a complex mixture of bioactive compounds ("venom") to entrap, subdue, and digest prey and to defend against predators. The tubules of the nematocysts, located on tentacles and body of the jellyfish, can penetrate the human dermis.

Cnidarians are divided into two subphyla: Anthozoa and Medusozoa. The members of medusozoan classes include Cubozoa, Hydrozoa, Scyphozoa, Anthozoa, and Staurozoa, which all have triphasic life cycles as a free-swimming planula larva, a sessile polyp, and sexual pelagic medusa. About 150 toxins have been identified in cnidarian venoms. These are protein enzymes (phospholipases A_2 and metalloproteases), pore-forming toxins (cytolysins), neurotoxins, and nonprotein bioactive molecules (serotonin, histamine, bunodosine, caissarone) (Jouiaei et al. 2015). The venom of no species has been fully elucidated, and few toxins have been well characterized.

Anthozoa (sea anemones, corals) venoms are rich in low molecular weight neurotoxic peptides. These are voltage-gated sodium (Nav) channel toxins, voltage-gated potassium (Kav) channel toxins and Kunitz peptides, small cysteine-rich peptides (SCRiPs), sodium-selective acid-sensing ion channel (ASICs) inhibitors, and nonselective cation channel (TRPV1) inhibitors. The voltage-gated ion channel toxins prolong the action potential in excitable and nonexcitable membranes of neuronal and muscle tissue resulting in release of neurotransmitters at synapses and neuromuscular junctions with a resultant hyperadrenergic state and paralysis.

Pore-forming (cell destroying) toxins are probably present in all cnidarian venoms, but have been identified principally in Cubozoan venoms. They are also present in Anthozoan and Hydrozoan venoms, in the latter as actinoporirns of approximate mass 20 kDa. Cubozoan pore-forming toxins (porins) have hemolytic, myotoxic, cardiotoxic, and dermatonecrotic effects and are of mass 40–46 kDa with α and β domains. The α domains polymerize on cellular membranes creating characteristic pores of inner diameter 12 nm.

Australian Box Jellyfish, *Chironex fleckeri*

The Australian box jellyfish, *Chironex fleckeri*, is the archetypal chirodropid and has killed approximately 80 people in subtropical Australian waters. Fatality is due to circulatory and respiratory failure occurring within minutes. It was identified and named in 1956 by Southcott, honoring Flecker who had spent many years investigating marine stings in Queensland waters.

Distribution and Seasonal Incidence

The Australian range of *C. fleckeri* extends from Port Curtis (latitude 24 °S) northwards along the Queensland coast and westward to Darwin and to as far as Exmouth in Western Australia (latitude 22 °S). In Queensland, the sting danger period is commonly regarded as October–May but only June and July have been sting free. In the Northern Territory and neighboring islands, stings have occurred in every month of the year with the peak prevalence in January (Fenner and

Harrison 2000). It probably also inhabits Southeast Asian seas to the immediate north of Australia.

Description and Habits

Chironex fleckeri has a white or translucent cubic or box-shaped bell up to 20 x 30 cm and may weigh more than 6 kg. It is a highly advanced species with sensory organs containing eyes, vibration sensors, and motion (gyration) sensors. It is attracted by light. It breeds in estuaries during autumn. Fertilized eggs develop into minute creeping polyps with two tentacles, which attach to estuarine rocks. These sessile polyps may reproduce by budding. In spring, the polyps metamorphose over 14 days and the immature jellyfish stage is carried to sea as small medusa. Its size at 2–3 months poses a threat to humans. It is an in-shore or littoral species and does not inhabit offshore coral reefs. It hunts small prawns in shallow beach water where human stings usually occur.

Envenomation

The jellyfish is rarely initially noticed by the victim. The tentacles are easily torn from the jellyfish, adhering to the skin of the victim on which they resemble earthworms of a pink, gray, or bluish hue. Severity of injury is related to size of the jellyfish and extent of tentacle contact. Most stings are quite minor. Over a 12-month period, 1999–2000, of 40 victims of jellyfish stings presenting to Royal Darwin Hospital, 28 were due to *C. fleckeri*. None of these was life-threatening and none required antivenom (O'Reilly et al. 2001). Injury from specimens having a bell of 5–7 cm is confined to painful local lesions which persist for several days, but stings from specimens with a bell of 15 cm or more are severe with death probable if the total length of weals on the victim is greater than 6 or 7 m (Barnes 1960, 1966), and may occur within minutes of stinging.

The unmistakable features of stinging are as follows (Barnes 1960): "Stings from large Cubomedusae (15 cm or more across the top of the bell) are extremely severe. During the first 15 minutes pain increases in mounting waves, despite removal of the tentacles. The victim may scream and become irrational. Areas of contact are linear and multiple, showing as purple or brown lines often compared to the marks made by a whip. A pattern of transverse bars is usually visible. Wealing is prompt and massive. Oedema, erythema and vesiculation soon follow, and when these subside (after some ten days), patches of full-thickness necrosis are leaving permanent scar perhaps with pigment changes."

Skin lesions caused by *C. fleckeri* are distinctive with transversely barred weals 8–10 mm wide usually in a crisscross fashion. These "frosted ladder patterns" match the bands of nematocysts on the tentacles. Lesions produced by another highly dangerous jellyfish, *Chiropsella bronzie,* are narrower and milder and the tentacle contact area far less.

Structure and Function of Venom Apparatus

Four bundles of up to 15 translucent extensile ribbon-like tentacles stream out from four pedalia (fleshy arms) under the bell, which is itself devoid of nematocysts. The tentacles may stretch 2–3 m and are covered with millions of nematocysts ("spring loaded syringes") whose tubules are tipped with small sharp denticles, enabling easy penetration of tissue contacted. The extruded tubules extend up to 1 mm, sufficient to reach into the dermis of human skin.

The explosive release of the tubule is enabled by sudden release of spring-like tension stored in the collagenous structural compartment of the nematocyst capsule and by a sudden increase in the osmotic pressure of the capsular fluid, due to the removal of bound calcium ions (Lubbock and Amos 1981; Tardent 1995). The intracapsular osmotic pressure reaches 150 bar. Another type of nematocyst contains a sticky substance which adheres the tentacle to the victim, while another, acting like a "grappling hook," pulls the tentacle closer to the victim, bringing into action more "batteries" of nematocysts, thus increasing envenomation of the prey or human victim (Rifkin and Endean 1983). The concentration of nematocysts is more than 1500 per mm^2. Nematocysts on detached tentacles remain active for long periods.

Rifkin and Endean (1983) proposed that toxins on the outside of the tubules are released as the nematocyst discharges. Thus venom may be injected continuously, not only when the tube is fully everted. Venom would be washed off the tubules as they transfix capillaries, contributing to the rapidity of envenomation.

Venom

The actions and character of toxins have been difficult to determine, perhaps influenced by the mode of venom collection. Recent investigators have used at least six different methods of venom collection and preparation (Yanagihara and Shohet 2012). Initial studies of extracts of frozen tentacles identified lethal, hemolytic and dermatonecrotic activities of the venom (Southcott and Kingston 1959). The different activities of the venom were due to separate and labile protein components. These findings were confirmed and amplified by Baxter and Marr (1969), using "milked" venom. Barnes (1967) obtained venom by "milking" – a technique in which venom is harvested from tentacles applied to an amniotic membrane and electrically stimulated. Today, CSL (Commonwealth Serum Laboratories) Pty Box Jellyfish antivenom is prepared from venom collected by this technique.

Lethal Effects

Endean et al. (1969) demonstrated that extracts from nematocysts were highly toxic to prawns, fish, rats, and mice. Prawns injected with the toxic extract of more than 50,000 nematocysts died within 5–90 s. Mice, which received venom from

approximately 35,000 nematocysts, died within 2 min of intravenous injection. Larger vertebrate animals (guinea pigs, rabbits, sheep, and monkeys) injected with a lethal dose died within 15 min (Baxter et al. 1973). Initially, control would be lost over the hind limbs and then the fore limbs, respiration soon ceased followed by cardiac standstill. The abrupt death was similar to that described in humans, but it was difficult to determine whether cardiovascular collapse was a primary event or secondary to respiratory failure.

Cardiovascular Effects

Extensive studies in animals by Endean et al. (1969) revealed that nematocyst extracts caused the heart to progressively fail to relax and it became paralyzed in systole. Numerous other investigators have reported lethal effects of venom on cardiac activity. Freeman and Turner (1969) showed respiratory arrest of apparently central origin was the terminal event in all species studied but clear evidence of cardiotoxicity was also obtained (Turner and Freeman 1969). Bradycardia developed, with varying degrees of conduction delay, and terminal atrioventricular block usually occurred. Biphasic blood pressure changes were seen and blood samples, taken before terminal apnoea developed, had varying degrees of hemolysis and raised serum potassium levels. Further work by Freeman and Turner (1971, 1972) suggested that the cardiovascular picture produced by cardiotoxicity and vasoconstriction might be complicated by baroreceptor stimulation.

Tentacle extract administered to mechanically ventilated piglets caused severe hypotension, cardiac dysrhythmias, and pulmonary hypertension (Tibballs et al. 1998) while mechanical ventilation similarly in rats did not prevent cardiovascular collapse caused by nematocyst-derived venom (Ramasamy et al. 2004). Winkel et al. (2005) observed that nematocyst derived venom contracted rat mesenteric artery, an action inhibited by CSL antivenom. (Winter et al. 2007) observed that nematocyst-derived venom of *C. fleckeri* and *C. bronzie* (previously *Chiropsalmus* sp.) produced sustained contraction of endothelium-denuded isolated rat aorta not affected by prazosin or CSL antivenom. A direct action on cardiac and vascular muscle was shown by Hughes et al. (2012): in rat isolated cardiac and vascular tissues, unpurified extracts from nematocysts caused negative inotropy and atrial standstill unaffected by propranolol, atropine, or CGRP8–37 and caused contraction of small mesenteric arteries unaffected by prazosin, bosentan, or tetrodotoxin. The investigators concluded that the venom did not involve autonomic nerves, postsynaptic α_1- or β_1-adrenoreceptors, muscarinic, endothelin, or CGRP receptors but may involve direct effects on cardiac and vascular muscle. Box jellyfish antivenom attenuated all effects of the venom. At high dose, the venom preparation caused lethal cardiovascular depression in ventilated rats. Pereira and Seymour (2013) showed in vitro that *C. fleckeri* venom, in contrast to *Carukia barnesi* venom, is highly toxic to human cardiac and skeletal muscle cells. Taken together, all these studies suggest that that severe hypotension caused by the venom is due to direct cardiotoxicity, which overwhelms any peripheral vasoconstrictor effect.

There is no doubt that cardiac effects are prominent. However, whether or not the venom toxins act by alteration of membrane permeability (and conduction), as originally suggested by Freeman and Turner (1969), by direct poration of cardiac tissue with or without calcium entry, or by hyperkalemia secondary to poration and lysis of erythrocytes (and other cells), remains still an open question.

Hemolysis

A hemolytic component (hemolysin) of venom was studied by Keen and Crone (1969a), and by Crone and Keen (1969, 1971) who concluded that it had a molecular weight of about 70 kDa. Crone (1976a) found that the hemolysin contained a disulfide bond which was essential for its activity, but it had no apparent phospholipase A or C activity. Hemolysis could be prevented by the presence of either divalent cations or nonspecifically by trace amounts of ganglioside (Crone 1976b). Previously, Endean and Henderson (1969) had found that extracts of nematocyst intracapsular material exhibited hemolytic activity but also had no phospholipase A or proteolytic activity. Further studies of extracts of isolated nematocysts and of tentacles from which nematocysts had been removed confirmed the presence of a hemolytic agent of molecular weight 70 kDa (Endean et al. 1993). Tibballs et al. (1998) later confirmed that infusion of extracts of tentacles in experimental animals caused hemolysis by observations of elevation of serum free hemoglobin levels. Curiously, although hemolysis has been observed in experimental situations, it has not *per se* been significant in clinical situations, suggesting that actions of toxins apart from hemolysis and its consequences, for example hyperkalemia, account for its significant cardiovascular effects.

Dermatonecrosis

A dermatonecrotic factor isolated from the venom produced rapid skin death in experimental animals, the changes being similar to those seen in human skin (Freeman and Turner 1969; Keen 1970).

Immunological Effects

The immunological aspects of *C. fleckeri* envenomation, and indeed of other jellyfish, have received little attention. Considering that envenomation involves deposition of numerous foreign proteins and bioactive components into the dermis and blood of a human, it is hardly surprising that immunological responses are not uncommon. For example, in 19 victims of *C. fleckeri* envenomation, 11 (58%) had delayed hypersensitivity reactions which resolved spontaneously or with oral antihistamine and topical corticosteroid (O'Reilly et al. 2001).

Envenomation deposits not only toxins but also structural elements of nematocysts. The tubules which deliver the venom are composed of collagens, glycoproteins, polysaccharides (Özbek et al. 2009), and chitin (a structural carbohydrate), which like the venom components may trigger antigenic, allergenic, and innate immune responses (Tibballs et al. 2011).

Toxins

Molecular Mass

Early study of toxins, generally acknowledged to be proteins, was hampered by their lability, tendency to aggregate, disaggregate, and to bind to nontoxic components and to adhere to apparatus (Olson et al. 1984). Moreover, the size of the proteins appeared to be dependent on the method of obtaining the venom. Crone and Keen (1969, 1971) found that a lethal component was a labile protein of molecular weight about 150 kDa but other investigators (Baxter and Marr 1969; Olson et al. 1984; Othman and Burnett 1990; Naguib et al. 1988) have suggested a lower molecular size.

Bloom et al. (1998), working with lyophilized nematocysts obtained by "beachside" autolysis of tentacles, was unable to demonstrate the existence of the 600-kDa toxin previously identified by Endean (1987) or by Endean et al. (1993). Instead, native polyacrylamide gel electrophoresis of crude venom yielded protein bands of 30–200 kDa. Using three different modes of preparations of venom, Wiltshire et al. (2000) identified proteins of variable size and illustrated the importance of venom extraction methodology in identification of toxins. With SDS-PAGE analysis of lyophilized tentacles, seven major proteins bands, spanning the full range of mass greater than 106 and less than 18.5 kDa, were identified. Nematocysts, grounded by mortar and pestle, yielded numerous protein bands with at least two major components larger than 106 kDa but the most abundant bands appeared at approximately 40 kDa. However, milked venom contained only one band at approximately 40 kDa and a doublet band at 17 kDa leading the authors to suggest that the toxin(s) of approximately 40 kDa were aggregates of 17 kDa subunits, as Endean et al. (1993) had similarly proposed for myotoxins. All other proteins were considered to be nematocyst structural or matrix components. Interestingly, Wiltshire et al. (2000) also showed that CSL box jellyfish antivenom bound strongly not only to box jellyfish proteins of 40 kDa proteins but also to an Irukandji jellyfish (*C. barnesi*) protein of around 50 kDa and to Blubber jellyfish (*Catostylus mosaicus*) protein of around 20 kDa, raising the undiscussed possibility that some components of cubozoan venoms were shared between jellyfish species although the clinical significance is doubtful since *C. fleckeri* antivenom is known to be ineffective against Irukandji envenomation (Fenner et al. 1986).

Myotoxins, Hemolysins, and Neurotoxins

Multiple lethal toxins are probably present in venom, or single toxins may have multiple actions. Endean (1987) isolated two myotoxins of approximate mass 150 kDa and 600 kDa from crude nematocyst venom. The toxins contracted skeletal, smooth, and cardiac muscle (Endean 1987; Endean and Sizemore 1987). In additional work, Endean et al. (1993) isolated a hemolysin of approximate mass 70 kDa and showed that the myotoxins were composed of aggregations of subunits of approximately 18 kDa mass. Working with tentacle extracts from which nematocysts had been removed, a hemolysin and a neurotoxin of approximate 150 kDa were isolated. All five toxins were lethal to mice on intravenous injection. Calton and Burnett (1986) isolated two components from a tentacle extract by immunochromatography using antivenom. They confirmed a lethal factor that had a molecular weight of 150 kDa. Naguib et al. (1988) generated monoclonal antibodies against tentacle extract, which suggested the presence of at least two hemolysins, two dermatonecrotic, and three factors lethal to mice. Collins et al. (1993) generated three monoclonal antibodies against hemolytic activity of tentacle extract.

Principal Mode of Action of Toxins: Cell Membrane Poration

Rapid lethality of cubozoan venoms is partially due to cell membrane pore formation (poration). Cellular death may result from calcium entry but the evidence does not favor direct activation of calcium channels (*infra*). In other species, e.g., *Physalia physalia*, calcium influx appears to be the result of toxin-induced membrane poration (Edwards and Hessinger 2000; Edwards et al. 2000). This is also probably a mode of action of *C. fleckeri* venom. Indeed, Bailey et al. (2005) observed, by electron microscopy, large numbers of circular lesions in the membranes of rat myocytes after exposure to *C. fleckeri* venom. Similarly, Yanagihara and Shohet (2012) observed abundant 12 nm transmembrane pores, rapid lysis, and potassium release from human red blood cells exposed to venom derived from *C. fleckeri* nematocysts. Mice injected with the same preparation showed rapid decline in cardiac ejection fraction with progression to electromechanical dissociation leading the investigators to suggest that acute hyperkalemia, resulting from lysis of red cells and possibly of other cells, was the mode of death. That acknowledged, simple disruption of cardiac membrane conduction by poration might also be expected. Wang et al. (2013) showed in vitro that lipid peroxidation is another potential mechanism besides pore-formation underlying the hemolysis of tentacle extract of *Cyanea capillata*. Chaousis et al. (2014) showed that two crude fractions of venom (CTF-α, CTF-β) extracted from nematocysts injured and decreased metabolism in human cardiomyocytes. The effect of one of the fractions was reversible leading the authors to suggest that poration was not the only mechanism of cytotoxicity.

Structure

The structures of two of the probable numerous toxins have been determined recently. In a new era, studies by Brinkman and colleagues have added significantly to knowledge of *C. fleckeri* toxins. Brinkman and Burnell (2008) identified powerful hemolytic proteins of approximately 370 and 145 kDa: the larger is an aggregate 2 subunits named CfTX-1 and CfTX-2 subunits which are antigenic for CSL *C. fleckeri* antivenom, while the smaller is an aggregate of 39 and 41 kDa proteins which are not antigenic. The structures and sequences of CfTX-1 and CfTX-2, derived from nematocysts, were determined by Brinkman and Burnell (2007, 2008). CfTX-1 and CfTX-2 have masses 43 and 45 kDa and 436 and 445 amino acid sequences, respectively, as determined by SDS-PAGE. These proteins are antigenic to CSL box Jellyfish antivenom and share significant homology with other known protein toxins from other cubozoan jellyfish: *Chironex yamaguchi* (*Chiropsalmus quadrigatus*) (CqTX-A), *Carybdea rastoni* (CrTXs), *Carybdea alata* (*Alatina moseri*)(CaTX-A), and *Malo kingi* (Cytotoxin A, Cytotoxin B) which all have pore-forming properties (Brinkman and Burnell 2009; Brinkman et al. 2012). Homologies also exist with toxins of non-cubozoan cnidarians *C. capillata* (Scyphozoa) and *Hydozoa magnipapillata*. The *C. fleckeri* toxins CfTX-1 and CfTX-2 cause rapid cardiovascular collapse in rats (Brinkman et al. 2014). Additional proteins with pore-forming effects in *C. fleckeri* venom, CfTX-A and CfTX-B, approximately 40 kDa and 42 kDa respectively, have strong hemolytic activity but minor cardiovascular effects (Brinkman et al. 2014).

Proteome Analysis

Proteome analysis of nematocysts, using SDS-PAGE and subsequent tandem mass spectrometry (Brinkman et al. 2012), identified 61 proteins including toxins and proteins associated with nematocyst development and structure. Further transcriptome and proteome analysis (Brinkman et al. 2015) predicted 263 proteins in the contents of nematocysts isolated from excised tentacles. More than 170 proteins were potential toxins predicted on the basis of homology with databanks of known toxins, grouped into functional groups including metalloproteinases (major constituents of spider venoms), an alpha-macroglubulins (coagulation-active proteins present in snake venoms), two SCRiPs, and a turripeptide-like protease inhibitor. Importantly, new members of an extended family of pore-forming toxins (CfTXs), previously identified (Brinkman and Burnell 2007, 2008; Brinkman et al. 2012) and known to be present in other cnidarians, were identified in the venom proteome.

Clinical Management of Envenomation

The severity and rapidity of envenomation mandate decisive action on the beach, during transport, and in hospital. Management requires:

- First-aid: Rescue of victim from the water to avoid further contact and to prevent drowning; basic life support; inactivation of undischarged nematocysts by pouring vinegar (4–6% acetic acid) over adhering tentacles for at least 30 s.
- Basic and if possible advanced cardiopulmonary resuscitation on the beach, during transportation, and in hospital where extracorporeal circulatory support and treatment to combat hyperkalemia may be required (not further discussed).
- Administration of antivenom.
- The administration of magnesium (unproven therapy) and possible zinc carbonate (untried therapy).

Inactivation of Nematocysts

There has been controversy over the first-aid treatment to prevent discharge of undischarged nematocysts deposited on the skin. Hartwick et al. (1980) observed that vinegar or acetic acid in solutions 3–10% produced rapid and complete inhibition, as tested by later exposure to methylated spirits which until then had been recommended erroneously to prevent discharge. A 10% formalin solution produced inhibition but it was slow. The commercial preparation "Stingose" (Hamilton Labs), which contains 20% aluminum sulfate (Henderson and Easton 1980), did not inactivate the nematocysts as well as vinegar. Thomas et al. (2001a) observed that that Sting-Aid (an aluminum sulfate solution) was no better than seawater in altering the pain of the Hawaiian "box jellyfish" *C. alata.* Of many substances tested, most inhibition of nematocyst discharge of *C. alata* was observed with vinegar (Yanagihara et al. 2016). Vinegar is actually an old remedy and has long been used, for example in The Philippines, for severe jellyfish stings (Williamson et al. 1996).

Vinegar-treated tentacles may be removed with safety. However, this is not necessary and consumes valuable time. If vinegar is not available, the tentacles may be picked off safely by rescuers since only a harmless prickling may occur on the fingers of the rescuer (Williamson et al. 1996). Nonetheless, detached live tentacles should be treated with caution. An unwitting 19-year-old lifesaver experienced respiratory difficulty when he mistakenly drank from a bottle containing tentacles (*The Age*, Wednesday 5 January, 2000), presumably due to pharyngeal edema.

Despite these observations, the use of vinegar is not without criticism but no clinically decisive studies have been performed. Welfare et al. (2014) suggested from studies in vitro that vinegar promotes further discharge of venom from already discharged nematocysts.

Use of First-Aid Pressure-Immobilization Bandage

This is also a controversial issue. This technique was adopted from the management of elapid snake bite where it retards the movement of venom from the bite site via

lymphatic channels (Sutherland et al. 1979). However, application of pressure simulating a pressure-immobilization bandage caused in vitro discharge of additional venom from partially discharged *Chiropsalmus sp*. (*Chironella bronzei*) nematocysts (Pereira et al. 2000) and from vinegar-soaked *C. fleckeri* nematocysts (Seymour et al. 2002). The possible advantage to be gained by retarding central movement of venom by compression of lymphatics or capillaries against the possible harm of inducing additional envenomation has yet to be tested in a realistic model of jellyfish envenomation. However, until evidence is presented which clarifies the situation, the Australian Resuscitation Council recommends (Guideline 9.4.5) (https://resus.org.au/guidelines) that first-aid treatment be confined to life support, dousing of the affected area with vinegar, and rapid transport of the victim to hospital.

Antivenom

Chironex fleckeri antivenom is the only jellyfish antivenom manufactured worldwide and has been in use since 1970 (Winkel et al. 2003). It is concentrated immunoglobulin isolated from serum of sheep hyperimmunized with venom. Each ampoule of antivenom contains sufficient activity to neutralize 20,000 intravenous LD_{50} mouse doses. The dose recommended is three or more ampoules (intravenously) on theoretical and on experiential grounds for resuscitation, analgesia, and skin-sparing cosmetic effects (Sutherland and Tibballs 2001). Allergic reactions to antivenom are infrequent.

Concerning the efficacy of the product, numerous studies have shown that the components in tentacle extracts are antigenic (Keen and Crone 1969b; Naguib et al. 1988; Collins et al. 1993). Keen and Crone (1969b) observed that both tentacle extract and "milked" venom were antigenic in rabbits whose antisera effectively reduced lethal and hemolytic effects and to a lesser extent the dermatonecrotic effects in rodents. Baxter and Marr (1974) showed in vitro that the ovine antivenom (raised against "milked" venom) neutralized the lethal, hemolytic, and dermatonecrotic effects of tentacle extract and of "milked" venom. Endean and Sizemore (1988) observed that this antivenom was less effective against crude nematocyst venom than against "milked venom" thus implying that venom prepared from nematocysts contained more or additional toxins compared with "milked" venom. Burnett et al. (1990) observed that the antivenom reduced mortality in mice injected with tentacle extract. Tibballs et al. (1998) observed that the antivenom incubated with tentacle extract prevented hemolysis and systemic hypotension but not pulmonary hypertension in mechanically ventilated piglets. Ramasamy et al. (2003) observed that antivenom mixed with venom prevented neurotoxic effects but not myotoxic effects in a chick biventer nerve-muscle preparation, but established neurotoxic effects, however, were not reversed. Antivenom administered 10 min before envenomation prevented cardiovascular collapse in only 40% of anesthetized rats leading the investigators (Ramasamy et al. 2004) to suggest that the venom used to raise antivenom may be lacking a lethal factor. In rats, antivenom incubated with

"milked" venom neutralized the cardiovascular depression usually caused by venom alone but failed to prevent the effects when administered 15 min before venom (Winter et al. 2009).

Indications for Antivenom

Antivenom should be given to a victim of suspected *C. fleckeri* stinging as soon as possible in the following circumstances:

- Unconsciousness, cardiorespiratory arrest, hypotension, dysrhythmia, or hypoventilation
- Difficulty with breathing, swallowing, or speaking
- Severe pain (parenteral analgesia is also usually required)
- Possibility of significant skin scarring

Antivenom should be injected intravenously, preferably by infusion. A dilution of 1 in 10 is advisable. The risk of serum reactions normally precludes the use of antivenom by lay persons but, if an emergency arises remote from medical aid, intramuscular injection by an informed layperson is justifiable. Indeed, several cases of severe envenomation have been treated successfully with intramuscular antivenom administered by ambulance personnel on the beach (Fenner et al. 1989; Beadnell et al. 1992).

The antivenom is effective in reducing pain and local tissue damage provided if given early. Williamson et al. (1984) for example reported two cases with remarkably dramatic relief of pain with antivenom. One patient had no relief with pethidine and the other responded neither to topical lignocaine nor iced water. In the latter case, the skin lesions were also seen to improve within 90 s of the antivenom. Topical corticosteroid or oral antihistamine (O'Reilly et al. 2001) or systemic steroids reduces the swelling and itchiness of the skin lesions.

Not unsurprisingly, the efficacy and effectiveness of the antivenom have been doubted (Winkel et al. 2003; Isbister 2010), Indeed, the variable experimental reports of neutralization of venom activity and of antigenicity of components and toxins underscore the concern for efficacy, while the rapidity of onset of toxicity in human victims compromises effectiveness. No other type of envenomation can be as rapidly fatal as that by *C. fleckeri*. It should be administered when indicated as soon as possible but its effectiveness cannot be assumed. Unfortunately, no efficacious rapid alternative treatment is available.

Laboratory Diagnosis

The simplest and quickest way to confirm *Chironex* envenomation is to examine, under a microscope, a piece of transparent sticky tape which has been applied to the sting site. A 4–8 cm length is applied, stroked several times, removed, and with its

sticky side up affixed onto a glass slide. This method was more successful than using skin scrapings to identifying characteristic *Chironex* nematocysts in patients presenting to The Royal Darwin Hospital with jellyfish stings (Currie and Wood 1995).

Microscopic examination of scrapings from the skin or sections of lesions may also confirm the diagnosis of jellyfish stinging. Skin scrapings may be suspended in sea water or saline and allowed to dry on glass slides. Examination of these scrapings may determine the origin of the nematocysts, but it is difficult to distinguish between those of *C. fleckeri* and *C. bronzei.* The undischarged nematocysts of these two jellyfish are very similar. If silver impregnation methods are used, penetrating nematocyst threads are seen. The toxin causes edema of the stratum corneum and death of cells. The histopathology of the skin changes are comprehensively described by Kingston and Southcott (1960), Cleland and Southcott (1965), and Williamson et al. (1996).

Calcium Channel Blockade

Apart from antivenom, calcium channel blockade is the only mode of specific treatment, which has been considered for *C. fleckeri* envenomation. Verapamil has been advocated in the management of serious *Chironex* envenomations (Burnett and Calton 1983; Burnett et al. 1990; Williamson et al. 1996), but it is not practical due to potential complications. Moreover, no successful clinical uses have been reported and combined evidence from animal studies indicates that it is in any case ineffective.

In the desperate setting of life-threatening *C. fleckeri* envenomation, the use of calcium channel blockade at first appeared reasonable and logical. Early experiments showed that the venom caused vasoconstriction (Freeman 1974), decreased coronary blood flow, heart rate, and amplitude of contraction in isolated perfused hearts (Turner and Freeman 1969). Moreover, venom preparations caused an influx of calcium ions into muscle fibers (Endean and Henderson 1969), inhibited uptake of calcium by the sarcoplasmic reticulum (Endean and Henderson 1974), and interfered with electrical events and contraction of myocardial tissue (Freeman 1974). All these events were assumed to be primarily due to opening of calcium channels.

Mustafa et al. (1995) confirmed that application of toxin to papillary muscle and isolated myocytes resulted in an increase in intracellular calcium and adverse symptoms of calcium overload (aftercontractions, spontaneous contractions, a decrease in developed force, and an increase in resting force). However, these events were all secondary to the influx of Na^+ into cells and importantly were not blocked by prior exposure to nifedipine, another calcium channel blocker. Neither were they prevented by exposure to Na^+ channel blockade, by inhibitors of sarcoplasmic reticulum, Na^+/K^+ ATPase, or Na^+/H^+ exchange. The responses of the toxin were however blocked by prior exposure to solutions devoid of Na^+ and by Ni^+. Olson et al. (1984) proposed that *C. fleckeri* toxin inserts into the myocyte sarcolemma and acts as a monovalent ionic channel. This echoes a previous similar suggestion by Freeman (1974) after observation that venom increases sodium conductance of

cardiac cell membranes, which is reversed by the sodium channel blocker tetrodotoxin. Bailey et al. (2005) observed that *C. fleckeri* venom causes a large elevation of cytosolic Ca^{++} in rat myocytes which is not prevented by verapamil but which is prevented by the transitional metal lanthanum (La^{+++}), a nonspecific channel and pore blocker. Yanagihara and Shohet (2012) observed that another metal, zinc (gluconate), prevented cardiovascular collapse in mice and suggested that it acted by preventing poration and lysis of red cells and consequent hyperkalemia. However, the effect of zinc on poration of myocytes remains untested.

Verapamil may be useful against the effects of venom on smooth and skeletal muscle. When applied before or after crude nematocyst venom, it blocked contraction of strips of ileal muscle but had no effect in blocking diaphragm contraction induced by crude venom. It did however block diaphragm contraction induced by a subfraction of crude venom (Endean and Sizemore 1987).

The efficacy of verapamil has been tested in vivo as a prophylactic (before envenomation) or rescue treatment (after envenomation). Burnett and Calton (1983) observed that verapamil administered 2–3 s after venom (1.25 lethal doses) did not prevent death but prolonged the time to death in mice. Although verapamil was ineffective when given before the same dose of venom, it was effective against larger doses in prolonging time to death but it did not prevent death.

Endean and Sizemore (1987) performed similar experiments in mice with venom and venom fractions prepared from nematocysts. In these, early death within 2 min was attributed to irregular cardiac activity while death after 6–7 min was attributed to pulmonary edema and respiratory difficulties. Death was not prevented nor was time to death prolonged by administration of verapamil 5 min prior to envenomation. Likewise, death was not prevented nor time to death prolonged by administration of verapamil 2 min after envenomation. Indeed time to death was shortened, but not significantly, in both prophylactic and rescue experiments. Similar observations were made with subfractions obtained by chromatography and ultrafiltration.

Subsequent experiments on the efficacy of rescue verapamil and CSL antivenom were carried out by Burnett et al. (1990). In these, survival was increased from zero to 27% with verapamil, 32% with antivenom, and to 65% with both verapamil and antivenom, and times to death were prolonged. In none of the above experiments were the cardiac and respiratory effects examined separately. Rescue experiments with verapamil and antivenom were repeated with venom prepared from isolated lyophilized nematocysts in which verapamil enhanced the survival time of mice previously injected with 2 LD_{50}s of venom (Bloom et al. 1999), but presumably not survival. However, the results were not convincing: antivenom alone failed to increase survival time in 3 antivenom dose schedules (0.15, 1.0, and 3.4 anti-lethal units/g) while antivenom in 3 dose schedules (0.15, 0.3, and 3.4 anti-lethal units/g) plus verapamil 1 μg also failed to increase survival times compared with venom alone. Antivenom in 1 dose, 1.0 anti-lethal unit/g, that is at a dose intermediate between doses that failed plus verapamil 1 μg did significantly prolong survival This observation was rationalized by postulating that an optimal dose of antivenom with verapamil was required to enable longer survival. The effect of verapamil alone was not reported.

Tibballs et al. (1998) examined the cardiac effects of tentacle extract and verapamil and antivenom in closely monitored mechanically ventilated anesthetized piglets, thus excluding possible complications of ventilation failure. They observed that verapamil, in the maximum (optimal) dose which did not depress blood pressure, did not prevent hyperkalemia or any of the cardiovascular effects of venom (cardiac output diminution, hypotension, dysrhythmias, or pulmonary hypertension) given in equal doses alone. Importantly, verapamil also increased mortality from zero, observed with equal doses of venom alone, to 100%. The dysrhythmias observed with higher fatal doses of venom alone were ventricular fibrillation, ventricular tachycardia, and slow idioventricular rhythm with pronounced conduction block. Similar dysrhythmias were observed in animals pretreated with verapamil and smaller doses of venom. No rescue experiments were performed. It was concluded that verapamil is not an effective prophylactic agent. The hyperkalemia observed in these experiments was probably due at least in part to hemolysis since high concentrations of plasma hemoglobin were measured. Destruction of other cellular membranes may also occur as observed with *Physalia* venom (Edwards et al. 2000; Edwards and Hessinger 2000) enabling release of potassium.

The ineffective and indeed deleterious cardiovascular effects of verapamil were confirmed in vivo and in vitro by other research groups. In rats, verapamil in the maximally tolerated dose did not alone have any effect on venom-induced cardiovascular collapse (Ramasamy et al. 2004). Moreover, verapamil negated the partially protective effects of antivenom. Bailey et al. (2005), investigating the role of calcium channels with venoms of *C. fleckeri*, *Chiropsalmus* sp., and *Carybdea xaymacana*, observed that verapamil had no effect on venom-induced calcium influx into myocytes. They concluded that there was no evidence that the venom of *C. fleckeri* exerted its effect by L-type Ca^{++} channels. On the other hand, Winter et al. (2007) observed that felodipine, a selective antagonist for peripheral L-type Ca ++ channels, significantly reduced the usual contraction of rat aorta caused by *C. fleckeri* venom but not that induced by *C. bronzie* venom.

Thus on an overall evidentiary basis, verapamil cannot be recommended as cardiac therapy for the seriously envenomated patient. Indeed, it is harmful and contraindicated. It has been useful as an aid to understand the mode of action of venom, but it is not in this circumstance a therapeutic agent. The well-known hypotensive action of this drug by its vasodilator and negative inotropic effects would prejudice resuscitation. In addition, neither verapamil nor any other calcium channel-blocker is recommended by authoritative resuscitative organizations for the treatment of life-threatening dysrhythmias in adults or children (International Liaison Committee on Resuscitation 2015). Other treatments are recommended for hypotension and dysrhythmias.

If indeed calcium channel blockade has a role in the therapy of envenomation, a calcium channel blocker of the T-type with less negative inotropic action would be more appropriate. T-type channel blockade however in experiments with *Physalia* venom did not prevent influx of calcium in *Physalia* experiments and whereas

transitional metals successfully prevented calcium influx the cytolytic effects of the venom were not prevented (Edwards et al. 2000). These and the earlier experiments suggest that calcium influx in jellyfish envenomation is a phenomenon secondary to sodium channel activation. Clearly, more research with *C. fleckeri* venom is required to understand the actions of the venom and to treat envenomation. While calcium channel blockers have a role in research in determining at least in part the action of venom, their role in clinical management of envenomation is nonexistent based on evidence presented to date.

Magnesium

Magnesium may be an important adjunctive therapy in envenomation but requires further evaluation. Prophylactic administration of magnesium alone did not prevent cardiovascular collapse induced by venom in rats but improved the effectiveness of antivenom from 40% to 100% (Ramasamy et al. 2004).

Zinc

Yanagihara and Shohet (2012), using an improved method of collecting cnidae from tentacles, observed that zinc carbonate, in a concentration of 5 mM, inhibited efflux of potassium and hemoglobin from red blood cells exposed in vitro to *C. fleckeri* and *Alatina moseri* venoms and to isolated porin toxins. A similar protective effect from *C. fleckeri* venom was afforded by CSL box jellyfish antivenom. In spontaneously breathing anesthetised mice, zinc carbonate administered before or after C. fleckeri venom prolonged survival but did not prevent death. In these animals, *C. fleckeri* venom caused rapid depression of myocardial contractility and onset of bradyarrhythmias and heart block with escape rhythms that were ameliorated but not prevented by zinc carbonate. The authors suggested that zinc, long known to block hemolysis by many agents, may be useful as therapeutic agent to inhibit porin assembly in human victims. The combined effects of antivenom and zinc have not been tested.

Skin Medications

Apart from vinegar no topical agents (anesthetic or steroid preparations) have been considered to be efficacious in case reports. Methylated spirits and ethanol cause nematocyst discharge and are contraindicated. Most inhibition of nematocyst discharge and reduction of venom-induced hemolysis of a related cubozoan jellyfish, the Hawaiian *Alatina alata*, was observed with use of a proprietary preparation (Sting No More ™) which contains copper gluconate, magnesium sulfate, and urea (Yanagihara et al. 2016). This preparation and tetracycline – a metalloproteinase

inhibitor which reduced dermal toxicity of jellyfish *Nemopilema nomurai* (Hydrozoa) in vitro and in vivo in rabbits (Kang et al. 2013) – requires testing on *C. fleckeri* nematocysts.

Application of Heat

This has not been investigated scientifically for *C. fleckeri* stings. However, in Hawaii, hot showers appeared to be analgesic for victims of Irukandji syndrome (Yoshimoto and Yanagihara 2002) while hot-water immersion of *C. alata* stings was better than applications of vinegar or papain meat tenderizer (Nomura et al. 2002). A randomized placebo-controlled trial of the analgesic effect of hot and cold packs on stings caused by *C. alata* showed a minimal trend toward pain relief after 10 min of hot pack application (Thomas et al. 2001b). It is well known that heat inactivates jellyfish venoms, for example, demonstrated with *C. fleckeri* venom by Pereira and Seymour (2013) using 44 °C for 20 min and by Carrette et al. (2002) using temperatures up to 58 °C – which are impractical for human treatment. However, immersion of *Physalia* stings at 45 °C does provide relief (Loten et al. 2006) although this study was assessed in a Cochrane review of first-aid interventions as of low quality (Li et al. 2013).

A systematic review (Wilcox and Yanagihara 2016) identified only three randomized/controlled trials (Thomas et al. 2001a; Nomura et al. 2002; Loten et al. 2006) of either hot water immersion or ice for treatment of Cnidarian stings. Although these trials were assessed as having serious errors, they support the use of hot-water immersion for pain relief and improved health outcomes.

Other Chirodropids

Several other members of the genus Chiropsalmus from the family Chiropsalmidae, previously known as *Chiropsalmus,* cause significant injury. Of the three species in the genus *Chiropsalmus*, *C. quadrigatus, C. buitendijki*, and *C. quadrumanus*, only the first named frequents in-shore waters of northern Australia. This species is now known as *C. bronzei* (Gershwin 2006) but here, discussion of studies prior to 2006 necessarily employs the species names used by Investigators.

Other members of the Chiropsalmidae are major causes of stings and death in the Indo-Pacific. Deaths have been attributed to *C. quadrigatus* in the Philippines and Japan (Williamson et al. 1996) but none have been documented in Australia where it is probably a different species (*C. bronzei*) and whose venom is considerably less potent. *Chiropsalmus quadrumanus* occurs elsewhere such as, for example, in Brazil, and in the Gulf of Mexico where it was responsible for the death of a child who died within 40 min of envenomation due to acute cardiac failure with pulmonary edema. Nematocysts retrieved from the skin were identified as belonging to *C. quadrumanus* (see Sutherland and Tibballs 2001) now reclassified as *Chiropsalmus alipes* (Gershwin 2006).

Chiropsella bronzie (Previously Australian *Chiropsalmus quadrigatus*)

Distribution

Barnes (1966) considered that the Australian *Chiropsalmus quadrigatus* (*C. bronzei*) had a much narrower distribution than *C. fleckeri*, possibly between Cooktown and Innisfail off the coast of Queensland. Multitentacled chirodropids have also been captured off beaches in Gove Peninsula on the northeast tip of Arnhem Land in the Northern Territory (Currie et al. 2002). Although the taxonomy remains unclear, it is distinct from *C. fleckeri* and closely resembles the Queensland *C. bronzei*. Contact with the tentacles during netting caused mild pain, redness, and itching.

Description and Habits

Mature *C. bronzei* have a maximum bell diameter of 10 cm and rarely more than nine tentacles attached to each of the four pedalia (fleshy arms). The tentacles are shorter, much finer, and rounded rather than flat compared with those of *C. fleckeri.* When seeking food (particularly shrimp, *Acetes australis*), they swim near the surface in shallow water.

Venom Apparatus

The tentacles carry bands of nematocysts with similar appearance and properties to those of *C. fleckeri*. Barnes (1966) estimated that, at comparable dimensions, the Australian *C. quadrigatus* had less than one tenth the stinging potential of *C. fleckeri.*

Venom

Extracts of whole tentacles from the Australian *C. quadrigatus* were found by Keen (1971) to have similar but less potent lethal, dermatonecrotic and hemolytic activities as extracts from *C. fleckeri* tentacles. Mice receiving lethal doses of *C. quadrigatus* extract died later than those receiving *C. fleckeri* extracts, but the mode of death (cardiovascular failure and respiratory arrest) was virtually identical. Freeman and Turner (1972) found the toxicity in mice of extracts from the Australian *C. quadrigatus* was approximately one sixth of a comparable *C. fleckeri* extract but had basically similar cardiovascular effects. Venom extracted from nematocysts of *Chiropsalmus* sp. was neurotoxic and myotoxic when added to a chick biventer nerve-muscle preparation (Ramasamy et al. 2005) while in rats, the venom caused cardiovascular collapse which was not prevented by CSL *C. fleckeri* antivenom, mechanical ventilation, or by magnesium (Ramasamy et al. 2003). Winter et al. (2007) observed that venom extracted from nematocysts of both *C. bronzie* and *C. fleckeri* caused contraction of rat aorta which was not affected by prazosin or CSL *C. fleckeri* antivenom.

Envenomation

Contact with the tentacles may produce sudden severe pain and shock, but the illness is usually mild compared with that which may be caused by *C. fleckeri.* After some minutes, the skin lesions become less painful, but discomfort may persist for at least

24 h. Swelling and redness develop immediately and tissue breakdown may occur. Any pigmentation and scarring has usually faded by 8 weeks.

Differential Diagnosis

Capture of the offending jellyfish is the only positive way a diagnosis may be made, as otherwise it is difficult to distinguish from mild injury due to *C. fleckeri.* Skin scrapings may reveal nematocysts similar to those from either jellyfish.

Antivenom

Box jellyfish (*C. fleckeri*) antivenom has been shown in mice to effectively neutralize the lethal, hemolytic and dermatonecrotic properties of Australian *C. quadrigatus* venom (Baxter and Marr 1974) and to prevent neurotoxic and myotoxic effects of *Chiropsalmus* sp. venom in vitro (Ramasamy et al. 2003), but neither cardiovascular collapse (Ramasamy et al. 2005) nor aortic contraction in vivo (Winter et al. 2007).

Conclusions and Future Directions

Knowledge of *C. fleckeri* and other chirodropid toxins is very limited, but it appears that they are proteins with their principal modes of action being cell membrane poration and probable disturbance of membrane ion channels for which clinical treatment is supportive. Calcium channel blockers are not recommended. Antivenom should be administered as soon as possible to the victim of serious envenomation. Apart from further characterization of toxins, research could be directed at new clinical treatments including administration of zinc and clarification of the first-aid roles of the application of vinegar to lesions, pressure-immobilization bandaging, and the use of heat as analgesia.

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Part III

Clinical Toxinology in Australia, Europe, and Americas: Envenomation in Europe

Pathophysiology and Treatment of Envenomation by European Vipers

13

Hans Persson

Contents

Abstract

Significant envenoming caused by European snakes is related to bites by species belonging to the family Viperidae, genus *Vipera*. The most common vipers in Europe are *Vipera berus berus*, *Vipera aspis*, and *Vipera ammodytes*. Vipers

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living in more restricted areas are, e.g., *Vipera latastei*, *Vipera xanthina*, *Vipera lebetina, Vipera ursinii, Vipera berus bosniensis*, and *Vipera palaestinae*. The venom of European vipers may cause anything from mild to severe and sometimes life-threatening reactions. Envenoming related to bites by European vipers may cause severe systemic symptoms and extensive local tissue damage. The symptoms caused by the different European vipers are rather similar, but there are a few important differences. Bites in the extremities result in local swelling and hemorrhagic discoloration. Parts or the whole of the bitten extremity might become affected, but the swelling may also gradually involve large parts of the trunk. The severity of the envenoming is related to the amount of venom injected and the age of the patient. Small children are more vulnerable as a certain dose becomes more noxious the smaller the victim is. Common systemic symptoms are abdominal pain, vomiting and diarrhea, hemodynamic instability, CNS depression – even coma and seizures have been observed in small children – and anemia that may become pronounced and require blood transfusions. Leukocytosis is common and if pronounced it may be a sign of severe envenoming. Also thrombocytopenia may occur. Some southern European vipers, e.g., *Vipera aspis* and *Vipera berus bosniensis*, may also cause neurological symptoms. A recent publication indicates that neurotoxicity also may develop after bites by *Vipera ammodytes*. Treatment of bites by European vipers includes both symptomatic care and specific therapy with antivenins. In Europe both equine and ovine antivenins are available. *Vipera berus berus* is considered to have the most widespread geographical distribution of all venomous snakes.

Introduction

This chapter is discussing medical aspects of bites by vipers that have their natural habitat in Europe. Bites by European vipers may result in local and systemic toxic reactions. Typical is an early damage to the vessel endothelium caused by proteolytic enzymes. This will result in a gradual swelling that – if a high dose of venom has been injected – may involve the whole extremity and even parts of the trunk. Hemorrhagic discoloration, gastrointestinal upset and – not least – circulatory instability might also follow. These symptoms are also related to effects of venom components. Some species may also develop significant neurological symptoms, e.g., *Vipera aspis* (de Haro 2012; de Haro et al. 2002), *Vipera berus bosniensis* (Westerström et al. 2010; Malina et al. 2011), and more recently also *Vipera ammodytes* (Sars et al. 2013). The geographical distribution of the different European snake species was described long time ago (Klemmer 1968). The most widely distributed snake is *Vipera berus berus* (Klemmer 1968) that occurs in large parts of Europe and eastward all the way through Asia to the Pacific Ocean – except for the most southern part of the European continent, the Mediterranean islands, Ireland, and land above the Arctic Circle. It hibernates normally from October until March. Recently, *Vipera berus bosniensis*, also named the Balkan adder, has called for attention. The effects of envenoming by this snake differ a lot from those exerted

by the common European adder (*Vipera berus berus)*. Bites by the Balkan adder have resulted in neurological symptoms in 20% of the cases (Westerström et al. 2010; Malina et al. 2011); further information on this will be given below. *Vipera aspis* and *Vipera ammodytes* are next to *Vipera berus berus* regarding occurrence. *Vipera aspis* is found particularly in the southeast of France, the whole of Italy, less frequently in southern Germany, the Alps, and the Pyrenees. The venom of a special branch of the asp viper, living in certain areas of its habitat, has proven capable to produce significant neurological symptoms as well. *Vipera latastei* is the snake of the Iberian Peninsula but it is also found in parts of North Africa. It spends a lot of its time in trees. *Vipera ammodytes* is mainly occurring on the Balkan Peninsula, in Greece, the northern part of Turkey, and in small parts of northern Italy and southern Austria. *Vipera lebetina* and *Vipera xanthina* are found in Cyprus, Turkey, the Middle East, and North Africa. *Vipera ursinii* is a smaller and presumably less toxic viper living in Southern and Eastern Europe and in parts of Asia.

Frequency of Bites

A number of studies have over the last years been performed in order to estimate the total annual number of bites related to European vipers. Also morbidity, mortality, and treatment of these poisonings have been subject to analysis and discussions. It has, however, been difficult to get entirely reliable results from these fairly heterogeneous studies. Many regional and a few national studies have been undertaken. The most recent study of the epidemiology has been published by Chippaux in Toxicon 2012 (Chippaux 2012). According to this extensive survey of the literature, the incidence of snakebites in Europe (including European Russia and Turkey) was estimated to be 7500–8000 bites per year. Around 15% of the patients in this study fulfilled the criteria of severe envenoming. Fatalities were rare (approximately 4–5 deaths/year). There are, however, often problems in interpreting collected data of this kind that inevitably are fairly heterogeneous. The number of fatal cases seems somewhat uncertain.

Circumstances

Most bites strike people when they come across snakes in the nature. The most common parts of the body being bitten are the upper and lower extremities. Bites in the feet and in the lower part of the legs mainly occur while people are walking in high grass, in the fields, in the forest, in the mountains, and along shores close to the water. Bites in the upper extremities may occur in connection with gardening and while harvesting flowers, wild berries, or mushrooms. Adders also swim and bites have, in fact, occurred in people who accidentally have come across a snake while swimming. The habit to pick up snakes with the hands to have a closer look at them is to be condemned. This maneuver results every year in a number of unnecessary bites, almost invariably in men – of all ages.

Children are comparatively more often hospitalized than adults, which relates to the fact that the venom injection is relatively larger in a small child compared to an adult. Hence, also the clinical response will in most cases be stronger in children.

Among the European snakes, *Vipera latastei* – with habitat mainly on the Iberian Peninsula – spend a lot of time in trees. This will explain the higher incidence of bites on the trunk, neck, and head by this snake, e.g., while people are harvesting fruits from trees.

Venom Contents, Kinetics, and Mechanisms of Toxicity

The venom apparatus normally consists of two venom glands, one on each side of the snake's head. The glands are through channels connected with the two bilateral fangs (sometimes there are, by exception, three fangs). The distance between the two fangs may vary from 6 to 10 mm.

The venoms contain a mixture of, in particular, proteins with enzymatic and toxic properties. Examples are hyaluronidase, enzymes with proteolytic and fibrinolytic activity, peptide hydrolases, phospholipase A2, phosphodiesterases, neurotoxins, and agents with myotoxic and anticoagulant effects.

Hyaluronidase may facilitate the local spread of venom components. Proteolytic enzymes will quite rapidly cause an endothelium damage which will allow an increasing leakage of plasma and erythrocytes into surrounding tissues. This will result in a gradually developing local swelling and discoloration. This reaction may be limited or it may progress to involve the whole extremity and even the trunk – depending on how much venom that has been injected and also with regard to the size of the bitten individual; children develop often more severe reactions than adults. There is also an enzymatic release of histamine, bradykinin, prostaglandins, and serotonin – agents that are responsible for a number of systemic effects. Other components in the venom are, e.g., amino acids, polypeptides, metalloproteins, and carbohydrates. There are, as mentioned above, also neurotoxins in the venom of certain European viper species (de Haro 2012; de Haro et al. 2002; Westerström et al. 2010; Malina et al. 2011; Sars et al. 2013); for further information about this, see under the section "Neurological Symptoms: Central and Peripheral." As mentioned elsewhere, also cardio- and neurotoxic agents have been isolated from different viper spp.

In severe envenoming, cardiac effects, e.g., arrhythmias and myocardial depression, have been observed after bites by many of the European viper spp. The genesis of this might be effects of a cardiotoxin that, however, has not been entirely identified.

As mentioned above, also neurotoxins are present in the venom of some viper species that have their habitat in the southern parts of Europe. Symptoms that have been observed are cranial and peripheral nervous system disorders. These neurological symptoms have been observed after bites by *Vipera aspis* and *Vipera berus bosniensis* (de Haro 2012; de Haro et al. 2002; Westerström et al. 2010; Malina et al.

2011), and recently neurotoxic effects have also been observed after bites by *Vipera ammodytes* (Sars et al. 2013).

The venom is in most cases injected subcutaneously, more rarely intramuscularly or intravenously. Intravenous venom injection is rare but may result in a more rapid development of systemic symptoms, whereas the local damage may remain minor. In most cases, however, the venom is initially dispersing around the site of the bite, whereupon it is further transported through the lymphatic system to the blood circulation. Muscular activity will hasten dissemination of the venom. That is why a bitten limb – and ideally the whole patient – should rest.

Venom has been detected in the blood around 30 min after the bite. The average top level in the blood is reached after around 2 h, whereupon the concentration is falling slowly with a half-life of around 8 h (Audebert et al. 1994). In practice, venom levels are not routinely analyzed. Clinical evaluation and treatment are anyway based on symptoms, not on serum levels of the venom.

In summary, the venom will induce a series of enzymatic actions that damage local structures like subcutaneous tissues, capillary endothelium, basement membranes, and even muscles (Lomonte et al. 1994). As important is, however, a venom-induced release of highly active, endogenous substances. These are responsible for the cascade of local and systemic symptoms with a great variation in terms of severity between bitten individuals.

Symptoms and Severity of Envenoming

Primarily, the injected venom dose may vary from zero to full dose which explains a most variable response. Around 30% (perhaps even more) of all snakebites are considered to be "dry bites" – no venom at all has been injected. The reason for this is most likely that the snake quite recently has emptied the venom storage in a prey. The occurrence of dry bites has, unfortunately, generated a misconception that the venom is weak and not dangerous, whereas the truth is that nothing or very little venom has been injected.

The severity of envenoming is variable and related to the amount of venom injected. Another important and self-evident parameter is the age and size of the "victim." Small children react with more alarming symptoms than adults after a bite. The reason is evident – high doses of venom versus a low bodyweight is most unfavorable. Also high age, general illness, and weakness may be predisposing factors in developing a more serious clinical reaction in relation to the venom injected.

Yet another parameter should be addressed, namely, the unfavorable and more rapid spread of venom that is generated by physical activity in general and strong movements of the bitten extremity in particular. This hastens the spread of the venom resulting in a more rapid local swelling, and it also accelerates the development of systemic symptoms. Therefore, it is generally agreed that physical activity shall be avoided as much as possible after snakebite. Cardiac arrest has been reported after

eager physical strain in two cases shortly after the bite (Pozio 1988; Persson and Irestedt 1981).

If no local reaction has developed around the site of the bite within a few hours and if there have been no systemic symptoms during this time lapse, it is relevant to draw the conclusion that no venom has been injected (Karlson-Stiber et al. 2006).

There is a continuously ongoing discussion whether the grade of toxicity varies for the different vipers in Europe. Comparisons between *Vipera berus berus* and *Vipera ammodytes* have been made, e.g., in Slovenia (Grenc and Groselj-Grenc 2012). Grading according to the poisoning severity score (Persson et al. 1998) was done. However, the material is small that is why the evaluation should be cautious.

Clinical Features

Overview

The principal clinical manifestations are local tissue damage with hemorrhagic swelling that might become extensive and involve the whole bitten extremity as well as parts of the trunk. Gastrointestinal disorders, circulatory instability, bronchospasm, and angioedema are common systemic effects. Certain southern European viper species may also cause important neurological disturbances. Other complications related to venom effects are, e.g., anemia, hemolysis, bleeding, pulmonary edema, transient kidney dysfunction, and infections. Small children and elderly people are at greater risk. Bites on the trunk, neck, and head may result in more rapid and serious reactions than bites on the extremities.

Systematic Presentation of Symptoms after Bites by European Vipers

Psychological Reactions

Once a bite has occurred, fright and anxiety will often set in. However, these symptoms will in most cases vanish relatively soon after adequate reassurance.

Local Symptoms

Fang marks, centripetal swelling of the affected extremity, and gradual onset of pain are typical local symptoms. The number of fang marks may vary between one and three – two is the most common. The swelling may involve just distal parts of the extremity. However, depending on the size of the venom dose injected, the local edema may gradually extend to involve the whole extremity and engage parts of the trunk – sometimes involving even the contralateral side. In these cases pain becomes considerable. Movements of the extremity – in some cases also just a touch – may cause pain. Bites in the face can impose a certain risk of airway obstruction related to local swelling of critical tissues, e.g., the mucous membranes in mouth and pharynx.

After around 24 h – sometimes earlier – the local swelling will take on a hemorrhagic discoloration related to leakage of erythrocytes from the damaged

vessels. This discoloration and swelling may continue for another 48–72 h. It could be remarked that the process of continued swelling and leakage of blood into the tissues can be avoided, or at least significantly reduced, through the administration of antivenin.

Blistering, ecchymoses, lymphangitis, and regional lymphadenitis are quite common local symptoms. Compartment syndrome has developed occasionally, but fortunately it seems to be a rare complication. Thrombophlebitis of the great saphenous vein has been observed in children (Bouquier et al. 1974).

The clinical deterioration of local symptoms may sometimes be insidious and therefore overlooked. If systemic symptoms are lacking, clinical observation of the local progress might easily become less cautious. This is not acceptable. Continuous monitoring of both general and local symptoms is important and mandatory also in cases where systemic effects are lacking. Steep progress of the local swelling alone is an indication for antivenin treatment – also in the absence of systemic symptoms.

Systemic Envenoming

Gastrointestinal Symptoms

Abdominal pain, nausea, vomiting, and diarrhea are common symptoms after bites by European vipers, and in many materials this is the most common systemic manifestation (Malina et al. 2011; Audebert et al. 1994; Pozio 1988; Karlson-Stiber et al. 2006). Onset of these symptoms varies a lot, but mostly gastrointestinal discomfort will appear with some delay, and in rare cases onset of gastrointestinal symptoms is delayed several hours. The intensity of symptoms is most variable. Occasionally, abdominal pain has been so intense that a gastric perforation or some other abdominal catastrophe was suspected.

Less common gastrointestinal symptoms that have been reported are acute pancreatitis (Kjellström 1989; Pande and Khan 2010), paralytic ileus (Persson and Irestedt 1981), and intestinal infarction (Beer and Musiani 1998).

Circulatory Disturbances

Tachycardia, hypotension, and circulatory shock indicate a severe envenoming (Karlson-Stiber et al. 2006). The etiology is multiple, involving release of endogenous agents causing vasodilatation (e.g., histamine, bradykinin, and prostaglandins). But there can also be a simultaneous development of hypovolemia related to loss of plasma and erythrocytes through the damaged capillary endothelium. Fluid loss caused by diarrhea, vomiting, and diaphoresis may further contribute to the circulatory instability.

Various ECG changes have been observed. Most common are T-wave flattening and negative T waves. Other registered aberrations are ST elevation, atrial fibrillation, brady-arrhythmias, and AV-block (Persson and Irestedt 1981; Karlson-Stiber et al. 2006; Karlson-Stiber and Persson 1994; Moore 1988). Development of myocardial infarction is rare but has been observed both in elderly and young people after viper bites (Karlson-Stiber et al. 2006; Aravanis et al. 1982).

Respiratory Dysfunction

Respiratory distress is less common than the effects on circulation. However, breathing difficulties related to mucous membrane swelling in the mouth and of the tongue, lips, pharynx, and larynx may occur sporadically. Bronchospasm may be severe and a problematic symptom; one death due to this complication has been reported (Karlson-Stiber et al. 1997). An uncommon complication is plasma leakage into the lung tissues causing an impaired oxygenation (Karlson-Stiber et al. 1997). It seems that respiratory distress is observed more frequently after bites by *Vipera aspis* and *Vipera ammodytes* than after *Vipera berus berus* bites.

Pleural exudates may develop with some delay due to a toxin-generated vulnerability of the pulmonary vessels, resulting in impaired pulmonary function. The most dreaded lung complication is, however, development of a pulmonary edema. This has occurred mainly in small children who have suffered severe envenoming with abundant extravasation of plasma. When all this fluid is reabsorbed into the circulation, a phase of heavy fluid overload might follow, sometimes resulting in a pulmonary edema. This latter complication has occurred in small children who have developed extensive edemas.

Other late, unusual but serious complications in the lungs are interstitial pulmonary bleedings, hemothorax, and pleural exudates (Gerrard and Pugh 1982, Swedish Poisons Information Centre – unpublished data).

Kidney Damage

Erythrocytes and protein are quite common but discrete findings in the urine after an adder bite. The hematuria is mostly microscopic and will vanish spontaneously after some time which is true also for mild proteinuria. None of these findings will be permanent and do not imply any special treatment. One case of acute glomerulonephritis has been described (Schabel et al. 1980).

In cases of severe envenoming where the clinical course includes circulatory instability, rhabdomyolysis, or systemic hemolysis, the kidneys might be damaged secondarily and develop a temporary insufficiency. The impaired renal function is reflected by an increase of serum creatinine and oliguria. This is a functional rather than toxic damage, and the renal dysfunction is fortunately transient.

Hematological Changes and Coagulopathy

Rather early after a venom injection, there will be a rapid onset of plasma leakage from the vascular system into surrounding tissues. This will on an early stage cause a marked hemoconcentration. Thus, the development of high hemoglobin levels on an early stage indicates that the actual envenoming is severe. Later on when erythrocytes start to penetrate through the damaged vessel endothelium and enter surrounding tissues, the hemoglobin concentration will gradually fall, and, instead, a true anemia will develop. This can be very pronounced and need blood transfusions. Furthermore, also intravascular hemolysis may in some cases add to the already substantial loss of erythrocytes.

It is generally agreed that an early, pronounced leukocytosis ($>18–20 \times 10^9$/L) is reflecting the severity of envenoming (de Haro 2012; Malina et al. 2011; Karlson-

Stiber et al. 2006; Karlson-Stiber and Persson 1994). So, this is considered to be a reliable prognostic parameter that may support the indication for antivenin, e.g., in borderline cases.

A mild thrombocytopenia is quite common, but it is generally of no clinical significance. However, there are always exceptions from the rules, and also a severe and clinically important thrombocytopenia may occasionally develop and enhance the tendency of bleeding into the tissues (Salmonsson et al. 2010).

Coagulation parameters may be pathological after bites by European adders and enhance the leakage of erythrocytes into the tissues surrounding the bite. Generalized systemic bleeding is, however, very rare after bites by the European vipers. But occasionally serious coagulopathy with subsequent, generalized bleeding has occurred after bites by *Vipera latastei* (González 1982) and *Vipera ammodytes* (Tiwari and Johnston 1986). In a Swedish series of 944 patients hospitalized because of *Vipera berus berus* bites, around 4% of the patients developed long-lasting, significant thrombocytopenia, however, without any systemic bleeding (Salmonsson et al. 2010). In Bulgaria severe coagulopathy developed in a patient after a *Vipera ammodytes* bite (Marinov et al. 2010). But, after all, as stated above, generalized coagulopathy is reported rarely after bites by European vipers.

Neurological Symptoms: Central and Peripheral

Central nervous system symptoms, such as transient unconsciousness and seizures have been reported sporadically after bites by European vipers in both adults and, particularly, in children. Milder CNS symptoms like dizziness, vertigo, fatigue, and somnolence are quite common after bites by any of the European viper species. The neurological symptoms mentioned so far have in most cases not been associated with any neurotoxin in the venom. Instead, they probably reflect the clinical response to circulatory instability associated with insufficient oxygenation resulting in CNS hypoxia.

True neurotoxicity related to specific venom components has during the last decades gradually been observed among a few European viper species. Recent publications address the fact that the venom of some snake species in Southern Europe contains genuine neurotoxins. Different neurological manifestations, related to the venom, have been observed.

Already in the 1990s it became clear that the venom of certain asp vipers – *Vipera aspis* – might contain a neurotoxin in species living in defined areas. Concerning the geographical distribution of this snake, see the section "Introduction." The asp vipers that cause true neurological symptoms are mainly found in the southeast of France (de Haro 2012; de Haro et al. 2002). The neurotoxin is presynaptic and may cause, e.g., ophthalmoplegia, ptosis, and paresthesia. Paralysis of the affected extremity may also occur. In severe cases both talking and swallowing difficulties have been described.

Quite impressive in this sense is also the Balkan adder – *Vipera berus bosniensis*. The first cases published on this topic came from Bulgaria (Westerström et al. 2010). In a more recent material (Malina et al. 2011), 54 patients bitten by the Balkan adder were thoroughly studied and graded according to the poisoning severity score

(Persson et al. 1998). Neurotoxic manifestations developed in 20% of the patients. The symptoms emanated from cranial nerve disturbances. Mainly cranial nerves III, IV, and VI were affected. The clinical symptoms were ptosis, ophthalmoplegia, diplopia, and reduced visual acuity. Ptosis was the most common symptom.

Recently, also a bite by *Vipera ammodytes* caused neurotoxic symptoms – dysarthria, ophthalmoplegia, ptosis, and distal neuromuscular weakness (Sars et al. 2013).

Some Other Occasionally Reported Manifestations

Fever and profuse perspiration are not uncommon symptoms. Secondary to circulatory instability a metabolic acidosis may develop. Deep venous thrombosis has developed in a bitten leg, but this is an uncommon complication (Karlson-Stiber et al. 2006). As stated earlier, thrombophlebitis of the saphenous vein has been observed in a number of children (Bouquier et al. 1974). Secondary infections after viper bites are uncommon in northern Europe but seem to occur more often in the southern parts of the continent. An exceptional reaction happened to a 60-year-old woman in Greece (Boviatsis et al. 2003). She was bitten by a local viper – exact which one is not clear – and developed multiple hemorrhagic infarcts in the brain.

Side Effects of Treatment and Toxic Sequels

Serum sickness may develop a couple of weeks after the bite. This is seldom related to the venom but might instead be a side effect of the antivenin. Fortunately, this is nowadays a rare adverse event as modern, highly purified antivenins are used.

Long-lasting or even permanent sequels may follow the bite. Examples are remaining edema but also a reappearing swelling. Sensory disturbances, pain, and stiffness may remain a long time after the bite.

Pregnancy

Bites during pregnancy constitute a risk, but severe complications are fortunately rare. The venom may, however, pass the placenta and weakening of fetal heart sounds has been observed. If severe systemic reactions appear after a venom injection in a pregnant woman, there is an apparent danger for the fetus and instant treatment with antivenin and optimum symptomatic support is mandatory. A few fetal deaths have been reported after bites by *Vipera berus berus* (11 and Swedish Poisons Centre, unpublished data).

Diagnosis and Prognostic Signs

The diagnostics aim at identifying the snake, assessing the circumstances around the accident and grading the severity of envenoming as interpreted from current symptoms.

The diagnosis is in most cases obvious from history and clinical features. However, it happens that the bite is not observed immediately by the patient but

instead interpreted as a scratch from a twig or some other sharp object on the ground. If it is not a "dry bite," symptoms will, however, follow sooner or later.

Continuous and cautious observation of the clinical course is mandatory for the judgment of severity and for applying adequate treatment. A critical issue is whether antivenin is indicated or not. Individual signs of envenoming that should be taken into account when grading severity are, e.g., long-lasting hypotension and circulatory shock, CNS depression, other central and peripheral neurological symptoms, pronounced and long-lasting gastrointestinal discomfort, rapid local swelling with the risk of involving the trunk, and also certain laboratory parameters – in particular a pronounced leukocytosis. All these clinical parameters are essential for making a proper evaluation of the severity of envenoming and for supplying an adequate treatment strategy. The poisoning severity score (PSS) could be used to grade severity (Persson et al. 1998).

Venom circulating in the blood can be detected with an ELISA (**e**nzyme-**l**inked **i**mmuno**s**orbent **a**ssay) method (Audebert et al. 1994; Audebert et al. 1992; Sjöström et al. 1996). It is, however, probable that this analytical method will be available only once during the 24 h – or not at all. This analysis is not accessible in most places where snakebites are treated. So, what always is important in snake envenoming is a continuous follow-up of those coagulation parameters that are available in the respective hospitals. This is what should guide the treatment.

Most hospitals have access to routine methods for assessing coagulation parameters around the clock. These analyses are important as a coagulopathy may develop, especially in patients presenting with other clinical signs of a severe, systemic envenoming. Coagulation disturbances are, in fact, mild after European viper bites, but there are exceptions.

Liver function tests are routinely performed and are often normal, but minor transaminase elevations have been observed. The liver is fortunately not an important target organ for toxins produced by European vipers. Echocardiography may be performed if there are any signs of cardiac failure. Methods for venom detection exist, but – as mentioned above – they are in most places not used routinely in clinical praxis. Instead, a continuous clinical evaluation will provide the parameters that are essential for a severity grading and for a relevant treatment. Thus, therapy is based on a cautiously and continuously performed analysis of the symptomatology.

Treatment

Dry Bites

Around one third – possibly slightly more – of all snakebites do not result in a venom injection ("dry bites"). If there is no reaction at all at the site of the bite after 2–3 h and no systemic symptoms whatsoever have occurred, it is justified to regard this as a dry bite. The patient could be discharged with an instruction to come back if, against all odds, symptoms would set in later on. However, if any uncertainty is at hand, the patient should be supervised in hospital for another few hours. Tetanus

vaccine should be given – even after dry bites – unless the patient already has adequate tetanus protection.

A Snakebite Has Happened

As a general principle, all people who have been bitten by a venomous snake should go to hospital for observation and evaluation. If the patient has not developed any systemic symptoms and there is no local reaction at all 2 h or more after the bite, it is probably "dry bite" – see above.

Instant Transport to a Medical Service is Motivated in the Following Cases

- Bites in children
- If vital functions are threatened – e.g., breathing difficulties, signs of circulatory failure, CNS depression, and bites on neck or head.
- When other evident signs of systemic poisoning appear – e.g., gastrointestinal discomfort and vomiting.
- If there is a local reaction with rapid progress.
- Signs of bleeding and coagulation disorders – however, these problems are not obvious on an early stage.
- For further information, see below under the section "Primary Treatment Measures at the Medical Service."

Primary Treatment Measures at the Medical Service

Some of the following treatment principles may, when appropriate and if possible, be relevant already on the place of the incident. Further treatment shall be implicated instantly when the patient is attending medical care.

- Avoid unnecessary movements. The patient should be lying down and rest.
- Reassurance – the patient is often scared.
- Immobilize the bitten limb and let it rest in a slightly elevated position.
- Remove rings, watches, bracelets, etc., from the bitten extremity.
- The site of the bite should not be manipulated in any way.
- Establish an intravenous line and start instantly an i.v. infusion.
- Check and support vital functions as required.
- Consider treatment with antivenin – see the below section "Antivenin Treatment."
- Avoid per oral intake initially.
- Tetanus prophylaxis should be supplied.
- Evaluate and register the development of the local swelling continuously.

Stay in Hospital

Adults with mild local symptoms and no systemic involvement do not necessarily have to stay in hospital for more than 6–8 h. If the local swelling is progressive or if systemic symptoms set in, the patient shall without any doubt remain in hospital for at least 24 h. In case of remaining or recurring systemic symptoms or a continuous progression of the local edema, the patient has to stay longer in hospital, often several days. Serious complications may prolong the stay for weeks – this is true both for children and adults.

Children bitten by a viper shall always be observed in hospital for a minimum of 24 h. If a child with generalized symptoms is not given early antivenin treatment, the systemic and not least local reactions may prolong the hospital stay for weeks.

Laboratory Investigations

The analyses given below are relevant for patients bitten by European vipers. The results partly reflect the grade of envenoming and may contribute as a guidance of the treatment procedures.

Hemoglobin concentration, hematocrit (EVF), leukocyte, and thrombocyte counts shall be registered regularly in all patients. In case of systemic symptoms or development of extensive edemas, these analyses shall be checked repeatedly and with rather short intervals. In severe envenoming analyses shall also be performed concerning acid-base balance, electrolyte balance, hemolysis, urine status, coagulation parameters, S-CK, S-creatinine, and hepatic transaminases.

The early occurrence of a significant leukocytosis ($>18–20 \times 10^9$/L) may indicate that the envenoming might be severe (Persson and Irestedt 1981).

A diagnostic ECG shall always be checked on arrival in hospital. Continuous ECG monitoring is relevant in all patients who develop severe or long-lasting symptoms.

The ELISA method mentioned above is rarely available in an emergency situation, and it is therefore less useful in clinical practice.

Symptomatic Treatment

During many years, antivenins for the treatment of envenoming caused by the European vipers were not available on the market or they were of a bad quality – in fact so bad that the antivenin was claimed to be more dangerous than the venom. Therefore, the treatment of envenoming caused by different European adders was for many years just optimum symptomatic care that certainly could save lives – but not all – and sequels were frequent and embarrassing. Nasty, widespread local reactions could not be influenced by symptomatic treatment only – and there were no antivenins of acceptable quality or no antivenins at all. Since the early 1980s, however, more specific antivenins of good quality gradually became available and

used more and more – to great benefit for the patients. It was, however, strange that it took quite a long time before physicians more generally realized that these new antivenins were effective and had few side effects, which could be handled if they would occur.

In spite of the undoubtedly good effect of modern antivenins, symptomatic care has still its role to play. And if the availability to antivenin is limited – or none – in certain regions, optimum symptomatic care is even more important.

Circulation

Treatment of venom-induced circulatory failure, related to a combination of hypovolemia and vasodilatation, includes, to start with, infusion of balanced electrolyte solutions combined with colloids. Acute hypotension and circulatory shock may also relate to cardiac failure which may motivate administration of, e.g., i.v. epinephrine. Intramuscular injection is only indicated if there are difficulties to insert an intravenous line. Administration of epinephrine is repeated if necessary. Cardiac stimulants and vasopressor agents may be indicated and given via infusion over a longer period of time – if negative inotropic effects and vasodilatation recur. Suggested pharmaceuticals for infusion are dobutamine when inotropic support is required, if necessary combined with an i.v. infusion of norepinephrine. This will provide both a positive inotropic effect and a reduction of vasodilatation.

However, the optimum treatment in circulatory failure is administration of a suitable antivenin (see below section "Antivenom Treatment"). That would in the majority of cases quite rapidly – often during the end of or just after the antivenin infusion – stabilize the circulation and eliminate also other non-desirable symptoms. While waiting for the complete effect of the antivenin, symptomatic treatment has to continue. It should also be remembered that a relapse into circulatory instability sometimes may occur many hours after the first antivenin dose and that this may motivate a second dose of antivenin.

Continuous ECG monitoring is mandatory in severe envenoming.

Respiration

Early breathing difficulties could be related to bronchospasm, angioedema, and mucous membrane swelling involving the lips, tongue, pharynx, and larynx. These symptoms may respond quite well to epinephrine and antihistamines. Corticosteroids have also been proposed and used in these situations, but they are nowadays not considered to contribute significantly in the acute situation. Occasionally symptoms may be so critical that an acute intubation and/or a tracheotomy must be undertaken.

In small children, who have been exposed to a heavy dose of venom but who have not been given antivenin, extensive swelling might gradually involve half the body and even more. In these cases there is a risk of development of a pulmonary edema. This complication might ensue when all the fluid in the extensive edemas start to get reabsorbed into the circulation. There will be a phase of fluid overload that the still damaged and vulnerable lung vessels cannot resist; so a pulmonary edema might develop necessitating intubation and controlled ventilation. These cases are few, but

dramatic. They indicate with emphasis that antivenin should be given already on arrival in hospital if the child presents with any sign of a severe envenoming. Early antivenin treatment should always be given in these cases, thereby preventing a nasty clinical course.

The Kidneys

Mild proteinuria and hematuria are common but transient findings. They are insignificant in a medical perspective. If more serious renal disturbances occur, they are secondary to systemic effects like circulatory shock and rhabdomyolysis. This may cause a phase of oliguria and – rarely – even anuria. Fortunately, these disturbances are reversible. A brisk diuresis and an alkaline urine are recommended in order to support and protect the kidneys and perhaps also enhance healing of the slightly damaged kidneys. Serious kidney damages – as in envenoming by, e.g., Russell's viper – do not develop after bites by European vipers.

Blood Disorders

After an initial hemoconcentration related to the leakage of plasma, an anemia may gradually develop in severe cases. This anemia is mainly related to leakage of erythrocytes into the extensive, hemorrhagic edemas. In these cases blood transfusions are often needed. Coagulation deficiencies alone are, however, less influential on bleedings into the tissues.

Local Symptoms

The local swelling may become considerable and involve the whole of the affected extremity and more than half the trunk. Immobilization and a moderately elevated position of the actual limb are recommended and might to some extent counteract the extensive swelling. It is relevant to avoid movements of the affected extremity.

The only effective weapon to prevent a threatening, heavy progress of the swelling is treatment with antivenin that shall be administered as early as possible – this is not least important in small children. Development of a compartment syndrome is rare, but it may occasionally develop and that is – certainly – an utterly troublesome complication. Fasciotomy is rarely indicated and very seldom undertaken. It shall not be performed unless tissue pressure exceeds 30–50 mmHg.

Infections

Antibiotics should not be given routinely after the type of snakebites discussed in this chapter. However, in case of an obvious or suspected infection verified by cultures, antibiotics shall be administered as required. In a Swedish study concerning 231 hospitalized patients in 1995 (the study covered the whole country) – six patients (2, 6%) developed verified infections and were treated with antibiotics. In spite of the fact that only 2, 6% of the patients had positive cultures, 26% of the patients were anyway treated with antibiotics, which does not seem to be relevant.

If the bitten patient has no tetanus protection, prophylaxis shall be applied after a snakebite, irrespective of where in Europe it occurs.

Some Other Aspects

Corticosteroids have in the past – and still in some places – been liberally used in the treatment of snakebites. The value of cortisone in snakebite cases has, however, become more and more questioned. There is no evidence of any effect on the proper venom. It might – together with antihistamines and adrenaline – provide some marginal benefit in preventing or mitigating symptoms of "allergic" character. However, also this is unclear. It is, however, clear that corticosteroids do not cure the serious systemic symptoms related to the venom, nor do they have any beneficial effects on the local edema.

Adults who have to be immobilized during some days because of a serious venom reaction including a widespread edema might benefit from thrombosis prophylaxis according to general routines, which means low-dose heparin (heparin 5000 IE × 2 s.c. or dalteparin 2500 IE × 1 s.c.). However, if there is an ongoing, significant coagulopathy, this treatment should be withheld and instead antivenin should be given to counteract the coagulopathy. Children are not given this type of prophylactic treatment.

Antivenin Treatment

Effective, purified antivenins have since around 30 years been available for the treatment of severe envenoming caused by European vipers. It is nowadays entirely obvious, and generally agreed, that patients with signs of serious envenoming after European adder bites should be given antivenin as soon as possible. Without any doubt, antivenin is more effective when administered early after the bite. No precise time limit for the efficacy has, however, been identified. It is interesting that coagulopathy has been cured promptly in two patients after antivenin treatment at 30 h and 7 days after the bite, respectively (Gerrard and Pugh 1982; Tiwari and Johnston 1986). But still – the sooner the better!

Suggested indications for antivenin treatment:

- Circulatory instability – severe or recurrent hypotension
- Protracted or recurring gastrointestinal symptoms
- Bronchospasm that does not easily respond to conventional treatment
- Mucous membrane swelling with a risk of airway obstruction and angioedema
- Continuous, evident, and rapid progress of the swelling around the site of the bite and with a likelihood of involving the trunk
- Central nervous system depression
- Cranial or peripheral nerve paralysis

In "borderline" cases, the following parameters may support the indication for antivenin:

- Leukocytosis ($>20 \times 10^9$/L)
- Pronounced hemoconcentration

- Metabolic acidosis
- ECG changes (e.g., arrhythmias, ST-T alterations)
- Significant coagulopathy

Special Cases and Aspects on Antivenin Indications

Toddlers and pregnant women are "risk groups" and shall as soon as possible get antivenin if there are obvious signs of envenoming. For these patient groups, it is especially important to have in mind that a progressive local reaction alone is an indication for antivenin treatment.

Circulatory disturbances vanish in most cases quite rapidly after or even during the end of the antivenin infusion. Gastrointestinal symptoms tend to last somewhat longer before disappearing. Neurological symptoms and the local swelling also respond to antivenin treatment. If symptoms recur or just continue, repeated dosing of antivenin might be indicated. Also continued progression of the local swelling may motivate repeated administration of antivenin.

Available Antivenins

(European Viper Venom Antiserum, Institute of Immunology, Zagreb: Equine serum of F(ab')$_2$ type. This antivenin has gradually become withdrawn and seems no longer to be available.)

Vipera Tab, Affinity purified, European Viper Antivenom (Ovine Fab for Infusion, MicroPharm LTD): Vipera berus-specific Fab fragments from sheep. Infusion concentrate 2 × 4 ml. An ampoule contains 100 mg Fab fragments.

It is used for bites by the most common European viper, *Vipera berus.*

For dosing and administration, see instructions in the package and/or call a poisons information center for advice and further information.

Viperfav Sanofi Pasteur (France): Equine European viper antivenom. Immunoglobulin F(ab')$_2$ fragments: Infusion concentrate 4 ml/vial.

The active ingredients are equine antivenom immunoglobulin F(ab')$_2$ fragments which neutralize the venoms of *Vipera aspis, V. berus*, and *V. ammodytes*.

For dosing and administration, see instructions in the package and/or call a poisons information center for advice and further information.

A quite new antivenin has recently appeared – "Viper venom antitoxin" from BioMed Co Ltd in Warsaw, Poland.

Conclusion and Future Directions

Bites by the various viper species living in Europe are fairly common and estimated to be 7500–8000 per year. However, further studies on the extent of this topic would be desirable. Most effects of the venom are quite similar for the different snake

species. There are, however, a few differences in symptoms and severity which must be taken into account. One major difference is that the venom of a few viper *spp.* in the southern parts of the European continent may cause neurological symptoms, which is not the case for the majority of European vipers. Mortality is nowadays low, which is related both to better treatment facilities with access to intensive care units and to the use of effective and safe antivenins. Most certainly the development and introduction of specific treatment with antivenins has meant a true turning point in the treatment of severe envenoming by snakes. It is also important to emphasize the fact that not only mortality but also morbidity has become reduced, and, because of that, the stay in hospital has become significantly shorter. Sometimes there has been a shortage of antivenins in certain regions, and patients with severe envenoming have had to wait too long, or they have not got any antivenin at all. It is an important and serious task for responsible authorities in the European countries to approach this problem and provide enough support to maintain sufficient production or import of antivenins – the optimum treatment of serious snake envenoming reads antivenin. This is a non-disputable issue, reading less morbidity and suffering, shorter time in hospital, fewer costs for the society, shorter sick leaves, and far less long-time sequels.

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Part IV

Clinical Toxinology in Australia, Europe, and Americas: Global Envenomation

Envenomations by Widow, Recluse, and Medically Implicated Spiders

14

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Abstract

Although there are many spider species throughout the world, most of which have venom, only about 60 are toxic enough to cause deleterious reactions in humans. In North America, only the widow and recluse groups are considered to be medically important. Widow envenomations are readily managed due to easily recognizable signs and symptoms; the pathophysiology of the venom effects is well known. Widow antivenom has been effective in reversing envenomation symptoms within a short period of time. In contrast, recluse envenomations are not as easily recognized and remedied due to a wide range of manifestation of venom effects and a still-developing understanding of the pathophysiology. Recluse antivenom is available in South America where envenomation manifestation appears more deleterious but not in North America where bites are less common and less dramatic. Adding difficulty in the recluse diagnostic process is the obfuscation due to a large number of non-spider-related dermonecrotic lesions that physicians too often misdiagnose as recluse spider bites, muddling the medical literature and preventing proper treatment. The overreliance by the medical community on spiders as causes of maladies has led to several other virtually harmless spider species around the world to be falsely incriminated in medical events.

Introduction

Spiders elicit much fear and anxiety from the general public due to proliferation of dynamic tales of morbidity and mortality from their bites. However, considering the ubiquity of spiders in and around homes and the probability of interacting with them, the risk posed by spider bite is small with only a few species of medical importance, predominantly spiders in the black widow and recluse taxonomic genera in North America. Although snake bites can often be much more serious, there is a strong psychological aspect to spiders because, in comparison to snakes, spiders are small and not so easily detected, they inhabit houses where bites can occur and are often nocturnal, the latter of which adds a nefarious agenda to their reputation with a perceived anthropomorphic nocturnal "stalking" of their victim. Nonetheless, the

outcome from spider envenomations can involve severe pain, dread feelings of doom, and, in rare cases, death. Hence, despite the rarity of a medically important encounter, the effects of spider venom are a concern and a subject of study for toxinologists.

Widow Spiders (Genus *Latrodectus*)

Distribution

The widow spiders belong to the genus *Latrodectus* with 31 species found worldwide (Garb et al. 2004). These spiders and their deleterious bites were well known to native human populations such that the spiders were given common names that often announced their infamous reputation (e.g., black wolf, Russia; black hag, Croatia; black widow, Europe (in several languages) and North America; button spider, South Africa). The genus name means "robber biter" or "secret biter." For some reason, many authors tend to misspell the genus as *Lactrodectus* which has never been an accurate scientific name for the widows. The medical syndrome involving widow bites is termed "latrodectism."

In the Western Hemisphere, widow species are well dispersed in North and South America, being more common toward the warmer regions of the continents. Many species exist in the circum-Mediterranean area and the Middle East as well as Africa. The redback, *L. hasselti*, is well distributed in Australia, and in New Zealand, the native katipo, *L. katipo*, is found close to coastal areas but is being ecologically displaced by nonnative spiders. However, in East Asia and Southeast Asia, indigenous *Latrodectus* spiders are sparse or nonexistent.

Several species have established elsewhere in the world such as the Australian redback in Japan and New Zealand. The brown widow, *L. geometricus*, is native to Africa but is now pantropical including South and Central America, southern North America, Hawaii, several Caribbean islands, Malaysia, India, Japan, Australia, and many remote islands such as Tahiti, Moorea, Guam, and the Galapágos.

Appearance and Identification

Widow spiders are web builders with a globose abdomen and typically a shiny appearance (Fig. 1). In their web, they hang with their ventral surface upward. A common form is mostly black spider with decorations of red. Many species have a ventral red hourglass which can vary from two perfect triangles connecting at their points to almost no red coloration at all. The Australian redback has a vivid longitudinal red stripe on the posterior half of the dorsal abdominal surface. The malmignatte, *L. tredecimguttatus*, in the Mediterranean region has no ventral hourglass but has up to 13 red dots on the dorsal abdomen. The brown widow is more cryptically colored with disruptive stripes, and the white widow, *L. pallidus*, in Israel and the Middle East, is mostly white, with a white to yellow shield on the ventral

Fig. 1 Female black widow spider

Fig. 2 Immature black widow spider showing the striped pattern of the juvenile which differs greatly from the mature female

surface where other species have the red hourglass. In addition, in many species, the spiderlings emerging from the egg sac look nothing like the adults, being bedecked with stripes and disruptive coloration (Fig. 2). This may cause confusion when immature spiders are presented by a bite victim.

Venom Components

Widow venom contains fractions with differential activity in target organisms. α-Latrotoxin is the main component of widow venom that affects vertebrates while having no or little effect on arthropods. It is a large molecule of 160 kDa size. Widow venom has additional components which are specifically detrimental to insects or crustaceans but have no deleterious effect on vertebrates. Although vertebrates are not a large constituent of widow spider diet, there are numerous accounts of the rare predation event of widows feeding upon lizards, snakes, frogs, and mice (Garb and Hayashi 2013).

Analysis of the venoms of many *Latrodectus* species shows that the genetic sequences are highly conserved among the black widow taxa (Garb and Hayashi 2013). Because of this venom similarity, antivenom developed for the Australian redback counteracted the lethal effect of venoms of two American black widow

species when tested in mice (Daly et al. 2007). However, α-latrotoxin is less well genetically conserved in the brown widow clade of species (Garb and Hayashi 2013), which may explain why brown widow bites are not nearly as dynamic as that of the species in the black widow clade (Müller 1993).

Laboratory comparisons seem to indicate that there is a correlation between color and toxicity with darker species of widow being more toxic than the lighter-colored species such as the brown widow and the white widow. However, one caveat that should be presented is that envenomation in humans is dependent on *both* venom toxicity *and* the amount of injected venom so LD_{50} studies alone are not completely demonstrable. Therefore, a series of verified bites with expert identification of the offending spider should still be the gold standard when discussing spider envenomation capabilities.

Mode of Action

α-Latrotoxin forms an ion channel in the presynaptic neuron of the neuromuscular junction allowing the influx of Ca^{++} ions which causes depolarization and neurotransmitter exocytosis (Garb and Hayashi 2013). It also prevents the reuptake of neurotransmitter back into the presynaptic vesicles which leads to various muscular aberrations.

Pathophysiology – Widow spider venom affects the sympathetic and parasympathetic nervous system.

Signs and Symptoms

In 2014, US poison centers received 1,692 cases reporting black widow spider bites, of which 765 cases were treated in a healthcare facility (Mowry et al. 2015). However, poison center data are comprised of spontaneous calls from patients (self-report) and healthcare providers. A case may range in severity from trivial to serious. The total number of cases for any poison is typically undercounted because patients may not call the center and physicians may not want assistance.

The bite of a black widow spider has been described as a pinprick although often the patient recalls no specific event. Bite symptoms start to manifest within 1 h, and patients present at medical facilities around the 6 h mark. Not every bite victim shows all the signs of latrodectism. The most common signs and symptoms include mild local pain without tenderness at the bite site with localized sweating followed by increasing pain and muscle cramps. The pain and muscle cramping often migrate centripetally (Clark et al. 1992) (Table 1). The combination of a bite on the arm with pain radiating centrally combined with the autonomic signs of mild hypertension and diaphoresis can appear similar to myocardial infarction. The same process starting in the leg can appear as an acute abdomen (appendicitis).

The patient may be restless and move incessantly to try to ameliorate pain symptoms; some reports state that these movements were so severe that it

Table 1 Signs and symptoms of western black widow spider bites (Clark et al. 1992)

Generalized abdominal or back pain	56%
Local or extremity pain	38%
Hypertension	29%
Diaphoresis	22%
Isolated abdominal pain	18%
Isolated chest pain	17%
Nausea and/or vomiting	11%
Tachycardia	11%
Headache	5%
Isolated back pain	4%
Shortness of breath	2%
Paresthesias	1%

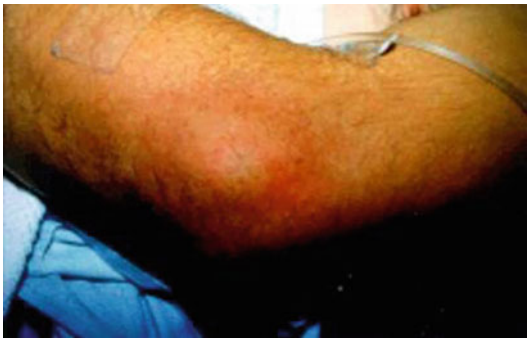

Fig. 3 Black widow spider bite. Note the lack of significant dermal damage. Signs that indicate a widow envenomation include minor erythema, piloerection, and localized diaphoresis

complicated taking patient history. Diaphoresis may be confined to the bitten appendage or expressed on just the face, particularly the nose and forehead, while other body areas remain dry. Patients may experience *facies latrodectismica* which includes a painful grimace, flushing, diaphoresis, trismus, and blepharitis (Maretic and Lebez 1979). Envenomation signs and symptoms may wax and wane where patients are sent home, only to return later for additional treatment.

There is minor dermatological evidence of a widow envenomation on the skin surface (Fig. 3). There may be the red streaking of lymphangitis from the bite site and the presence of a target lesion; however, latrodectism does not involve dermonecrosis. Bacterial infection is exceptionally rare, as it was conspicuously absent as an envenomation sign in several large series of a combined total of more than 3,000 latrodectism victims from around the world (Vetter et al. 2015).

Despite the effects of widow venom on muscle activity, pregnant women suffered no more significant reactions when bitten by widow spiders when compared to nonpregnant women of the same reproductive age; there were no documented pregnancy losses (Wolfe et al. 2011).

Epidemiology

When human populations were primarily rural, widow bite victims were predominantly males (70% of 152 bite victims) with bites occurring predominantly on the genitalia and buttocks (Wiener 1961) because of the prevalence of widows living under the seats in primitive outhouses. With the advent of indoor plumbing, the sex ratio of bite victims equalized somewhat (61% male in 163 bite victims; Clark et al. 1992), and bites shifted to the extremities (48% lower, 28% upper; Clark et al. 1992) as people stuck their hands and feet into gloves and footwear that had been stored in garages and sheds.

Treatment

The recommended treatment of latrodectism in the early 1900s included an array of ineffective modalities: whiskey, strychnine, nitroglycerine, and mercury bichloride. This later gave way to muscle relaxants such as calcium gluconate, which was shown to be ineffective (Clark et al. 1992). Currently, the primary treatment of latrodectism is an opioid analgesic combined with a muscle relaxant like a benzodiazepine.

Antivenom

Antivenom for black widow spider bite was first licensed in the United States in 1936 as antivenin *Latrodectus mactans* Merck (black widow spider antivenin). It is produced by partial purification of serum immunoglobulin G (IgG) from horses immunized with *L. mactans* venom. Although considered effective, it has not been tested in a prospective clinical trial. In a retrospective analysis, patients receiving the antivenom reported symptom resolution in an average of 31 min (Clark et al. 1992) with 50 of 58 patients (86%) requiring only one vial; this study was done in Arizona so presumably the species involved was the western black widow, *L. hesperus*. Antivenom has shown benefit for a latrodectism victim, treated after a 90 h delay, whose inability to walk due to pain and spasm was reversed 10 min after completion of antivenom treatment (O'Malley et al. 1999). Of patients receiving antivenom, only 12% were hospitalized compared to 52% of patients not receiving antivenom (Clark et al. 1992).

Antivenin *Latrodectus mactans* is recommended for patients with a diagnosis of black widow spider bite who have had an insufficient response to modest doses of a parenteral opioid analgesic. If repeated or large doses are needed to control pain, use of the antivenom is justified. The dose is one-vial intravenously administered as described in the package insert and in a facility capable of treating anaphylaxis. Typically, one dose is sufficient although one repeat dose may be necessary in unusual cases. The antivenom should not be administered intramuscularly because of erratic absorption and evidence that intramuscular administration does not produce adequate blood levels of antivenom (Isbister et al. 2008). If the patient does not respond to antivenom, another diagnosis should be sought.

However, the Merck antivenom is an old, partially purified product that can cause acute allergic reactions. Severe anaphylactic reactions including death have been reported (Clark et al. 1992). The use of the Merck antivenom is also decreased

because of limited supply provided by the manufacturer (American Society of Health-System Pharmacists. Black widow antivenin (*Latrodectus mactans*) [Web site]. Available at https://www.ashp.org/DrugShortages/Current/bulletin.aspx?id_670. Accessed June 8, 2012).

A new antivenom, antivenin *Latrodectus* (black widow) equine immune F(ab)2, is currently being tested in clinical trials. The antivenom is commercially available in Mexico but has not been approved for use in the United States. This antivenom is a highly purified equine $F(ab)_2$ antibody preparation that is expected to produce fewer acute allergic reactions. Two studies have been completed with favorable results (Dart et al. 2013, 2016).

These studies stand in contrast to a recent study involving Australian redback spider antivenom that reported no difference in pain relief between antivenom and placebo groups. Given the remarkable similarities of the black widow and redback spider venoms and animal research indicating cross-reactivity, the contrasting results are difficult to reconcile. However, there are major differences between the US studies and the Australian study that likely explain the differences (Dart et al. 2013; Isbister et al. 2014).

False Widow Spiders (Genus *Steatoda*)

The genus *Steatoda* is closely related to the *Latrodectus* spiders and can be confused with them (Fig. 4). They are typically shiny brown in color, some with light abdominal markings. Many species are very small and of no envenomation concern; however, the larger species can cause bites with mild to moderate envenomation symptoms. The false black widow, *S. grossa*, is a European immigrant that has spread through areas of the United States and Australia. They are commonly found in homes, in closets, and in the toe kick areas under kitchen and bathroom cabinets. Because of their proximity to humans, they end up biting people. Most signs and symptoms are minor; however, rare severe reactions have been documented (Graudins et al. 2002). Other species of large size and potential concern are

Fig. 4 False black widow of the genus *Steatoda*. Besides the similar physical appearance, they also share many similarities in venom composition with black widows

S. paykulliana in Europe and *S. nobilis* which has spread from continental Europe into England, California, and Chile.

Steatoda venom contains the widow venom component α-latrotoxin but with a less conserved genetic sequence; hence, this is thought to be the reason why *Steatoda* bites are not as medically important (Garb and Hayashi 2013). Although most *Steatoda* bites are minor with mild erythema, edema, and pruritis, manifesting in mild latrodectism symptoms, a treating physician could easily misidentify the spider as *Latrodectus* and overmedicate. Administration of *Latrodectus* antivenom in one case appeared to effectively diminish *Steatoda* envenomation symptoms (Graudins et al. 2002).

Widow Summary for Clinicians and Patients

Make sure of the diagnosis: Widow envenomations are typically pathognomonic and, hence, rarely misdiagnosed. However, there are two concerns with misdiagnosis. The first is mistaking pain for myocardial infarction or acute abdomen. The second is misidentifying less toxic *Steatoda* spiders (which are more common inside homes than are widow spiders) as verified biting widows if presented by patients, which may lead to unwarranted use of antivenom.

Treat the pain: Mild bites typically resolve by themselves after a few days. Antivenom, if available, is highly successful at reversing severe envenomations.

Reassure: Due to the infamy of widows, their high recognition factor by the general public and an overwhelming feeling of impending death in some envenomations, patients should be reassured that the probability of a negative outcome is highly unlikely with current medical care.

Prevent future bites: Widow spiders are predominantly found in garages and around homes but rarely are considered "house" spiders. Negative encounters can be minimized by various proactive precautions such as cleaning up clutter around the outside of homes, using large plastic bags to store rarely used or seasonally used clothing and sports gear in the garage, moving wood piles away from the house, wearing gloves for gardening or dealing with a wood pile (after checking the gloves for widows if gloves are not stored in spider-proof bags), and checking underneath children's molded plastic playthings. However, if one is using a still-extant outhouse, the age-old advice of "bang the lid before sitting down" still has utility.

Recluse Spiders (Genus *Loxosceles*)

Distribution

Recluse spiders (also known as fiddle-back or violin spiders) are found worldwide. As of the writing of this chapter, there are 114 species but that number will most likely increase in the future as more taxonomic attention is given to the genus. Many species are only known from a few specimens from the one location where the

original spiders were collected with several being strictly cave residents with extremely limited distribution. This cave-dwelling aspect may be a primitive character for the genus because when a non-cave-dwelling species establishes itself outside of its indigenous range, it often only infests the one building which it colonizes and does not spread beyond that unless aided by humans.

The greatest number of currently known *Loxosceles* species occurs in Central and South America. In South America, the main spider of medical concern, *L. laeta*, allegedly has the most toxic bite and is the largest of the *Loxosceles* species with females being up to 14 mm in body length. Other South American entities of medical importance include *L. gaucho* and *L. intermedia*. Most of the others are not often found in association with humans or have limited distribution.

In North America, the *Loxosceles* spider of greatest concern is the brown recluse, *L. reclusa*, which is found in the Midwest and parts of southeastern United States. There are five other widespread species in the southwestern American deserts (Fig. 5). One important behavioral proclivity is that the brown recluse is synanthropic, meaning that its populations increase in association with humans similar to such pests as cockroaches and rats. Hence, it is often found in homes and presents an elevated envenomation threat in comparison to the other American *Loxosceles* spiders which are more likely to only be found in homes surrounded by native desert habitat.

The Mediterranean recluse, *L. rufescens*, is a worldwide tramp, found in many places but typically as a spot infestation so even though it would not be surprising to find it anywhere, its distribution is still sporadic and highly circumscribed outside of

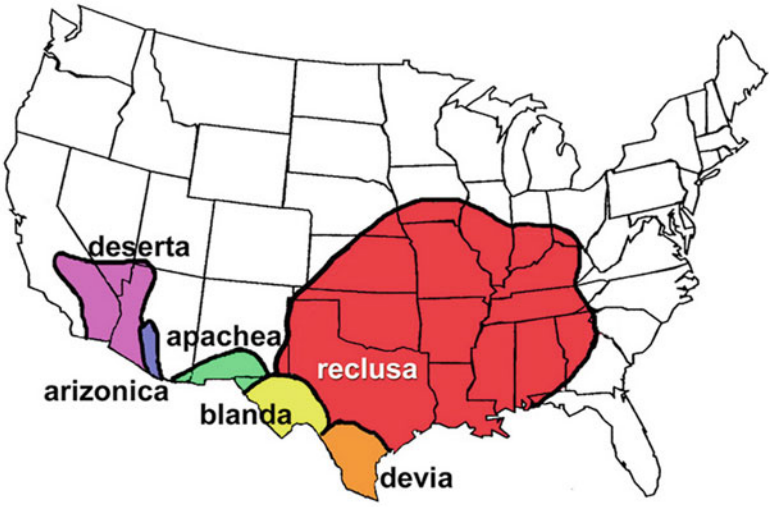

Fig. 5 Approximation of the distribution of the brown recluse spider and five closely related widespread native species in North America (Reprinted from *The Brown Recluse Spider*, by Richard S. Vetter. Copyright © 2015 by Cornell University. Used by permission of the publisher, Cornell University Press)

its indigenous habitat. It is only established in a very localized area in Adelaide, Australia, but causes more damage in a psychological manner as people spread rumors about the presence of the spider rather than the actual envenomation threat that it poses. In the circum-Mediterranean area, currently all specimens are considered to be *L. rufescens*; however, recent research suggests that there are many cryptic species that may eventually be separated out and given new scientific names (Duncan et al. 2010). Several *Loxosceles* species are found in Africa, with more being discovered and named in undercollected areas like Asia.

Appearance and Identification

Many *Loxosceles* spiders are nondescript tan and brown and medium-sized with body lengths of 5–14 mm in mature specimens (Fig. 6). The genus name *Loxosceles* means "crooked or slanted legs" for the position the spider takes when at rest; the genus name is the basis for the medical syndrome associated with recluse bites ("loxoscelism"). Several North American species have a distinct violin shape on the cephalothorax (the body part to which the legs attach); however, this is not a consistent marking in all *Loxosceles* spiders. Female Chilean recluses, *L. laeta*, are cinnamon colored which makes the violin difficult to see, North American species in the southwestern American deserts are often devoid of pigment in the violin area, and some South African species have multiple dark markings on the cephalothorax and abdomen. In addition, because many non-arachnologists (including authorities such as physicians, entomologists, and pest control personnel) are aware that some recluse species have violin markings, they creatively interpret dark markings on

Fig. 6 The conspicuous violin shape on the dorsal surface of the cephalothorax of the brown recluse spider. Although many people are aware that brown recluses have violin patterns, similar markings cause many harmless spider species to be misidentified as recluse spiders (Reprinted from *The Brown Recluse Spider*, by Richard S. Vetter. Copyright © 2015 by Cornell University. Used by permission of the publisher, Cornell University Press)

Fig. 7 The six-eye pattern is a diagnostic feature that helps identify a recluse spider. However, it is not the only spider that has this pattern (three pairs of eyes with a space separating the pairs) (Reprinted from *The Brown Recluse Spider*, by Richard S. Vetter. Copyright © 2015 by Cornell University. Used by permission of the publisher, Cornell University Press)

other spiders as violins and, hence, misidentify harmless spiders as recluses. This can lead to unwarranted overtreatment of alleged bites as well as overzealous pest control measures by home owners and increased anxiety in families of patients diagnosed with loxoscelism (Vetter and Isbister 2008).

Therefore, the violin marking should not be used as a reliable identifier for these spiders. Instead, one should look at the eye pattern. Most spiders have eight eyes, typically arranged in two rows of four (although some non-recluse spiders have aberrant eye patterns of three rows but still with eight eyes). Recluse spiders have six eyes, with an anterior pair (dyad) and two lateral pairs, with the dyads separated by a space (Fig. 7). Recluse spiders are not the only taxon that has six eyes, but the majority of spiders encountered have eight eyes and not in this pattern. In addition, recluses have:

- Monochromatic legs devoid of stripes, rings, or spots (although in some *Loxosceles* males, anterior legs will be darker or a different monochromatic color than the others)
- Fine recumbent hairs on their legs (whereas many other spiders have conspicuous spines)
- Monochromatic abdomens (whereas many other spiders have multiple abdominal pigments)

Venom Components

The destructive recluse venom components are in the range of 5–40 kDa and predominantly consist of phospholipases, astracin-like metalloproteases, and inhibitor cysteine knots. For many years, the main destructive component of interest in recluse venom was simply described as sphingomyelinase D. However, recent research has shown that there are several isoforms of the enzyme, and therefore, venom toxinologists now refer to the group as phospholipases D (Gremski et al.

2014). These enzymes target erythrocytes, platelets, and the kidneys (Gremski et al. 2014) and are principally responsible for the dermonecrotic reaction in humans, possibly with metalloproteases triggering endogenous cell destruction. In addition to *Loxosceles* spiders, this venom component is only found in some species in the closely related spider genus *Sicarius* and, curiously, outside of spiders, is found in several bacteria. When tested against insects, phospholipases D generated almost equivalent LD_{50}s to whole venom indicating that the function of the enzymes is to kill the spider's prey. The fact that it also has effect on humans is just a vagary of venom chemistry. In at least one South American recluse species, *L. intermedia*, females have more potent venom than males, causing more dermonecrosis in rabbits and showing sexual differences with venom analysis (de Oliveira et al. 2005). Another component of the venom includes spreading factors (hyaluronidase).

Although laboratory studies with animal models are surely critical for loxoscelism research, results obtained from animal studies must also be taken with caution. Humans, rabbits, and guinea pigs develop dermonecrotic lesions when exposed to *Loxosceles* venom although rabbits heal faster and do not develop the chronic necrosis seen in humans (da Silva et al. 2004). On the other hand, rats and mice do not develop dermonecrosis at all. Pigs have been used as models for loxoscelism studies because of similarities of mostly hairless skin to humans but present their own limitations as their skin is much thicker.

Pathophysiology and Expression of Envenomation

Many *Loxosceles* species share the same destructive enzymes responsible for injury to humans, and, therefore, information regarding a few species will probably be applicable to some extent for bites from all species of *Loxosceles* spiders. However, effects of envenomations, especially systemic manifestations such as hemolysis, are subject to considerable variation depending upon the *Loxosceles* species involved. Manifestations of cutaneous and systemic loxoscelism are arbitrarily divided into an artificial dichotomous distinction, yet many patients will experience both local and systemic manifestations of loxoscelism.

Throughout history, medical authors have often presented graphic images of verified and alleged loxoscelism; however, it is underreported that many recluse spider bites have mild to moderate manifestation. In North America, about 90% of loxoscelism events are unremarkable or of minor consequence with self-healing, causing merely inflammation (Tutrone et al. 2005). However, these minor events do not garner attention and are overlooked in the literature as well as by the public media. Hence, the manifestation of loxoscelism can range from minor rash-like symptoms to massive necrotic skin lesions (Fig. 8). In addition, very rare systemic loxoscelism can occur causing hemolytic anemia although apparently more commonly in South American than North American incidents.

The most common loxoscelism symptom is tenderness or pain at the bite site. This increase is in accord with the delay in the release of the cytokine interleukin 8 (IL-8). After experimental inoculation of recluse venom in human epithelial cells,

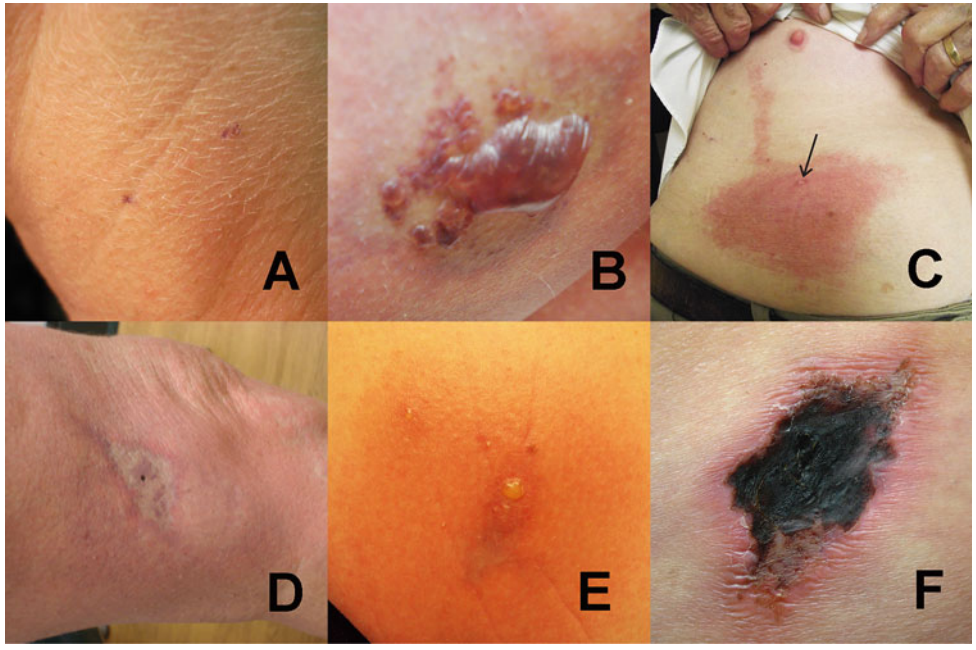

Fig. 8 Examples of recluse bite wound progression over the first 2 weeks. All except the first image had positive venom ELISA test; the patient in (**a**) brought in a spider identified as a recluse before an ELISA test was available. All were clinically likely recluse bites; all except **c** had significant pain. Cases (**b**) through (**e**) were acquired while sleeping. Note that no cases had early or large areas of ulceration or necrosis, as sometimes reported in cases without documentation. (**a**) **Day 1** – 26-year-old female, lateral neck, minimal purpura and erythema, acquired while moving furniture. (**b**) **Day 4** – 21-year-old female, lateral thigh, purpuric bulla with surrounding pallor and purpura. (**c**) **Day 4** – 89-year-old male abdomen, pallor but no necrosis. A rash that follows a lymph vessel toward the lymph node signals inflammation here, not infection. The *arrow* points to the recluse bite site. (**d**) **Day 5** – 50-year-old female, thigh, pallor, purpura, patchy rash prominent at *right*. (**e**) **Day 8** – 27-year-old female, forearm, vesicle, and *red*, *white*, and *blue sign*. (**f**) **Day 14** – 37-year-old female, lateral upper thigh, acquired cutting down tree, eschar thinning at edges and exterior lines show that wound is healing

IL-8 levels were more than ten times higher at 8 h than at 0.5 or 2 h, the latter of which did not differ from background (Gomez et al. 1999). Pain increase over the first day is so characteristic of recluse bites and aligns so well with the rise of cytokines that this has been called "the cytokine pain pattern" (Payne et al. 2014).

Cutaneous

What is considered the classic loxoscelism event is development of a dermonecrotic lesion which was first associated with *Loxosceles* spiders in South America in the 1940s (known as "gangrenous spot"), and in North America, spider association was confirmed in 1957 (Vetter 2008).

Recluse venom causes destruction of the endothelial tissue manifesting in constriction and destruction of the capillary bed within minutes of envenomation. Phospholipases D cause hemolysis resulting in endogenous and exogenous leakage into surrounding tissue. Decreased circulation to the area occurs when polymorphonucleocytes infiltrate the bite site within 48 h and platelet aggregation sets up a zone of ischemia, the latter of which is thought to contribute to pain. Cytokines are released as is evident by erythema surrounding the bite and intense pain. In nearly all cases, pain is minimal at the start with the average pain level of 2.4 on a scale of 10 at the time of the bite with levels increasing significantly over the next 8 h (Payne et al. 2014).

Systemic

Loxoscelism can also generate a predominantly systemic response with most victims being children; it is estimated that systemic loxoscelism occurs less than 1% of the time in North America (Anderson 1998) but with higher prevalence in South America. However, despite its rarity at least in North America, systemic loxoscelism is of great concern because it can develop swiftly, prior to dermonecrotic development, reducing diagnostic accuracy, and can result in pediatric death within 12–30 h.

In systemic cases, bite victims may exhibit severe hemolytic anemia and acute kidney injury due to the free hemoglobin in the blood such that victims produce dark brown urine indicating hemoglobinemia and hemoglobinuria. Hemolysis can be direct and caused through a complement-mediated reaction. Thrombocytopenia is also a manifestation, and there may be direct nephrotoxicity (Chaim et al. 2006).

Signs and Symptoms

Cutaneous

Diagnosis of dermonecrotic loxoscelism is challenging because of the great variation in expression in loxoscelism victims, the different forms in progression of the lesion through manifestation, and the many medical conditions with which it can be confused. Loxoscelism initially involves an area with compromised blood flow so lesions will boast purple, blue, and white colors at the bite site. Target lesions (blue center, white, red peripherally) are good indicators but are not pathognomonic; Lyme borreliosis can also produce a target lesion.

At 24 h, a fluid-filled bleb may form at the focal bite site with fluid being clear or slightly red but never pus filled. Morbilliform eruptions, usually pruritic, can occur on the trunk around 36 h but usually dissipate coincident with ischemia by the end of the first week. At least some degree of malaise and anxiety are universal; nausea is common. The bite site shows erythema; if the lesion is small, the erythema can be central.

More extensive envenomations show central pallor and peripheral erythema, which is often firm. The central area is flat, except for envenomations on soft tissue areas such as on distal extremities, head, neck, and genital areas (Figs. 9 and 10). These soft tissue areas are usually edematous and swollen such that for bites above the neck, respiratory passages can be compromised. Purpura/ecchymosis can appear as a bruise with pallor and bluish pallor indicating incipient necrosis. Additional indications of impending necrosis are that red areas near the bite site do not turn white when pressed or if they become white, do not refill with red when pressure is removed. An encouraging sign is if the lesion retains its red coloration, indicating that the lesion will probably not turn necrotic. By the third or fourth day, necrosis sets up with eschar formation occurring typically by the end of the first week but could also occur as early as a few hours or as late as 2 weeks. By the second week, the eschar becomes dark, slightly sunken, numb, necrotic, and indurated. The eschar sloughs away as the edges thicken. The lesion is dry due to decreased circulation; hence, recluse bites do not ooze blood, pus, or serum. Of 23 lesions with necrosis, the average necrotic area was 1.5 cm^2 (range = 0.04–33.6 cm^2) (Payne et al. 2014). These findings are similar to reports of necrosis in 11 of 19 (57.9%) documented bites with the median necrotic area of 1.95 cm^2 (Sams et al. 2001).

Debridement may proceed around 14–21 days when the edges of the lesion have stopped increasing and granulation has begun. Necrosis can be extensive in obese victims as recluse venom can cause great detriment in poorly vascularized adipose tissue. Gravitational spread of the lesion can indicate loxoscelism. Although massive hemolysis is a sign of systemic loxoscelism (see below), it may be underappreciated that mild hemolysis may be present in cutaneous loxoscelism as well (Malaque et al. 2011).

Systemic

For the astute physician, systemic loxoscelism may be easier to diagnose than the dermonecrotic form. The clinical presentation in cases of systemic loxoscelism depends upon the degree of hemolysis. Of recluse bite cases presenting to clinical facilities, the great majority will show no evidence of hemolysis although the exact incidence remains unknown. Persons with mild systemic loxoscelism may present with a diffuse pruritic rash, nausea, and fatigue. Dark urine is an indicator of hemoglobinuria. Icteric sclera and jaundice are potential manifestations. Hemolytic anemia typically lasts 4–7 days, but if it does not occur by 96 h, it is unlikely to develop. Other signs and symptoms include joint and muscle pain, pruritis, emesis, nausea, and malaise. During the first week, a systemic loxoscelism victim may express scarlatiniform, morbilliform, or petechial eruptions on the trunk. Disseminated intravascular coagulation is a potential development. A urine test strip serves

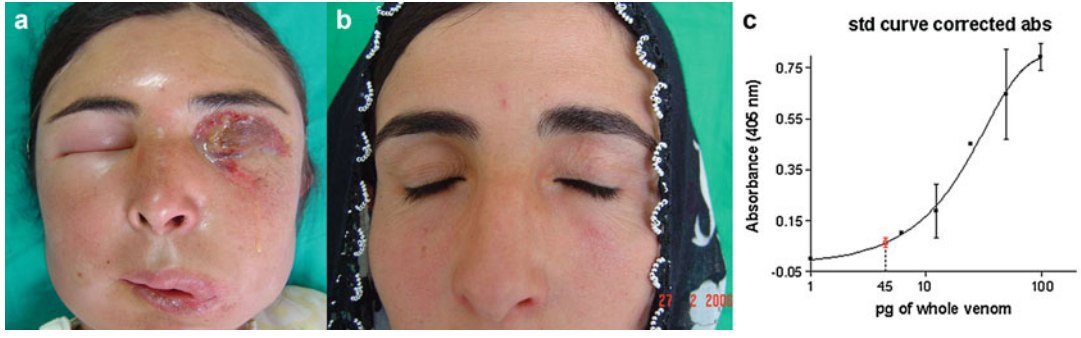

Fig. 9 (**a**) Patient with significant eyelid edema and necrosis. (**b**) The wound healed without significant sequelae. (**c**) The associated ELISA venom standard curve showing 4.5 pg venom recovered at the bite site (Creative Commons @ Univ. Calif. Davis)

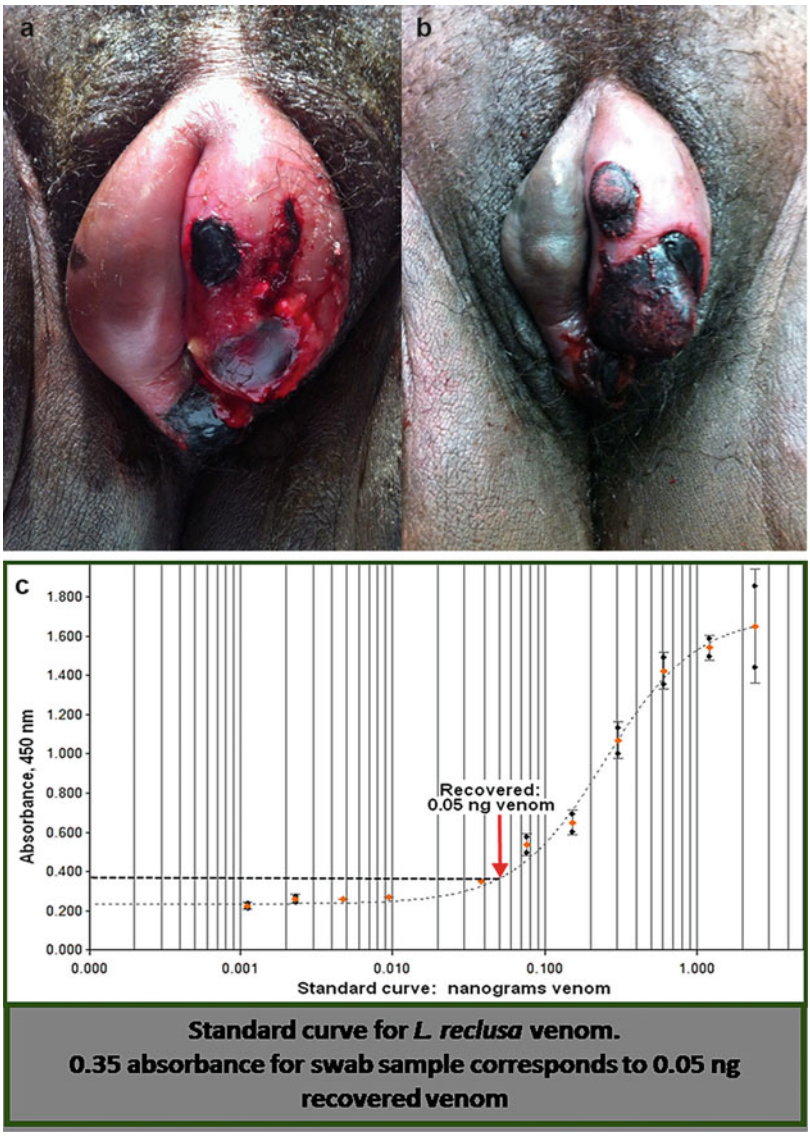

Fig. 10 (**a**) Necrotic genital bites on the labia minora of a 12-year-old girl. (**b**) Near recovery at 12 days. (**c**) Associated ELISA standard curve for recovery of 50 pg (0.05 ng) venom

to identify significant hemolysis by showing elevated urobilinogen (indicating extravascular hemolysis) or red cells (indicating intravascular hemolysis).

Anecdotal evidence supports an inverse relation between recluse wound severity and severity of systemic manifestations (Fig. 11). Patients presenting with acute hemolytic anemia are often unaware of their spider bites which were only discovered

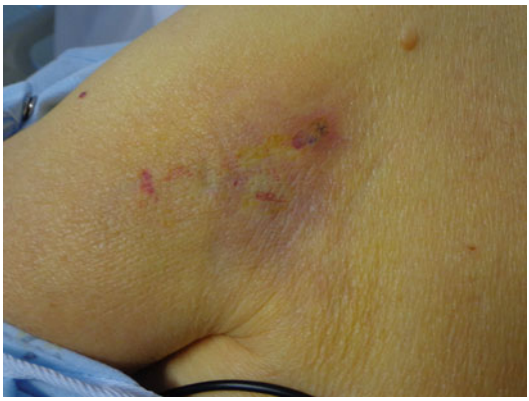

Fig. 11 A 79-year-old male, 10-day-old recluse bite on shoulder, severe hemolysis, myocardial infarction and obtundation. Neurological symptoms were relieved with transfusion. Venom ELISA: 9.8 pg venom (Reprinted with permission *Missouri Medicine*)

after thorough examination (McDade et al. 2010). The most common symptoms reported in 25 patients in Brazil with significant hemolysis were a diffuse morbilliform rash (72%) and fever (68%) (Malaque et al. 2011). Jaundice was present in 32% of these patients (Malaque et al. 2011). Fatigue, nausea, malaise, and headache are commonly present in milder cases; these symptoms do not necessarily correlate with the degree of hemolysis (Anderson 1998; Malaque et al. 2011). The reported incidence of hemolysis varies with geographic region and its indigenous spider species: 7.3% and 15.7% with *L. laeta* (Chile), 4% with *L. reclusa* (USA), and 0% with *L. rufescens* (Israel).

A peripheral blood smear may show fragmented erythrocytes and microspherocytes. Other laboratory findings in these cases include elevated bilirubin, elevated lactic dehydrogenase (LDH), and evidence of either intravascular hemolysis (hemoglobin in the urine) or extravascular hemolysis (urobilinogen in the urine). The urine test strip will demonstrate hemoglobin, but microscopic urinalysis may not show intact red blood cells, because of erythrocyte lysis. A drop in hemoglobin to a level of 7 gm/dL is typical in cases of severe hemolysis (Anderson 1998; McDade et al. 2010). Many patients without anemia will show indirect evidence of hemolysis with other laboratory studies. In Brazil, 41% of patients showed elevated total bilirubin, and 50% had elevated LDH (Malaque et al. 2011). Other laboratory abnormalities reported in this series included leukocytosis (51%), elevated C-reactive protein (85%), and plasma fibrinogen (34%). Leukocytosis without clinical evidence of infection may reach a level of 37.4×10^9/L (McDade et al. 2010). In one study, the direct antiglobulin test was positive for surface complement component C3 in six of six patients and positive for IgG in three of six cases (McDade et al. 2010).

Detection of Loxoscelism

The advent of a swab ELISA (enzyme-linked immunosorbent assay) test for brown recluse venom has helped obtain a clearer picture of recluse bites (Akdeniz et al.

2007) because it is allowing documentation of recluse bites in the estimated 93% of cases for which the spider is not retrieved (Sams et al. 2001). Early ulceration, large areas of tissue damage resembling necrotizing fasciitis, and secondary infections early in the course are examples of signs that are not part of the loxoscelism syndrome when cases are properly documented. In a series of documented cases (Sams et al. 2001), approximately 40% of cases seen in medical facilities show no necrosis; when necrosis is present, the median area of necrosis is less than 2 cm^2. In cases with no purpura and no necrosis, minimal-change bites such as these closely mimic other arthropod bites, except for the great amount of pain present with many loxoscelism cases. Healing of these typical small lesions should occur within a few weeks after a recluse bite. Because some North American *Loxosceles* bites can result in severe life-threatening hemolysis, practitioners should remain vigilant for possible hemolysis. In one case, a 10-year-old child's hematocrit rapidly fell from 38 to 10; only careful support in an intensive care unit (ICU) allowed the child to survive (Boyer et al. 2000). Thus, vigilance is urged regarding the occasional case of loxoscelism with severe hemolysis yet conservative management of the great majority of recluse bites that lack serious sequelae.

Because of difficulties in diagnosing spider bites, a reliable sensitive and specific test for *Loxosceles* envenomation has long been sought (Gomez et al. 2002; Stoecker et al. 2006; McGlasson et al. 2009). Several attempts have been made through the decades with continual improvement. The lower limit for *Loxosceles* venom ELISA sensitivity in 2002 was around 100 picograms (pg) (one part in 20,000 of the 2 μg venom estimated in a recluse bite). A minimally invasive swab test has been developed to recover venom from the lesion (Stoecker et al. 2006; McGlasson et al. 2009). Venom was recoverable for at least 7 days in experimentally inoculated rabbits. Venom detection becomes increasingly unreliable more than 1 week after envenomation, in agreement with experimental results. Continued modifications to the procedure resulted in detection of as little as 3 pg venom. The assay has an estimated 97% specificity (for competing non-spider-bite conditions) and 87% sensitivity for cases rated "probable" for up to 1 week after envenomation. The ELISA can recognize the venoms of multiple *Loxosceles* species including *L. reclusa*, *L. arizonica*, and *L. rufescens*. The complete protocols for collection of the sample collection and venom detection are available at derminfo.org/brown_recluse_assay/protocols.

Epidemiology

Most bites occur on the leg, arm, or torso. As it is for almost all spiders, bites are inflicted as a last-ditch defensive response as a spider is being near-fatally squashed. Therefore, envenomations occur predominantly when a sleeping person rolls over on a recluse in bed or dons clothing left hanging in a closet or tossed on to the floor and the spider is pressed against flesh. Bites have been recorded while cleaning the house, especially in secluded areas such as basements and closets, supporting the

hypothesis that many bites are acquired while disturbing the spider in a secluded area. Bites occur most frequently from April to October in North America (Rader et al. 2012).

Treatment

Cutaneous Wound Care

In the early era just after *Loxosceles* spiders were proven to cause dermonecrotic lesions, excision of the wound to stop or limit dermonecrosis was advocated. However, a consensus on excising a recluse spider wound has been reached: wounds should granulate in and not be excised. Conservative management is urged because of variable response to the spider venom which may delay healing time. Once the acute phase is over, and an open ulcer has appeared, debridement of devitalized tissue speeds wound healing. At this stage, at approximately 2–3 weeks, a recluse bite should be treated like any other chronic wound, with attention to tissue, inflammation/infection, moisture, and edge (TIME) factors. Healing of a recluse bite on the upper arm is shown in Fig. 12.

Cutaneous Pain Management

The period following a loxoscelism event can involve considerable suffering with excruciating levels of pain, often augmented by anxiety engendered by dire outcomes due to patient Internet "research." The average pain score on a scale of 10 after 24 h was 6.7, but 7 of 23 loxoscelism victims had pain levels of 9 or 10 (Payne et al. 2014) (Fig. 13). Management of this pain can employ physical, local pharmacologic, and systemic pharmacologic therapy. Local physical therapy with rest, ice, compression, and elevation (RICE) is the most commonly recommended local therapy (Sams et al. 2001). Injury from ice or ice packs may be rare but has been noted. Local pharmacologic therapy with lidocaine-releasing patches has been advocated where some patients experienced a sensation of cooling and rapid pain relief. If possible, a trial patch should be placed on the lesion on the clinic visit. Drawbacks of the lidocaine patch include significant expense and the 12-h per day limit for application. For small wounds, lidocaine patches may be divided. Systemic pharmacologic therapy can include mild non-prescription pain relievers such as acetaminophen and prescription acetaminophen with codeine. Physicians should be aware that in some cases, a brief course of Schedule II narcotics containing either hydrocodone or oxycodone is indicated, generally confined to the first 2 weeks after a recluse bite.

Systemic

Although fatal outcome is possible in systemic loxoscelism, adequate treatment of corticosteroids, transfusions of packed red blood cells but not whole blood, dialysis, and hydration are typically sufficient to counteract negative development. Tests for kidney and liver function are useful to monitor the progression of reaction.

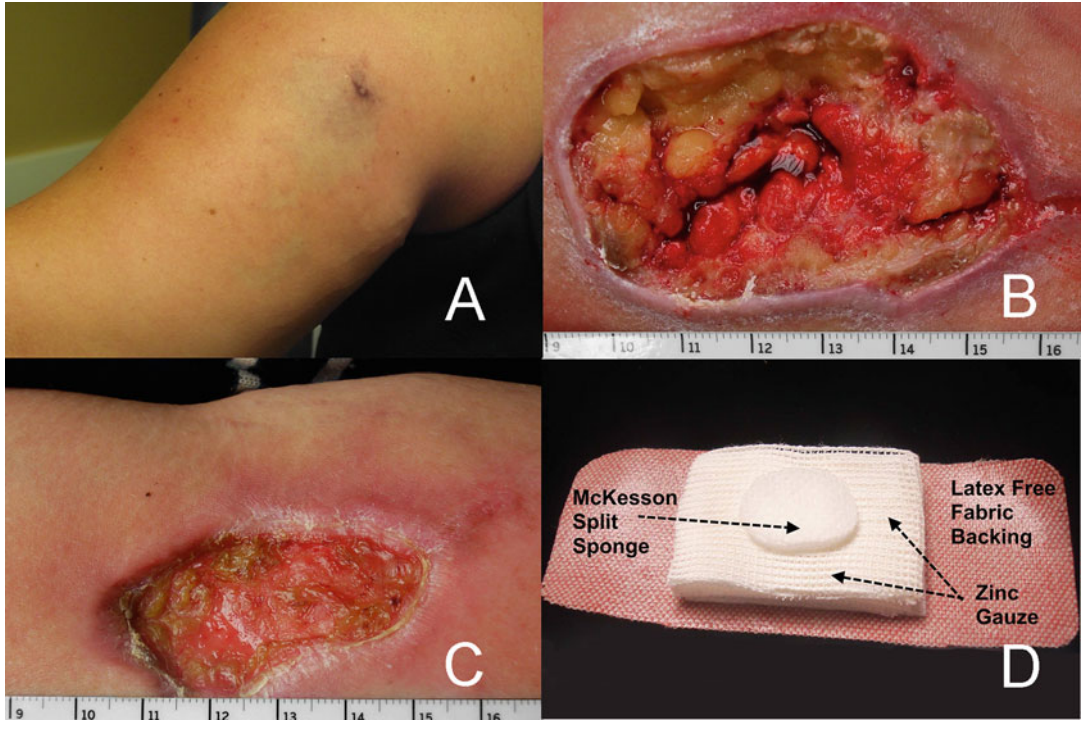

Fig. 12 Wound healing with debridement and zinc gauze technique on upper arm of 35-year-old female. (**a**) **Day 1** – recluse bite on arm, 6 by 5 cm necrosis, throbbing pain. (**b**) **Day 39** – after debridement (eschar remnant lower right). (**c**) **Day 63**. (**d**) A zinc rim bandage with structure of the bandage. The central gauze allows drainage via elliptical pores; the peripheral Unna gauze speeds healing via the favorable effect of zinc oxide on the actively healing wound rim

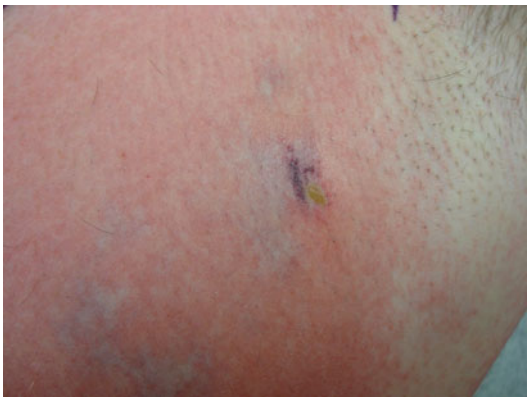

Fig. 13 A 35-year-old female with 3.5-day-old spider bite on leg; *red*, *white*, and *blue sign* and small blister are seen. After 24 h, the pain was rated at 10 of 10; the recluse spider recovered was identified by an arachnologist. Venom ELISA: 131.5 pg venom

Once hemolytic anemia is demonstrated, close observation is mandatory, preferably in the ICU. Rapid hemolysis, volume depletion, and hypotension may necessitate dopamine infusion. The time course of hemolysis varies significantly and can be sudden in onset; therefore, hematocrit should be monitored at least every 6 h. To avoid trauma from blood draws, urine free hemoglobin may be monitored. Transfusion is indicated, depending upon the clinical situation, when the hematocrit drops below 25–30%.

Antivenom and Other Remedies

Antivenom is available in South America (Pauli et al. 2006) but not in North America. Although antivenom works best when given within the first 24–48 h post-bite, most bite victims do not present until later because the initial stages of loxoscelism do not appear serious to patients. Despite a time delay, it has been argued that even late antivenom administration has benefit in reduced hospital stay and reduced lesion development (Pauli et al. 2006). Antivenom also appears to work on all *Loxosceles* species on which it has been tested. Recommendations of early debridement are now contraindicated as it can lead to greater scarring and longer healing times. Several additional remedies have been recommended (e.g., hyperbaric oxygen, electrical shock, nitroglycerine), but none have been proven effective (Swanson and Vetter 2005) in part due to the lack of untreated control groups in any of the studies; additionally, withholding treatment in order to perform clinical research would be unethical. The leprosy drug, dapsone, was commonly used in loxoscelism cases but has been shown to be of little benefit (Elston et al. 2005); in addition, dapsone causes a drop in hemoglobin (similar to a loxoscelism sign), can be detrimental to those with glucose-6-phosphate dehydrogenase deficiency, and can induce methemoglobinemia. An interesting development is the benefit ascribed to topical application of tetracycline to experimentally injected rabbits; the thought is that tetracycline inhibits metalloprotease activity that can lead to dermonecrosis (Paixão-Cavalante et al. 2007).

Differential Diagnoses and Overdiagnosis of Loxoscelism

The notoriety of the brown recluse spider, along with many reports of "spider bites" and the lack of physician awareness of the limited distribution of recluse spiders in North America, can result in failure to appropriately diagnose and treat other non-arachnid conditions (Vetter 2008). Some of these conditions can have rapid development with serious consequences including death (Fig. 14); they include a spectrum of diverse afflictions such as infections of bacterial, fungal, and viral nature, blood disorders, circulatory issues, topical contaminations, lymphoproliferative diseases, factitious injury, etc. (Table 2, Fig. 15).

Once the idea of a "spider bite" is entertained, the patient and the physician often consider this to be the preferred diagnosis. A classic description of the expected local findings of loxoscelism (Anderson 1998) is summarized here with added case experience. These findings include pain in the area, often with pallor, bruising, or a vesicle, sometimes showing gravitational spread of venom effects, with erythema surrounding a central area, not exudative or very large, not red and swollen in the center, acquired in a manner consistent with disturbance of a secluded spider (Anderson 1998). A description of characteristics that are not consistent with a recluse bite could be of use in stemming the tide of reports of recluse spider bites lacking supportive evidence of spider involvement that continue to appear in the medical literature. The mnemonic term NOT RECLUSE may be useful for findings that are NOT typical of recluse envenomation (Stoecker et al. 2017).

N – Numerous. A typical recluse bite is a single focal lesion (Anderson 1998). Bites most often result from a defensive response when a spider is compressed or crushed. Occasionally two bites may occur.

O – Occurrence. Circumstances surrounding recluse bites involve disturbance of secluded spider, whether the spider has secluded itself in a bed, in clothes long unused or left on the floor, or in a dark closet.

T– Timing. In North America, credible bites are unlikely that occur before the last week in March or after the first week in November, i.e., falling significantly outside this normal recluse activity season (Rader et al. 2012). This time window is reversed for the Southern Hemisphere. Uncommonly, recluses may be encountered when disturbing secluded areas during the winter holiday season.

R – Red center. Except for mild recluse bites without necrosis, recluse bites are not red in the center.

E – Elevated. Recluse bites are flat or slightly sunken; if the focal area is raised more than 1 cm above the normal skin level, a different diagnosis should be entertained.

C – Chronic. Only the largest recluse bites are not healed within 3 months. In two studies of documented loxoscelism cases, only 1 of 53 (Payne et al. 2014) and 2 of 19 bites (Sams et al. 2001) exceeded 3 months in healing time.

L – Large. The largest recluse wounds typically do not exceed 10 cm in greatest dimension or 33 cm^2 in area (Sams et al. 2001). Although erythema may extend several times this distance around a wound, there is no credible documentation of

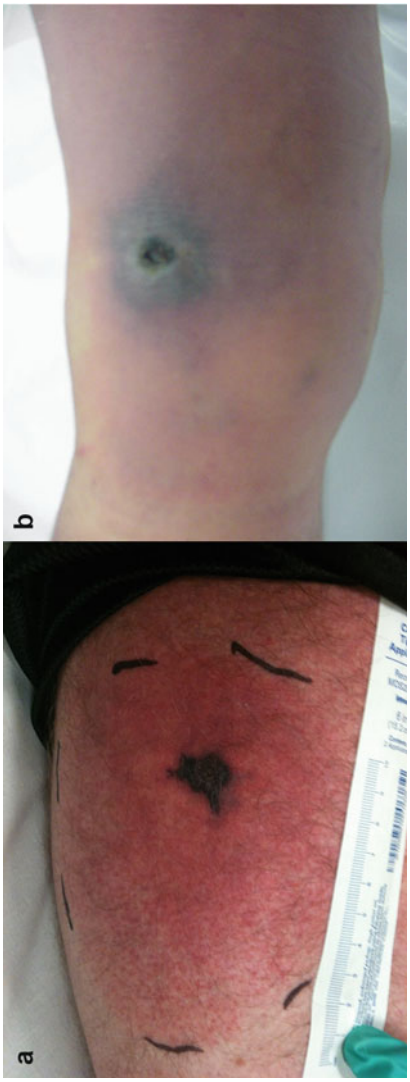

Fig. 14 Lesions on the legs of two patients, both displaying similar necrosis. (**a**) 28-year-old Oklahoma male 3 days after a lesion was noticed on his thigh upon awakening; venom ELISA: 110 pg. (**b**) A 10-year-old Texas boy with a fatal case of documented streptococcal sepsis; venom ELISA: 0 pg

Table 2 Conditions that have been or could be mistaken for loxoscelism (Modified from Swanson and Vetter 2005)

Infections
Atypical mycobacteria
Bacterial
Streptococcus
Staphylococcus (especially MRSA)
Lyme borreliosis
Cutaneous anthrax
Syphilis
Gonococcemia
Rickettsial disease
Tularemia
Deep fungal
Sporotrichosis
Aspergillosis
Cryptococcosis
Ecthyma gangrenosum (*Pseudomonas aeruginosa*)
Parasitic (Leishmaniasis)
Viral (herpes simplex, herpes zoster (shingles))
Vascular occlusive or venous disease
Antiphospholipid-antibody syndrome
Livedoid vasculopathy
Small-vessel occlusive arterial disease
Venous stasis ulcer
Necrotizing vasculitis
Leukocytoclastic vasculitis
Polyarteritis nodosa
Takayasu's arteritis
Wegener's granulomatosis
Neoplastic disease
Leukemia cutis
Lymphoma (e.g., mycosis fungoides)
Primary skin neoplasms (basal cell carcinoma, malignant melanoma, squamous cell carcinoma)
Lymphomatoid papulosis
Topical and exogenous causes
Burns (chemical, thermal)
Toxic plant dermatitis (poison ivy, poison oak)
Factitious injury (i.e., self-induced)
Pressure ulcers (i.e., bed sores)
Other arthropod bites
Radiotherapy
Other conditions
Calcific uremic arteriolopathy
Cryoglobulinemia

(*continued*)

Table 2 (continued)

Diabetic ulcer
Langerhans cell histiocytosis
Pemphigus vegetans
Pyoderma gangrenosum
Septic embolism

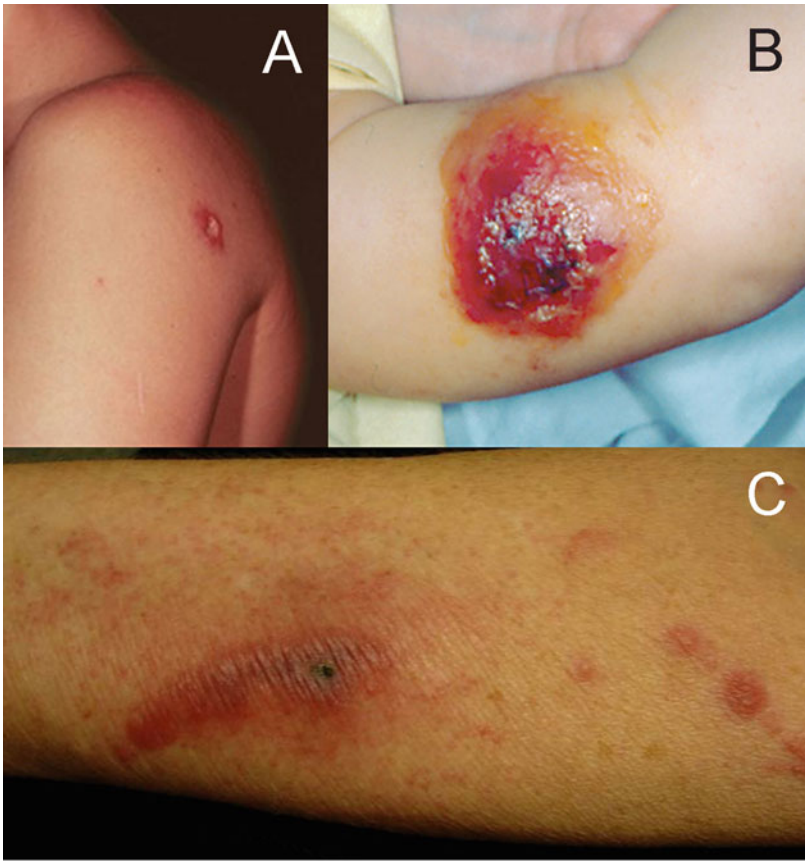

Fig. 15 Conditions misdiagnosed as loxoscelism by medical personnel. (**a**) A 10-year-old boy with confirmed ulceroglandular tularemia on the back after tick bite, misdiagnosed as loxoscelism by his pediatrician. (**b**) Anthrax infection in a 7-month-old infant with white cell count of 28,100 per cubic mm that was diagnosed with polymerase chain reaction; the lesion drained 10 ml of dark red fluid. This lesion was initially diagnosed as loxoscelism; the case received national attention and was reported in the *New England Journal of Medicine* (Copyright © 2002 Massachusetts Medical Society. All rights reserved). (**c**) A case of "black-spot poison ivy" with necrosis caused by urushiol. This case was misdiagnosed as a recluse bite by a nurse practitioner

larger areas of necrosis. Recluse wounds appear to be larger in areas of greater body fat and in morbidly obese persons.

U – Ulcerates too early. Recluse bites typically do not develop their ulceration until 7–14 days post envenomation.

S – Swollen. Recluse bites typically do not cause massive swelling below the neck. However, bites to the head and neck, especially the eyelids (Akdeniz et al. 2007), distal extremities, and genital areas develop significant edema (Figs. 9 and 10).

E – Exudative. Recluse bites are not exudative (Anderson 1998), moist, or purulent initially except on the eyelids and feet. There may be an initial blister at the bite site which contains clear fluid or blood, but open exudative wounds are not expected. Pus formation indicates an alternate diagnosis – primarily bacterial infection. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is currently a very common condition mistaken for brown recluse spider bite (Vetter 2008; Stoecker et al. 2017).

Recluse Summary for Clinicians and Patients

Make sure of the diagnosis: If a patient presents with a spider, get confirmation from a qualified entomologist, if possible. Many non-arachnologists are familiar with a recluse spider having a violin marking, thereby leading to misidentification of harmless spiders as recluses. The more diagnostic identification feature is the six-eye pattern (Fig. 7).

At the bite site, central pallor will develop with surrounding bruising and erythema at the periphery, i.e., the red-white-and-blue sign (also known as the bull's eye sign); even two colors would indicate a partial red-white-and-blue sign. A red, raised center or an early-ulcerating lesion is "NOT RECLUSE" signs. The cytokine pain pattern, i.e., pain increasing from negligible to severe over the first day, is usually present. A patient history regarding the alleged envenomation locale can be instructive, e.g., did the lesion occur in bed? A patient's confidence in a loxoscelism self-diagnosis may influence a later medical diagnosis, but skepticism about any "spider bite" diagnosis is warranted even with referrals from physicians.

Monitor the urine: If no urobilinogen or red blood cells are found with the urine test strip, hemolysis is insignificant. A urine test strip is superior to microscopic urinalysis because split erythrocytes are not easily observed. Because the time of hemolysis onset is unpredictable, patients should monitor for tan- or dark-colored urine for the first 2 weeks; beyond 2 weeks, hemolysis is unlikely.

Treat the wound and the pain: RICE therapy, as outlined above, can be critical. Loxoscelism victims were able to perform normal work duties employing ice for better pain relief than lidocaine patches or using zinc oxide gauze dressings, saline soaks, and oral NSAID therapy.

Reassure: Patients researching loxoscelism on the Internet can convince themselves of dire outcome including amputation and death. False information should be countered with reassurance based on hundreds of cases with adequate

documentation in the medical literature. If hemolysis is lacking, positive outcome is expected. Large wounds can heal with granulation tissue with little or no loss of function, even on the eyelid.

Prevent future bites: Negative encounters can be minimized by various proactive precautions such as pulling beds away from the wall, avoiding leaving clothes on the floor overnight, banging shoes before wearing to dislodge spiders, and inspecting seasonally used clothes and sports gear that have been in months-long storage. During the active recluse spider season, check the bed while pulling back bed covers. Sticky traps may reduce ambulatory spider populations.

Erroneous Elevation of Spider Species to Medical Importance

In the earlier period when little was known about spider venom effects on humans, it was difficult to catch a spider in the act of biting. Therefore, it became acceptable for authors to publish articles blaming particular species without solid evidence associating the spider with injuries to humans. This led to several instances of virtually harmless spiders being incriminated in causing severe medical injury and, in some cases, development of an ineffective antivenom, widespread overreaction to spiders, and, no doubt, misdiagnoses of skin lesions as spider bites. Currently, it is advocated that publication of spider bite reports must involve verified spider bites where the spider is caught in the act of biting and presented to a qualified expert (preferably an arachnologist) for identification.

Wolf Spider

In South America in the 1930s, wolf spiders were blamed for causing necrotic skin lesions due to injections of large doses of venom into test animals and poor reporting of the results (Ribeiro et al. 1990). Antivenom was developed and used ineffectively for decades. A study of 515 verified wolf spider bites revealed no necrosis. It is thought that these lesions were actually caused by *Loxosceles* spiders, but because recluses were not known to be toxic when the initial wolf spider study was done, the actual culprit was overlooked.

Yellow Sac Spider

In the 1960s, yellow sac spiders of the genus *Cheiracanthium* were implicated as causing dermonecrotic lesions in the United States and South Africa. Although in the United States these spiders caused necrosis when pressed into guinea pig flesh, all associations to necrosis in humans were circumstantial as the authors readily admitted (Spielman and Levi 1970). In South Africa, several papers blamed yellow sac spiders for dermonecrosis when none of the bites were verified and the alleged characteristic fang mark spread on bite victims was an anatomical impossibility

(Vetter et al. 2006). Studies involving 30 verified yellow sac spider bites showed pain, mild edema, mild erythema, and pruritis but no necrosis (Vetter et al. 2006; McKeown et al. 2014).

White-Tailed Spider

In Australia in the 1980s in a short abstract, white-tailed spiders of the genus *Lampona* were suggested to be the cause of necrotic arachnidism. This ignited a frenzy of publications with corroborative opinions in the Australian medical literature which was further exacerbated by the media. Once again, incrimination was made with circumstantial evidence and speculative bites. The few verified bites that had minor symptom development were dismissed as aberrant. Requests were made to develop antivenom. However, a study with 130 verified bites and no necrosis (Isbister and Gray 2003) reversed 20 years of incorrect attribution and showed that, once again, publications lacking evidence of spider involvement led to incorrect conclusions.

Hobo Spider

In 1987, hobo spiders, *Eratigena agrestis* (formerly *Tegenaria agrestis*), originating from Europe, were forcibly applied to test rabbits to inflict bites and were implicated in necrotic skin lesions in the Pacific Northwest of North America. However, the signs and symptoms of its alleged envenomation in humans were based on circumstantial evidence. Subsequently, cleanly collected venom injected into the same strain of rabbits resulted in no necrosis (Binford 2001). An editorial summarizing the literature of alleged hobo spider bites severely questioned the validity of hobo spider venom toxicity (Vetter and Isbister 2004). Neither the hobo spider nor its closely related congenerics are known to be toxic in their native Europe. Currently, the hobo spider is no longer considered to be a dermonecrotic agent unless future work can provide substantial evidence otherwise.

Spiders Are Not Vectors of Bacteria

Along with the diagnostic improvement of cases of suspected loxoscelism that has occurred with greater awareness of the list of differential diagnoses and recluse spider distribution, another area is the realization that spiders do not vector bacteria when they bite. Although this connection had been speculatively made in earlier literature, it was not supported by evidence. Studies have shown that bacteria does not survive on spider fangs and mouthparts long enough to be transferred (Atkinson et al. 1995) and that when rubbed in bacteria, spiders do not contaminate clean agar plates (Gaver-Wainwright et al. 2011). A study that data-mined 23 spider envenomation reports involving over 4,000 bite victims showed a conspicuous paucity of

infection (Vetter et al. 2015). Additionally, an American loxoscelism expert stated that recluse bites are "not exudative" even in patients not already treated with antibiotics (Anderson 1998) and pus in a wound is the first sign that should exclude loxoscelism in a dermonecrotic differential diagnoses (Rader et al. 2012). In general, the venoms of many animals (snakes, bees, scorpions, spiders) have antibacterial and antimicrobial activity. The evolutionary supposition is that for animals such as spiders, the venomous bite clears the prey of bacterial fauna before siphoning up nutrients when feeding.

Conclusions and Future Directions

Bites by widow spiders appear to be manageable with the current level of medical knowledge; signs and symptoms are fairly diagnostic with small chance for misdiagnoses. Black widow antivenom works well to ameliorate the effects of a widow bite. On the other hand, bites by recluse spiders are more difficult because of the wide range of manifestation of their envenomations and the lack of complete knowledge of the underlying pathophysiology of dermonecrosis. Diagnostic accuracy should improve with the ongoing development, expansion, and use of ELISA swabs for verifying recluse venom in dermonecrotic wounds along with the dissemination of the awareness that 90% of loxoscelism cases are of minor manifestation, that there are many non-spider etiologies that can be misdiagnosed as loxoscelism, and that there are dermatologic signs not typically found in recluse envenomations that have led to misdiagnoses in the past. The widespread recognition of MRSA as a major cause of skin and soft tissue injury by the medical community has helped stem the tide of loxoscelism misdiagnoses.

In addition, there has been a history of erroneous elevation of virtually harmless species to that of dynamic medical importance. Better dissemination and awareness of the aspects of these misdiagnosis scenarios should improve healthcare, especially if journals would only publish envenomation reports where a spider has been unequivocally proven to be involved in an envenomation (i.e., caught in the act of biting and identified by a qualified arachnologist or entomologist) instead of endorsing the speculation of idiopathic wounds as spider bites.

The next big challenge in the assessment and treatment of latrodectism will be to determine whether the antivenoms available are effective and whether this efficacy extends to all *Latrodectus* species. Newer products in development are likely to be expensive, so another need will be to assess the cost-effectiveness of these products. Unlike snakebite where fatalities or serious permanent injury often occurs, essentially all widow spider envenomations improve eventually (although it may take weeks in some cases).

For loxoscelism, the area that will probably be most illuminating in the future is in regard to the continued noteworthy research being performed by South American toxicologists, further elucidating the pathophysiology of the development of dermonecrotic and systemic reactions to the destructive enzymes.

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Envenomations Caused by Aquatic Animals in Europe and South America

15

Vidal Haddad Jr.

Contents

Abstract

The contact between humanity and coastal areas has increased in recent decades and thus also increased injuries by aquatic animals. In South America continent, which is bathed by the Atlantic and Pacific Oceans, there are injuries and envenomations caused by various aquatic animals. The animals that cause envenomations in Europe are similar to those that cause in the Western Atlantic in the American coast. Furthermore, the freshwater Basins in South America is very wide and have a unique fauna also associated with a series of injuries and envenomations. The highest percentages of injuries that occur in marine

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environments are associated with invertebrates such as sea urchins, jellyfish and Portuguese man-of-war (echinoderms and cnidarians), and venomous fish. In this chapter, we discuss the clinical manifestations caused by marine and freshwater animals of Europe and South America and emphasize therapeutic and preventive aspects of injuries.

Introduction

Until recently, studies on injuries caused by aquatic animals were sparse and insufficient for the achievement of effective diagnostic and therapeutic measures. Today we know that this type of injury is common throughout the world (about 1 in 1000 patients at the Emergency Centers in coastal cities) and protocols of treatment are capable of guiding Health teams in emergency situations (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

In South America continent, which is bathed by the Atlantic and Pacific Oceans, there are injuries and envenomations caused by various aquatic animals. The animals that cause envenomations in Europe are similar to those that cause in the Western Atlantic in the American coast. Furthermore, the freshwater Basins in South America is very wide and have a unique fauna also associated with a series of injuries and envenomations (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009). The injuries by marine animals in about 3500 patients in the of southeastern Brazil coast (Atlantic Ocean) were mainly caused by sea urchins (50%), cnidarians (25%), and toxic and traumatic fish of various species (25%) (Haddad 1999, 2000). About 2500 injuries caused by freshwater animals were also observed on the Tietê, Paraná, Paraguay, Araguaia, and Negro Rivers, in Brazil, of which almost all was caused by venomous and traumatic fish (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

Etiology and Pathophysiology

The injuries can be caused by various animals: Porifera or marine sponges can cause irritative dermatitis in the human skin and some European species are associated with injuries also in South America, such as the *Tedania* genus (Haddad et al. 2007). Cnidarians have radial structure with tentacles, most of which are carriers of the defense cells, the cnidocytes. These specialized cells contain shooting structures and inoculate into the skin of the victim neurotoxic and cardiotoxic toxins and allergenic proteins (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2002a, 2003a, 2007, 2009, 2010, 2013; Risk et al. 2012; Resgalla et al. 2011). The species present in the Atlantic Ocean and Mediterranean Sea associated with injuries in humans are the Portuguese man-of-war (*Physalia physalis*) and the scyphomedusas *Pelagia noctiluca* and *Aurelia aurita* (Haddad et al. 2007). In the Atlantic Ocean (America), the medical interest is on the Portuguese man-of-war, the cubomedusas

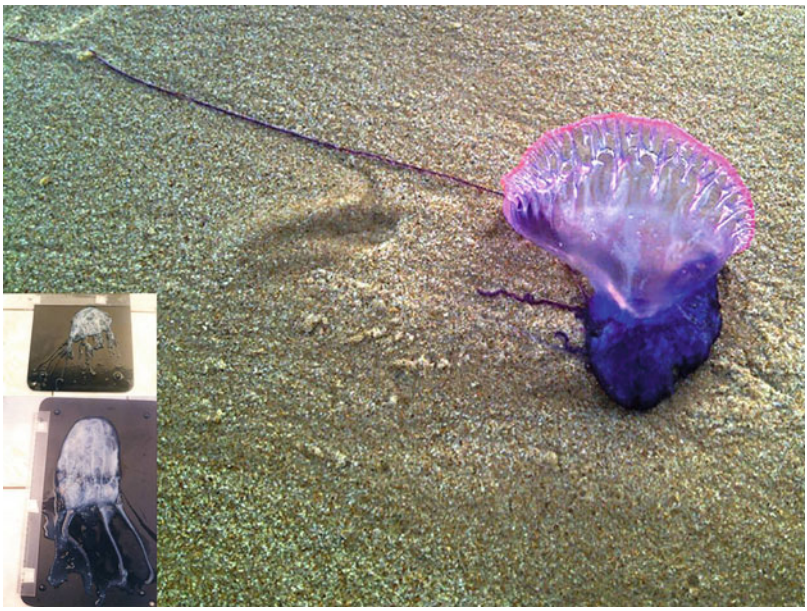

Fig. 1 *Left*, in detail: cubomedusas (*Chiropsalmus quadrumanus* and *Tamoya haplonema*). In the bigger image: the Portuguese man-of-war are common cnidarians of the South American Atlantic coasts and cause major accidents and systemic involvement potential (Photo: Vidal Haddad Junior)

(*Chiropsalmus quadrumanus* and *Tamoya haplonema*), and the species *Chrysaora lactea* and *Olindias sambaquiensis* (Fig. 1) (Haddad et al. 2002a, 2003a, 2007, 2010, 2013; Risk et al. 2012; Resgalla et al. 2011).

The sea urchins have a round body and hard calcium carbonate spines that penetrate deep into the feet of bathers in shallow waters. These spines may or may not inoculate toxins (Haddad 1999, 2000; Haddad et al. 2007). The most common species in the Atlantic Ocean are *Echinometra lucunter*, *Arbacia lixula*, *Arbaciella elegans*, *Centrostephanus longispinus*, *Diadema* sp., and *Paracentrotus lividus*. In Europe, closer to the swimmers, there is a greater likelihood of an injury by the species *Arbacia lixula* and/or *Paracentrotus lividus* while in South America (Atlantic coast) the main cause of injuries is the *Echinometra lucunter*, a species that does not cause envenomation (Fig. 2) (Haddad et al. 2002b, 2007; Resgalla et al. 2011; Rossetto et al. 2006; Haddad 2012).

Venomous fish have cells, producers of toxins in the skin, with a large percentage of these covering the stingers, fin rays, and other traumatogenic body structures. The most important venomous fish in Europe belong to Dasyatidae and Myliobatidae families (marine stingrays), Trachinidae (weeverfish), and Scorpaenidae (scorpionfish) families (Haddad et al. 2004, 2007, 2014; Garrone Neto and Haddad 2010). The main European species of stingrays are *Dasyatis centroura*, *D. violacea*, *Myliobatis aquila*, which are species of the Dasyatidae and Myliobatidae families (Fig. 3) (Haddad et al. 2004, 2007, 2014; Garrone Neto and Haddad 2010).

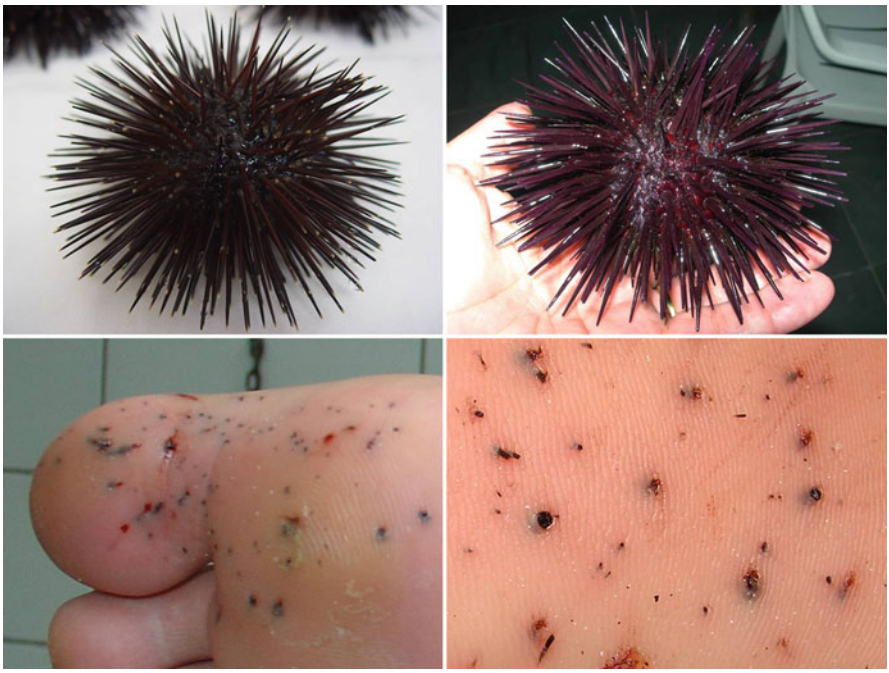

Fig. 2 Black sea urchins (*Echinometra lucunter*) and injuries in bather. Note the black spines penetrating the skin (Photo: Vidal Haddad Junior)

The catfish (suborder Siluroidei) are found in marine and freshwater environments around the world. They come in many shapes and sizes, their mouths have barbels, and they have no true scales on the skin. A great number of catfish have three serrated bony stings on the dorsal and pectoral fins which are used for defense against predators. The punctures caused by catfish are always painful, but when the fish present venomous stings, the pain is intense and it persists for longer. The pain can irradiate. There is local inflammation, but the manifestations cease after about six hours, without other complications (Fig. 3) (Haddad and Martins 2006; Haddad et al. 2008; Sazima et al. 2005).

Weeverfish are the main venomous fish in Europe, due to the frequency that cause envenomations, mainly in bathers. Although the venom is not as potent as the venom of the scorpionfish, they live in shallow waters and frequently are stepped by bathers, causing extremely painful wounds in humans. They are present in all the coastal waters of North Sea, in the Mediterranean and the North of Africa (Haddad et al. 2007).

Several species of the Scorpaenidae family can cause envenomations in South America and Europe, especially those of *Scorpaena* genus. The manifestations of these envenomations are very severe due to their venoms present systemic effects (Fig. 4) (Haddad et al. 2003b, 2015; Boletini-Santos et al. 2007).

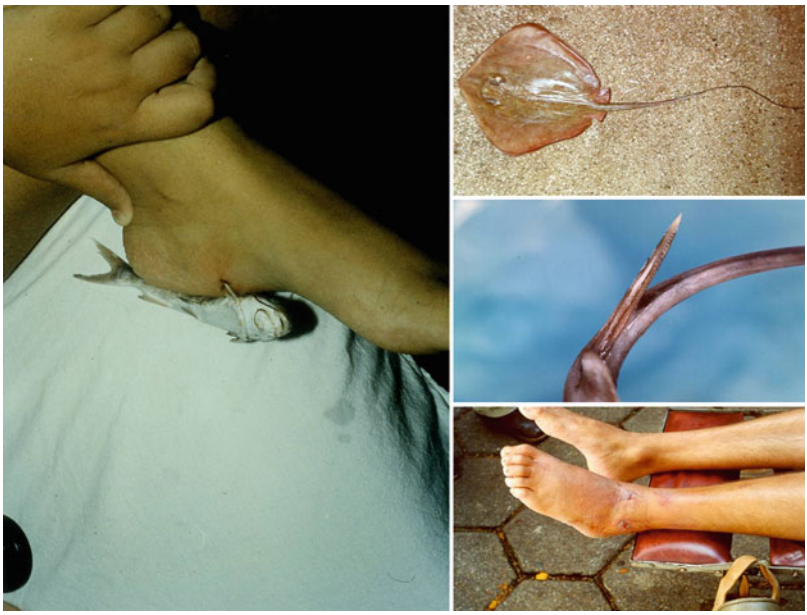

Fig. 3 Marine catfish and a typical accident in a bather. Small catfish are thrown on the beaches for fishermen causing injury to vacationers (Photo: Vidal Haddad Junior)

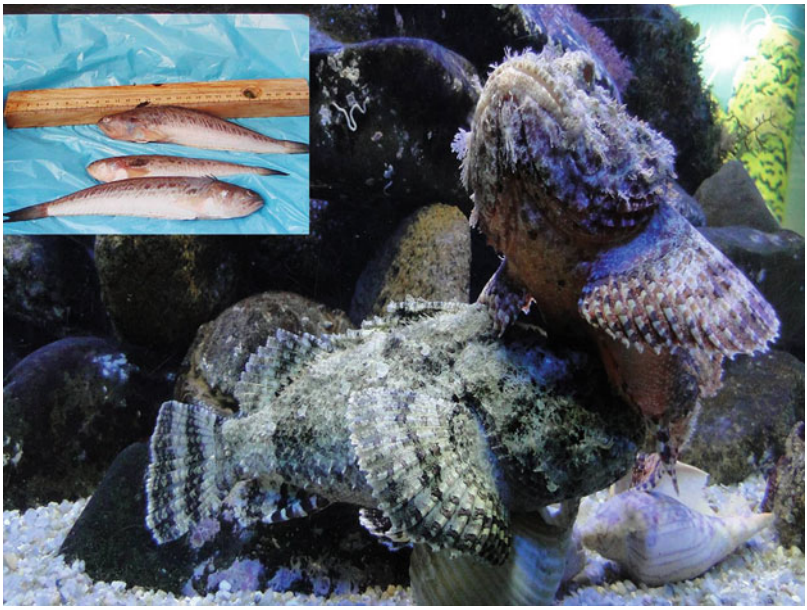

Fig. 4 Scorpionfish (*Scorpaena* sp.) and weeverfish (*detail*). The second venomous fish only exists in Europe and North of the Africa (Photo: Vidal Haddad Junior)

Clinical Aspects

Phylum Porifera (Sponges)

Marine sponges are animals that have a "skeleton" of calcium carbonate, espongin, silica, and irritant lime in their surface (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009). The sponges can cause an eczematous rash and the main species associated with the problem are of *Neofibularia* genus and the species *Tedania ignis* (fire sponge) and *Microciona proliferates*, the red sponge.

The skin disease caused by contact with marine sponges reminds an eczematous process, appearing 1–3 h after contact. The site shows inflammation, and blisters can occur with intense itching. The rash disappears within about 2 weeks. The most common location is on the hands (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

Freshwater sponges cause similar manifestations, but the rash is disseminated (Haddad 2008). Belonging to Demospongiae class, the freshwater sponges are popularly called *cauxi* in the Amazon regions and "powder-monkey of water" in the *cerrados* (savannah-like areas). The lesions caused by freshwater sponges are manifested by papular inflammation disseminated with exulcerations and crusts (Haddad 2008). The exposed areas of the skin and the mucous membranes can present the dermatitis, which occurs after the baths in the lakes and rivers.

Phylum Cnidaria (Jellyfish and Portuguese Man-of-War)

Cnidarians present a free stage of life, the jellyfish or medusa, with sexual reproduction and a fixed stage, the polyps, which reproduce asexually. Four classes are associated with human envenomations: Anthozoa (corals and anemones, which do not present the jellyfish stage), Hydrozoa, Scyphozoa, and Cubozoa (cubomedusas).

The cnidocytes are defense cells that internally have an organelle called nematocyst. These are small distal spicules kept rolled under pressure and that shoot by pressure changes and/or osmosis, inoculating their content on the victims (Haddad et al. 2002a).

The nematocyst's venom contains tetramine, 5-hydroxytryptamine, histamine, and serotonin, as well as thermolabile toxins of high molecular weight capable of changing the ionic permeability and to cause cardiac dysfunction (Haddad et al. 2002a, 2003a, 2010, 2013; Risk et al. 2012; Resgalla et al. 2011). The venom may still cause late hemolysis and renal failure (Haddad 1999) and to develop non-toxic protein can trigger allergic processes of variable severity (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2002a, 2003a, 2007, 2009, 2010, 2013; Risk et al. 2012; Resgalla et al. 2011).

The clinical manifestations of the envenomations depend on an immediate toxic effects and allergic actions that can be immediate and delayed. In the point of contact

with the cnidarian immediately arises intense pain with burning sensation and erythematous, urticaria-like, intersecting lines. After a few hours, there may be blisters and superficial necrosis. The pain persists for hours and systemic phenomena can arise with heart failure, respiratory failure, hypovolemic shock, and death in some cases.

Another complication is the immediate allergic reactions, such as angioedema and anaphylactic shock. The most frequent allergic observation is late allergic reactions, such as the persistence of lesions after 48 h, appearance of new lesions at distance, recurrent reactions (four or more), or onset of late contact dermatitis. Ingestion of jellyfish is observed in Oriental cuisine and has been associated with skin and gastrointestinal allergic conditions (Haddad et al. 2009, 2010).

The larvae of the thimble jellyfish (*Linuche unguiculata*), a small scyphomedusa, is involved in the genesis of the seabather's eruption, a papular, erythematous, and pruritic rash that develops in swimsuits covered areas of bathers (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

Envenomations by anemones show a localized, erythematous, irregular, and painful dermatitis, due to the short tentacles of anemones. False coral or fire-corals (*Millepora* sp.) are Hydrozoans which cause serious and extensive envenomations and may be cause of medical emergencies. True corals (Anthozoa) cause minor accidents but can cause deep wounds in bathers.

Accidents by true corals, anemones, and fire corals have no typical pattern. The lesions are irregular and may be round, oval, or a clear pattern (Haddad 2008). These contacts should be reminded to divers who approach the submarine substrate, and they are manifested by pain and local burning, in addition to rapid onset injuries.

The most common complication of injuries caused by cnidarians is the residual hyperpigmentation but may arise keloids, subcutaneous tissue atrophy, and skin gangrene. Cuts by corals can develop a granulomatous foreign body reaction (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

Phylum Annelida (Leeches and Polychaetes)

Leeches are worms that can reach 10 cm in length and that are found in fresh and salt water and even trees. Leeches firmly adhere to animals through oral suction, and jaws equipped with sharp teeth feed on blood of the victim but without compromising the health of the victim. In the saliva of the worms there is an anticoagulant (hirudin) which contributes to preventing the blood clotting in their gut. Skin allergies and infections can also occur in the point of the fixation (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2009).

Marine worms are similar to land worms. The most representative are marine "brush" worms. These worms can hurt unsuspecting divers by bites of powerful jaws with chitinous teeth or by introducing the spicules of the body, causing edema, papules, pain, and itching, which can cause skin necrosis (Haddad 2000, 2003, 2008; Haddad et al. 2009).

Phylum Mollusca (Octopuses and *Conus* Snails)

The Gastopoda of the *Conus* genus cause serious envenomations, inoculating toxins by a harpoon-like structure. The venom of the *Conus* genus is composed of low molecular weight neurotoxins capable of inducing neuromuscular blockade and progressive paralysis, including respiratory muscles, which can cause the death of the victim (the approximate number of human deaths caused by *Conus* snails is 50). The action is very rapid and occurs by blocking sodium, potassium, and calcium channel receivers present in the muscles and nerves (Haddad 2000, 2003, 2008; Haddad et al. 2009). There are no descriptions of envenomations by *Conus* snails in European coast (the *Conus mediterraneus* species is small and probably slightly toxic), but recently, an accident by *Conus regius* was described in Brazil (Haddad et al. 2009).

The author observed a case of poisoning by an octopus, mollusk present in the European and American coast, especially the common octopus (*Octopus vulgaris*). The patient presented neurotoxicity manifested by generalized paresthesias (including perioral tingling), malaise, dizziness, diarrhea, and muscle weakness after eating raw meat octopus (Japanese cuisine) (Haddad et al. 2009).

Phylum Echinodermata (Sea Urchins, Starfish, Sea Cucumbers)

The echinoderms are animals that present a rounded and hollow body covert by calcium carbonate spines. A species of starfish (the "crown-of-thorns") and some sea urchins have venoms with hypotensive, hemolytic, cardiotoxic, and neurotoxic effects (Haddad 2008, 2012; Haddad et al. 2002b; Rossetto et al. 2006). Sea cucumbers (*Holothuria*) produce holothurin, an irritatant of the skin and mucous membranes (Haddad 2008). The envenomation causes skin inflammation manifested by erythema, edema, papules, vesicles, and occasional necrosis.

The majority of injuries by sea urchins in Europe and South America are traumatic because the spines penetrate the human skin and break and displayed as dark points on the skin and there is no inoculation of the venom. The pain is moderate and occurs after compression (Haddad 2008, 2012; Haddad et al. 2002b; Rossetto et al. 2006). The plantar regions are commonly affected and secondary infections may arise, including the tetanus. Most of the spines is eliminated, but there may be formation of erythematous nodules (foreign body granulomas), a problem difficult to solve.

The black sea urchin (*Echinometra lucunter*) is the most common species in Brazil and there are no signs of envenomation after the injuries. Bathers are the main victims, but it is possible to observe scuba divers with spines or late nodules in hands, contrasting with the bathers who are injured on the feet when walking in shallow water between rocks.

Phylum Crustacea (Blue Crabs, Crabs, Shrimps, Barnacles, Lobsters, and Mantis Shrimps)

Crustaceans do not produce venoms, but they can cause traumatic injuries and severe allergic reactions. The lacerations caused by the claws of the animal are common, but contact dermatitis also can be observed, including urticaria and anaphylactic reactions. The traumatic injuries related to crustaceans are not serious and the majority is caused by crabs and blue crabs. The lesions provoked by mantis shrimp can be very serious, with extensive lacerations and intense bleeding (Haddad 2000, 2003, 2008; Haddad et al. 2007, 2009).

Phylum Insecta

Venomous aquatic insects are rare. The Balastomatidea are carnivores hemiptera, popularly known as Giant Water Bugs or Toe Biters, They present toxic saliva capable to kill frogs and small fish. There are species that can reach 10 cm in length. There are descriptions of envenomations caused by these arthropods in humans, manifested by intense pain and segmental paralysis (Haddad 2008).

Phylum Chordata

Venomous Fish

Venomous fish are those that present glandular structures producers of venom and an apparatus capable to inoculate these secretions, as rays of fins, bony stings, body spines, or teeth. The marine stingrays and catfishes are good examples of venomous fish.

Evolution of the Venom

The skin of fish is composed of a layer of scamous cells stratum of varied thickness and presents other cells, including secretory cells as the goblet cells, responsible for the cuticle, and the mucous external layer that reduces the turbulence during swimming and also protects against infections once it presents antibodies and the proteinaceous cells, responsible for the production of toxins. The main proteinaceous cells are the clavate cells (Haddad 2003, 2008; Haddad et al. 2009).

The toxins produced by the proteinaceous cells in the skin are the crinotoxins and they are not associated with venom apparatus. Crinotoxins stay in the fish's skin. The fish that present crinotoxins can kill another fish when in the same aquarium (Haddad 2003, 2008; Haddad et al. 2009). The functions of the crinotoxins are the protection against other organisms, as bacteria, fungi, and other invertebrates or predators (Haddad et al. 2009). The thickening of the epidermis with increase of number of the proteinaceous cells in the proximity of acute and harmful structures, as

for example the rays of fins of some fish, conferred greater effectiveness to the defense of the fish and to the system of inoculation of toxins, with obvious advantages for the venomous fish in their environment. The crinotoxins passed to be called of venoms and the adapted structures of inoculation, venom apparatuses (Haddad 2003, 2008; Haddad et al. 2009).

Marine Fish

A. GIMNURIDAE, MYLIOBATIDAE, RHINOPTERIDAE, and DASYATIDAE FAMILIES

The marine stingrays are cartilaginous fish that present one to four bone-like stings in caudal position, constituted of vasodentine (Fig. 3). The stingray's injuries are common when the bather step on the animal, which whip with the tail in defense. In Europe and South America, however, the injuries caused by stingrays occur in professional fishermen. The trauma is important and the epithelium sheath break and the venom, present in a thick mass glandular in the groves of the sting flows for the wound (Haddad et al. 2004, 2014; Garrone Neto and Haddad 2010).

The envenomation caused by marine stingrays is severe, presenting intense pain, local edema, erythema, and systemic manifestations associated to the pain, as malaise and cold sweating. The cutaneous necrosis is not rare. If the necrosis is established, the result can be chronic ulcers that only cure after months, what brings serious damages to the activities of the fishermen (Haddad et al. 2004, 2014; Garrone Neto and Haddad 2010).

B. ARIIDAE FAMILY

The catfish are the most important fish associated to human injuries, both in marine or freshwater environments. The accidents occur in fishermen.

Marine catfish cause injuries through serrated bony stings localized in anterior position to the dorsal and pectoral fins, covered by integumentary sheath with glandular venomous tissue (Fig. 3). The envenomation caused by a catfish presents intense pain, local pallor, malaise, and vomits (Haddad et al. 2004, 2008, 2014; Garrone Neto and Haddad 2010; Haddad and Martins 2006; Sazima et al. 2005) (Fig. 3). The envenomation is considered of moderate gravity, not compromising internal organs, but it impedes the work of the victim for some days, and it presents possibility of serious complications, as break and retention of fragments of the stings and severe bacterial infections (Haddad et al. 2004, 2008, 2014; Garrone Neto and Haddad 2010; Haddad and Martins 2006; Sazima et al. 2005).

C. SYNANCEIIDAE AND SCORPAENIDAE FAMILIES

The fish of the Synanceiidae and Scorpaenidae families are the most venomous fish in the world. The *Scorpaena* genus (Scorpaenidae) is present in South America and Europe and the *Pterois* genus is now an invader fish in Atlantic Ocean (Fig. 4) (Haddad 2008; Haddad et al. 2003b, 2015; Boletini-Santos et al. 2007).

The venom apparatus of the family Scorpaenidae are composed by 12–13 rays of the dorsal fin, three rays of the anal fin, and two rays of the pelvis spines.

The envenomation by scorpionfish is very serious and it causes intense, excruciating pain. There are discrete alterations in the point of the puncture(s) and the signs and symptoms are systemic, on the contrary of the other venomous fish of the Atlantic Ocean (Haddad 2008; Haddad et al. 2003b, 2015; Boletini-Santos et al. 2007). It is possible to observe malaise, fever, local adenopathy, respiratory and cardiac alterations, and hallucinations and seizures. In a series of 23 injuries, all the patients presented intense pain and systemic alterations, confirming the gravity of the injury (Haddad 2008; Haddad et al. 2003b, 2015; Boletini-Santos et al. 2007).

D. Batrachoididae Family

There are various species of fish of Batrachoididae family in Brazil, but the Thalassophryne genus is the most common, especially the species Thalassophryne nattereri, the niquin or miquim, the most associated with human envenoming in North and Northeast regions.

The envenoming caused by toadfish causes intense pain, local erythema and edema, cutaneous necrosis (rare), and it do not causes systemic manifestations, beyond those associates to the painful process (Haddad 2000, 2003, 2008).

E. Batrachoididade Family

All species of weeverfish have a similar envenomation apparatus formed by spines covered by venomous epithelium in the anterior part of the dorsal fin (4 to 8 spines) and preopercular ones (one on each side). Spines are not hollow and the venom flows to the wound when the epithelium is stretched (Fig. 4) (Haddad et al. 2007).

The victims have intense pain, cutaneous edema and erythema, nausea, vomit, and joints pain. Some reports also refer convulsion, cardiac arrhythmias, paralysis, arterial hypotension, and death, although these are associated to secondary bacterial infection. The only recorded death occurred in 1927, when a British fisherman suffered multiple stings. However, there is some suspicion that the victim may have died of other medical causes eventually exacerbated by the stings (Haddad et al. 2007).

Freshwater Fish

Pimelodidae Family

The freshwater catfish are Siluriform fish common in South American rivers and lakes.

The main genus associated to envenoming are the *Pimelodus* sp. The structures and mechanism of envenomation are very similar to those caused by marine catfish.

The penetration of the stinger and inoculation of the venom causes intense pain, edema, and erythema. The painful process is intense and fades after near 6 h, but the complications are common, as retention of fragments of the sting or bacterial infections (Haddad et al. 2004, 2008, 2014; Garrone Neto and Haddad 2010; Haddad and Martins 2006; Sazima et al. 2005).

Potamotrygonidae Family

The elasmobranches of the family Potamotrygonidae are the unique stingrays adapted to freshwater environment and only exist in South America. The envenomation causes intense pain and local inflammatory phenomena, ranging from erythema and edema to large skin necrosis (Haddad et al. 2003b, 2004, 2008, 2014, 2015; Garrone Neto and Haddad 2010; Haddad and Martins 2006; Sazima et al. 2005; Boletini-Santos et al. 2007).

Treatment

The pain caused by injuries by cnidarians can be controlled by cold sea water compresses or cold-packs applied alternate on the skin with vinegar compresses. It is important to remember that freshwater baths on the place shoots intact nematocysts adhered to the skin by osmosis and increases the envenomation (Table 1). If cardiac arrhythmias or respiratory phenomena are observed, there is urgent care indication once there is possibility of cardiogenic shock (rare) (Haddad 1999, 2000, 2003, 2008; Haddad et al. 2002a, 2003a, 2007, 2009, 2010, 2013; Risk et al. 2012; Resgalla et al. 2011).

The immediate extraction of the spines of sea urchins in the hospital is the correct behavior for such injuries, superficially scarifying the entry point with hypodermic needle caliber and using fine forceps to remove the spikes (Table 1). This injury can cause tetanus (Haddad et al. 2002b; Rossetto et al. 2006; Haddad 2012). There are no antivenom **sera** used regularly in South America or Europe for any kind of fish venom. The most effective measure is the immersion point engaged in hot water, but tolerable for 30–90 min (the venom seems to be heat labile and the hot water promotes vasodilatation in ischemia areas by the vasoconstriction caused by the venom). There is possibility of retention of fragments of barbs or spines and severe bacterial infections (Table 1) (Haddad et al. 2003b, 2004, 2008, 2014, 2015; Garrone Neto and Haddad 2010; Haddad and Martins 2006; Sazima et al. 2005; Boletini-Santos et al. 2007).

Conclusions

Venomous cnidarians (jellyfish and Portuguese man-of-war) cause severe pain and instantaneous linear urticaria-like dermatitis that reproduces the shape of the tentacles.

Accidents by sea urchins can be traumatic and not always provoke envenomations, but the permanence of the spines cause foreign body granulomas manifested by hyperkeratotic nodules.

Marine catfish cause the most common envenomation by the venomous fish, but it is possible to observe envenomations by stingrays (various genera), scorpionfish (*Scorpaena* sp.), toadfish (*Thalassophryne* sp.), weeverfish (*Echiichthys* and *Trachinus* sp.) and other.

Table 1 Algorithm for identification and treatment of injuries caused by aquatic animals of Europe and South America

Puncture wounds			Skin eruptions		Lacerated wounds	
Presence of stinger[a]	Presence of local spines[b]	Spines rarely present[a]	Urticariform plaques, edema, erythema, vesicles, necrosis[a]	Eczema-like lesions[b]	Cianotic or pale edges Fragments of stingers[a]	Lacerations with pain proportional to the wound[b]
Marine and freshwater catfish, stingrays	Sea urchins	Scorpionfish/ Toadfish	Jellyfish, Portuguese man-of-war, corals, anemones	Marine and freshwater sponges, marine worms, sea cucumbers	Marine and freshwater stingrays and catfish (occasionally punctures)	Sharks, barracudas, moray eels, piranhas and other traumatogenic fish.
1	**1**	**1**	**2**	**2**	**1**	**3**

Adapted from Haddad Jr V – Medical Emergencies Caused by Aquatic Animal: a zoological and clinical guide. Springer, New York, 2016

[a]Intense pain

[b]Moderate pain

1 – Immersion in hot water (test with your hand) for 30–90 min (about 50 ° C). Remove spikes, stinger, or glandular epithelium fragments and infiltrate local anesthetic. Persistence of symptoms in late stages: radiologic examination. Evaluation of tetanus prophylaxis

2 – Wash the injured site and make compresses with cold seawater (DO NOT USE FRESHWATER!). Apply vinegar (wash the area and make compresses). Analgesia (dipirone 1 amp. intramuscular)

3 – Intensive washing and surgical exploration. Use of antibiotics/prevention of the tetanus

In all cases of lacerated wounds, evaluate antibiotics: Cephalexin 2.0 g/day for 10 days or Amoxycillin clavulanate 1.5 g/day for 10 days

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Envenomation by Caterpillars

16

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Contents

Abstract

In recent years, injuries resulting from human contact with Lepidoptera species have become an increasing health problem in different regions around the world, occasionally with epidemic features. The majority of medically significant contacts with Lepidoptera occur with exposure to the larva form. Adverse effects are

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often treated with antipruritic and oral antihistamines. In the case of South American *Lonomia obliqua* caterpillar, however, it was necessary to develop a specific antivenom to neutralize the severe coagulation disorders in patients. Toxic principles related to the envenoming are found in setae secretion and hemolymph in larvae (erucism) and, in a few cases, in urticant hairs in adult moths (lepidopterism). These compounds are responsible for many toxic or allergic reactions in different organisms, such as local pain, inflammation, itching, irritation, and, in more severe cases, deforming arthritis, consumption coagulopathy, and hemorrhage. Despite clear documentation of adverse reaction in humans due to some species of Lepidoptera, very few studies were carried out as an attempt to understand the pathophysiological mechanism of the envenoming and toxins involved. This chapter reviews currently available information about Lepidoptera's toxic species and relates, when possible, its properties with clinical data that have been described so far.

Introduction

The order **Lepidoptera** includes **moths** and butterflies, with 125,000 to 150,000 described species on the planet (Gullan and Cranston 2005). Many **moths** and butterflies are recognized as agricultural pests, which infest several plantations on which their **larvae** feed and store food supplies (Mullen 2002). However, a number of **Lepidoptera** species, notably **moths**, can cause significant health problems in humans and other animals. From a total of 80 families of **Lepidoptera** which cause **envenoming**, approximately 12 families can inflict serious injuries after contact with humans. Among them, more than 150 species of **caterpillars** are known to cause adverse reactions (Diaz 2005; Gullan and Cranston 2005).

Most of syndromes caused by **Lepidoptera** are consequence from direct contact with the **venomous hairs** or **setae** (also called nettles, spicules, flechettes, and arrows) of the larval stage known as **caterpillar. These hairs or setae** act as a biological defense mechanism against natural predators. Exposure to **caterpillars** can result in a variety of reactions depending on the family and species involved, the degree of contact, the nature of the **toxin**, and the physical condition of the victim, e.g., health, age, and body weight (Diaz 2005; reviewed by Hossler 2009; Mullen 2002).

The order **Lepidoptera** undergoes a complete metamorphosis (holometabolous) during the biological life, divided in four phases that present distinct duration: egg, **larvae** or **caterpillar**, pupa or chrysalis, and adult form (imago) (Cardoso and Haddad 2005). In most species, **venomous setae** appear in the second and later **instars** of **larvae**. In some species, larval **setae** are transferred and incorporated into the **cocoons**, which may have a higher concentration of them than **caterpillars**, becoming a greater source of irritation. Emerging from the **cocoons**, adults of many species lose their irritating **setae** and become harmless (Lee et al. 1999).

Contacts normally occur by **accident** (Diaz 2005). Some factors can facilitate **accidents** with **Lepidoptera** species, such as environmental changes (reduction of

natural parasites by natural phenomena), human intervention (introduction of new species in a region without natural predators, deforestation, and intensive use of agrochemicals, among others), climatic change (seasonal warmth, strong winds, and dry weather), and artificial illumination (it congregates **moths** and, thus, causes **outbreaks**). Even inherent characteristics of some **caterpillars**, such as gregarious habits and beautiful colors, may increase the chances of direct contact and **outbreaks** (Diaz 2005; reviewed by Hossler 2009).

Lepidopterism is the term used to describe systemic signs and symptoms with or without **cutaneous reactions** (reviewed by Hossler 2009). **Erucism** is the term used to describe the **urticaria** (a vascular reaction of the skin in the form of papules or wheals) caused by the contact with specialized **setae**, toxic hemolymph, **cocoon**, or parts of certain species of **caterpillars** (Balit et al. 2003; Mullen 2002).

In general terms, **caterpillar envenomation** presents common symptoms that typically include local and systemic effects such as burning pain, erythema (blood-colored spots), swelling, nausea, itchiness, abdominal distress, and headache (reviewed by Hossler 2010a). In most severe cases, apart from **cutaneous reactions**, patients may develop conjunctivitis, bronchitis, anaphylactic reactions, **deforming arthritis**, or even bleeding disorder (Diaz 2005; Zannin et al. 2003). The ample range of effects makes it difficult to identify the **caterpillar involved** in the **envenoming**. Table 1 lists the most studied species of **Lepidoptera** in Australia, the Americas, and Europe, their taxonomic classification, distribution, and adverse effects on humans.

Caterpillar and their **toxins** have not yet been studied, nor their morphology and taxonomy, with the exception of *Euproctis*, *Thaumetopoea*, the South American *Lonomia* (*L. achelous* and *L. obliqua*) and *Premolis semirufa* (Diaz 2005). This chapter reviews the toxic aspects of secretions from some families of **moths and butterflies** found in Australia, the Americas, and Europe, frequently involved in human **accidents**.

Most species of medical interest belong to the Saturniidae, Limacodidae, Megalopygidae, Lasiocampidae, Arctiidae, Notodontidae, and Lymantriidae families (Diaz 2005; reviewed by Hossler 2009; Mullen 2002). There are at least five additional families with one or more of their members reported to cause limited adverse reactions in humans.

Arctiidae

Adults of this family known as tiger **moths** and **larvae** are usually covered with fairly dense **setae** of varying colors arising from raised warts in contrast to the normally bare, shiny head. Even though the family is cosmopolitan, the largest diversity of arctiid **moths** occurs in the Neotropical and Oriental regions, and several species are known to cause **erucism** or **lepidopterism** (Mullen 2002).

In severe cases, **caterpillars** from this family can cause occupational **erucism** among field workers, as in the case of the Brazilian caterpillar of *Premolis semirufa*, usually called as **pararama by natives** (Fig. 1), which feeds on *Hevea brasiliensis*, the rubber tree found in the Amazon forest. The caterpillar envenomation is known as "**pararama**-associated phalangeal periarthritis." Due to its importance as an

Table 1 **Lepidoptera** species commonly involved in human **accidents** and their distribution around the world

Lepidoptera family	Representative genus and species	Distribution	Adverse reaction in humans
Arctiidae	*Arctia* sp.	Worldwide	**Urticaria**
	Arctia caja	Holarctic	Pruritic papular eruptions and ophthalmia nodosa
	Estigmene acrea	Canada, United States, Central America	**Dermatitis**
	Euchaetes egle	United States	**Dermatitis**
	Eutane terminalis	Australia	**Dermatitis**
	Hyphantria cunea	United States, Europe, Japan	**Dermatitis**
	Lophocampa caryae	United States	Pruritic rush, oral contact require laryngoscopy to remove **setae**
	Manuela replana	Australia	**Dermatitis**
	Premolis semirufa	South America	**Pararamose** (**arthritis** and joint deformity)
	Pyrrharctia isabella	United States	**Dermatitis**
	Spilosoma glatignyi	Australia	**Dermatitis**
	Spilosoma lutea	United Kingdom, Europe	**Dermatitis**
	Spilosoma virginica	United States	**Dermatitis**
Lasiocampidae	*Dendrolimus* sp. (*also cocoons*)	China	**Dendrolimiasis**, **ophthalmia nodosa**, osteoporosis, sclerosis, and joint destruction
	Eriogaster lanestris	Palearctic	**Dermatitis**
	Malacosoma spp.	United States, southern Canada	**Dermatitis**
	Lasiocampa quercus	United Kingdom, Europe	**Dermatitis**
Limacodidae	*Acharia stimulea*	United States	Intense stinging with **urticaria** and vesiculation
	Adoneta spinuloides	United States	Mild to severe stinging, **dermatitis**
	Darna pallivitta	Hawaii, Southern Asia	Itch and wheal formation
	Doratifera sp.	Australia	Itch, **dermatitis**
	Euclea delphinii	Eastern United States	Mild to severe stinging, severe **dermatitis**
	Isa textula	United States	Mild to severe stinging, **dermatitis**

(*continued*)

Table 1 (continued)

Lepidoptera family	Representative genus and species	Distribution	Adverse reaction in humans
	Latoia lepida	Japan	Severe **dermatitis**
	Natada nasoni	United States	Itch, erythema, wheals
	Parasa chloris	United States	Itch, erythema, wheals
	Parasa indetermina	Eastern United States	Mild to severe stinging
	Thosea penthima	Australia	Crushing chest pain, severe local reaction
	Phobetron pithecium	North America	**Dermatitis**
	Sibine stimulea	United States	Severe **dermatitis**
Lymantriidae	*Acyphas leucomelas*	Australia	**Urticating dermatitis**, conjunctivitis, wheezing, bronchospasm
	Dasychira sp.	Europe	
	Euproctis sp.	Asia, Australia, Europe, United States	**Urticating dermatitis**
	Leptocneria reducta	Australia	**Urticating dermatitis**
	Lymantria dispar	Europe, United States	**Dermatitis**
	Orgyia sp.	Australia, United States, Canada	**Urticating dermatitis**
Megalopygidae	*Megalopyge opercularis.*	United States	Called pus caterpillar. Induce intense burning pain that can cause pseudoparalysis; also edema and erythema in the local of a characteristic grid-like hemorrhagic eruption
	Norape ovina	United States	Mild **stings**
	Podalia sp.	Central and South America	Severe dermatitis
Morphoidae	*Morpho* sp.	Mexico, Central and South America	**Dermatitis**
Notodontidae	*Anaphe venata*	Africa	Seasonal ataxia
	Epicoma sp.	Australia	Pruritic **dermatitis**
	Ochrogaster lunifer	Australia	Intense pruritic **dermatitis** or **ophthalmia nodosa**
	Thaumetopoea pityocampa	Asia, North Africa, Europe	**Dermatitis**, allergic reactions, anaphylaxis
	T. processionea	Europe	**Dermatitis**, conjunctivitis, bronchitis, ophthalmia nodosa, anaphylaxis
	T. wilkinsoni	Israel, Turkey, Asia	Severe vomiting, abdominal pain, and hypertension

(*continued*)

Table 1 (continued)

Lepidoptera family	Representative genus and species	Distribution	Adverse reaction in humans
Nymphalidae	*Hypolimnas misippus*	Asia, Africa, Central and South America, Australia	Mild **stings**
	Nymphalis antiopia	Holarctic	Mild **stings**
Saturniidae	*Automeris io*	United States	Stinging sensation, pruritic papulourticarial eruptions
	Dirphia sp.	South America	**Urticaria**, **inflammatory** response. Similar pathology than *H. maia*
	Hemileuca oliviae	United States	Stinging, itching, and swelling. Sneezing, rhinitis, bronchitis, or asthma due to inhalation. **Ophthalmia nodosa**
	Hemileuca oliviae	Northeastern Mexico, United States	Contact causes stinging, itching, and swelling. Inhalation of **hairs** can cause sneezing, rhinitis, bronchitis, or asthma. Ophthalmia nodosa
	H. maia	United States	Painful **dermatitis**, vesiculation, ecchymosis, edema, and occasional adenopathy or secondary bacterial infection
	Hylesia metabus (adult)	Central and South America	**Dermatitis**, pruritic papulourticarial or vesicular eruptions
	Lonomia achelous	Venezuela, Peru, Bolivia, Southern Brazil, French Guiana, Colombia	**Hemorrhagic syndrome**: consumption coagulopathy and **fibrinolysis**
	Lonomia obliqua	Argentina, Brazil, Uruguay	**Hemorrhagic syndrome**: consumption coagulopathy and secondary **fibrinolysis**

occupational disease, since the morbid condition tends to specially affect rubber tappers in jungle areas of the Brazilian State of Pará, this **caterpillar envenomation** was inserted into the "Manual of diagnosis and treatment of **envenomations**," released by the Brazilian Ministry of Health in 1992 (Saúde 1992).

The **accidental** contact with **caterpillar** small **setae** or with those of the **cocoon**, in most cases, instantly causes an intense itching, followed by symptoms of an acute **inflammation,** such as pain, heat, and redness, which resolve within hours or days, after the first **accident**. Chronic symptoms frequently occur in individuals after

Fig. 1 *Premolis semirufa* caterpillar in the last instar. On the *left* is the caterpillar with its small and large setae and on the *right* its cocoon covered with setae (Photograph: personal collection of Giselle Pidde)

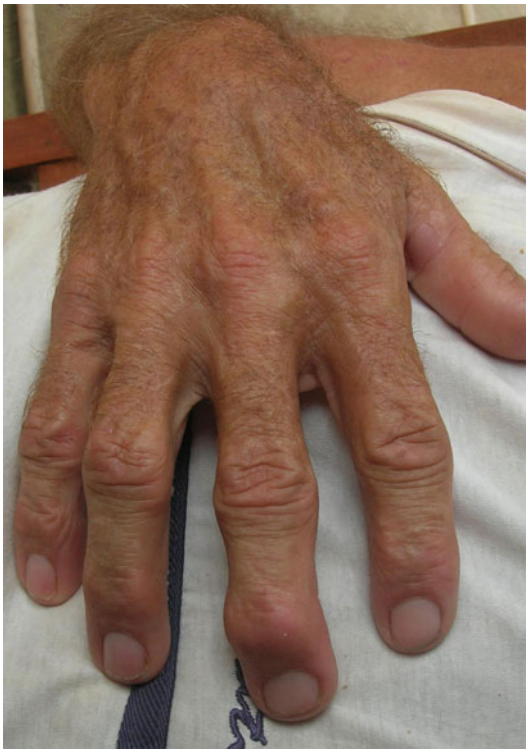

Fig. 2 Photo of a patient with pararamose. The *middle* finger of the *left* hand shows thickening of the distal phalange as a result of prolonged contact with the caterpillars in rubber plantation found in the Belterra, count of the city of Santarém, state of Pará, Brazil, during the 1970s (Photograph taken in 2007: courtesy of Fan Hui Wen, MD)

multiple **accidents** and are characterized by synovial membrane thickening, joint deformities, and chronic synovitis (mono or oligoarticular), which may result in ankyloses (Fig. 2). This chronic **inflammatory** reaction is called "**pararamose**" (Costa et al. 1995; Costa 2003).

Studies about "**pararamose**" are scarce not only regarding the characterization of toxic substances released by the **caterpillar setae** but also regarding molecular mechanisms involved in its pathogenesis. Recently, it was demonstrated that *Premolis semirufa* **setae**'s crude extract presented strong proteolytic activity and intense **inflammatory** process, characterized by the presence of neutrophils in the paw tissues of injected mice and a strong and specific antibody response (Villas-Boas et al. 2012). Following up this study, authors confirmed that the strong cellular and humoral immune responses induced by **setae**'s toxic components in a murine model consisted of an intense infiltration of neutrophils and macrophages to the **envenomation** site, proliferation/migration, and activation of T and B lymphocytes to the draining of the lymph nodes and elevated plasma levels of IL-6, IL-10, IL-12, IL-17, and IL-23 (Villas-Boas et al. 2013). More recently, the authors demonstrated that the extract was able to activate the human complement system, generating biologically active fragments, such as C3a, C4a, and C5a anaphylatoxins, and that it contains a serine protease, named Ps82, with similar activities to those of the whole extract. Thus, the complement system can play an important role in the **inflammatory** process presented in humans after **envenomation** (Villas-Boas et al. 2015).

So far, there is no effective treatment for **accidents** with **pararama**, since neither toxic components of the caterpillar's **setae** nor the **venom's action pathway** are completely known. However, systemic corticosteroids treatment has been used, in the belief that this would prevent the onset or attenuate the chronic disease (Cardoso and Haddad 2005).

The *Lophocampa caryae*, more commonly called the hickory tussock **moth**, occurs from Southeast Canada through United States' Maine and Minnesota, South to North Carolina, and Southwest Texas. It feeds on the leaves of hickories, birch, elm, and black locust trees. Toxic substances released by the **caterpillar setae** have not been elucidated. **Accidental** contact is thought to produce an irritant or **histamine** reaction in humans, although the exact mechanism and chemical composition have not yet been determined. This caterpillar caused 365 cases of pruritic rash over a two-year period in Pittsburgh, Pennsylvania, in 2001. Eight percent of children also had direct oral contact with **caterpillars**, resulting in crying, drooling, refusal to drink, and oral or lip irritation. Six children required direct laryngoscopy to remove **setae** as well as overnight hospital observation. In all cases, including those with oral involvement, reactions subsided within 24 h (Kuspis et al. 2001).

Ingestion of **caterpillars** has previously been reported in the literature. The insect identified in the majority of cases was *Lophocampa caryae*. In a more recent **accident** involving *L. caryae*, the first detailed case report describing the operative and anesthetic management of a 14-month-old baby girl after ingestion of a moth **cocoon** resulted in dramatic symptoms of irritability, drooling, and anorexia. Direct laryngoscopy, bronchoscopy, and esophagoscopy under general anesthesia revealed copious, tenaciously adherent, barbed **setae** embedded in her tongue and buccal mucosa. She received intravenous hydration overnight and increased her oral intake on the following morning, with a preference for solids over liquids. Approximately 25% and 95% of the **setae** disappeared within 24 h and 48 h after the ingestion, respectively, and the girl fully recovered with supportive care (Tripi et al. 2010).

The treatment is based on the removal of **setae** to decrease the intensity of symptoms. Therapy after removal focuses on pain control and alleviating the resultant rash, using oral antihistamines and/or corticosteroid administration. Further complications were not identified (Kuspis et al. 2001).

The caterpillar of the garden, tiger moth *Arctia caja*, grows up to 6 cm in length and is covered with stiff black **setae** that may cause a pruritic red scaly papular eruption and **ophthalmia nodosa**. This moth is found in Eurasia as well as in North America (Henwood and MacDonald 1983). The toxicity of *A. caja* was first suspected when a small child appeared to be severely stung on the leg by a specimen with which she was playing. The sensation resembled that of a wasp sting, and the skin and subcutaneous tissues became red and swollen. These effects persisted for several days, and, in one instance, subsequent vesiculation failed to heal for between 1 and 2 weeks and left a slight pigmentation (Bisset et al. 1960).

Ophthalmia nodosa is a well-documented chronic ocular condition characterized by initial conjunctivitis with subsequent panuveitis caused by corneal penetration and subsequent intraocular migration of **urticating hairs** or **setae** from any arthropod, typically **caterpillars, moths**, or tarantulas. It may be worsened by chorioretinitis, corneal granulomas, cataracts, glaucoma, and reduced visual acuity and should always be managed by an ophthalmologist (Sengupta et al. 2010).

Some components of *A. caja* have been isolated, such as acetylcholine, found in **caterpillar setae**, **moths**, and eggs (Frazer 1965). In addition, a pharmacologically active choline ester is present in very high concentration in the cervical (prothoracic) defensive **glands** and in the strongly smelling fluid secreted by the **glands**. It is generally assumed that these **glands** function in defense and that their secretion acts primarily through an effect on the taste or smell receptors of predators. Another substance, non-dialyzable and heat labile, that increases capillary permeability on intradermal injection and causes death on intravenous injection into guinea pigs is present in the abdominal tissues of *A. caja*. Its lethal action appears to be due to the constriction of bronchial smooth muscle (Bisset et al. 1960). Recent studies on the composition of the content found in the **setae** of *Arctia caja* are not described in the literature.

There are many other Arctiidae **caterpillars** that may cause **erucism** or **lepidopterism**. Mullen specifically mentions the genera *Eilema*, *Lithosia*, *Adolia*, *Callimorpha*, and *Parasemia* (Mullen 2002). In Australia, *Manulea replana*, *Eutane terminalis*, and *Spilosoma glatignyi* can cause **dermatitis**. In the United States, other potentially offensive species are *Hyphantria cunea* (fall webworm moth), *Spilosoma lutea*, *Spilosoma virginica*, *Euchaetes egle*, *Pyrrharctia isabella*, and *Estigmene acrea*.

Lasiocampidae

In its family, special attention is given to the **caterpillar** that causes a similar reaction to *Premolis semirufa* (**pararama**), characterized by prominent **arthritis** in association with pruritic **dermatitis**. The syndrome is a chronic form of **lepidopterism**

caused by direct contact with the hemolymph, **cocoon**, or **urticating setae** of the central Asian pine tree lappet **moths** or **caterpillars** *Dendrolimus* sp. This caterpillar has sharp **setae** associated with **venom glands** under their base (Huang 1991; Lawson and Liu 1986).

Clinical effects of the **dendrolimiasis**, which may be noted within 2 weeks of contact, include a maculopapular **dermatitis**, migratory polyarthritis, **inflammatory** polychondritis, **osteoarthritis**, and rarely acute scleritis. Generalized symptoms consist of pyrexia, anorexia, malaise, rigors, headache, or dizziness. The clinical course of this illness suggest an IgE-mediated acute phase secondary to **hypersensitivity** to foreign proteins followed by an autoimmune-mediated chronic phase involving bones and joints. Multiple factors are believed to be involved in the pathogenesis; however, the mechanisms of these reactions and the nature of the toxic substances are still poorly understood, such as in the case of **pararamose** (Huang 1991; Lawson and Liu 1986).

Ophthalmitis has also been reported in **accidents** with *Dendrolimus punctatus*, which is very common in Taiwan. In the report case, a 26-year-old experienced intense pain and tearing when the **caterpillar** fell into his right eye. Severe conjunctival injection, chemosis, and erosion of the cornea developed immediately. Numerous **setae** fragments were found to be embedded into the palpebral conjunctiva and deep cornea, extending into the anterior chamber near the anterior iris surface. After partial removal of the **setae**, the **inflammation** subsided and visual acuity returned to the preinjury level (Horng et al. 2000). The **urticating** protein of the pine processionary **caterpillar** has been identified as **thaumetopoein**, described in *Thaumetopoea pityocampa*, and a similar protein may exist in other **caterpillars** (reviewed by Vega et al. 2011).

Two European lasiocampids, the oak eggar *Lasiocampa quercus* and the small eggar *Eriogaster lanestris*, have been reported to cause widespread papular **urticaria** after direct or indirect exposure to the **caterpillar setae**. There are other species that cause adverse effects in humans worth mentioning: *Malacosoma americanum*, *Tolype* sp., and *Macrothylacia rubi*.

Limacodidae

This is a large, mostly tropical and subtropical family of **moths**, which occurs widely throughout the Neotropical, Ethiopian, Indo-Australian, and Palearctic regions. Approximately 50 species can be found in North America. The **larvae** are called slug **caterpillars** or nettle grubs, because of their shape and the fact that most species have **stinging setae** (Mullen 2002).

In the family Limacodidae, the most well-known **caterpillar** is the saddleback *Acharia stimulea*, which is common in the United States, from Texas to Florida. **Stings** are potent, and the contact causes immediate intense stinging associated with urtication. Vesiculation has also been reported (reviewed by Hossler 2010a).

Less intense **stings** are caused by other members of the Limacodidae found in the Eastern United States: *Natada nasoni*, *Parasa chloris*, *P. indetermina*, *Euclea*

delphinii, *Isa textula*, and *Adoneta spinuloides* (Mullen 2002). *Natada nasoni* **stings** cause moderately painful blanching erythema and wheals, followed by follicular prominence, as well as increased perspiration in the affected area. These symptoms remain for approximately 5 h and leave residual blanching red macules at the site of **envenomation** (reviewed by Hossler 2009).

In Hawaii, the stinging nettle **moth** *Darna pallivitta* was introduced in 2001 from Southeast Asia. The contact causes immediate stinging and wheal formation, which may take up to 5 days to heal. In Japan, *Latoia lepida* has caused several cases of **dermatitis**. The genus *Doratifera* contains several Australian **caterpillars** capable of human **stings,** and exposures to these **setae** have been reported to cause a reaction consisting of severe stinging, erythema and wheal formation at the site of the sting. This reaction is resolved within 24 h with 80% resolving within 12 h. Another Australian limacodid, the billygoat plum stinging **caterpillar** *Thosea penthima*, caused crushing chest pain and severe local reaction in one patient. The stinging ability of the genera *Microleon*, *Monema*, and *Scopelodes* was reported (Mullen 2002).

Treatment is entirely supportive with immediate washing of the site to remove toxic hemolymph, stripping of the affected region with adhesive duct tape, application of ice packs and administration of topical and oral antihistamines or intramuscular corticosteroids. Anaphylactic reactions are rare (Diaz 2005).

There are a few studies about the **venoms** of limacodid **caterpillars**. The **venom** of *Doratifera oxleyi* from Australia contains **histamine**, while the pain-producing activity of *Latoia consocia* has been separated into low molecular (LM) weight and high molecular (HM) weight fractions. The two LM fractions contain **histamine** and a polypeptide that produced slight pain in humans, and the two HM fractions contain one that caused wheal formation with erythema (HM-I) and the other that induced pharmacological effects consistent with endogenous mediator release (HM-II) (Kawamoto and Kumada 1984).

Lymantriidae

This family includes several genera spread worldwide, but not all species possess **urticating hairs**. Species of medical importance are found in the United States and Australia. Infestations have been reported for a few Lymantriidae species such as *Lymantria dispar*, several *Euproctis* species, and *Orgyia pseudotsugata* (reviewed by Hossler 2009). *L. dispar* and *E. chrysorrhoea* are two species introduced accidentally in the United States from Europe in 1869, becoming a serious forest pest facilitated by the lack of natural enemies (Mullen 2002).

Caterpillars of the genus *Euproctis* are distributed worldwide and are commonly known as brown-tail moth **caterpillars** (Diaz 2005). Despite cutaneous reactions caused by contact with the **hairs** of *E. chrysorrhoea* in the United States, such as erythema and edema within 5 h, allergic people may develop more serious reactions, including bruising, conjunctivitis, or rhinitis (Balit et al. 2001; reviewed by Hossler 2010b). In Southeastern Australia, *E. edwardsi* is the best-known caterpillar of

medical importance that causes pruritic **urticarial** wheals, papular eruptions, and **ophthalmia nodosa**. Similar symptoms were reported after the contact with the **cocoon**. Moreover, the caterpillar **hairs** are small enough to be airborne and affect exposed skin of people or by contaminating clothes. Similar to *Megalopyge opercularis* treatment, the primary treatment recommended is the removal of the fine **hairs** using a sticky tape, a very useful technique even as a diagnostic tool. After the **hair** removal, the treatment of **caterpillar dermatitis** is essentially symptomatic and supportive. The application of topical aspirin paste in the affected area appears as an effective treatment to reduce **histamine**-induced rash, presenting improvement within hours (Balit et al. 2001; reviewed by Hossler 2009).

The gypsy moth *Lymantria dispar* is well known for causing epidemic **dermatitis** in the Northeast United States when their population is large, especially from May to June (reviewed by Hossler 2010a; reviewed by Hossler 2010b; Mullen 2002). The gypsy moth feeds on a wide range of trees affecting large tracts of forest. Contact with the **setae** of this caterpillar may induce mild to moderate reactions within 8–12 h after the contact, ranging from erythema and papule formation to eye irritation, **inflammation** of the nasal passages, and shortness of breath. Rash may last 4–7 days. An **outbreak** involving this species was reported in 1981 in the Northeastern United States, resulting in many cases of pruritic **dermatitis**.

From Canada to Texas, *Orgyia leucostigma* was reported to cause **dermatitis** after contact with human skin, while the Douglas-fir tussock moth caterpillar *Orgyia pseudotsugata* caused several **outbreaks** of papular **urticaria** and **dermatitis** in the Pacific Northwest of the United States. In Australia, there are other lymantriid species able to cause **erucism:** *Acyphas leucomelas*, *Leptocneria reducta*, *Porthesia lutea*, and *Orgyia anartoides*. In Japan, *E. flava* caused an **outbreak** of pruritic **dermatitis** in 1955, and *Dasychira pudibunda* was reported to provoke **dermatitis** in Europe.

Literature about the **toxin** content of lymantriid species is still scarce. **Histamine** has been isolated from **caterpillars** of *E. chrysorrhoea* and *L. dispar* (reviewed by Hossler 2010b; Mullen 2002). Kallikrein-like serine esterases, able to activate the kinin sequence, were described as the major factors in the elicitation of clinical symptoms observed after contact with *Euproctis* **caterpillars** (Bleumink et al. 1982). **Venoms** of *E. chrysorrhoea* and *E. subflava* **caterpillars** have partially been characterized and contain ester hydrolases, phospholipase, and serine proteases, including kallikrein (Cardoso and Haddad 2005; Mullen 2002). It is interesting to notice the presence of fibrinolytic, hemolytic, and anticomplement activities in **hair** extracts of *E. chrysorrhoea* **caterpillar**. Moreover, the presence of phospholipase A and esterase in both species is probably related to the **cutaneous reaction** observed in patients (De Jong et al. 1982).

Megalopygidae

Called flannel **moths**, referring to the densely hairy adults and **larvae**, this family is widely distributed throughout the New World and inflict neuropathically painful **stings**, often during seasonal **outbreaks** in the late summer or early fall in temperate

regions and during both spring and fall in tropical regions. Although there are many species of **venomous** *Megalopyge* **caterpillars**, *Megalopyge opercularis* – popularly known as the puss caterpillar, given its extremely hairy appearance, or wooly slug ("el perrito" or "little dog" in Central and South America) – is the most widely distributed and carefully studied species in the United States and Latin America. These **caterpillars** feed on many trees and shrubs, including apple, elm, hackberry, maple, pecan, oak, sycamore, and most citrus trees. Upon contact, that may be multiple when the **caterpillars** fall from trees, the hollow **setae**, connected to a **venom gland** at their base, release toxic **venom** with uncharacterized proteolytic components (Diaz 2005).

Similar to the **stings** of the saddleback **moth caterpillar**, puss caterpillar **stings** are instantaneously painful and quickly followed by localized edema, erythema, and pain radiating proximally toward regional lymph nodes. Following the initial sting with radiating pain, the puss caterpillar sting site develops into a distinct lesion surrounded by an erythematous halo with an inner footprint or grid-like pattern reflecting each broken **setae** hypodermic injection point. The individual puncture sites often become hemorrhagic and vesicular and may later become pustular and coalesce into large bullous lesions. Although no deaths have been reported, systemic manifestations of puss caterpillar **stings** may occur and include headache, fever, nausea, vomiting, tachycardia, hypotension, seizures, and, rarely, acute abdominal pain and myospasm mimicking acute appendicitis or latrodectism (Diaz 2005; Pinson and Morgan 1991).

The treatment is supportive and includes adhesive stripping of retained broken **setae**, topical and systemic antihistamines, and parenteral corticosteroids for severe, prolonged reactions. Subcutaneous epinephrine may be indicated for anaphylactic reactions with peripheral vasodilation and hypotension (reviewed by Hossler 2009; Pinson and Morgan 1991). There is still little knowledge about the puss **caterpillar**'s **venom** and its specific effects on human tissue. A closely related species of the puss caterpillar, the *Megalopyge crispata* or *Lagoa crispata* (flannel **moth caterpillar**), is more widespread throughout the United States. *M. crispata* is similar to *M. opercularis* in appearance, but it lacks the taillike structure found on the puss caterpillar. The sting of *M. crispata* is much less severe than that of *M. opercularis*. Cases of **ophthalmitis** caused by **caterpillar setae** were reported by Shibui et al. (1997). In one case, vitrectomy with surgical removal of the **setae** was required to eliminate the **inflammation**.

The major food source of *M. crispata* is oak leaves (shin oak). The high tannin content of this food source results in **setae** extracts rich in oak tannins. These extracts loose enzyme-**toxin** activity and form inactive gel filtration protein components. No hyaluronidase, protease, or phosphohydrolase activity was detected in protein fractions (Lamdin et al. 2000).

Other *Megalopyge* species, including *Megalopyge urens*, *Megalopyge lanata*, and *Megalopyge krugii*, are found in Central and South America and are also capable of severe **stings**. The **caterpillar** of the flannel **moth**, *Norape ovina*, found from Virginia to Missouri and across the Southeastern United States, also causes mild **stings** (Henwood and MacDonald 1983; reviewed by Hossler 2009).

Another puss **caterpillar** involved in **accidents** is the genus *Podalia*. A case of a 33-year-old man that had an **accident** with this caterpillar was reported in Brazil. The diagnosis was obtained from what the patient told the doctors, clinical manifestations and the identification of the **caterpillar.** The patient presented severe pain on the spot of the sting, with itching from the fourth to the seventh day after contact. Treatment involved oral medication of anti-**inflammatory** and analgesic. Pain persisted for 24 days, accompanied by hyperemia in the forearm and pectoral region, followed by intense **dermatitis**, interpreted as related to the toxic effect of the **venom** (Espindula et al. 2009).

Morphoidae

This family includes neotropical species all of which are butterflies as adults. Adult wings are covered with bright blue-colored and iridescent scales. **Caterpillars** of several species of *Morpho* are known to cause **urticarial dermatitis,** even though only a few cases have been reported involving *Morpho achillaena*, *M. anaxibia*, *M. cypris*, *M. hercules*, *M. laertes*, *M. menelaus*, and *M. rhetenor*. These species of *Morpho* butterflies are found in Mexico and Central and South America. The **accidental** encounters with the **caterpillars** are high in summer when larval population is largest. Little is known about the nature of the **urticating structures**.

Notodontidae

Known as processionary tree **caterpillars**, because of the way in which the **larvae** move head to tail from their nests at the bottom of the tree to the top of the tree, the European *Thaumetopoea* **caterpillars** and the Australian *Ochrogaster lunifer* are all gray-black with slightly raised dark red to brown tubercles and fine **setae** varying from white (*T. pityocampa*) to black (*O. lunifer*). The **venom** is drawn by capillary action into the tip of the **setae** from tiny **glands** in the epidermis (Diaz 2005).

The European pine processionary **caterpillar** (*T. pityocampa*) is found in all Mediterranean countries, including those of North Africa. The European oak processionary **caterpillar** (*T. processionea*) has a wider distribution and ranges from Northern Europe to North Africa. The American pine processionary **caterpillar** (*T. wilkinsoni*) ranges from Southern Canada to Mexico and is an unusual cause of **ophthalmia nodosa** and blepharoconjunctivitis in the United States. The Australian processionary **caterpillars** include coastal ground-dwelling *Ochrogaster* species that feed on *Acacia* species shrubs and inland canopy-nesting *Ochrogaster* species that feed on *Acacia* and *Eucalyptus* trees (Diaz 2005).

C**aterpillars** of this family frequently cause **outbreaks** of **caterpillar dermatitis**, **ophthalmia nodosa**, contact **urticaria**, and rarely respiratory signs and anaphylactic reactions through IgE-mediated or non-IgE-mediated mechanisms after exposures to their **urticating setae**, which can be airborne. Despite having few studies on the composition of the **caterpillars**' **venom**, it was demonstrated that the extracts from

the **setae** of the *T. pityocampa* contain a non-IgE-mediated mast cell degranulator protein, **thaumetopoein**, with a molecular weight of 28 kDa, composed of two subunits of 13 and 15 kDa, and that it is a potent skin irritant and systemic **histamine** and kinin releaser. Subsequently, the presence of IgE directed to **allergens** from the pine processionary caterpillar venom extract was reported, and **thaumetopoein** was described as an 18-kDa protein (reviewed by Vega et al. 2011). Moneo et al. (2003) isolated a 15-kD IgE-binding protein in crude larval extracts of this **caterpillar**, Tha p 1, which is a major **caterpillar allergen**. A third protein named Tha p 2, unrelated to Tha p 1, was identified, and the cDNA encoding the complete polypeptide was amplified and sequenced (Rodriguez-Mahillo et al. 2012).

Other important members of Notodontidae include the Australian **caterpillar** *Ochrogaster lunifer* (bag-shelter **moth** or processionary **caterpillar**) and *Ochrogaster contraria* of New Guinea and Australia, which can cause an intense pruritic **dermatitis** or **ophthalmia nodosa**, and the Australian genera *Epicoma* and *Trichiocercus*.

The treatment of the processionary **caterpillar envenoming** is entirely supportive and follows generally recommended management strategies of washing loose **urticating setae** off the skin, stripping off skin-embedded **setae** and applying cooling icepacks and topical antihistamines and corticosteroids. Nebulized and parenteral bronchodilators may be indicated for asthmatic bronchitis with bronchospasm and wheezing following exposures to aerosols containing **urticating setae** (Diaz 2005; reviewed by Vega et al. 2011).

Nymphalidae

Like Morphoidae family, species of the Nymphalidae family are butterflies as adult. Most of the butterflies in North America belong to this family, but only the caterpillar of *Nymphalis antiopa* (*mourning cloak butterfly* in the United States; *Camberwell beauty* in the United Kingdom) was reported to contain **urticating setae** capable of mild **stings**. This species was accidentally introduced in United States from Europe and can now be found throughout Eastern United States and Canada. Another **stinging caterpillar** from this family is *Hypolimnas misippus* (Danaid eggfly), found in Southeast Asia, Africa, the tropical Americas, and Australia.

Saturniidae

This family presents about 2,400 species around the world (Van Nieukerken et al. 2011), but only a few have medical importance, specially associated to the larval stage (**erucism**). Despite being rare, cases of **lepidopterism** occur in tropical areas in South America and Caribbean region, especially during the rainy season, and most of the reported cases involve adult moth of the genus *Hylesia* (Mullen 2002). Approximately 110 species of the *Hylesia* genus have been described in the Americas. Although rare, contact with abdominal **hairs** of *Hylesia* moth causes a

severe pruritic, erythematous and papulovesicular **dermatitis** and eye lesions. Inhalation of body **hairs** can also cause irritation and **inflammation** of the respiratory tract. Atopic bronchitis and acute pharyngitis may appear in some cases. Concerning *Hylesia* species, only female **moths** can cause the disease, especially during mating season, when **hairs** easily detach from the body, or during oviposition to protect the eggs. Males do not possess **urticating hairs**. The dermal reaction lasts typically from 5 to 10 days. However, in some cases, the **dermatitis** may persist for months. In Brazil, skin contact with *H. paulex* adult moth causes severe itching with maculopapular lesions. Moreover, histopathological evaluation of the papular lesions revealed the presence of leukocytoclastic vasculitis, an antibody-mediated **inflammatory** process associated with necrosis of small vessels with either deposition or formation in situ of immune complexes in the vessel wall and complement activation (Benvenuti et al. 1998). In most of the cases of **accidents** by adult moth, spontaneous recovery took place. Usually, treatment is symptomatic and medical literature has diverse therapeutic options. For example, **accidents** by *Hylesia* can be controlled by oral antihistamine drugs and topical corticosteroids to alleviate symptoms. In extreme cases, it is recommended to use systemic corticosteroids (Cardoso and Haddad 2005).

Most of the **stinging caterpillars** responsible for **erucism** in the Saturniidae family belong to the subfamily Hemileucinae (reviewed by Hossler 2009; Mullen 2002). In North America, the genera *Automeris* and *Hemileuca* are the most important species causing **urticating dermatitis**. In Eastern United States and Canada, *Automeris io* causes a **stinging** sensation and pruritic papulourticarial wheals, healing few hours later. All *Automeris* genera are **urticating,** causing different degrees of pain depending on the type of **setae**. In the United States, contact with the buck moth *Hemileuca maia* and other *Hemileuca* species also causes a painful **dermatitis**, often followed by local swelling and **histamine**-induced edema. Moreover, *H. maia* also represent a serious threat to cattle. In some cases, inhalation of aerosolized **hairs** can cause respiratory problems like bronchitis and asthma. In more severe cases, **ophthalmia nodosa** has also been reported. The recommended treatment for system symptoms caused by *Automeris* or *Hemileuca* generally includes topic use of cooling icepacks, antihistamines, and corticosteroids (Diaz 2005).

In South America, especially in Brazil and Peru, some *Automeris* species cause **urticating dermatitis**. In the same region, other Saturniidae species that cause **urticaria** in larva form are *Dirphia multicolor* and *D. Sabina* (Mullen 2002). Regarding the characterization of toxic substances released by Saturniidae, little is known about the toxic content released by **caterpillars** and the mechanism involved in the pathogenesis of **envenoming**. However, it seems that **histamine** may play a role in the symptoms, like itching sensation and local edema. That is why it is recommended to the majority of cases of **erucism** the use of anti**histamine**s (Valle et al. 1954). **Histamine** has been isolated from **caterpillar setae** of genus *Dirphia* and from **hairs** of adult *Hylesia* **moth** (Dinehart et al. 1987).

In spite of the fact that most lepidopterans may cause dermatologic or systemic symptoms in humans, the Saturniidae family includes one of the most important genera of medical interest in the world, responsible to cause dramatic damages in the

Fig. 3 *Lonomia obliqua* caterpillar in the 5th instar. Accidents involving *L. obliqua* larvae occur within instars 4–6. The caterpillar has a *green-brownish* color and prominent structures named *scoli* formed for multiple setae (Photographs: personal collection of Miryam P. Alvarez-Flores)

human blood coagulation. Contact with *Lonomia* caterpillar **setae** (Fig. 3) leads to a **hemorrhagic syndrome**, and, if the victim is not quickly treated, pulmonary and intracranial hemorrhages and acute renal failure frequently lead to death. **Envenomation** by *Lonomia* genus is known as **lonomism.** Although 26 species of the genus *Lonomia* are spread in South America, only two species were reported to provoke severe coagulation disorders in human beings (Alvarez-Flores et al. 2010; Chudzinski-Tavassi and Alvarez-Flores 2013; Zannin et al. 2003).

In 1989, an **outbreak** of **accidents** with *L. obliqua* became a serious public health threat in Southern Brazil due to high fatality rates and to the **hemorrhagic syndrome**, the most important clinical complication (Saúde 1992). *L. achelous* is found mainly in Venezuela and in Northern Brazil, whereas *L obliqua* is found in Southern Brazil. Both species provoke a **consumption coagulopathy**, similar to a disseminated intravascular coagulation (DIC), which takes place between 6 and 72 h after **envenomation. Accidents** involving *L. obliqua* **larvae** occur within **instars** 4–6. The first symptoms after the contact with the caterpillar **setae** are an intense burning sensation followed by erythema, edema, heat, and blisters.

Several studies have demonstrated differences in the toxic content of *L. obliqua* and *L. achelous* caterpillar **setae**. Although the **venoms** of both species provoke similar clinical effects, the mechanism by which they cause adverse effects seems to be different. Several studies have shown that the effect of *L. obliqua* **venom** is mediated mainly by thrombin formation secondary to the action of procoagulant **toxins** at different levels of the coagulation cascade, whereas *L. achelous* **venom** activates both **fibrinolysis** and clotting pathways (Alvarez-Flores et al. 2010; Arocha-Piñango et al. 2000; Zannin et al. 2003). While coagulation factor XIII is slightly reduced in *L. obliqua* patients, FXIII is drastically reduced in *L. achelous* patients due to a factor XIII degradation component present in that **venom**. In the case of envenomed patients by *L. achelous*, Arocha-Piñango et al. (2011) have suggested that the hemorrhagic syndrome is primarily caused by the activation of **fibrinolysis** and a mild disseminated intravascular coagulation that can be corrected with the addition of normal plasma. However, *L. obliqua* **venom** is not responsible

for factor XIII inhibition or degradation, and those differences can be observed in patients as stated above (Chudzinski-Tavassi and Alvarez-Flores 2013).

Several compounds ("lonomins") were isolated from fluids of *L. achelous*, including a plasminogen activator and a direct fibrinolytic **toxins**, as well as **toxins** that activate factors II, V, and XIII. In that case, antifibrinolytic drugs could neutralize either fibrinolytic **toxins** or the primary activation of **fibrinolysis** (Arocha-Piñango et al. 2000).

L. obliqua **setae** extract does not contain enzymes capable of degrading cross-linked fibrin in vitro and does not activate plasminogen (Alvarez-Flores et al. 2010; Chudzinski-Tavassi and Alvarez-Flores 2013). Two procoagulant factors were purified from *L. obliqua* caterpillar **setae**: a factor X activator (Losac, *Lonomia obliqua* Stuart factor activator) and a prothrombin activator (Lopap, *Lonomia obliqua* prothrombin activator protease (Alvarez-Flores et al. 2006; Reis et al. 2001a). Lopap seems to contribute with the **consumption coagulopathy** observed in vivo (Reis et al. 2001b). Clinical and experimental studies suggest a combination of events, which lead to hemorrhagic syndrome in *L. obliqua* patients (Zannin et al. 2003): (i) a disseminated intravascular coagulation due to thrombin generation, (ii) an intravascular fibrin formation, and (iii) a secondary activation of the fibrinolytic system.

Treatment after contact with *L. achelous* consists of administration of antifibrinolytic drugs such as ε-amino caproic acid and aprotinin (Arocha-Piñango et al. 2011). However, antifibrinolytic agents are not recommended in *L. obliqua* **envenomation**, because those drugs do not neutralize the procoagulant **toxins** present in *L. obliqua*, and, when coagulation consumption is taking place, they could even exacerbate coagulation disturbances (Saúde 1992). The only effective treatment for reestablishing the coagulation parameters in poisoned patients by *L. obliqua* is the specific **anti-lonomic antivenom** produced by the Butantan Institute in Brazil (Da Silva et al. 1996).

Other Families

There are other **Lepidoptera** families including species reported to cause adverse effects in humans: Zygaenidae, Anthelidae, and Pyralidae (Balit et al. 2004; reviewed by Hossler 2009). *Neoproctis floridana* and *Harrisina americana* from the Zygaenidae family can cause mild **stings**. Several Australian genera within this family may also cause **stings**. Scales from the Australian moth *Scirpophaga innotata* (family Pyralidae) can cause hay fever-like symptoms in exposed individuals.

The Anthelidae family contains species responsible to cause severe skin irritation. They are found throughout Australia. The caterpillar and the **cocoon** of *Chelepteryx collesi* (white-stemmed gum moth) bear stiff **hairs** capable of penetrating the skin causing mechanical injuries and pain in different intensities during the first hour, followed by swelling, popular **dermatitis**, **urticarial**, or angioedema (reviewed by Hossler 2010b). A systemic allergic reaction was reported only in two cases, and the complete removal of the **hairs** is not recommended, especially in young children, due to the difficulty of removing the hairs (1–2 mm) that can become very stressful

for them. Moreover, the majority of hairs eventually fell out and the remaining hairs caused no sequelae (Balit et al. 2004). *Anthela nicothoe*, an Australian **anthelid** known as *hairy mary caterpillar*, causes **urticating dermatitis**. Several members of this genus have also been implicated in **ophthalmia nodosa**.

Conclusion and Future Directions

C**aterpillars** use their colorful body, odd shapes, and **setae** to protect and conceal themselves in nature; contact with humans is **accidental**. There are about 12 families of **moths** and butterflies worldwide that can cause serious injuries in human, among which are **urticating dermatitis**, atopic asthma, **deforming arthritis**, **consumption coagulopathy**, renal failure, and intracerebral hemorrhage. Children suffer more often than adults from airway problems and general symptoms, such as fever and malaise. However, most **caterpillar** exposures can be prevented by caution and individual protection taken during peak larva seasons.

Some studies suggest that mechanical irritation, **hypersensitivity**, and **pro-inflammatory** reactions to antigens from the **setae** that may be barbed or branched can contribute to the reaction. However, little is known about the composition of the **venoms**, which varies among different species of **caterpillars** and ample studies are yet to be done in order to discover the mechanisms of local and systemic reactions caused by the **setae** in the human body.

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Part V

Clinical Toxinology in Australia, Europe, and Americas: Exotic Envenomation

Exotic Envenomation in the United States 17

Steven A. Seifert

Contents

Abstract

Venomous snakes not native to the USA exist in zoos, aquariums, serpentariums, academic, public and quasi-public institutions, and private collections, which may or may not be legal, may or may not be known to healthcare providers in their region, and for which there may or may not be preparations for an envenomation. Envenomations by nonnative species pose challenges to every element of the healthcare system. A variety of response systems have been developed in the USA and other countries. A preexisting response system can optimize patient outcomes and lessen stresses on health delivery systems. The specifics of an optimal systematic nonnative envenomation response program will differ by

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geographic region and healthcare system considerations but should address common issues encountered in these cases regarding provider unfamiliarity, antivenom acquisition and expert consultation. In the USA, a system of antivenom availability through zoos, strategic antivenom geographic placement, information resources through the Antivenom Index and regional poison centers, as well as a national expert consultant panel constitute the current response system.

Introduction

Zoos, serpentariums, aquariums, academic, public and quasi-public institutions, and private collectors acquire and house nonnative also called "exotic" venomous species. These collections may or may not be legal, may or may not be known to healthcare providers in their region, and there may or may not be preparations for an envenomation. Among these various collections are numerous and varied species of venomous animals. In general, preparing for and responding to envenomations by nonnative species pose many challenges to those who keep venomous animals as well as to healthcare providers and their institutions. This is a global problem, with reports of nonnative envenomations found worldwide. A variety of response systems have been developed, usually at a national level. This chapter will review the experience of nonnative envenomations in the United States (USA), mostly through reports to the National Poison Data System (NPDS), the current USA response system, as well as those in other countries.

Scope of Nonnative Envenomations in the USA

Epidemiology of Nonnative Envenomations in the USA

Overview

Zoos, serpentariums, aquariums, academic, and other public and quasi-public institutions collect, house, and display venomous animals legally and openly, so that healthcare providers in their regions are aware of the variety of species that may be involved in an envenomation and can prepare for such eventualities by the creation of species-specific response protocols.

Bites at Zoos, Academic Institutions, and by Private Collectors

A recall survey of North American Zoos found 21 of 30 responding zoos reporting 31 staff bites over a 26-year period (Card and Roberts 1996). In a similar recall survey of USA academic institutions, 74 of 130 queried institutions responded. Forty reported working with venomous animals and 18 institutions reported 42 envenomations and six dry bites. Of the 48 bites, 24 (50%) involved nonnative species. Importantly, 61% reported having no training protocols, 48% had no emergency response protocols, and 33% made no provisions for the availability of

antivenom. Despite some areas for improvement, neither zoos nor academic institutions are a major contributor to nonnative envenomations (Ivanyi and Altimari 2004).

Private collections in the USA, however, appear to be the major source of such envenomations. They may or may not be legal and are often clandestine, and the specific species only become known when an envenomation occurs, making advance planning more difficult (Warrell 2009).

The USA is the largest importer of reptiles in the world, with an estimated 1.5–2.0 million households housing a reptile specimen. Snakes account for approximately 11% of these reptiles, and it is estimated that up to 9% are venomous (McNally et al. 2008). There is a large community of individuals who collect and trade in venomous animals. For example, in the Berks County Reptile Show in October 2004, 22 species of Elapidae, 17 species of Old World Viperidae 57 species of New World Viperidae 5 species of venomous Colubridae, and 2 species of venomous lizards (Heloderma spp.) were available for purchase (Keyler, Dan, personal communication, 2004).

The Incidence of Non-native Envenomations in the USA

In the latter portion of the twentieth century, Sherman Minton and Findlay Russell were both frequently consulted in serious or unusual envenomations. Minton reported consulting on 54 nonnative venomous bites in the USA between 1977 and 1995. Of those, 48% involved Elapidae, approximately 88% of which were cobras, 41% were Viperidae spp., 7% Colubridae spp., and 2% Atractaspididae (Minton 1996). Russell similarly reported on 85 consultations in nonnative envenomations, involving 20 separate genera in the Crotalidae, Viperidae, Elapidae, and Hydrophiidae families (Russell 1980).

Two studies reviewed data in the National Poison Data System (NPDS), collected by US poison centers. One study covered the period 1995–2004 (Seifert et al. 2007), and a follow-up study reviewed data from 2005 to 2011 (Warrick et al. 2014).

In the first study, there was a 60% rate of coding error, primarily because some native snakebites were coded as nonnative due to similar common or Latinate names. There were also issues of case duplication, as multiple poison centers would occasionally be independently contacted and then all report a case as the primary center, unaware that others had also been consulted. Cases had to first be manually confirmed as truly being a nonnative envenomation and then as being a unique case before further data analysis was possible (Seifert et al. 2007). The importance of this for other epidemiologic surveillance systems is that similar methodological issues may exist in other data systems. It is important to have accurate data on the incidence and types of nonnative envenomations in order to assess need and to develop appropriate response systems.

Over the combined 17 years of NPDS data, between 33 and 52 nonnative envenomations were reported to US poison centers per year. At least 87 different species were involved in envenomations. The location of envenomation was the patient's own residence in 73% of cases, confirming that the large majority of nonnative envenomations involve private collectors. Gender and age distributions closely paralleled that for native envenomations. The involvement of 10% of

envenomations in those less than 12 years old and 8% between 13 and 19 years of age underscored the dangers of in-home private collections to children.

Although deaths were small in number, their percentage of envenomations was seven-fold higher than in native snakebite. Only 34% of patients received antivenom, reflecting the logistical difficulties in locating and acquiring it in a timely manner, and this may have contributed to morbidity and the mortality rate (Seifert et al. 2007; Warrick et al. 2014).

Availability of Antivenoms with Efficacy Against a Given Snake's Venom

An antivenom may or may not exist for any given venomous snake. If a venomous snake is not clinically important in its usual geographic range, antivenom may not have been developed, even for potentially fatal species, such as Atractaspididae (Wagner et al. 2009). Ideally, an antivenom would be species specific and be designed to neutralize all of the possible venom components of that snake. Although there may be paraspecific cross-genus venom neutralization, as with *Notechis scutatus* (tiger snake) antivenom against *Micrurus* spp (coral snakes) (Wisniewski et al. 2003), such coverage may not provide complete venom neutralization. Even antivenoms made to a species within a genus may not be completely effective against other species within that genus and may not even be effective against other snakes of that species from other geographic ranges (Calvete et al. 2014; Gutiérrez et al. 2013; Fahmi et al. 2012). Even if a species-specific antivenom exists, unless it has been already imported into the USA, it generally will not be possible to obtain it in a clinically relevant time frame in the event of an acute envenomation.

Elements of the Healthcare System Impacted

When an envenomation occurs, multiple elements of healthcare system become involved in its management. First responders may encounter a chaotic scene, which may be at an institution or in a home setting. The offending venomous animal, or others, may not be contained, posing the risk of additional envenomations to the victim and/or responders. Genus and species identification of the offending animal may or may not be available or reliable. Zoos are highly likely to have a properly identified specimen and procedures in place to identify the envenomating animal. Private collectors, although usually quite knowledgeable, may not be accurate in their snake genus/species identification and, if incapacitated, may not have procedures in place to identify the likely offending animal. Emergency Services Personnel, although trained in the assessment and management of native snakebite patients, may have no idea what to do in the variety of possible nonnative envenomations. The proper prehospital management of these envenomations may differ considerably from native snakebite and "standard" managements applied to

native snakebites may be ineffective or even harmful when applied to nonnative envenomations (Seifert et al. 2011).

Similar issues will be faced when the patient arrives at a healthcare facility, first in the emergency department, then as an inpatient and finally postdischarge. Healthcare providers are unlikely to be familiar with the clinical management of a nonnative envenomation and, as in the prehospital setting, may make incorrect medical decisions based on the normal management of native species. For example, the US coral snake, belonging to the Elapidae Family, does not typically produce significant local tissue injury. In approximately half of the cases, pain and parasthesias are the only local symptoms and severe local effects are not generally seen even in major envenomations (Wood et al. 2013; Kitchens 1987). However, elapid snakes from other parts of the world may produce very significant local injury, including extensive necrosis (Sennwald 1992; Habib et al. 2001). A second example is the thrombotic effect of some South American Viperidae (Malbranque et al. 2008), which is generally not seen in North American Viperidae envenomation. Thus, the management of a nonnative snakebite as though it were from a native bite may be inappropriate and may result in potentially preventable adverse outcomes. Postdischarge management is also likely to provide challenges in finding a clinician with the experience to provide appropriate outpatient follow-up.

The US Non-native Envenomation Response System

System Overview

The systematic response to nonnative snake envenomations in the USA has developed and evolved over decades. Zoos, which import antivenoms for the species in their collections, have traditionally made their antivenoms available for the treatment of exotic envenomations of private collectors, usually without compensation. Federal grants have allowed the development of information resources and strategic antivenom deployment, but it is not so much a system as a set of response elements that have been cobbled together by need and availability.

Antivenoms for Nonnative Species

Species-specific snake antivenom, if available, is the definitive management for an envenomation. After basic first aid and resuscitation, if needed, priorities will include determining (1) the need for antivenom, (2) the appropriate antivenom for the specific snake species, (3) the location of the antivenom, and (4) arranging its transportation to the treating facility. Once the antivenom has arrived, other issues that may be encountered including packaging and administration information in a foreign language, pharmacy concerns regarding prior storage conditions, transport conditions, "expiry" status and administration protocols, and national, state, and institutional regulations and reporting requirements governing the off-protocol use of experimental drugs. Although there are other venomous animals, such as *Loxosceles* (brown spiders) species, nonnative *Latrodectus* (widow spiders) species, and various nonnative marine organisms (e.g., *Synanceia* [stonefish] and *Chironex*

fleckeri [jellyfish]) for which antivenoms exist, their use in the USA is rarely, if ever, needed.

The Role of Poison Centers and the Antivenom Index

In the USA, there is a system of regional poison centers, which provide consultative services to healthcare providers managing poisonings and envenomations. Poison Centers have access to the Antivenom Index, a joint project of the Association of Zoos and Aquariums and the American Association of Poison Control Centers. It is an online resource developed under DHHS/HRSA federal grants (DHHS/HRSA Grants) that includes information on the management of snake and other envenomations, including to nonnative species, the location of nonnative antivenoms stored at US zoos, which is the *de facto* source of antivenoms to nonnative species envenomations in the USA, as well as a list of medical consultants experienced in the management of nonnative snake envenomations and who make themselves available to provide expert consultation to either poison center toxicologists or directly to treating clinicians. Access to the Antivenom Index website is password protected and limited to poison centers and US zoos and aquariums, which list the types and expiry dates of antivenoms at their institutions as well as providing 24/7/365 contact information for zoo personnel in the event of an emergent need for an antivenom (Fig. 1) (Antivenom Index URL). Hospitals in the USA are not allowed to purchase or store non-US Food and Drug Administration (FDA)-approved drugs,

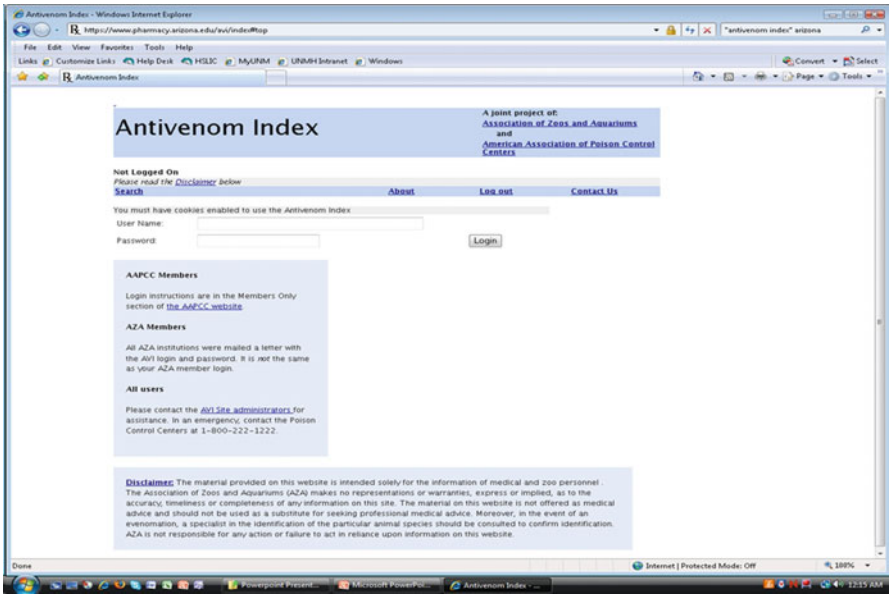

Fig. 1 Antivenom Index Login screen. Each US poison center and participating zoo has a unique User Name and Password

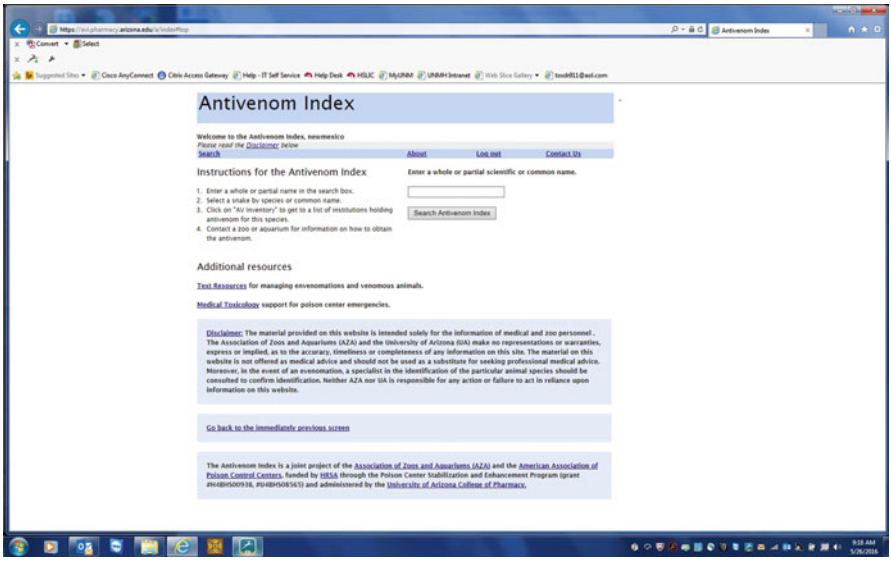

Fig. 2 The search screen allows entry of partial or complete common or scientific names. It also includes links to text resources regarding appropriate antivenom selection, management protocols, and a listing of experienced clinicians available for consultation

which includes antivenoms to all nonnative species. Hospitals are also governed by rules and regulations regarding the use of investigational drugs, which the category under which zoos import them into the country. The Antivenom Index includes informational resources and a robust search feature (Figs. 2, 3, 4, and 5) and additional information on antivenom availability and location (Fig. 6).

Strategic Placement of Antivenoms for Faster Response

In 2011–2012, as part of the same federal DHHS/HRSA grants that created the online Antivenom Index, certain antivenoms which were not optimally, geographically distributed, were purchased and strategically placed at various zoos to shorten transportation time for future envenomated patients.

Expiry Status of Non-FDA-Approved Antivenoms and Prior FDA Approval For Use

"Expiry" status of foreign, non-FDA approved antivenoms is one aspect that can pose difficulties, as zoos often keep, and then of necessity or preferentially send, their "expired" antivenom when requested for an off-site envenomation. Antivenoms are expensive, purchased by zoos for the benefit of their employees, are unlikely to be recompensed when sent for the treatment of private collectors, and expire over relatively short periods of time. Yet, they are known to retain their efficacy over a much longer time frame than their manufacturer designated expiration dates (US FDA Extension of Coral Snake AV URL; O'Leary et al. 2009).

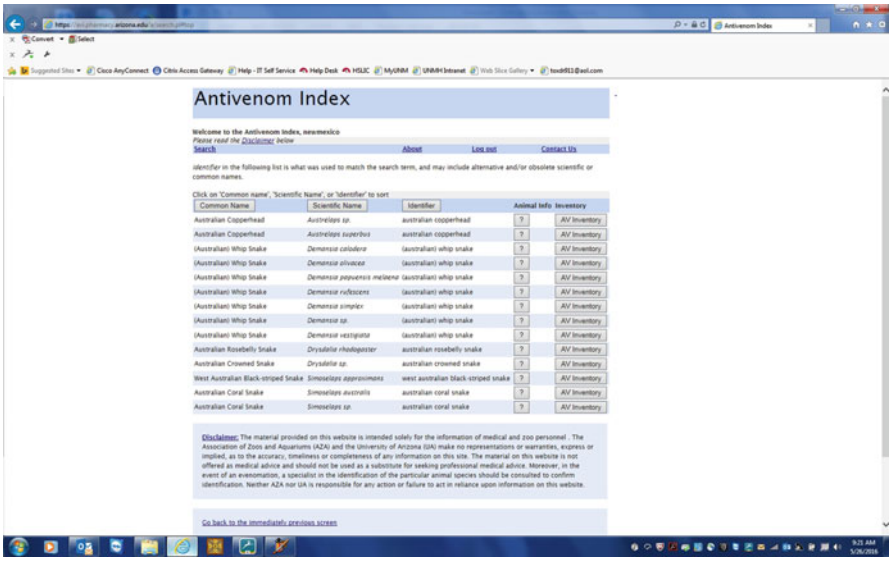

Fig. 3 Results of a search for the term "Australian." This returns a list of all snakes with that term in a snake's common name, those snakes' scientific names, and links to animal information and current US inventories of antivenoms against those species

In the USA, except under specific circumstances, FDA-approved drugs may not be administered beyond their expiration date (US FDA Expiration Data Guidance URL). Ideally, in-date, foreign antivenom would be preferentially be obtained and given. Foreign manufactures list "expiration" dates on their antivenoms, which are primarily established in regard to efficacy. Any loss of efficacy over time can be compensated for by the administration of additional antivenom, if needed. Since the FDA does not approve INDs – they are simply allowed to go into effect if a hold is not placed on them – manufacturer-applied "expiration dates" would not appear to carry the force of law.

Theoretically, administration of any experimental drug, off-protocol, should have prior approval by the FDA Office of Biologics. The FDA Office of Biologics can be reached during business hours (8:30–4:30 M-F, EST) at 240-402-8010 and after hours at 301-796-8240. Aside from this requirement, there are advantages from both regulatory and local logistical perspectives of such prior approval. For example, it is likely that a hospital's IRB will require notification within 48 h of the use of an experimental product off-protocol and prior FDA approval for use will be helpful in that process. The zoo and/or the treating physician will also have reporting requirements regarding an investigational antivenom being used. Prior FDA approval for its use may also be helpful in subsequent dealings with the FDA. Similarly, a hospital pharmacy may be more comfortable administering a product that is non-FDA approved and may be beyond its stated "expiration" date if prior FDA approval has been received. Regardless, it is common practice to administer foreign,

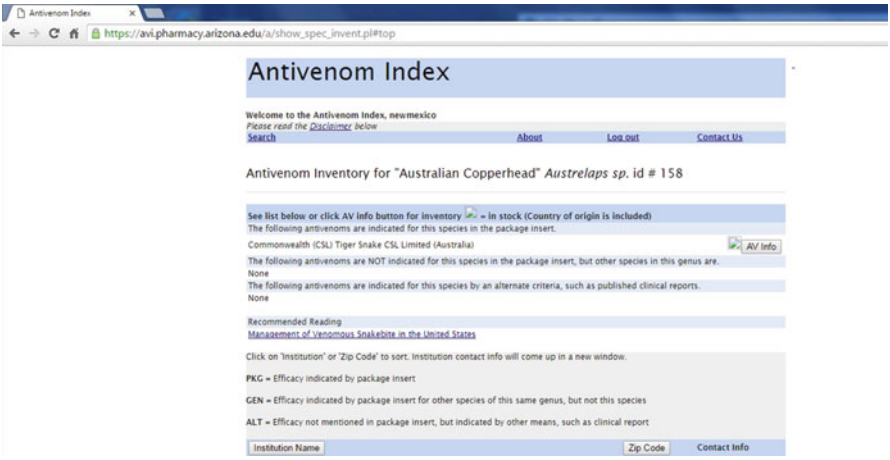

Fig. 4 A listing of antivenoms indicated for "Australian Copperhead" (*Austrelaps superbus*) snakes. Included are antivenoms which list the specific species in their package inserts, as well as antivenoms, if any, that list other species within that snake's genus as well as antivenoms that may be indicated for that snake by alternative criteria (e.g., case reports). In this instance, only the Commonwealth (CSL) Tiger Snake CLS Limited (Australia) antivenom is indicated, and an icon indicates whether there is a supply of this antivenom currently in the USA. In addition, there is also a link to a recommended text resource. At the bottom of the screen begins the listing of participating US zoos that have this (and other indicated) antivenom in their inventory. The list can be sorted by Institution name or Zip Code, which allows rapid determination of the closest source of antivenom to the patient

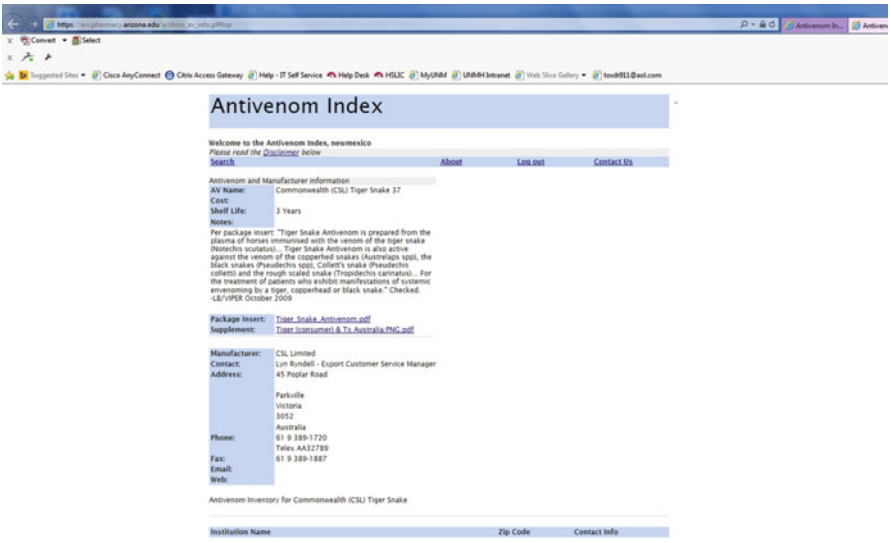

Fig. 5 PDF files of package inserts are available along with notes of special importance and manufacturer contact information

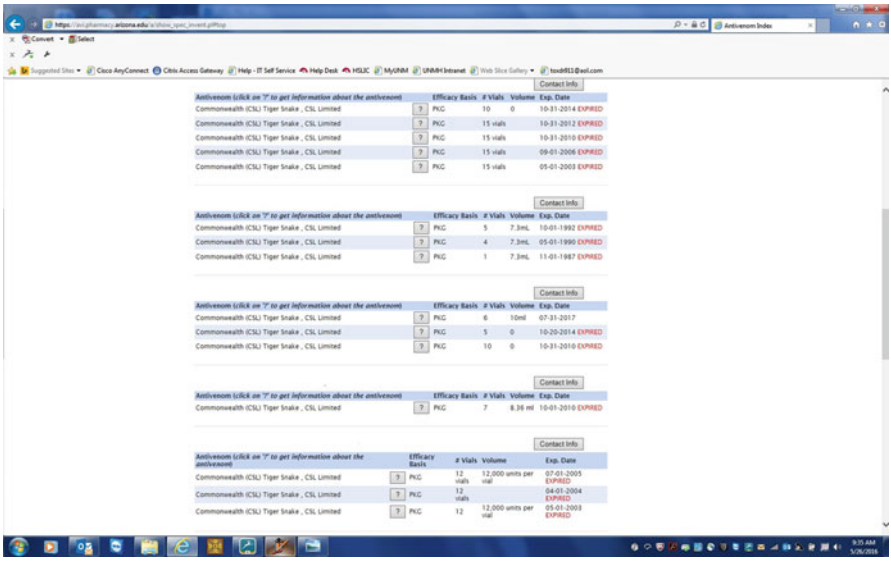

Fig. 6 A partial listing of zoos having CSL Tiger Snake Antivenom (zoo names and Zip Codes redacted), including the efficacy basis for inclusion (e.g., listing of species in the package insert), the number of vials in inventory, expiration dates, and links to zoo contact information. As can be seen here, zoos maintain expired antivenoms in their inventory. The third zoo down the list is the only one with in-date antivenom and may be geographically more remote from the patient than sources of expired antivenom. See the text for a discussion of issues related to expiry status of antivenoms

non-FDA-approved antivenoms without prior FDA approval and to administer "expired" antivenoms, as long as they appear to have maintained sterility. No additional adverse effects beyond that expected for biologic products should be anticipated.

Postdischarge Clinical Management

Post-discharge management will likely be performed by community clinicians similarly unfamiliar with nonnative envenomations. The consulting poison center toxicologists are likewise available to provide guidance and support for ongoing clinical case management.

Guidelines for Institutions Housing Venomous Animals

A published guideline exists for zoos and other institutions that house venomous animals. This guideline addresses: (1) the capability of facilities to properly house and care for venomous animals, (2) acquiring antivenom prior to acquisition of the animal, (3) proper storage of antivenom, (4) procedures for venomous animal handling and identification, (5) preparations, policies, and procedures in the event of an envenomation, (6) involvement of the regional poison center, (7) having a patient transport plan, (8) protocols at anticipated medical receiving facilities, (9) obtaining prior hospital IRB approval for the use of non-FDA-approved antivenoms, and (10) participation in the Antivenom Index (Seifert et al. 2006).

Zoos obtain antivenoms for the nonnative snakes in their collections for the purpose of treating potential envenomations of their workers. They legally import these medications under a special Investigational New Drug Application (INDA) process of the US FDA (US FDA URL). Zoos usually maintain physical control of these antivenoms and the guideline recommends storage of these antivenoms in accordance with manufacturer recommendations, which may include refrigeration and those of a hospital pharmacy, including continuous temperature monitoring and a backup power supply for refrigerators. Since envenomations at the zoo can be anticipated, policies and procedures for the participation of a local consultant, the regional poison center, and the hospital to which an envenomated individual would be transported can all be arranged in advance.

Determining the Correct Antivenom, Locating, and Transporting it to the Patient

Since zoo antivenom supplies are also the *de facto* supply for private collectors who have been bitten; however, many of these contingencies cannot be planned for in advance since their locations, preenvenomation, are not know. The envenomated individual may be quite remote from the nearest source of appropriate antivenom, so the regional poison center, the receiving hospital, and local clinicians will not have advance warning of the potential for an envenomation by any given snake. If a private collector is envenomated and needs an antivenom not located in the same city, once an appropriate antivenom is determined and located, arrangements for transport of the antivenom to the patient are made by the zoo and the treating physician. But air transportation of antivenom as cargo can be problematic. It may need to be shipped in a cold pack or on dry ice, which may result in temperature excursion, both above and below manufacturer recommendations. Since 9/11, if unaccompanied by a passenger, cargo on US air carriers can only be sent by a "trusted shipper," which may not apply to all antivenom sources. And not all carriers have cargo receiving facilities. As a result, there may be delays of many hours to days in the patient receiving an antivenom, with the potential to increase morbidity and mortality.

Nonantivenom Management of Envenomations

Although species-specific antivenom is the definitive management of snake envenomations, it may not be available or may not arrive in a timely manner. Other managements may be adjunctive or may provide sufficient therapeutic benefit even without antivenom. The specific management of snake envenomations is beyond the scope of this chapter, but some adjunctive and alternative therapies are summarized below.

Some paralytic, neurotoxic venom components may be neutralized by antivenom while still in circulation but, if a presynaptic toxin, will not be available for neutralization or have their effects reversed by antivenom. Some elapid venoms, including some *Micrurus* (coral snake) species (Carbajal-Saucedo et al. 2014; Brazil and Vieira 1996), *Naja* (cobra) (Machiah and Gowda 2006) species, *Pseudechis* (black snake) species (Hart et al. 2013), *Bungarus* (krait) species (Nirthanan et al. 2003), and others have postsynaptic venom components that inhibit the acetylcholine receptor.

An acetylcholinesterase inhibitor, such as neostigmine, used in standard doses for reversal of pharmaceutically induced paralysis, may be capable of increasing acetylcholine activity on the postsynaptic membrane and reversing or partially reversing venom effects. Since presynaptic toxins prevent the release of acetylcholine, neostigmine would be expected to have greater benefit with venoms with wholly or predominantly postsynaptic toxins (Brazil and Vieira 1996; Nirthanan et al. 2003). Whether this is sufficient therapy will depend on the degree of reversal of paralysis and other venom effects.

There are a variety of hemotoxic components and effects in snake envenomation, including thrombotic sequelae, venom-induced consumptive coagulopathy (VICC), and clotting factor and platelet inhibition, dysfunction or consumption, with hemorrhagic sequelae. Antivenom is usually the definitive management to stop and reverse these effects, with blood products (e.g., fresh frozen plasma or platelets) reserved for acute hemorrhage and then only given with additional antivenom, as they will likely be ineffective and quickly consumed in its absence (Maduwage and Isbister 2014; Lavonas et al. 2011). Heparin is not effective in preventing procoagulant-associated consumptive coagulopathy (Maduwage and Isbister 2014) but may play a role in preventing thromboembolism in certain snake envenomations, once the acute, hemorrhagic phase of envenomation has passed (Chani et al. 2012). Although plant-based venom inhibitors, low-voltage electrical current, and metal chelators may have some future role in mitigating venom-related hemotoxicity, currently, none are in clinical use (Panfoli et al. 2010). Similarly, alternative therapies for the cytotoxic effects of venom have been investigated, including plant-based substances with anti-PLA2 activity (Silva et al. 2016), but symptomatic and supportive local wound care is the mainstay of therapy.

Scope of the Problem in other Parts of the World

Overview

The problem of snakes and other venomous animals being out of their normal geographical ranges is not unique to the USA.

Systematic Reviews

A review of 404 exotic envenomations in France and Germany between 1996 and 2006 involved exotic snakes in 39%, aquatic animals in 30%, and arthropods in 27%. All severe envenomations involved venomous snakes, but there were no fatalities (Schaper et al. 2009).

A review of snakebite enquiries to the United Kingdom (UK) National Poison Information System (NPIS) between 2004 and 2010 revealed that 132 of 510 cases (26%) were known to involve exotic species and another 92 (18%) were of unknown type (Coulson et al. 2013). UK National Health System data between 2004 and 2011 showed an increase in the number of homes with reptiles. Contact with a venomous snake or lizard occurred in 307 of 760 (40%) of episodes and resulted in 287 hospital

admissions and 463 hospital bed days. Additional injuries and hospital admissions were associated with ownership of other exotic reptiles, crocodiles, alligators, and scorpions (Warwick and Steedman 2012).

In the Czech Republic between 1999 and 2013, there were 72 nonnative envenomations involving 34 different venomous snake species, of which 31 were reliably identified.

Case Reports

Aside from these systematic studies, numerous case reports of envenomations by venomous animals out of their normal geographical ranges, and the logistical challenges they present, can be found. Examples include an African, green mamba (*Dendroaspis viridis*) envenomation in France (LeClerc et al. 2008), an Australian, broad-headed snake (*Hoplocephalus bungaroides*) envenomation in Scandinavia (Chew et al. 2003), and a US Western Diamondback (*Crotalus atrox*) envenomation in Malaysia, in which, in all cases, there were delays in locating, obtaining, and administering the appropriate antivenom. In addition, the management of Western Diamondback envenomation included a fasciotomy, something rarely, if ever done in modern times in managing such cases in the USA (Adnan, Anisah, personal communication, 2012).

Response Systems in other Countries

Overview

Response systems, usually at a national level, and similarly to that in the USA, have often developed piecemeal and as a result of cases or studies demonstrating the logistical difficulties of treating such patients and suboptimal outcomes.

France

In response to logistical difficulties encountered in the management of nonnative envenomations, a national antivenom bank was created in France in order to provide prompt delivery of antivenom for exotic snake envenomations throughout the country. The system is based in two poison centers, one in Angers and the other in Marseille, with regional hospital pharmacies stocking a selection of nonnative antivenoms chosen by the French Ministry of Ecology, the French drug agency, in consultation with snakebite specialists. In 2010, the antivenom bank had the capacity to treat about thirty different kinds of envenomation. It is unclear what percentage of potential envenomations this covers, as there were 135 exotic venomous snake species officially registered in France at that time. However, cross-species and paraspecific activity would likely provide treatment capability for most such envenomations (Darsonval et al. 2010; de Haro 2012).

UK

In the UK, institutional and private collections are supposed to possess the necessary antivenoms. There is an exotic antivenom depot system under control of the National

Poisons Data System and an informational resource and procedural guidance under National Operating Procedure 17. An online information resource, TOXBASE, also contains information on nonnative envenomations and is intended for use by poison specialists (Bateman, Nicholas, personal communication, 2014).

Switzerland and the Netherlands

In Switzerland (Stadelmann et al. 2010) and in The Netherlands (Dijkman et al. 2012), national antivenom depots have been created. In The Netherlands, the depot contains 20 different snake antivenoms, four scorpion antivenoms, three spider antivenoms, and 1 fish antivenom.

Germany

In Germany, the Munich Poison Centre created a database of relevant information on nonnative envenomations (Munich Antivenom Index: MAVIN), which contains information regarding where nonnative antivenoms are stored and can be obtained (Schaper, Andreas, personal communication, 2015).

Australia

In Australia, an online Clinical Toxinology Resource is available to assist in managing envenomations of all kinds. There are two tiers of access, with a highly detailed, password-protected database available to clinicians (Clinical Toxinology Resources URL)

Features of an Exotic Venomous Animal Response System

Elements of a Response System

The elements of an exotic venomous animal response system will vary based on the historical context, existing healthcare entities and systems, governmental and institutional rules and regulations, geography, and other factors. In general, an integrated and coordinated system should contain regional or national Informational resources for lay individuals and clinicians faced with a nonnative envenomations. Such resources should be available online and by knowledgeable personnel in real-time and should include at a minimum:

1. Clinician training
2. Information and advice regarding first aid measures
3. A system for importing nonnative antivenoms and their availability to treat
4. Exotic envenomations
5. Selection of transport modalities and appropriate receiving facilities
6. Management protocols and/or management resources
7. Procedures for antivenom selection, location, delivery, and expert consultation to hospital pharmacies and treating clinicians
8. Training in and periodic testing of the response system (Othong et al. 2012)

Conclusion and Future Directions

Envenomations by snakes outside of their normal geographic range create many challenges in healthcare response and involve every element in a healthcare system. A preexisting response system, from first responders to postdischarge follow-up care can optimize outcomes for these individuals and lessen stresses on health delivery systems. The specifics of systematic nonnative envenomation programs will differ by geographic region and healthcare system considerations but should address common issues encountered in these cases. In the USA, a system of antivenom availability through zoos, some strategic antivenom placement, clinical information, and clinical assistance available through regional poison centers as well as a national expert consultant panel constitute the current response system.

Cross-References

► Injury and Envenomation by Exotic Snakes and Other Venomous Pets in Europe

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Injury and Envenomation by Exotic Snakes and Other Venomous Pets in Europe

18

Luc de Haro

Contents

Abstract

In the Western world, there is an undeniable increase in the trend for exotic pets among which there are many venomous species. Over the past few years, tarantula bites and exotic fish stings have become frequent in several countries; however snakes are unquestionably the most dangerous of all these unusual pets. Breeders of these exotic snakes are collectors in search of rare specimens for which toxicity is poorly known. Furthermore, some breeders do not hesitate to interbreed these species, thus creating new breeds for which it is difficult to predict toxicity. The European and North American medical practitioners are not trained to deal with patients who have been envenomed by exotic animals. It is therefore advised to consult a specialized department in order to assess risk and draw up a therapeutic protocol according to the venomous species concerned and the clinical picture observed. Finally, it must also be made clear that antivenoms, often the only solution for a quick recovery, are not available in most cases. In order to get out of this unsatisfactory situation, several antivenom banks have

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been set up by well-informed teams and medical toxicologists from different countries.

Introduction

"Exotic pets" have become a trend or even a social phenomenon in Europe and North America (de Haro and Pommier 2003; Warrell 2009). It is now common to find in pet shops, next to the usual dogs, cats, and hamsters, more original species such as reptiles, amphibians, or exotic birds. Furthermore, part of the general public seems fascinated with the danger of certain species (wild cats, crocodiles, etc.), and this has caused the chaotic apparition of a dangerous animal market including venomous taxa. Since there is no such thing as zero risk, all venomous animal owners will be exposed 1 day or another to a bite or sting causing a proper envenomation (Lubich and Krenzelok 2007; Warwick and Steedman 2012). Such envenomations can rapidly put the victim's life at risk, depending on the animal in question (many snakes and several scorpions). It is also necessary to highlight the fact that the European and North American medical practitioners are practically untrained to care for such patients who present multiple medical problems (Chew et al. 2003). In order to understand the venomous pet issue better, the main notions that medical staff, brought to deal with such patients, should know will be developed.

Who Breeds Venomous Exotic Pets?

Finding venomous exotic pets for sale in pet shops is not surprising. These reptiles or arthropods, which people usually find repulsive, also fascinate part of the population. There is, therefore, a "venomous" market, and several shopkeepers explain that they are under a certain amount of pressure from clients to supply potentially dangerous species. Pet shop assistants have not always been trained to deal with such residents. This exposed and inexperienced profession represents a population at high risk of envenomation (de Haro and Pommier 2003). The staff in professional vivariums open to the public are as exposed but much better informed. To be profitable, a display of live reptiles or arthropods has to include spectacular species. To attract a sensation-seeking public, there can be no zoo without a lion, aquarium without a shark, and of course vivarium without a cobra or rattlesnake. Thus, people in charge of terrariums declare that they are under a lot of pressure from the public who want to see dangerous animals that frighten them. To meet this demand, some sensible professional breeders make lists of venomous species to choose from, according to the availability of antivenoms from hospitals or antivenom banks: with two or three North American and European antivenoms covering venom from several types of viper and rattlesnake, it is possible to have a few spectacular species, highly demanded by the public, without permanently taking excessive risks for the keepers (de Haro 2009).

Amateur breeders have the completely opposite approach: collectors are rarely interested in classical species. They are generally after rare taxa; however, these exceptional species practically cause no envenomations in the wild. Moreover, certain snake species, although highly venomous, are not considered to be public hazards in their countries of origin, and thus no specific antivenom may have been developed for them (Othong et al. 2012). In case of envenomation, therapists must turn to polyvalent antivenoms capable of neutralizing venom from closely related species (de Rudnicki et al. 2008). These antivenoms, however, are created in far-off countries (Brazil, Costa Rica, India, Thailand, etc.) and are difficult to obtain in Europe or in North America (Darsonval et al. 2010; Dijkman et al. 2012). In some extreme cases, the species involved is not even known to be toxic, this still happens frequently with some poorly studied tarantulas or snake species. The medical team in charge of the patient can only prescribe symptomatic treatments. The example of the « Swamp Viper » (*Proatheris superciliaris*) clearly illustrates this problem: the formidable toxicity of this Tanzanian snake was only revealed after three fervent breeders were envenomed in search of an exceptional resident (one case in the USA, one in Czech Republic, and one in France) (Warrell 2008). In its country of origin, there are no medical reports of bites from this snake that very few humans come across. Consequently, the antivenom capable of neutralizing this viper's venom does not exist since it causes no problems in Africa. In Europe and in North America, however, the physicians in charge of the envenomed breeders were confronted with a multitude of problems when hematological and renal complications developed; they had no specific treatment and one patient contracted renal insufficiency for life since there was no antidote (Warrell 2008).

It is impossible to draw up an identikit image of the average venomous exotic pet breeder. A lot of collectors are serious people who are extremely well informed about the weird world of reptiles or arthropods. These amateurs are capable of conveying their love of these initially unattractive animals to neophytes. Unfortunately, the dangerous side to these venomous pets makes them attractive to people known as dropouts. Classically, there are two types of individual among envenomed patients: either adolescents who have dropped out of school or society and who are often attracted by morbid trends where venomous animals play an important symbolic role (hard rock, gothics) or adults suffering from exclusion (unemployment, alcoholism) and who almost always have behavior problems (aggressiveness, negativism, social isolation). It is impossible to generalize, but usually accidents happen more often to these breeders than to experienced collectors (de Haro 2009).

Exotic Snakes

Exotic snakes that are imported into countries where they are not naturally present do not usually have a venom apparatus. They are quite primitive snakes such as constrictors from the **boa** or **python** families. Some specimens become very large and their bites can cause severe lesions due to their sheer size. The fangs of these snakes allow them to immobilize their prey for a few minutes while they suffocate

them with their powerful muscles. The wounds are deep and each sharp fang punctures the skin which easily becomes infected due to the abundant bacterial flora present in the reptiles' mouths. These constrictor snakebites should be treated in the same way as large dog bites while paying particular attention to systematically prescribe broad-spectrum antibiotics (note that this is not the case with venomous snakebites). It should also be noted that as well as snakebites, there have also been cases of constriction with humans who have been suffocated by their "pet" python or anaconda. People should be advised against breeding specimens over 3 or 4 m long which are capable of swallowing a child or suffocating an adult (Omalu et al. 2003).

Colubrids are a lot less worrying because they are smaller and cannot inflict serious wounds. The main colubrid species that are for sale represent no toxic danger to their owners. They are lively and sometimes colorful reptiles which are perfect for terrarium lovers. Among the many species of colubrids (the largest family of snakes), some taxa should be treated with caution. It was in fact grass snakes that evolved to become the greatly feared venomous elapids and vipers. This evolution was not sudden, but rather progressive: transformation of saliva composition into venom and then anatomical modifications of the head in order to inject this venom. The limit between saliva and venom is unclear. It is therefore wrong to consider grass snakes as nonvenomous. Some species have small rear fangs at the back of the snake's mouth (species known as opisthoglyphous snakes with some taxa that are dangerous to man, such as the genus *Dispholidus* or *Thelotornis*). These snakes must be considered to have venom (Pommier and de Haro 2007). Of course the venom system will only harm the victim if they put their finger in the snake's mouth, something that is practically never seen in the wild but can happen when the snake is in captivity and there is close contact in order to feed a snake that is not inclined to eat on its own. Other species of grass snake, the greater majority, do not have a venom injection system (known as aglyphous snakes), but this does not mean that their saliva is completely harmless. The complete absence of an inoculation system means that the bite has to be prolonged for the saliva to penetrate the tissues, something that is only possible under particular circumstances such as the victim being drunk. It is worth retaining that, while many species of colubrids have never envenomed anybody in the wild, close and prolonged contact that comes from breeding them in captivity can reveal toxicity that was previously unheard of. This was the case with the Japanese species *Rhabdophis subminiatus* which was thought to be harmless until there were reports of unwary collectors being envenomed. If the toxicity of one venom is unknown, it implies that there is no specific treatment against it. Thus, apart from two exceptions (South African antivenom against the boomslang *Dispholidus typus*, Japanese antivenom against the Yamakagashi grass snake *Rhabdophis tigrinus*), there are no antivenoms against colubrids venom. In cases of envenomation, the only treatments available are symptomatic (de Haro and Pommier 2003; Schaper et al. 2009).

Three groups of snake, among which there are many species sought after by collectors, must be considered as particularly dangerous: viperinaes, crotalinaes (two subfamilies of the viper family), and elapids. In Africa and the Near East, several species of **viperinae** have been responsible for a large number of envenomations

with a high mortality rate. For example, there are desert vipers such as *Cerastes*, their *Echis* cousins, and of course puff adders such as *Bitis* (including the largest and most dangerous vipers, *Bitis gabonica*, *Bitis nasicornis*, and *Bitis arietans*). There are fewer vipers in Asia than in Africa with some steppe vipers such as *Pseudocerastes* and *Eristicophis*, without forgetting the formidable large taxa such as *Macrovipera* and *Daboia* (including Russell's viper *Daboia russelli* which is the only dangerous viper present in Southeast Asia, where it is responsible for a large proportion of serious snake envenomations). Generally speaking, the dangerous African and Asian vipers have venom that is extremely rich in enzymes which are responsible for a genuine exo-digestion of their victim's tissues and also for major coagulation disorders that can quickly become life-threatening (de Haro 2009; Larréché et al. 2008; Meggs et al. 2010). On the contrary it contains little or no neurotoxins, this explains why neurological disorders appear late after envenomation and are not the direct consequence of venom toxicity (neurological disorders are due to other actions of the venom such as convulsions and/or comas following cerebral hemorrhage). When venom is injected, the pain is intense and immediate. Huge swelling develops rapidly and becomes necrotic with blister formation far from the bite site (typical of envenomation by tropical vipers). In a hot and humid atmosphere, secondary infections are practically systematic. Coagulation disorders rapidly cause internal and external widespread hemorrhages. If the patients survive, thanks to appropriate medical care, the aftereffects are among the worst after envenomation by such a snake. From a treatment point of view, if the protocol for viper bites is now well known in Europe (purified antivenom available in hospitals likely to receive envenomed patients), this is unfortunately not the case for tropical viper bites (de Haro 2012). This is even more paradoxical, because envenomation by these snakes is the most problematic: impossibility to slow down exo-digestion of tissues without an antivenom, impossibility to restore a correct level of coagulation without an antivenom (all blood clotting factors are made useless because they are immediately destroyed by venom enzymes), impossibility to avoid frequent infectious complications without antibiotics, impossibility to recuperate integrally when tissue necrosis is already widespread without a rapid and adapted medical intervention, etc. For all of these reasons, it is advised to rapidly set up, in collaboration with a specialized department (Poison Center), a protocol adapted to the snake species concerned and to localize, as soon as possible, vials of antivenom potentially capable of neutralizing toxins and enzymes following assessment of the severity of envenomation (Darsonval et al. 2010; Dijkman et al. 2012; Lubich and Krenzelok 2007).

Snakes belonging to the **crotalids** subfamily (usually called pit vipers) can be anatomically distinguished from their viper cousins as they possess thermoreceptive loreal pits between their nostrils and eyes on each side of the head (these organs allow the snake to detect the slightest variations in temperature of their environment and thus detect the presence of homoeothermic animals, mammals, and birds, which, depending on their size, are either potential predators or prey). These snakes originate from Southeast Asia, but it is in America that they have spread the most. There are four important types of American pit vipers: moccasins or copperhead (genus *Agkistrodon*) which are terrestrial and aquatic; American fer-de-lance which

are most often tree climbers (about 40 species in several genera: *Bothrops*, *Porthidium*, *Bothriopsis*, *Bothriechis*, *Atropoides*, and *Cerrophidion*); rattlesnakes (genera *Sistrurus* and *Crotalus*) which are the only snakes to have a rattle; and finally the "bushmaster" *Lachesis muta*, a rare but dreadful species that lives in the Brazilian forest. Some of these species are small and not very aggressive, but as a general rule, all American pit vipers should be considered as potentially dangerous. In Asia, two groups represent this subfamily: old-world ancistrodons (genera *Calloselasma*, *Deinagkistrodon*, *Hypnale*, and *Gloydius*) and Asian fer-de-lance, locally known as banana snakes (about 30 green species from genera *Trimeresurus*, *Ermia*, *Ovophis*, and *Tropidolaemus*). These lively and colorful snakes are particularly sought after by collectors, and many species of *Trimeresurus* can now easily be found in pet shops and people's homes (these pit vipers represent a large percentage of the snakes responsible for envenomation of snake lovers) (de Haro and Pommier 2003; Schaper et al. 2004). Crotalids venom is very rich in digestive and coagulation-perturbing enzymes, generally inducing a major local syndrome with unbearable pain and diffusing/compressing swelling (the risk of ischemia due to compression is very high with American species). There are, however, few cases of necrosis, the opposite of what is observed with viper bites. Coagulation disorders are constant and important though, with each venom containing a cocktail of hemorrhagins and procoagulants as well as hemolytic phospholipases. Venom may also contain an inhibitor of the angiotensin converting enzyme (genus *Crotalus*). *Crotalus durissus*, the South American forest rattlesnake, must be considered separately as it produces beta-neurotoxic phospholipases A2 which cause ascending paralysis with a high risk of respiratory paralysis. White or dry bites are less frequent with pit vipers than with adders, and the injection of venom is systematically followed by immediate and intense pain which diagnoses envenomation. Envenomation by pet pit vipers must always be considered as being potentially serious (Lonati et al. 2004). Indications for antivenom administration are the same as for vipers: extensive local effects and/or general signs of venom spread and/or major coagulation disorders. It would be pointless to try and stop huge swelling or a hemorrhage with symptomatic treatments alone: only specific antivenom can neutralize the toxic effects of venom. This is the reason why American epidemiological data show that since purified antivenoms with few side effects have been put on the US market, use of these antidotes has rapidly expanded in the country. In fact surgical operations such as fasciotomy are no longer used to treat viper envenomations (there is never any real compression with adder bites) but are still controversial for pit viper bites where the venom is likely to rapidly induce genuine compartment syndrome (Schaper et al. 2004).

Finally, the **Elapidae** family has characteristic, short, rigid, front fangs (proteroglyphous snakes) the size of which depends on the capability of the snake to shut its mouth. The venom system of these reptiles is undeniably less efficient than that of solenoglyphous snakes (vipers and rattlesnakes); however it is this family which has the most formidable ophidian venom. It is in Asia that these snakes are responsible for the highest number of envenomations and deaths (several tens of thousands deaths per year in the south of the continent). This concerns mainly Indian cobras (genera *Naja* and *Ophiophagus*) and nocturnal kraits (genus *Bungarus*). Asian coral snakes (genera *Maticora* and *Calliophis*) are only occasionally

responsible for bites. In Africa, elapids come second to local vipers. It is mostly cobras (genera *Naja* and *Hemachatus*) and mambas (genus *Dendroaspis*) which are to blame for envenomations. Several other species from the genera *Boulengerina*, *Elapsoidea*, *Walterinnesia*, and *Aspidelaps* are also only rarely responsible for bites. The only elapids in America are coral snakes from the genera *Micrurus*, *Micruroides*, and *Leptomicrurus*. Even though they have such deadly neurotoxic venom, these elapids are only responsible for a small percentage of serious or lethal envenomations on this continent where the majority of morbidity-mortality due to snakes is from rattlesnake bites. In Australia, elapids are the only venomous snakes and, in the absence of competition, this family has developed into a large number of species. The genera *Acanthophis*, *Notechis*, *Pseudonaja*, *Pseudechis*, *Tropidechis*, and *Oxyuranus* are the most venomous snakes in the world. Surprisingly, mortality due to snakebites is very low in Australia for several reasons: the human population is predominantly urban, medical facilities are highly proficient, and efficient antivenoms are available everywhere. Venom from elapids characteristically contains large amounts of neurotoxins, reaching 50–70% of the venom's dry weight in this family. These snakes produce several different varieties of neurotoxin: α-neurotoxins are curare-like toxins that bind to acetylcholine nicotine receptors on the postsynaptic junction and prevent transmission of the neuromuscular impulse. β-neurotoxins are presynaptic toxins that prevent acetylcholine from being recycled in synaptic vesicles, and thus they also inhibit this impulse. Both types of neurotoxin can be present in the same venom; in this case they potentiate each other. These molecules are responsible for the ascending paralysis syndrome. Other neurotoxins can also be found in elapid venom. Let us take the example of κ-neurotoxins which are postsynaptic neurotoxins close to α-neurotoxins but with tropism for the central nervous system (molecules inducing cortical symptoms such as drowsiness). Also, to mention depolarizing neurotoxins (characteristic of species from the genus *Dendroaspis*) which can be anti-acetylcholinesterase fasciculins or dendrotoxins that increase presynaptic acetylcholine release, both types of neurotoxin potentiate one another and induce a transitory muscarinic syndrome. Other toxic proteins can also be found in elapid venom, but they are generally limited to a few species: myotoxins in some cobras and Australian elapids, cardiotoxins in some cobras, and coagulation-perturbing factors acting at an infra-clinical level as a rule, with Australian species being the exception. The clinical picture of envenomations by elapids is characterized by the near-constant presence of a group of neurological disorders at the origin of an ascending paralysis with a high risk of respiratory depression (de Haro 2009; Richardson et al. 2006). All species from the elapid family are capable of inducing such a syndrome, as well as snakes from other families such as some rattlesnakes or some species or subspecies of viper. Ascending paralysis syndrome begins with early signs associating minor to moderate local symptoms (often with locoregional paresthesia and/or dysesthesia) and signs of cranial nerve damage: bilateral ptosis, diplopia, ophthalmoplegia, orbicularis oris paralysis, and dysgeusia. Drowsiness is sometimes observed at this stage. After 10 min to a few hours without any evolution in clinical feature, signs of severity may begin to appear: dysphonia, dysarthria, and dysphagia which predict the apparition of motor disorders like areflexia, followed by

ascending paralysis, leading more or less quickly to respiratory arrest due to paralysis of the diaphragm. Any complications due to anoxia may then appear. It is because of this neurotoxic syndrome that over 50% of envenomations by elapids in India end in death of the patient before they arrive at the emergency department. Depending on the species of elapid, the neurotoxic syndrome may also be accompanied by other disorders: hemorrhages for Australian and New Guinean species, inaugural and transitory muscarinic syndrome with trembling for mambas from genus *Dendroaspis*, and disorders of the cardiac conduction system with some African cobras (e.g., *Naja nigricollis*). When African spitting cobra (*Naja nigricollis*, *Naja mossambica*, and *Hemachatus haemachatus*) spit venom in the eye, it only causes local lesions (intense pain, blepharospasms, conjunctivitis, or even keratitis) which must be treated symptomatically. Systemic damage to the eye in such cases is rare and anecdotal. Unlike what is observed with solenoglyphous snakebites, the absence or benignity of local symptoms must not reassure the emergency team into thinking that it is a dry bite while in fact venom, poor in enzymes but rich in neurotoxins, has been injected. An elapid bite must therefore always lead to systematic hospitalization with at least 24 h of medical surveillance (de Haro 2009; Larréché et al. 2008; Richardson et al. 2006). In cases of respiratory depression and unavailability of the appropriate antidote, symptomatic treatments are necessary to maintain sufficient oxygenation: endotracheal intubation, mechanical ventilation, and, even in some critical cases, tracheotomy. Antivenom is the only specific treatment. It is advised to use these antidotes if signs of severity are observed (dysphonia, dysarthria, dysphagia), in order to avoid respiratory paralysis. When the patient arrives in the hospital, already intubated and under ventilation, antivenom reduces intubation duration and so it must be injected as quickly as possible, even if the patient seems to no longer be in any danger thanks to a good oxygenation. Indeed, with elapid neurotoxins, the quicker the neutralization, the more efficient it is (Richardson et al. 2006; Warrell 2009). This also helps to limit the risks due to other toxins (coagulation disorders, cardiotoxicity).

It is also necessary to dwell on a recent phenomenon: hybridization between neighboring species. Some breeders, genuine "sorcerer's apprentices" in search of novelty, experiment with hybridization which occasionally results in snakes with venom about which we know nothing. It is easy to find such hybrid snakes for sale on the web; they are the joy of some collectors, but despair for toxicologists. In the event of envenomation by such a snake, the medical staff can only observe and give symptomatic treatment. It is not possible to ensure that antivenom produced for wild snakes will have any effect on the venom of animals which do not even exist in the wild (de Haro 2009).

Other Venomous Exotic Pets

Exotic poisonous **fish** kept in an aquarium are much less toxic to human than snakes. Stings by lionfish of the genus *Pterois* are common in Europe and North America as these species are frequently sold to specialist in sea aquarium. The induced clinical

feature is characterized by intense pain with syncope that may be associated with local signs such as swelling or bleeding (de Haro 2009). Symptoms regress quickly after home treatment by local thermal variation method, i.e., exposure of the puncture site to heat for 2 min followed rapidly by application of a cold pack. Other venomous fish species are less frequent in pet shops. Some of them can be at the origin of more severe stings: stonefish species of the *Synanceia* and *Inimicus* genus are for the moment limited to professional aquariums; freshwater Amazon stingrays of the genus *Potamotrygon* can induce extensive swelling and systemic symptoms (trembling, tremor, and digestive symptoms) and are since the 1990s easily available for nonprofessional fish lovers. Several poisonings after contact with these South American rays are published in Europe and North America (Brisset et al. 2006; Schaper et al. 2009).

Recent advances in aquarium technology now enable home hobbyists to simulate **mini coral reef** systems in which numerous invertebrate species can develop. Reef aquariums usually contain few or no fish. The main interest is to keep colorful coral, shellfish, starfish, and anemones. Unlike their inoffensive kin living in temperate waters, several invertebrate species from tropical waters can cause skin reactions after the slightest contact. The experience of several European or American poison centers includes frequent cases in which unknowing novice reefkeepers were injured by handling shortly after purchasing harmless-looking animals such as diadem urchins and Polynesian sea cucumbers. Local signs (erythema, pain, and blisters) must be treated like heat-related burns. In general, symptoms persist for 1–10 days before a complete recovery (Schaper et al. 2009).

Tarantulas have recently gained rapid popularity as pets because they require little care and can be easily kept by people with little training. In the early 1990s, the first spider-related accidents reported in Europe and North America involved species imported from South America. Although the venom of these spiders is often mildly toxic for humans (local signs and isolated noninfectious fever), some species have urticating hairs that can cause serious eye lesions (keratitis that may require several months of treatment). More recently, Indian ornamental species of the *Poecilotheria* genus have become fashionable among amateur collectors because they are not sluggish like their American cousins. Asian tarantulas are aggressive and their particularly toxic venom can produce neurological disturbances in man (trembling and convulsions). As a result, keeping these old-world spiders should be strongly discouraged (Schaper et al. 2009). One actual fear of clinical toxicologists in Europe and North America is the possibilities of importation of the Australian funnel-web spiders. Funnel-web spiders are the most dangerous spiders in the world and continue to cause severe envenoming in eastern Australia. They belong to the family Hexathelidae (Atracinae subfamily) and comprise about 40 species in two genera (*Atrax* and *Hadronyche*). Funnel-web spider antivenom was first used in 1981. It is produced against *A. robustus* venom and, based on case reports and small case series, appears effective for severe envenoming. Although it is assumed to have few adverse effects, there are few studies of its safety (Isbister et al. 2005). This antivenom is only available in Australia, and in case of envenomation elsewhere, only symptomatic treatments can be prescribed.

Experienced tarantula keepers are often interested in **other arachnids**, such as higher spiders or scorpions. However, there is in the medical literature very few cases of envenomations induced by dangerous species (*Latrodectus* or *Steatoda* spiders, *Centruroides* or *Androctonus* scorpions) kept in captivity, proving that tarantulas are really the most frequent arachnid pets (Schaper et al. 2009).

Problems Posed by Antivenoms

Concerning antivenom, there is a deep belief in the general public: envenomation is potentially serious and can be life-threatening, but as long as one antivenom (incorrect terms generally used are "serum" or even "vaccine") is injected, everything heals by magic, thanks to this miracle medicine, capable of neutralizing venom within minutes. It is clear that this myth needs to be refuted (de haro 2009). Antivenoms are made up of more or less purified antibodies from animals (horses for the most part, sometimes sheep or goats) which have been immunized against one or several types of venom. They are proper antidotes which can be spectacular when they are correctly used (Dijkman et al. 2012; Warrell 2009). They have, however, several limits which must be known.

Firstly, antivenom available internationally is only for snakebites. In Europe it is impossible to obtain antivenom against scorpions, spiders, fish, or jellyfish. This type of antivenom is exclusively available in local hospitals and is not exported. Everyone who breeds dangerous scorpions, stonefish, or Australian tarantulas has to know that in the case of an accident, European and North American practitioners could never obtain the appropriate antidote (Darsonval et al. 2010; Schaper et al. 2009).

Another important point is the cost of antivenoms. The more one antivenom is purified, the more reliable it is and the more expensive it is. The price per vial can easily exceed 1,000 euros. Considering the cost of 1 day's hospitalization on a reanimation ward in a European or American country, this remains acceptable. However, the cost of setting up an antivenom bank can rapidly reach exorbitant prices that few facilities can financially bear. Furthermore, these products sometimes have a short shelf life, which complicates stock management even more (Darsonval et al. 2010; Dijkman et al. 2012).

As with any drug made from heterologous proteins, there is a non-negligible risk of an immediate (anaphylaxis) or delayed (serum sickness) allergic reaction. This risk is greatly reduced with antivenom produced according to current reference methods; but this is not always the case since several antivenoms are produced in emerging nations (Brazil, India, Thailand, etc.). In general, these antidotes should always be restricted to hospital use in order to be able to correctly handle any side effects (Darsonval et al. 2010). On the contrary, the risk of allergy should never be used as a pretext for not using the available antivenom if it is called for. In the hospital, it is always possible to manage allergic complications, whatever their degree: they will always be less problematic than a grade 2 or 3 envenomation (de Haro 2009; Larréché et al. 2008).

The most important problem with antivenoms against exotic snake venom is their availability (Othong et al. 2012). These products are foreign drugs which do not have the authorizations necessary to be considered as drugs or antidotes in Europe or North America. In the absence of an official authorization, the use of these antivenoms made from heterologous proteins or protein fragments is under the full responsibility of the prescriber, which is enough to discourage more than one: given the incidence of immediate or delayed allergic reactions with some of the less purified antivenoms, it is easy to understand the reticence of some doctors to use a drug that is not recognized as such in their country, especially one with such a high probability of side effects (including the risk of court procedures which can be complex). From now on, however, in some countries such as the Netherlands and France, antivenom banks have managed to obtain authorizations from their higher administrations to use these antidotes, albeit with a limited usage, nevertheless allowing the injection of foreign antivenom without risking their career (de Haro 2009; Dijkman et al. 2012). Such antivenom banks have been established in different countries (Germany, Switzerland, France, and Netherlands) under the authority of various organizations (breeder's associations, poison centers, toxicologist network). These initiatives have contributed to making foreign antivenom available for treating breeders who have been envenomed by exotic snakes that do not normally live in these countries (de Haro 2012; Stadelmann et al. 2010). Unfortunately, it would be unwise to think that these antivenom banks will solve all the problems linked to envenomation by exotic snakes: exorbitant cost of medical care for these accidents, lack of training for medical staff, breeders and medical staff being repeatedly and almost constantly on bad terms, etc.

Finally, clinical toxicologists have often been confronted with great deception on behalf of exotic snakebite victims who have been treated with specific antivenom. Indeed, the miracle medicine image is such that many breeders wrongly believe that they are not running a great risk if the antivenom is available (which is, in itself, a genuine victory). When antivenom is properly used, it can rapidly neutralize venom toxins and enzymes: this means it is possible to observe, for example, a return to normal in terms of blood coagulation within a quarter of an hour or so or a return of spontaneous breathing (de Haro 2012). However, the tissue lesions are often definitive: impossibility to recuperate tissues after necrosis caused by exo-digestion or ischemia and difficulty in obtaining rapid regression of lesions due to cerebral hemorrhage or hypoxemia following respiratory arrest. Patients have to understand that antivenom is able to stop the damage from the time of the antidote injection.

Conclusion and Future Directions

The "venomous exotic pet" trend is at the origin of important problems in health care when collectors or their family are envenomed (Othong et al. 2012). Furthermore in many countries nothing has been planned for such patients: little or no inspections of illegal breeding of venomous species, absence of training during studies for medical staff, frequent unavailability of the only efficient treatment, etc. Worldwide

specialists admit that the risks generated by venomous exotic animals are not sufficiently under control in the majority of European or North American countries faced with this problem. There is, however, no point in fooling oneself: despite all recommendations and warnings to collectors (multiple operations among the breeder's associations, terrarium magazines, or specialist fairs), exotic pets are definitively present, and clinical toxicologists have to face the fact that there is a proportion of the population that is exposed on a daily basis to animals with potentially dangerous venom. The challenge is to be prepared to care for an increasing number of envenomed patients, following the example of the fortunate initiative of setting up antivenom banks in several different countries (Germany, Switzerland, Netherlands, France, etc.).

Cross-References

► Pathophysiology and Treatment of Envenomation by European Vipers

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Part VI

Clinical Toxinology in Australia, Europe, and Americas: Veterinary Envenomation

Management of Clinical Snake Bite in Dogs and Cats

19

Raegan J. Wells and Kate Hopper

Contents

Abstract

The major clinical effects of Australian elapid snake envenomation are neurotoxicity and coagulopathy. Human patients appear to have greater susceptibility to the coagulopathic effects but are less susceptible to the neurotoxic effects, when compared to dogs and cats. The envenomation syndrome in dogs tends to be different than cats. Dogs often demonstrate clinical signs prior to the onset of paralysis, such as vomiting, and can have very rapid progression of neurotoxicity

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while cats do not tend to have preparalytic signs and have a much slower progression of clinical signs overall. The optimal approach to diagnosis and treatment of Australian snake bites in dogs and cats has yet to be determined and a true understanding of incidence, current management practices, and outcome is needed.

Crotalinae and less common, Elapidae, envenomations occur in dogs and cats throughout the United States. The most common clinical signs of Crotalinae envenomation include local pain and swelling as well as coagulopathy, while the most common symptoms of Elapidae envenomation include neuromuscular weakness and hemolysis in the dog. In general, rattlesnake bites are more severe than copperhead or water moccasin bites. Veterinary antivenoms are regulated by the USDA, however human antivenoms are used in an off-label manner. Survival following envenomation is good; however, morbidity may be improved with antivenom administration.

Introduction

Snake bite in dogs and cats is a common clinical presentation to veterinary facilities in areas of the world endemic to venomous snakes. While management of snake envenomation in dogs and cats has many similarities to that of human envenomation, there are also significant differences due to variability in species susceptibility to venom constituents as well as resource and financial restrictions in veterinary medicine. This chapter reviews the key clinical features of snake envenomation in dogs and cats in the United States and Australia.

Australian Snake Envenomation in Dogs and Cats

The common venomous snakes of Australia belong to the elapid family. These snakes have small, front fangs and the bite is often painless. The envenomation syndrome associated with elapids is one of the systemic abnormalities with minimal to no local signs at the bite site. The major species of elapids responsible for envenomation in pets and humans in Australia are the Eastern brown snake (*Pseudonaja textillis*), the Eastern tiger snake (*Notechis scutatus*), Black snakes (*Pseudechis* sp.), the Death Adder (*Acanthopis antarcticus*), and Taipan (*Oxyuranus scuttelatus*). (Heller et al. 2007). The distribution of snake species in Australia varies geographically. Approximately 550 people are treated for snakebite annually in Australia while the true incidence of envenomation in dogs and cats is unknown (Liew and Braitberg 2017). In 1998 a survey of Australian veterinarians estimated that over 6000 cases of snake bite were treated annually (Mirtschin et al. 1998). It is likely that this underestimates the number of cases of snake bite currently managed by veterinarians in Australia.

There are significant differences in the effect of elapid envenomation between species. Human patients appear to have greater susceptibility to the coagulopathic

effects but are less susceptible to the neurotoxic effects when compared to dogs and cats.

Presentation

The two main clinical effects of Australian elapid snake venom are lower motor neuron paralysis and venom-induced consumptive coagulopathy (VICC). Death in dogs and cats is most commonly due to respiratory paralysis. The speed of onset and other concurrent clinical abnormalities will vary between snake species responsible for the bite, severity of the bite, and species of the victim. Animals can develop clinical signs of envenomation minutes to hours after the bite. Severe bites can lead to death within 1 h in dogs, while the progression of clinical signs tends to be slower in cats. Due to the small size of elapid fangs and the lack of local signs, bite sites are rarely identified in animals with elapid bites. There is some variability of the clinical effects of envenomation by specific Australian snake species. An overview of the envenomation syndrome seen in dogs and cats with some of the more common Australian snakes is provided.

- Eastern brown snake: This snake is ranked as the second most venomous snake in Australia and is the most common cause of serious snake bite in the country. Brown snake envenomation of dogs and cats is usually characterized by severe, generalized, flaccid paralysis and VICC. Cardiovascular collapse is often reported and bites can have a high fatality rate if not treated promptly (Tibballs et al. 1992). There is geographical variability in toxicity of the Eastern brown snake. Snakes from the Southeast Queensland region have been found to be larger, to have a larger venom yield and the venom has greater procoagulant effects than in Eastern brown snakes from elsewhere in the country. Veterinarians from Southeast Queensland report higher rates of respiratory paralysis necessitating mechanical ventilation than other regions. In addition, the cases of catastrophic pulmonary hemorrhage have been reported in dogs following Eastern brown snake envenomation in Southeast Queensland in recent years (personal communication, Dr. Ellie Leister, Veterinary Specialist Services, QLD, Australia).
- Tiger snake: Envenomation tends to cause neurotoxicity with myotoxicity, and VICC is common. Myotoxicity appears to be more severe in dogs and cats than in people following Tiger snake bite (Indrawirawan et al. 2014).
- Red-bellied black snake (*Pseudechis porphyriacus*): Envenomation by the red-bellied black snake in humans is described as causing hemolysis, variable myotoxicity, and minimal neurotoxicity or VICC. Envenomation in dogs is reported to cause weakness, ataxia, and paralysis as the more common presenting signs (Heller et al. 2005). One case report of a dog bitten by a red-bellied black snake described hemolysis and rhabdomyolysis (Heller et al. 2006). In human medicine, bites from the red-bellied black snake are considered milder than many of the other Australian snakes and antivenom therapy is not always required (Churchman et al. 2010). In contrast, one veterinary study of a large group of

practices estimated the survival of dogs following envenomation to be 84% and antivenom therapy is commonly given (Heller et al. 2006).

- Death adder: The venom from the Death adder has been shown to cause predominantly neurotoxic effects, with little to no myonecrosis or VICC. Death adder bites have been rarely reported in animals (Sutherland and Tibballs 2001).
- Taipan: The Taipan is considered the third most venomous snake in Australia but bites by this snake are rarely reported. One case report of Taipan envenomation in a dog described coagulopathy, neurotoxicity, and myotoxicity. The dog required mechanical ventilation despite antivenom therapy (Judge 2015).

Dogs

Dogs commonly have preparalytic signs following snake envenomation. Preparalytic signs are clinical signs that can develop prior to the onset of paralysis. These signs usually occur within 30 min of the bite and can serve as an early warning sign to owners that envenomation may have occurred. As the dogs can recover for a period following these signs and prior to onset of paralysis, appropriate education of dog owners is important. Preparalytic signs can include vomiting, trembling, collapse, salivation, and lethargy. The cause of transient collapse following envenomation is believed to be pulmonary outflow tract obstruction from thrombus formation leading to systemic hypotension, pulmonary hypertension, and myocardial ischemia (Tibballs et al. 1992). Dogs often recover from these signs for a period and appear relatively normal, before succumbing to the effects of the venom. The presence of preparalytic signs has been associated with a potentially lethal Tiger snake envenomation in dogs (Lewis 1994a). Common presenting clinical signs include vomiting, defecation, muscle fasciculation, weakness, mydriasis, and pigmenturia. Flaccid paralysis usually starts in the hind limbs and ascends to become generalized, although the progression of paralysis can be very rapid. Evidence of clinical bleeding maybe present but is considered uncommon (Heller et al. 2005; Indrawirawan et al. 2014).

Cats

Cats do not commonly show preparalytic signs. Presenting clinical signs in cats include ataxia, hind limb weakness, obtundation, respiratory distress, pigmenturia, coma, and bleeding from the bite wound.

Diagnosis

A snake bite maybe suspected in dogs and cats given the history of a witnessed bite or close interaction with a snake. In animals not witnessed to have interacted with a snake, envenomation should be suspected based on the clinical signs, the geographical location, and the time of the year. Australian snake bites occur most commonly in the warmer months (October to March) (Barr 1984). A survey study of 399 veterinary clinics from one state in Australia reported that the diagnosis of snake bite was made solely based on clinical signs in 26% of practices (Heller et al. 2005).

In an animal with a history and/or clinical signs suggestive of snake bite, the presence of a coagulopathy is generally considered a strong indication of envenomation. Coagulopathy is identified by prolongation of an activated clotting time or activated partial thromboplastin time. In the absence of either of these tests, many veterinarians rely on evaluation of a whole blood clotting time. Animals can have a significant snake envenomation without a coagulopathy, so normal clotting times do not rule out the diagnosis (Heller et al. 2007; Indrawirawan et al. 2014). In addition, there is evidence that dogs and cats are less susceptible to the coagulant effects of Tiger and Brown snake venom (Crawford and Mills 1985; Lewis 1994b).

Although the diagnosis of a snake bite maybe strongly suspected, confirmation of a bite and identification of the species of snake responsible is ideal. This allows the administration of a species-specific antivenom. The ability of lay people or medical professionals to correctly identify Australian snake species is very poor and should not be relied upon for antivenom selection (Morrison et al. 1983). When the species of the snake involved is unknown, a polyvalent antivenom may need to be given, if more than one snake species is present in the local region. The snake venom detection kit (SVDK) produced by the Commonwealth Serum Laboratories can test samples from the bite site, blood, or urine for the presence of Tiger, Brown, Black, Death Adder, and Taipan snake venom. Blood samples are not recommended for testing due to nonspecific binding with plasma proteins. There are concerns regarding the accuracy of the SVDK and the results of this test need to be considered in context of the entire clinical picture (Isbister et al. 2013a).

Treatment

Emergency veterinary care is essential if an animal is suspected of suffering a snake bite and antivenom is routinely recommended for the treatment of systemic envenomation in dogs and cats. There is some discussion in the human literature regarding the efficacy of antivenom to reverse all clinical syndromes associated with envenomation (Johnston et al. 2013). This has raised the question when do the risks of antivenom administration outweigh the potential benefits in human patients. In veterinary medicine, the limited ability to provide sustained high-level supportive care and the cost associated with such care can result in owners electing euthanasia for these pets. Given the possibility that antivenom therapy could reduce the severity and/or duration of clinical signs and severe adverse effects have been uncommonly reported, antivenom therapy is considered indicated in cases of moderate to severe snake envenomation of dogs and cats in Australia.

In Australia, there is antivenom available for Brown snake, Tiger snake, Mulga Snake, Death Adder, and Taipan snakes. Polyvalent antivenoms are also available including a combined Brown and Tiger snake antivenom and combined Brown, Tiger, Mulga, Taipan, and Death adder snake antivenom. Where possible, a species-specific antivenom is given, but this requires confident identification of the species of snake responsible. It is important to note that envenomation due to many of the Black snakes, and Copperhead snakes are best treated with Tiger snake antivenom.

In severe envenomations, two or more vials of antivenom are often given to dogs. In general, cats are considered less susceptible to envenomation and are commonly treated with a single vial of antivenom (Indrawirawan et al. 2014). There is evidence in human medicine that a single vial of the appropriate antivenom will neutralize the venom of the average Tiger or Brown snake in adult patients (Brown et al. 2012; Isbister et al. 2013a). And as the signs of envenomation can take time to resolve, repeat dosing of antivenom based on persistence of clinical signs is not recommended. The ideal dose of antivenom in dogs and cats is not known.

It is recommended to dilute the vial of antivenom 1:10 or more with an isotonic crystalloid fluid and administer it over 15–30 min. The animal should be monitored closely during the administration period for signs of anaphylaxis or worsening of the clinical signs that may require an increased rate of antivenom administration. The use of premedication prior to antivenom administration in an attempt to prevent anaphylaxis is controversial. There is limited evidence that premedication with epinephrine, antihistamines, and/or glucocorticoids will reduce the incidence of hypersensitivity reactions and they are not generally recommended at this time. (Isbister et al. 2008).

In addition to antivenom therapy, dogs and cats often require intravenous fluid therapy, oxygen therapy, and mechanical ventilation as part of the management of snake envenomation. Animals with significant myonecrosis require fluid therapy to minimize the development of acute kidney injury, as well as analgesia and general supportive care. The neurotoxic effects of Australian snake venoms can lead to respiratory failure and require mechanical ventilation to prevent death. The incidence of respiratory failure in dogs and cats with snake envenomation has not been well described. In one study from Melbourne, of primarily Tiger snake bites, 8 out of 104 envenomated dogs and 1 out of 45 envenomated cats required ventilation (Indrawirawan et al. 2014). While in South East Queensland, approximately one-third of Eastern brown snake envenomations in dogs require mechanical ventilation (personal communication, Dr. Ellie Leister, Veterinary Specialist Services, QLD, Australia).

The use of blood products, in particular plasma products for the treatment of coagulation abnormalities in Australian snake bites, is controversial. The major aim of plasma administration is to treat afibrinogenemia, which occurs as a consequence of VICC. A randomized control study in human patients with VICC compared the efficacy of fresh frozen plasma (FFP) administration within 4 h of envenomation versus no FFP administration. Although the patients that received FFP had a more rapid restoration of coagulation function, there was no change in time to discharge from hospital (Isbister et al. 2013b). There are no studies evaluating the role of FFP administration in clinical veterinary patients. An experimental study of Brown snake venom in research dogs found afibrinogenemia persisted longer after FFP administration than it did in the dogs that did not receive FFP (Jelinek et al. 2005). Although there are reports of FFP administration being used as part of treatment of Australian snake envenomation in dogs, there is limited availability of canine FFP at most Australian practices and little to no availability of feline FFP, so it is unlikely to be a major part of management in many cases (Indrawirawan et al. 2014).

Complications

Myonecrosis following snake envenomation can be severe and may reach peak severity several days after the bite, perhaps when the animals become more active once the neurotoxicity resolves (Judge 2015). It can result in prolonged hospitalization and animals may take weeks to regain normal muscle function. Megaesophagus has been reported in dogs following Tiger snake envenomation, which can require feeding tube placement and significant additional cost and challenges for management (Hopper et al. 2001).

Acute kidney injury is a reported complication following Australian snake bite. The potential mechanisms may include pigment nephropathy from myoglobinemia and/or hemoglobinemia, renal thrombosis or hemorrhage, and possibly thrombotic microangiopathy (Casamento and Isbister 2011; Heller et al. 2006). In veterinary patients, anuric kidney failure is usually fatal given the lack of availability of hemodialysis and the cost and potential complications of peritoneal dialysis. As such, appropriate early management of these cases is essential. Immune-mediated hemolytic anemia has also been reported following elapid envenomation in dogs. The single published case series described four dogs that were all suspected to have sustained Tiger snake bites, but a definitive diagnosis of the snake species was not made for all dogs (Ong et al. 2015). Three of the four dogs had evidence of disease resolution in weeks to months after the envenomation, one dog was lost to follow up.

Prognosis

Reported survival rates following snake envenomation in Australia range from 30% to 95% of dogs and 66% to 97% of cats that received treatment (Barr 1984; Heller et al. 2005; Hill and Campbell, 1978; Indrawirawan et al. 2014; Mirtschin et al. 1998). It is important to note that these results include several older studies and represent a bites of several different species of snake as well as a varying standard of veterinary care. The duration of hospitalization following snake envenomation has not been well reported in the literature. A more recent study of primarily Tiger snake envenomation reported an average period of hospitalization of just over 3 days for both dogs and cats (Indrawirawan et al. 2014).

Snake Envenomation in Dogs and Cats in the United States

Snake envenomation is a clinically significant cause of presentation to veterinary hospitals for small animal patients in the United States. Approximately 162 snake taxa are native to the United States, about 27 of which are front-fanged venomous taxa, with the majority of these belonging to the family Viperidae, Subfamily Crotalinae. Pit vipers (Crotalinae), including rattlesnakes (*Crotalus* spp.), copperheads and water moccasins (Agkistrodon spp.), and pygmy rattlesnakes and massasaugas (*Sistrurus* spp.), are responsible for approximately 99% of the venomous

bites sustained in the USA (Peterson 2006). Two genera of Coral Snakes (family Elapidae) are indigenous to the United States: *Micruroides* euryxanthus (Sonoran Coral Snake) and *Micrurus* which contains three medically relevant subgenera; *M. fulvius* fulvius (Eastern Coral Snake), *M. fulvius tenere* (Texas Coral Snake), and *M. fulvius barbouri* (South Florida Coral Snake). There are no published reports of clinical envenomation in the dog or cat by *M. euryxanthus.* Crotalinae and *M. fulvius* envenomation in the United States will be discussed.

A common misconception among veterinarians is that young pit vipers cannot control the amount of venom injected with a bite, therefore resulting in a larger dose of venom. Studies evaluating the flow and volume of venom injected with various types of bites (predatory or defensive) have confirmed that there is a percentage of bites without measureable venom delivery, and there may be a trend toward lower volumes injected with defensive bites (Young and Zahn 2001). Coral snakes have short fixed front fangs and require chewing action in order to deliver venom. Coral snake envenomation in dogs and cats in the United States is rare. While the mechanics of venom delivery are fascinating, the clinical reality is that most veterinary patients will present with clinical signs of envenomation. The decision to treat these symptoms will be directed by the severity of clinical signs and available resources. Snake envenomation can occur at any time of year, depending upon the activity of the snakes and exposure of veterinary patients. In very warm climates, such as the Sonoran Desert, crotalinae envenomation occurs year-round (Witsil et al. 2015).

Presentation

The main clinical effects of Crotalinae envenomation in dogs and cats are local tissue damage and coagulopathy. Crotalinae venom toxins have both local and systemic effects ranging from tissue necrosis, pain, and vascular endothelial damage, to induction of coagulopathies and other hematological changes, effects on the kidneys secondary to hypoperfusion, primary nephrotoxins, and rhabdomyolysis due to myotoxins, as well as cardiac effects and direct assault on the nervous system via neurotoxins (Gopalakrishnakone et al. 1980; Powell and Lieb 2004). A general rule of thumb with Crotalinae venom toxicity in dogs and cats is that rattlesnakes are the most severe, followed by water moccasins and copperheads.

There is relatively more information available on Crotalinae envenomation in dogs compared to cats. The published mortality rates for Crotalinae envenomation are low, ranging from 1.8% to 24% in dogs and 6% to 18% in cats (Witsil et al. 2015; Julius et al. 2012; McCown et al. 2009; Willey and Schaer 2005; Carr and Schultz 2015; Katzenbach and Foy 2015; Peterson et al. 2011; Hackett et al. 2002; Hoose and Carr 2013; Pashmakova et al. 2013). Nonsurvivors typically suffer envenomations to the head, including the eye and tongue, which may provide a more direct route to the central nervous system or predispose to asphyxiation. Dogs that have suffered distal limb envenomations and acutely died are suspected to have experienced intra-arterial envenomation. Envenomations to the trunk may lead to profound clinical signs, including hemoperitoneum and acute respiratory muscle

paralysis (Julius et al. 2012; Istvan et al. 2015). Relatively speaking, cats appear more susceptible to profound muscle weakness (Julius et al. 2012; Pashmakova et al. 2013). Dogs with advanced age and increased time from envenomation to treatment are risk factors for death (Witsil et al. 2015). It is well accepted that crotalinae envenomation has an overall low mortality rate, but patient suffering and morbidity may be profound, requiring significant and costly therapies. Nearly any body system may be affected following Crotalinae envenomation.

The classic clinical signs of Crotalinae envenomation involve pain, swelling, regional ecchymosis, and one to two small puncture wounds. It is reported that the Mojave rattlesnake may have pure neurotoxins, therefore making identification of a wound difficult. Most animals presenting for evaluation following envenomation will exhibit local disease at the bite site, in addition to systemic clinical signs. Dogs often suffer bites to their muzzle, with extremity as the next most common site. Cats will often suffer bites to multiple regions of their body. These patients may present anywhere along the spectrum of compensatory to decompensatory shock. Bites to the tongue or mouth may swell rapidly, leading to upper airway obstruction (Fig. 1). Intraocular envenomation may result in acute death, and nearly always leads to permanent vision loss and may require enucleation if the globe is ruptured (Fig. 2). Hyperglycemia and hypokalemia may be appreciated as a consequence of catecholamine surge. Cardiac arrhythmias are common and should be monitored for. Pigmenturia may occur due to hemolysis, rhabdomyolysis, or both. Anemia may occur due to hemorrhage, hemolysis, or both. Thrombocytopenia may be observed, with or without prolonged bedside coagulation times (PT, aPTT). Hyperlactatemia is common, and likely due to both tissue damage and hypoperfusion. Widespread hemorrhage may occur, leading to hematemesis, hematuria, melena, epistaxis, pulmonary infiltrates, or any combination thereof. Neurotoxicity may lead to seizures, nystagmus, or paralysis. Hypoventilation is a risk factor for patients with profound weakness and/or central nervous system involvement. Patients may exhibit any combination or degree of severity of these clinical signs.

Fig. 1 Severe swelling resulting in upper airway obstruction from *Crotalus atrox* lingual envenomation

Fig. 2 Intraocular envenomation resulting in globe rupture from *Crotalus atrox* envenomation

The most common clinical effects of *M. fulvius* envenomation in dogs and cats are lower motor neuropathy. Hemolysis has been reported in dogs, but this is not observed in cats following envenomation. Dogs envenomated by *M. fulvius* are most commonly reported to experience lower motor neuron weakness (requiring mechanical ventilation), ptyalism, vomiting, hemolysis, and pigmenturia. In cats, mental depression, acute ascending flaccid quadriplegia, and hypothermia are the most common reported clinical signs (Perez et al. 2012).

Diagnosis

The most common clinical signs of Crotalinae envenomation in dogs and cats include painful swelling and one or two very small wounds draining blood or serosanguineous fluid. Many dogs suffer envenomation to the face and mouth, with foot/distal extremity wounds next most common. *M. fulvius* bite wounds are difficult to impossible to identify, thus making recognition of clinical signs critically important in regions where dogs and cats share a habitat with these snakes.

In general, Rattlesnake envenomations result in more severe clinical signs in both dogs and cats. These symptoms vary by region and may include pain and swelling at the site of envenomation, hypovolemic shock, arrhythmias, hemolysis, hemorrhage, profound muscle weakness, and seizures. Peer-review literature describing symptoms of Agkistrodon envenomation in dogs and cats is lacking, but symptoms are reportedly limited to mild local pain and swelling at the site of envenomation.

Clinicopathologic changes that have been reported in dogs and cats with Crotalinae envenomation include red blood cell membrane morphology changes, hemolysis, hypokalemia, muscle enzyme elevation (creatine kinase, alanine aminotransferase, aspartate aminotransferase), thrombocytopenia, prolonged activated prothrombin time and activated partial thromboplastin time, hypofibrinogenemia, and anemia. Type III echinocytosis has been reported in dogs envenomated by

C. viridis viridis in Northern Colorado, and is generally observed in most envenomations in Maricopa County, Arizona (Hackett et al. 2002). Anemia and hemolysis have been reported in the dog following *M. fulvius* envenomation (Perez et al. 2012).

Treatment

Treatment for snake envenomation in dogs and cats will vary based upon suspected snakebite and patient clinical signs. Patients with clinical signs of *M. fulvius* have the best chance of survival if they receive antivenom, as many will require mechanical ventilation. Antivenom administration may hasten clinical recovery and decrease overall cost of care. The most current practical option for *M fulvius* antivenom is Coralmyn (Polyvalent Anti-Coral Fabotherapeutic, Instituto Bioclon S.A. de C.V., Mexico), which requires an import permit from the USDA. Coralmyn has been documented to be safe and clinically effective in dogs and cats, and it is recommended at the earliest possible time following envenomation.

There are currently four USDA approved antivenoms for dogs in the United States. Other formulations of antivenoms may be administered to dogs and cats in an off-label manner, which is commonplace in the practice of veterinary medicine. Antivenin Crotalidae Polyvalent (ACP) is concentrated, lyophilized antivenom consisting of whole IgG molecules from horses that have been immunized with venom from four snake species (Table 1). There has been a failure to reverse the effects of neurotoxins with this antivenom. Acute and delayed immunologic reactions have been documented with this product (Witsil et al. 2015; Peterson et al. 2011; Pashmakova et al. 2013). The time to reconstitute this antivenom can be prolonged and averages 1 h in human studies. Boeringer Ingelheim Vetmedica distributes ACP antivenom and is USDA approved for use in veterinary medicine.

There is one USDA approved F(ab')2 antivenom available at the time of writing, Venom Vet™. This product is labeled to neutralize the venom of all North American Crotalinae snakes, and is a collection of purified pooled immunoglobulins from healthy horses immunized against multiple species (Table 1). The product comes as a liquid, must be refrigerated and has a 3-year shelf life. There are no peer-review publications describing the clinical efficacy or use of this antivenom.

Another widely used F(ab')2 antivenom is Antivenom (*Crotalus durissus – Bothrops asper*), imported from Mexico and distributed by Veteria Labs. This antivenom has been described in multiple peer-review publications and appears to be safe and effective (Witsil et al. 2015; Julius et al. 2012; Carr and Schultz 2015; Katzenbach and Foy 2015; Hoose and Carr 2013; Pashmakova et al. 2013). It is not approved for use by the USDA, therefore requires an import permit. The product comes as a sterile, lyophilized product with a 3-year shelf life at room temperature storage. The reconstitution occurs instantaneously. The author (RW) finds this antivenom to be effective at clinical improvement with neurotoxins and myotoxins. One vial has been shown to be sufficient to neutralize clinical signs of rattlesnake envenomation in most dogs. Dogs with lower body weight and increased time from

Table 1 Comparison of various *Crotalinae* antivenoms used in dogs and cats

Immunoglobulin type	Formulation	Supplied as	Venoms used in production	USDA approval status as of May, 2017
IgG – Equine Longest $T_{1/2}$ 150 kDa Two venom binding sites	Antivenom Crotalidae polyvalent (ACP) Distributed by Boehringer Ingelheim Vetmedica	Lyophilized Powder Slow Reconstitution Room temperature storage	*Crotalus atrox, C. adamanteus, C. Terrificus, Bothrops asper*	USDA approved for use in veterinary medicine
Fab – Ovine Shortest $T_{1/2}$ 50 kDa One venom binding site	CroFab®	Lyophilized Powder Fast Reconstitution Room temperature storage	*Crotalus Atrox, C. Adamanteus, C. scutulatus, Agkistrodon piscovorus*	FDA approved for use in human medicine Off-label use in veterinary medicine
F(ab')2 Longer $T_{1/2}$ than fab, shorter than IgG 110 kDa Two venom binding sites	Venom vet™ Produced by Instituto Biologico, Argentino S. A.I.C.	Liquid No reconstitution necessary Refrigeration necessary	*C. durissus, C. C. simus, Lachesis muta, Bothrops Asper, B alternatus, B diporus*	USDA approved for use in canine
F(ab')2 Longer $T_{1/2}$ than fab, shorter than IgG 110 kDa Two venom binding sites	Antivenom – Bothrops Asper & Crotalus durissus Produced by Veteria labs, S.A. de C.V.	Lyophilized Powder Slow Reconstitution Room temperature storage	*C. durissus, C. oreganus, C. o. helleri, C. adamanteus, C. scutulatus, C. atrox, C. horridus, Agkistrodon contortix, A. piscivorus, Bothrops asper*	Pending USDA approval for use in veterinary medicine Import permits required for experimental use

bite to presentation require more antivenom (Witsil et al. 2015). A safety study reported that up to six vials could be administered intravenously within 1 h safely to healthy dogs (Woods and Young 2011). There is a pooled equine plasma product called Rattler Antivenin (MG Biologics) that was recently labeled and approved by the USDA as an antivenom, and is marketed as a less costly option for antivenom in dogs and cats. The author (RW) does not currently recommend this product for dogs and cats, as it is a xenotransfusion of plasma lacking peer review evidence of safety or efficacy. The author has consulted on a case with documented Type III allergic reaction following single infusion in a canine patient.

CroFab® has been administered to dogs and cats, but is typically cost prohibitive and not commonly pursued. Petersen and colleagues reported that an average of 1.2 vials were sufficient to neutralize clinical signs in dogs with Crotalinae envenomation in Arizona, California, and Texas (Peterson et al. 2011).

Local wound treatment with LASER (Light Amplification by Stimulated Emission of Radiation) therapy has been promoted by some veterinarians. Peer-review evidence of this therapy is lacking. Nonsteroidal anti-inflammatory medications are not recommended, as these patients are at risk for kidney injury due hypoperfusion, coagulopathy, nephrotoxins in the venom, and pigmenturia. Additionally, gastrointestinal ulceration is possible secondary to hypoperfusion and coagulopathy. Routine administration of glucocorticoids is not recommended. No morbidity or mortality benefit has been documented with use of glucocorticoids in dogs envenomated by Crotalinae spp., and potential risks of use outweigh potential benefit.

The only reliable means of envenomation prevention is avoidance. Common attempts at envenomation prophylaxis include aversion training, and use of a rattlesnake vaccine, *Crotalus atrox* Toxoid manufactured by Hygieia Biological Laboratories and distributed by Red Rock Biologics. This vaccine claims efficacy against *C. atrox* venom, and the manufacturer also notes possible protection against venoms of many other Crotalinae snakes. Canine challenge studies evaluating postvaccine antibody titers in dogs, or support of clinical efficacy are lacking. A retrospective evaluation of dogs suffering rattlesnake envenomation reported no measureable benefit of vaccination (Witsil et al. 2015). There is no peer-review evidence supporting prophylaxis of snakebite by using avoidance training, behavioral modification, or prophylactic vaccination.

Complications

Crotalinae envenomations may result in severe local tissue necrosis requiring significant wound management interventions, including surgical debridement or open wound management (Fig. 3). Severe muscle weakness with evidence of rhabdomyolysis (elevated CK with myoglobinuria) has also been observed following Crotalinae envenomation in dogs and cats. Dogs that suffer envenomation to the trunk, as opposed to the face or extremity, experience more severe clinical signs, often resulting in rapid clinical deterioration. The precise mechanism of this observation remains unknown, but it is thought to be related to close proximity of venom delivery to diaphragm and resultant paralytic effects. Dogs that have been treated with the rattlesnake vaccine may experience profound anaphylactoid-like response when naturally envenomated by Crotalinae species. These symptoms include profound vomiting, diarrhea, and cardiovascular collapse and are not the classic symptoms of envenomation. *M. fulvius* envenomations may result in delayed clinical signs as late as 48 h following exposure.

Some patients may require transfusion of red blood cells to treat secondary anemia due to blood loss, hemolysis, or both. Hemolysis with spherocytosis may be observed, sometimes as late as 72 h following initial envenomation. It is most

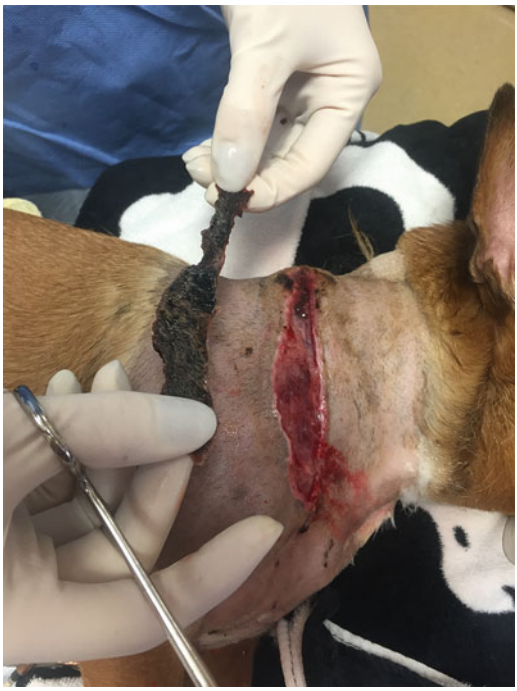

Fig. 3 Necrosis at site of bite following *C. atrox* envenomation in a dog

likely that these patients are experiencing ongoing envenomation, and treatment with antivenom should be prioritized over immune suppression. In patients experiencing hemorrhagic complications of envenomation, treatment with fresh frozen plasma is not indicated. The mechanism of coagulopathy in most cases is not due to factor deficiency, rather a complex syndrome of factor inhibition, activation, platelet inhibition, and endothelial dysfunction. As such, neutralization of circulating venom with antivenom is the treatment of choice.

Prognosis

Prognosis for survival following Crotalinae envenomation in dogs and cats is relatively good. The published mortality rates for Crotalinae envenomation are low, ranging from 1.8% to 24% in dogs and 6% to 18% in cats (Witsil et al. 2015; Julius et al. 2012; McCown et al. 2009; Willey and Schaer 2005; Carr and Schultz 2015; Katzenbach and Foy 2015; Peterson et al. 2011; Hackett et al. 2002; Hoose and Carr 2013; Pashmakova et al. 2013). It is well accepted that crotalinae envenomation has an overall low mortality rate, but patient suffering and morbidity may be profound, requiring significant and costly therapies. Prognosis for survival following M. fulvius envenomation is variable, with a poor prognosis once ventilatory failure occurs without access to mechanical ventilation.

Conclusion and Future Directions

Australian elapid snake envenomation is very common in dogs and cats and is associated with a high fatality rate if not treated appropriately. The optimal approach to diagnosis and treatment has yet to be determined and a true understanding of incidence, current management practices, and outcome is needed.

Overall survival following snake envenomation in dogs and cats is good. Dogs and cats suffering *M. fulvius* envenomation have a good prognosis with treatment. One retrospective study out of Florida reports an overall survival to hospital discharge of 70% (Perez et al. 2012). Multiple studies have documented survival following pit viper envenomation to exceed 90%, with and without antivenom administration. The morbidity with and without antivenom is not well documented, and it is possible that antivenom administration reduces patient morbidity.

Snake envenomation in the United States is a common regional problem for dogs and cats, but is associated with a low fatality rate. There are ongoing developments in antivenom options with increasing safety and efficacy. The optimal approach to treatment is quite variable between region and individual patient clinical signs. The needs for antivenom in dogs and cats include long shelf life, polyvalent, low risk of antigenicity, room temperature storage, rapid reconstitution, affordability, and lowest possible dose to achieve sustained clinical improvement. These needs provide an opportunity for companion animals to serve as a model for development of human antivenoms in third-world countries.

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Snake Envenomation in Domestic Animal Species in Australia

20

A. M. Padula, Hui Mei Ong, and Kylie Kelers

Contents

Abstract

Snakebite is a significant animal health problem for domestic animals in Australia. Animalsare more frequently envenomed than humans, perhaps due to their innate hunting instincts. The Australian continent is home to many highly venomous snakes. The most common snake species responsible for animal envenomation are the tiger snake (*Notechis scutatus*) and eastern brown snake (*Pseudonaja textilis*).

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Notable, but less frequently reported snake species include the red bellied black snake (*Pseudechis porphyriacus*), taipan (*Oxyruanus scutellatus*) and death adders (*Acanthophis* sp.). There is significant geographic variation between regions of Australia and the venomous snake species that occur. Dogs and cats are the most frequently reported animal species requiring veterinary treatment for clinically significant envenoming, however horses and other animal species are occasionally envenomed. A range of clinical syndromes have been reported for envenomed animals and are dependent upon the snake species. Animals may present with generalized paralysis, myopathy, coagulation disorders or local bite site reactions. The chronology and severity of clinical signs appear to be related to total venom dose received, bodyweight and elapsed time. Death may occur rapidly following envenomation from some species of snakes, particularly in low bodyweight dogs. Veterinarians commonly diagnose snakebite by interpretation of clinical signs, and application of hematological, biochemical and coagulation tests. The principle treatments used by veterinarians are antivenom, hospitalization, supportive care, and respiratory support measures. Research to improve diagnostic efficiency, better understanding of cost-benefit of various treatment approaches are required to improve outcomes for animals and owners.

Introduction

Australia is home to many of the most venomous snakes in the world. The hunting instincts of dogs and cats mean that they frequently encounter and are bitten by these snakes. Though less frequently an occurrence, herbivorous animals may inadvertently come into contact with snakes whilst grazing. The incidence of and death rate from snake envenomation in Australian domestic animals is far greater than that in the Australian human population (Heller et al. 2005; Mirtschin et al. 1998; Sutherland and Leonard 1995). Despite the significance of snake envenomation in Australian domestic animals, there remains a paucity of literature on this subject. Knowledge relating to the pathophysiology and treatment of envenomation in Australian animals has largely been extrapolated from human literature. This review on snakebite in Australian domestic animals summarizes the current veterinary literature on this topic.

Snakebite in Animals in Australia

Epidemiology of Snakebite in Animals

The epidemiology of snakebite in animals is not easily studied. Not all animals bitten by a snake will receive veterinary attention, and of those that do, some cases will go undiagnosed while some other conditions will be incorrectly diagnosed as snakebite.

Incidence in Dogs, Cats, Horses and Other Animal Species

In 1993, 10% veterinarians listed with the Australian Veterinary Association were asked for the details of snakebite cases recorded in the previous 5 years. Eighty practising veterinarians responded to the survey: 61 reported cases and 19 had no records of snakebite. From the data recorded it was estimated that approximately 6240 cases of snakebite would be presented to veterinary clinics in Australia each year (Mirtschin et al. 1998). Veterinarians in this survey reported that cats and dogs were more frequently presented to veterinarians with snakebite than other domestic species (Table 1). In 2005 a mailed questionnaire was sent to veterinary clinics in one state of Australia (New South Wales, N.S.W.), with the objective of obtaining data on the incidence of elapid snake envenomation in dogs. Sixty-four percent (253) of the returned questionnaires noted that they had encountered snake envenomation cases in dogs. From their data the authors estimated that the yearly incidence of snake envenomation within in the population of dogs in New South Wales was 0.31% (Heller et al. 2005). These surveys may give us some idea of the incidence of snakebite; however, the statistics derived from the information gathered cannot be taken as being definitive as there was no control over the accuracy of information received.

Geography

Venomous snakes are found throughout the continent of Australia. Based survey results, (Mirtschin et al. 1998) it was reported that 88% of snakebites in domestic species occur in rural areas. A retrospective study in Western Australia (W.A.) compared numbers of multivariate poisoning presenting to two veterinary clinics: one on the outskirts of a city and one in a rural area. In this study there were more snake envenomed dogs and cats presenting to the urban clinic however numbers were small ($n = 26$) (Robertson et al. 1992a). A subsequent prospective survey was sent to 66 veterinary practices in W.A. with the objective of reporting the epidemiology of poisons affecting dogs and cats. Thirteen cases of snake envenomation were reported from rural or semi-rural areas compared with just one case reported from an urban area (Robertson et al. 1992b). Despite these findings, the incidence of snake envenomation continues to be a significant issue in urban areas as demonstrated by a recent publication that reviewed 149 cases of snake envenomation in dogs and cats that had presented to a city practice over a 9 year period (Indrawirawan et al. 2014).

Table 1 Snakebite cases recorded over a five-year period (1988–1993) by 80 practicing veterinarians in Australia (Adapted from Mirtschin et al. 1998)

Animal	Number of snakebite cases recorded (%)
Cat	821 (51.8%)
Dog	702 (44.3%)
Horse	27 (1.7%)
Cattle	36 (2.3%)
Total	1586

Seasonality

Given that snakes are ectotherms, it is no surprise that the incidence of snake envenomation in Australia is affected by season with an increased incidence of snakebite over the warmer months (Barr 1984; Hill 1979; Hill and Campbell 1978; Robertson et al. 1992b). Barr compared the presentation of tiger snake envenomed dogs and cats to weather data obtained from the Bureau of Meteorology. Barometric pressure, temperature, rainfall and humidity results were examined in relation to snake envenomation over a 10-year period in southern Australia. In this study, 80% (240) of snake envenomation cases occurred in the warmer months of the year (October until March). The highest incidence for dogs was in the months of December and January whilst the highest incidence for cats was in the months of October and November. Of all of the weather factors assessed, only daily maximum temperature influenced the incidence of snake envenomation in this population. Cases of snake envenomation in dogs were most likely to occur when the maximum air temperature was above 20 °C. This phenomenon was not observed in cats. Overall year to year incidence of snake envenomation trended in the direction of the average air temperature of each year (Barr 1984).

Envenomation Syndromes by Venom Type in Dogs, Cats and Horses

The signs associated with snake envenomation in dogs, cats and horses are related to the effects of their venom components and are generally similar to those in humans. A number of cases of Australian snake envenomation have been reported though it is often not possible to accurately identify the species of snake that has been responsible for envenomation in animals (Lewis 1994c; Mirtschin et al. 1998; Shea 1999). In many cases the snake responsible for the envenomation is not witnessed (Indrawirawan et al. 2014; Kelers 2005). Animals present to veterinarians with a range of clinical signs that are typical of snake envenomation. Geographical location, venom detection, and identification using scale counting are all methods used in order to ascertain the genera of snake responsible.

Tiger Snake (*Notechis* sp.)

The systemic effects of tiger snake (*Notechis scutatus*) envenomation in dog and cats have been researched more extensively than in any other species of snake in Australia. Both retrospective reports and experimental studies have been published (Hopper et al. 2001; Indrawirawan et al. 2014; Lewis 1994b, c; Moisidis et al. 1996; Ong et al. 2010). The venom of tiger snakes contains components that cause pre- and post- synaptic paralysis, consumptive coagulopathy, rhabdomyolysis and mild haemolysis.

The systemic effects of tiger snake venom in animals include early collapse, vomiting, salivation, coagulopathy, neuromuscular paralysis and myopathy. Clinically tiger snake envenomation in dogs and cats ranges from a peracute onset of paralysis and death, to a slowly progressive myopathy that develops over several days. The severity and rapidity of the clinical signs observed in tiger snake

Table 2 Dose dependent clinical signs recorded after SC injection of TSV in dogs (Lewis 1994a)

LD of TSV	Clinical signs
0.25–0.5	Quiet demeanour, ataxia and mydriasis from 5 h
0.75–1	Pre-paralytic signs within 2 h Inability to close the mouth, weakness, ataxia, mydriasis and myolysis from 2 h
8–16	Pre-paralytic sign within 20 min Paralysis and mydriasis from 2 h Death within 5.5 h
32	Pre-paralytic signs within 20 min Death within 1.5 h

The LD of TSV in dogs has been determined to be 0.03 mg/kg by SC injection (Lewis 1994a)
TSV tiger snake venom, *LD* lethal dose in a dog, *SC* subcutaneous

envenomed dogs is directly related to the dose of venom that the victim receives; with a greater dose of venom leading to more rapid onset and more severe signs (Lewis 1994c) (Table 2). A multiple lethal dose of tiger snake venom (TSV) will result in death within 1 h due to paralysis and respiratory arrest and a sub-lethal dose of tiger snake venom will result in a slowly progressive myopathy (Lewis 1994b). Though a dog or cat may receive a sub-lethal dose of tiger snake venom, it is possible for them to die from ventilatory failure due to severe rhabdomyolysis several days after the snakebite.

Clinical signs in the dog have been grouped into three categories (pre-paralytic, paralytic and sublethal) to reflect the range of presenting signs that may occur after tiger snake envenomation (Lewis 1994c). Pre-paralytic signs are those signs that occur within minutes of the bite. They include sudden collapse, vomiting, salivation, defecation, trembling and tachypnea (Lewis 1994c). Pre-paralytic collapse is followed by an apparent recovery with the ability to walk again (Lewis 1994c). The onset of pre-paralytic signs occur between two and 30 min after subcutaneous injection with venom and last for up to 30 min (Lewis 1994c). Paralytic (or lethal) signs include paralysis, respiratory difficulty, continued hemorrhage from the bite site, and hemoglobinuria (Lewis 1994c). Hemoglobinuria occurs in the acute stages due to hemolysis and is seen when three or more lethal doses of venom are injected (Lewis 1984). Sub-lethal (or delayed) signs include mydriasis, slow or absent pupillary light reflex, ataxia or stiffness of gait, and inability to completely close the jaw (Lewis 1994c).

In contrast to dogs, cats appear to be more resistant the effects of TSV. In an experimental study TSV was injected subcutaneously into cats and the clinical effects observed (Moisidis et al. 1996). Four cats were injected with the following doses of TSV whilst being kept under a light plane of anesthesia: 0.5, 0.1, 0.02 and 0.004 mg/kg body weight. Those cats injected with 0.02 and 0.004 mg/kg of TSV showed no signs of envenomation. The cat injected with 0.1 mg/kg of TSV exhibited flaccid paralysis 21 h after the injection followed by cardiac arrest 2 h later. The cat injected with 0.5 mg/kg of TSV died within 4 h. A further six cats were injected with 0.1 mg/kg (four cats) and 0.025 mg/kg (two cats) then observed without anesthesia for 24 h (Figs. 1, 2, and 3).

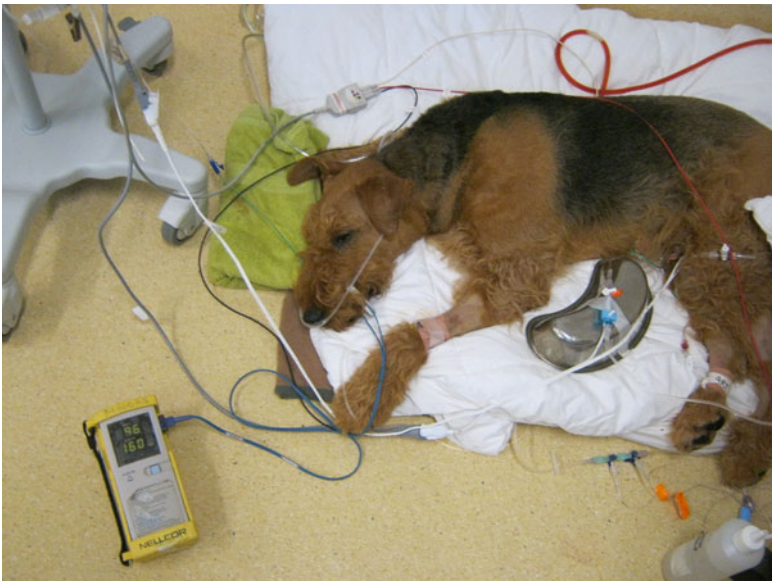

Fig. 1 Tiger snake envenomation in a dog with resulting myopathy and paralysis (Photo credit K. Kelers)

Fig. 2 Myoglobinuria in a cat associated with severe myopathy due to tiger snake envenomation (Photo credit K. Kelers)

Signs such as mydriasis, vomiting and pupillary light reflex were inconsistent and intermittent and thus may have been missed if continuous observation had not taken place. Weakness and ataxia were noted to be important early signs of a (lethal dose) LD of TSV in cats (Moisidis et al.,1996) (Table 3).

In clinical veterinary practice, dogs commonly present for veterinary attention within 4 h of a snakebite (Hill 1979; Holloway and Parry 1989; Kelers 2005); thus the acute signs are often observed in this species. Cats are more likely to present 12 or more hours after a snakebite (Hill and Campbell 1978; Holloway and Parry 1989; Kelers 2005). The significance of this is that cats, unlike dogs, will often

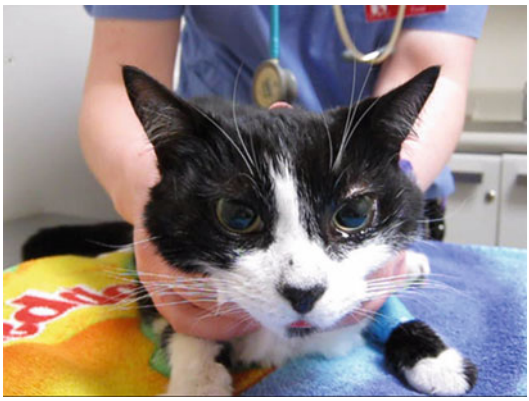

Fig. 3 Cat with dilated pupils and inability to fully close the mouth following tiger snake envenomation (Photo credit K. Kelers)

Table 3 Signs of tiger snake envenomation in cats injected with venom subcutaneously (Adapted from Moisidis et al. 1996)

	Time of onset in hours Venom per kg bwt					
Signs	0.025 mg		0.1 mg			
Cat No. Bwt (kg)	1 5.2	2 4.9	3 4.5	4 4.3	5 4.1	6 3.9
Depression/weakness	–	15	7.5	19	1	15
Vomiting	16	–	2	1	–	–
Ataxia	–	–	16	–	8	2
No PLR	8	15	7.5	7.5	–	15
Mydriasis	24[a]	23	–	–	–	–
Tachypnea/dyspnea	–	–	16	23	7	–
Muscle tremors	–	–	23	23	8	18[b]
Flaccid paralysis		–	–	–	–23	–
Death	24[c]	23[c]	24[c]	24[c]	23[c]	22

Bwt/bwt body weight, *PLR* pupillary light reflex
[a]Slight mydriasis, one eye lid twitching
[b]Convulsions
[c]Euthanized

present when neurotoxic paralysis has taken full effect and with significant myopathy (Hill and Campbell 1978; Indrawirawan et al. 2014).

The records of confirmed tiger snake envenomation in dogs ($n = 21$) and cats ($n = 4$) were retrospectively examined and the clinical features reported (Indrawirawan et al. 2014). Clinical signs recorded for dogs were variable with no one sign occurring in more than 43% of cases. Difficulty breathing or respiratory arrest was the most commonly recorded sign (9/21, 43%). Vomiting (8/21, 38%), mydriasis (6/21, 29%), and collapse (6/21, 29%) were also commonly recorded signs. Of the four cats reported, three of them presented with ataxia and two with respiratory difficulty (Indrawirawan et al. 2014).

Venom induced consumptive coagulopathy (VICC) occurs with similar frequency in tiger snake envenomed dogs and cats as it does in humans (Indrawirawan et al. 2014; Isbister et al. 2013a; Kelers 2005). Lewis reported that VICC occurred when a dog was injected with 10 LD of TSV, but did not occur when a dog was injected with 5 LD of TSV (Lewis 1984). In a retrospective study, coagulopathy as assessed by prolonged aPTT, ACT, occurred in 12/21 (57%) of dogs and 2/4 (50%) of cats (Indrawirawan et al. 2014). A clinical observational study that measured ACT in tiger snake envenomed dogs and cats on arrival at a veterinary clinic found that 14/19 (73.4%) dogs and 5/7 cats (71.4%) of cats had a coagulopathy (Kelers 2005). Despite the majority of dogs and cats presenting clinically with prolonged clotting times, clinical haemorrhage is uncommon (Indrawirawan et al. 2014).

Megaesophagus is a feature of tiger snake envenomation in dogs that is not seen in other animal species with tiger snake envenomation (Hopper et al. 2001). Esophageal dysfunction coincides with clinical myopathy. It is likely that extensive skeletal muscle within a dog's esophagus predisposes this species to this unique envenomation syndrome. Histopathology of a case of megaesophagus post tiger snake envenomation has revealed extensive myonecrosis of the esophagus (Hopper et al. 2001).

Acute microangiopathic hemolysis occurs after tiger snake envenomation in humans (Isbister et al. 2012). This condition has not been described in dogs and cats. In contrast, dogs may suffer from a delayed immune-mediated hemolysis (IMHA) after tiger snake envenomation (Ong et al. 2015). In a case series of four dogs, IMHA was diagnosed 3–9 days after envenomation in previously non-anemic dogs. IMHA was diagnosed based on the presence of morphologically abnormal red blood cells which included spherocytes, and a low packed cell volume (Ong et al. 2015). Whilst the presumed immune-mediated response appeared to have causality with snake envenomation, the authors concluded that the exact trigger for the reaction was unclear. Possible triggers were; snake venom, antivenom, fresh frozen plasma transfusion, concurrent morbidity and administered drugs (Ong et al. 2015).

Occasional cases of tiger snake envenomation in horses have been described (Cullimore et al. 2013; Fitzgerald 1975). Sweating and diffuse muscle fasciculation appear to be the dominant early clinical signs. Experimental subcutaneous injection of tiger snake venom into three horses produced rapid onset of clinical signs with death occurring 6–50 h post- envenomation following a single dose of 0.005, 0.011 and 0.022 mg/kg (Kellaway 1929). Extensive sweating was observed in all cases with muscular twitching preceding the onset of paralysis. Death was considered due to neurotoxicity and paralysis of the diaphragm (Kellaway 1929). Horses appear to be very susceptible to tiger snake venom and Kellaway estimated the lethal dose to be 0.005 mg/kg.

Brown Snakes (*Pseudonaja* sp.)

Members of the brown snake (Pseudonaja) family have been commonly reported to result in significant envenomation in animals. In a survey of 256 veterinary clinics in the state of NSW in Australia 40.4% of all envenomed dogs were suspected to be from brown snakes (Heller et al. 2005). The eastern brown snake (*Pseudonaja*

textilis) appears to be responsible for the majority of animal envenomation (Best 1998; Mirtschin et al. 1998). Members of the brown snake family are typically found in drier areas of Australia and are not present in Tasmania (Lewis 1978; Heller et al. 2005).

Venom from *P. textilis* is extremely toxic to mice with an LD50 of 0.05 mg/kg (Broad et al. 1979) making it one of the most toxic venoms of any snake in the world. Animals appear to experience a neurotoxic syndrome of progressive paralysis, likely to be the result of the action of pre- and post-synaptic neurotoxins. Coagulation disturbances commonly occur, with venom induced consumption of clotting factors quickly leading to depletion of fibrinogen (Judge 2013). In contrast to animals, the envenomation syndrome in humans appears to rarely result in neurological signs with the major signs being coagulopathy without myopathy and this has been referred to as the "brown snake paradox" (Barber et al. 2012). Despite the relatively common presentation of snakebite in animals caused by eastern brown snake to veterinarians, there are few detailed case studies describing outcomes and much of the information that is known is derived from descriptive reports (Furneaux 1967). A complication from coagulation disturbances induced by brown snake venom was described in a dog whereby an extradural hematoma formed following antivenom therapy and discharge from a veterinary hospital; subsequent additional antivenom and surgical drainage of the hematoma resulted in uneventful complete recovery (Ong et al. 2009).

A cluster of twelve cases of horses and foals envenomed by brown snakes in Queensland has been described (Pascoe 1975). Unusual seasonal weather conditions, resulting in cracks in the ground, and the feeding of horses Lucerne hay off the ground appear to have been risk factors for snakebite. Clinical signs of sweating, recumbency and generalized flaccid paralysis were described and eight of twelve horses died despite antivenom treatment, which the author acknowledges may have been too delayed to be effective (Pascoe 1975). Deaths occurred up to 10 days post-envenomation and botulism was a differential diagnosis (Pascoe 1975) (Fig. 4).

Black Snakes (*Pseudechis* sp.)

The red bellied black snake is the most commonly reported member the black snake (*Pseudechis* sp.) family causing envenomation of companion animals in Australia (Heller et al. 2005; Mirtschin et al. 1998). The effects of intravenous injection of the venom of *Pseudechis porphyriacus* on prolongation of the coagulation of dog blood of the were first reported over a century ago (Martin 1893). The clinical effects of subcutaneous injection of *Pseudechis porphyriacus* venom in various animal species demonstrated much lower toxicity than found in brown and tiger snakes (Kellaway 1930). A dose of 10 mg/kg proved certainly lethal to the cat whilst doses of 2–3 mg/kg were survived (Kellaway 1930). The most prominent clinical finding from Kellaway's studies in animals was marked hemoglobinurina and plasma hemolysis. The typical venom yield of *Pseudechis porphyriacus* is approximately 30 mg (Kellaway 1930). Subcutaneous injection into a 550 kg horse of 0.19 mg/kg (74.7 mg) of venom caused no significant clinical effect other than hematuria during the first 24 h and mild swelling at the injection site. However, intravenous injection of

Fig. 4 Cat showing signs of total body paralysis following eastern brown snake (*Pseudonaja textilis*) envenomation (Photo credit A. Padula)

20 mg of *Pseudechis porphyriacus* venom into a 840 kg horse resulted in death less than 24 h after injection with intense hemolysis observed at post-mortem dissection (Kellaway 1930).

Despite the high frequency of occurrence of *Pseudechis porphyriacus* envenomation in companion animals reported in N.S.W. (Heller et al. 2005) there are very few detailed descriptions of the resultant clinical syndrome. In a case series of dogs bitten by *Pseudechis porphyriacus* a characteristic feature was edematous swelling at the bite site and a relatively mild envenomation syndrome that was responsive to tiger snake antivenom (Gordon 1958; Padula and Winkel 2016b). The venom of *Pseudechis porphyriacus* has been shown to have powerful direct hemolytic effects, which presumably explains the marked hemoglobinuria and hemolysis (Padula and Winkel 2016b; Trigg and McAlees 2015). A single case of acute renal failure secondary to envenomation by *Pseudechis porphyriacus* and subsequent pigmenturia has been reported in a dog. The authors of this case study noted that the dog was elderly, and whilst it appeared to be clinically healthy, its renal function prior to envenomation was unknown (Heller et al. 2006). *Pseudechis* sp. venom also has active phospholipase A2 enzyme activity with a weak neurotoxic and myotoxic activity. Elevation of the muscle enzyme creatine kinase (CK) is commonly associated with bites from this species (Padula and Winkel 2016b; Trigg and McAlees 2015). Hemolysis caused by the venom may result in clinically significant anemia requiring blood transfusion (Trigg and McAlees 2015).

Other members of the black snake family appear to be only occasionally involved in envenomation of companion animals. In a survey of veterinary practices in N.S.W., 8/253 (3.2%) of veterinary practices reported treatment of blue bellied black

snake (*Pseudechis guttatus*) and 17/253 (6.7%) reported treatment of Mulga (*Pseudechis australis*) snakebite (Heller et al. 2005).

In summary, the clinical envenomation syndrome in animals induced by snakebite from *Pseudechis porphyriacus* is relatively mild, although potentially fatal, and is characterised by oedematous bite site swelling, myotoxicity, a mild anti-coagulant coagulopathy, marked plasma hemolysis and hemoglobinuria.

Taipan (*Oxyuranus* sp.)

Of our companion animals, only the dog has been reported to have been envenomed by the taipan, more specifically, the coastal taipan (*Oxyuranus scutellatus*) (Judge 2015). The venom of the coastal taipan was ranked as the third most venomous of Australian snake venoms in mouse toxicity studies (Broad et al. 1979). In this case report, the dog initially presented with vomiting, coagulopathy (ACT > 300 s, reference range 75–130 s) and neuromuscular weakness. The dog had reduced pupillary light reflexes, an absent gag reflex, reduced jaw tone and on inspiration, minimal thoracic wall movement and increased abdominal effort. The dog's clinical signs progressed to paralysis and respiratory failure. There was a mild anemia (PCV 31%, reference range 35–55%), which was attributed to hemolysis, and myolysis with CK and AST values peaking at 60 h post-envenomation (CK 6360 U/L, normal <84 U/L; AST 316 U/L, reference range 10–40 U/L). This dog was treated successfully and survived to hospital discharge (Judge 2015).

Death Adders (*Acanthophis* sp.)

Little information exists in the literature regarding the envenomation of domestic animals by death adders (Acanthophis sp.). There is a case series of four dogs that responded to treatment with death adder antivenom (Swindells et al. 2006). Based on the geographical location of the envenomation events and occurrence of snake species in those areas, three of the dogs were believed to have been envenomed by the common death adder (*Acanthophis antarcticus*) while one was thought to have been envenomed by the bardick (*Echiopsis curta*). Bardick venom has been shown to cross react with death adder antivenom such that bardick venom gives rise to a positive death adder result on the snake venom detection kit (CSL), and death adder antivenom is effective in the treatment of bardick envenomation (Marshall 1985; Steuten et al. 2007). All four dogs in this case series developed severe and progressive lower motor neuron paralysis without evidence of hemolysis, coagulopathy or significant myopathy. These findings are similar to those reported in humans (Johnston et al. 2012). Two dogs suffered respiratory muscle paralysis and required mechanical ventilation and one dog developed megaesophagus. One dog initially presented with excitement, which underscores the fact that snake envenomation can present atypically. Despite the severity of their signs, all four dogs lived to discharge following treatment with antivenom and intensive supportive care.

Whip Snakes

There is a single case report describing envenomation of a dog by a whip snake, which a herpetologist identified as a black whip snake (*Demansia papuensis* species

group) from a photograph provided by the dog's owner (Fawcett et al. 2014). The dog presented with pain, bruising and necrosis of the bite site, not unlike that which has been described in humans (Isbister and Currie 2003). CK was not measured and coagulation was not assessed in this case. The dog remained systemically well and was discharged on day 3 of hospitalization. Even though the venom of the black whip snake has been demonstrated in vitro to have neurotoxic and myotoxic effects (Kuruppu et al. 2006), the black whip snake has not been reported to cause serious envenomation in humans (Isbister and Currie 2003). This may be because the neurotoxins and myotoxins exist in small quantities in the venom, that the toxins have weak activity, or that the black whip snake has an inefficient biting mechanism or low venom production (Kuruppu et al. 2006).

Diagnosis

Differential Diagnosis, Clinical Pathology

A diagnosis of elapid snake envenomation is made based on a combination of supportive history, clinical signs and laboratory test results. Clinical signs alone are not reliable alone for diagnosing snake envenomation because of similarities shared with other diseases causing neurotoxicity, myotoxicity and coagulopathy. Lower motor neuron diseases such as tick paralysis, botulism, polyradiculoneuritis and fulminant myasthenia gravis can present with weakness in much the same way as elapid snake envenomation. Likewise, myopathies such as those of infectious, autoimmune, hypokalemic and exertional nature may mimic the rhabdomyopathy of snake envenomation. Coagulation anomalies are not uncommon in domestic animals with disorders such as rodenticide toxicity, disseminated intravascular coagulopathy (DIC), hepatic failure and inherited factor deficiencies all potentially leading to prolonged coagulation times. Clinical signs alone also do not allow for differentiation of envenomation syndromes because of the overlap in clinical manifestations. Local knowledge of snake species and the appropriate history, paired with information gathered from laboratory tests, can aid diagnosis. Some of the tests and their findings are described below.

Clotting Times, Fibrinogen, Platelet Count

The presence of coagulopathy can be assessed by measuring activated clotting time (ACT) or activated partial thromboplastin time (aPTT). A recent study of 149 snake-envenomed animals comprising 104 dogs and 45 cats found that dogs (69%) were more likely to display coagulopathy than cats (36%), as evidenced by a prolonged ACT or aPTT (Indrawirawan et al. 2014). Only 5% of animals had clinical bleeding, of which all of them were dogs. Similar findings were observed in an earlier study assessing coagulation in 6 dogs and 7 cats with suspected or confirmed tiger and brown snake envenomation (Holloway and Parry 1989). In this study, 83% of dogs exhibited marked prolongation of aPTT. In contrast, there was minimal to no prolongation of aPTT in the cats. 31% of animals (4/13) displayed clinical bleeding, and once again, they were all dogs. Of these dogs, two had evidence of spontaneous

bleeding. The remaining three dogs developed hematomas only after venepuncture. Possible reasons for the species difference include a smaller dose of venom received as a consequence of the increased agility of cats compared to dogs, apparent inherent resistance of cat plasma to the toxic effects of tiger snake venom (Maduwage et al. 2016) and the delay in presentation often seen in cats which allows for normalization of the coagulation profile (Barr 1984; Kellaway 1929; Lewis 1994c).

The effect of snake venom on coagulation is dose-dependent. An experimental study investigating the toxicity thresholds of tiger snake venom in the dog showed that venom doses ten times the lethal dose or greater led to a detectable prolongation in whole blood clotting time (Lewis 1994c). It should be noted that the lack of coagulation dysfunction on testing does not preclude the possibility of envenomation.

(Ireland et al. 2010) demonstrated in their research on humans that coagulation studies, in addition to CK concentration measurements, and serial neurological examinations, allow for the reliable detection of envenomed patients within 12 h. In their study, 96% of severely envenomed patients were identified by 6 h and 99% by 12 h (Ireland et al. 2010). In this same study, coagulation testing was the most helpful early laboratory parameter as the major clinical syndrome was venom-induced consumptive coagulopathy (VICC). Due to the presence of an anticoagulant coagulopathy in black snake envenomation and VICC in tiger snake and taipan envenomation, where there is often a concurrent myotoxicity, aPTT was an early detector of myotoxicity. Although it is widely practised, the clinical utility of coagulation testing has not been specifically assessed in snake envenomed veterinary patients (Indrawirawan et al. 2014; Judge 2015; Padula and Winkel 2016a; Swindells et al. 2006; Trigg and McAlees 2015).

Fibrin(ogen) degradation products (FDPs) occur with fibrinolysis and are increased with procoagulation, a common feature of envenoming syndromes caused by the tiger snake, brown snake, and the taipan (Gulati et al. 2013; Holloway and Parry 1989; Isbister et al. 2012; White 2005). As with secondary hemostatic testing, there are no clinical veterinary studies assessing the diagnostic value of FDPs in the diagnosis of snake envenomation.

It would appear that thrombocytopenia is less of a feature of Australian elapid snake envenomation compared to rattlesnakes (*Crotalus* spp.) and vipers (*Vipera* spp.) (White 2005). The little information that is available regarding the effect of Australian elapid snake venom on platelet count in domestic animals relate to canine experimental studies. Thrombocytopenia and leukocytosis associated with procoagulation and thrombosis formation has been described following injection of tiger snake venom (Tibballs 1998). The thrombocytopenia was transient, with platelet numbers almost normal within 30–40 min of venom administration. Thrombocytopenia has similarly been observed in dogs as early as within 5–10 min of brown snake venom administration (Tibballs et al. 1991). Improvement in platelet numbers is reported to occur independently of antivenom dose, fresh frozen plasma administration or fibrinogen regeneration (Jelinek et al. 2005). The degree to which thrombocytopenia contributes to clinical bleeding is unclear, although it may be presumed to be small. This is in view of the fact that the majority of animals that

present with snake envenomation in Australia do not display clinical bleeding (Barr 1984; Holloway and Parry 1989; Indrawirawan et al. 2014; Ong et al. 2016). In human tiger and brown snake envenoming cases, thrombocytopenia has been observed as part of the syndrome of thrombotic microangiopathy (Casamento and Isbister 2011; Isbister et al. 2007a). This has not been described in veterinary patients.

Serum Enzymes (CK, AST, ALT)

Myotoxicity is a feature of some envenomation syndromes, such as those caused by tiger snake, black snakes, and the taipan. There is no clear definition for snake venom induced myolysis in veterinary medicine; it is sometimes assessed to be a CK concentration of >1000 U/L (Indrawirawan et al. 2014; Ong et al. 2016). Other cytosolic enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may also reflect muscle damage if increased, however, CK is the most specific (Boyd 1983, 1988; Preston et al. 1990; Valentine et al. 1990). In many species, CK is found at much higher concentrations in skeletal muscle as compared to AST (Boyd 1983). However, because skeletal muscle comprises a relatively large proportion of the body's mass, an increase in AST activity can also reflect myofibre damage (Valentine et al. 1990).

Creatine kinase values can be markedly elevated in elapid snake envenomation. One of the largest Australian veterinary snake envenomation studies to date, reported median CK concentrations of 1878 U/L (range: 100–62,586 U/L) in tiger snake-envenomed dogs and 22,396 U/L (range: 1,058–216,147 U/L) in tiger snake-envenomed cats (Indrawirawan et al. 2014). These values are comparable to those of other published cases of tiger snake-envenomed dogs, cats and humans (Hopper et al. 2001; Isbister et al. 2012; Ong et al. 2016; Padula and Winkel 2016a, c).

In the study by Indrawirawan et al. 2014, the percentage of tiger snake-envenomed dogs with elevated CK values was found to be higher compared to humans (Isbister et al. 2012). This was attributed to the increased susceptibility of the dog to notexin and/or to a delay in presentation (Lewis 1994b; Sharp et al. 1993). A delay in presentation is likely to be the reason why cats have a higher median CK concentration (Barr 1984). Although serum CK concentrations increase soon after envenomation even in sub-lethally envenomed dogs (Lewis 1994c) they take time to rise to significant levels. Thus, animals that present with acute envenomation may not initially have a markedly elevated CK concentration. This is supported by the findings of a study investigating the pharmacokinetics and pharmacodynamics of the venom of *Pseudechis australis* in rats that demonstrated a delay venom absorption and CK elevation (Churchman et al. 2010; Hart et al. 2014; Johnston et al. 2013).

The use of CK values to determine the extent of tissue damage needs to be interpreted with caution. With some snake venoms, the magnitude of CK activity has been demonstrated experimentally to parallel the amount of venom injected, the time elapsed from venom administration, and the degree of myonecrosis (Nakada et al. 1980; Nakada et al. 1984). However, with tiger snake venom, CK concentrations did not necessarily correlate to the degree of muscle damage (Mebs et al. 1983; Preston et al. 1990).

It is important to continue to monitor CK activity especially if myotoxicity has been identified. Free myoglobin is believed to cause renal injury through direct cytotoxicity, renal vasoconstriction and the formation of casts leading to tubular obstruction (Petejova and Martinek 2014). A CK concentration greater than 5,000 U/L has been found to be associated with renal failure (Brown et al. 2004). A more recent study showed that even though CK correlated with the severity of rhabdomyolysis, it did not correlate as well with acute myoglobinuric kidney injury. Instead, serum myoglobin concentrations served as a better early predictor and marker of acute myoglobinuric acute kidney injury (Premru et al. 2013).

Hematocrit

Diagnosis of snake envenomation in dogs does not rely on hematology although the presence of hemolysis and anemia may be supportive. Tiger snake venom, even at low doses, has been shown in canine experimental studies to cause as initial decrease in red cell count. The reduction in red cell count was attributed to venom-induced hemolysis, as evidenced by the presence of pink or red plasma, although hemodilution caused by fluid shifts was considered a possibility (Lewis 1994c; Tibballs 1998). Taipan envenomation has also been reported to cause hemolysis and anemia in the dog (Judge 2015). Even though hemolysis is a known feature of envenomation by these snakes, the resulting anemia is often not severe (Judge 2015; Lewis 1994c; Tibballs 1998). In dogs, clinically significant venom-induced anemia necessitating the transfusion of blood products has only been reported following red-bellied black snake envenomation (Heller et al. 2005; Padula and Winkel 2016b; Trigg and McAlees 2015). The incidence of anemia secondary to hemolysis is unknown in cats although it would seem less common. Cat erythrocytes are allegedly more resistant to venom-induced hemolysis compared to the dog due to their different lecithin to sphingomyelin ratios (Condrea 1979).

In addition to hemolysis resulting from a direct effect of the venom, immune-mediated haemolytic anemia (IMHA) is another potential cause of anemia (Ong et al. 2015). Immune-mediated haemolytic anemia should, therefore, be considered as a differential diagnosis for a persistent or worsening anemia in snake envenomed animals.

Urinalysis

A urinalysis should be performed to assess for the presence of myoglobinuria and/or hemoglobinuria (Callens and Bartges 2015). The former occurs with rhabdomyolysis and the latter with hemolysis. On gross examination, the urine may be pink, red, brown or black, and will have a positive occult blood reaction on dipstick semi-quantitative chemical analysis (Callens and Bartges 2015; Lewis 1994c). The reagent test strip cannot differentiate between hemoglobin and myoglobin (Callens and Bartges 2015). However, there should be no red blood cells present on microscopic examination (Callens and Bartges 2015). It can be difficult to differentiate hemoglobinuria from myoglobinuria in a normal laboratory setting although plasma colour and serum CK and AST concentrations can provide additional clues. The plasma will be pink or red with hemoglobinuria, and CK and AST concentrations

will be elevated with myoglobinuria (Callens and Bartges 2015). There is no staining of the plasma with myoglobinemia because of the kidney's ability to rapidly clear myoglobin (Biörck 1949; Knochel 1982). Both pigments may be present simultaneously, causing pigmenturia. To monitor renal function, it is important to serially assess urine for the presence of casts, and to assess for changes in urine output and serum creatinine (Murray et al. 2014).

Urea, Creatinine, Electrolytes

The venom of Australian elapids has not been shown to have direct nephrotoxic effects. However, acute kidney injury may occur indirectly, as a result of rhabdomyolysis, hemolysis, procoagulation and hypoxemia/ ischemia (Ross 2011). Factors such as hypovolemia and aciduria can contribute to the development of renal dysfunction (Ross 2011). The incidence of kidney injury with elapid snake envenomation is not known but has been reported in the literature (Heller et al. 2006; Ong et al. 2016). Accordingly, it is important to assess for renal dysfunction, especially if the patient's presentation has been delayed or if there is a clinical suspicion. Baseline bloodwork and urinalysis obtained at presentation will aid diagnosis and help chart the patient's progress during the course of treatment. The following may be seen with renal dysfunction: renal azotemia (elevated serum urea and creatinine) with poorly concentrated urine, hyperphosphatemia, hyperkalemia and metabolic acidosis (Ross 2011). Rhabdomyolysis by itself can also cause hyperkalemia and hyperphosphatemia (Zutt et al. 2014).

Snake Venom Detection Kit

Rapid immunological diagnosis of Australian elapid snakebite can be achieved in humans and animals using a commercial snake venom detection kit (SVDK, bioCSL, Parkville, Australia). The SVDK utilises venom specific rabbit antibodies coated to polystyrene microplate wells to capture free venom. Detection is achieved using a novel freeze-dried horseradish peroxidase conjugate and the addition of TMB as the chromogen (Cox et al. 1992). The sensitivity is reported as being at least 10 ng/mL in clinical samples (Cox et al. 1992). Like many other immunoassay formats the SVDK is susceptible to reporting low or zero venom concentrations when venom is present in very high concentration, which can be found in bite site swabs (Steuten et al. 2007).

In a study evaluating SVDK sensitivity, domestic cats were injected subcutaneously with either tiger or brown snake venom and SVDK results compared to a venom specific microplate based ELISA (Moisidis et al. 1996). The results demonstrated that venom could be reliably detected by the SVDK in serum within 1 h and for up to 8 h after injection, and in urine for up to 24 h (Moisidis et al. 1996). The SVDK was not used as per the manufacturer's directions in this study. An additional 'stop' step consisting of 50 μL of 0.5 M H_2SO_4 was applied and wells were not read visually, but with a spectrophotometer. The addition of the acid has been shown to increase assay sensitivity.

The clinical utility of the SVDK was evaluated in a study of naturally envenomed dogs and cats presenting for veterinary treatment (Kelers 2005). Urine and serum

samples were tested in the field with the SVDK and also using a modification of the SVDK by use of a stop solution and quantification of the well optical density. In the study by Kelers the SVDK was found to be most useful when animals presented within 4 h of envenomation. In these cases, the majority of serum and urine samples returned positive results that were consistent with the patient history and clinical presentation. The study found that cats were typically presented to veterinarians more than 12 h post envenomation. The SVDK was highly likely to result in negative results in this cohort, despite the clinical presentation being consistent with snakebite (Kelers 2005). SVDK results are often negative in cats and this is consistent with the excretion and metabolism of venom to non-detectable levels after a delay in presentation (Moisidis et al. 1996).

The SVDK was also evaluated for its sensitivity in detection of snake venom in horse urine (Church and Forbes 2011). The larger bodyweight of horses was suspected to result in lower serum and urine levels of venom in clinical cases of snakebite. The SVDK was found to be less reliable for detection of tiger snake venom spiked into horse urine at 10 mg/mL and completely unable to detect horse urine spiked with brown snake venom at 5 mg/mL (Church and Forbes 2011).

The specificity of the SVDK in clinical cases of envenomation in animals appears to be satisfactory. Urine samples that had been previously collected from 50 unwell dogs and 25 unwell cats that were not suffering from snake envenomation were tested using the SVDK. No false positive reactions occurred. This suggests that the SVDK is highly specific for snake venom in the clinically unwell dogs and cats (Ong et al. 2010).

In a study of naturally envenomed dogs and cats by either tiger or brown snake, all of the dogs ($n = 6$) suspected of snakebite had a positive SVDK test within 2 h of the suspected snakebite (Holloway and Parry 1989). In contrast, four out of a total of seven cats suspected of snakebite were positive for SVDK test. Unlike the dogs tested, all cats presented more than 10 h after the bite. All SVDK negative cats had presented more than 12 h post bite. The study did not state whether the SVDK was performed on urine or on blood, however the results of this study support that it may be more difficult to detect venom using the SVDK in a cat with a delayed presentation compared to that of an acutely presenting dog (Holloway and Parry 1989).

Although the SVDK would appear to be a useful tool in diagnosing snakebite in animals it seems to be not widely adopted by veterinarians in Australia; this may be due to the limited time window for diagnosing envenomation from serum samples and cost of the test kits (Mirtschin et al. 1998).

Treatment

Antivenom

The principal treatment utilised by veterinarians for snakebite in animals is the intravenous administration of specific snake antivenom to neutralise circulating venom. The intravenous route of administration is essential because due to the

high molecular weight of immunoglobulin G (IgG) absorption from the subcutaneous and intramuscular routes is considered too slow to be clinically effective (Riviere et al. 1997).

Safe administration of antivenom to animal patients is generally performed by dilution in sterile saline and intravenous (IV) administration via an intravenous catheter. Care must be given to observing for the potential signs of anaphylactic reaction to the foreign protein being administered. Despite the relatively common occurrence in humans of immune-mediated acute reactions to antivenom this appears to be a much less common phenomenon in animals. Minor adverse reactions to equine $F(ab')_2$ antivenom administration were reported in 7% (4/54) of dogs intravenously antivenom for European viper envenomation (Lund et al. 2013). The acute reactions consisted of facial swelling, vomiting and profound panting although no cases of delayed reactions consistent with classical serum sickness were documented (Lund et al. 2013). In another controlled experimental study, no adverse signs were observed, early or late, when purified whole IgG equine antivenom was administered by rapid intravenous infusion (<1 min) in healthy non-envenomed animals (20 cows and 47 horses) at a dose of 0.4 mL/kg (Estrada et al. 2010). However, when the same antivenom was used in humans early adverse reactions occurred in approximately 20% of patients and 0.5% of reactions were described as severe (Estrada et al. 2010). Administration of reconstituted lyophilised equine $F(ab')_2$ antivenom directed against *Crotalus durissus* and *Bothrops asper* to normal healthy dogs did not result in any adverse reaction in 10 dogs administered three vials over 30–45 min (Woods and Young 2011). The typical therapeutic dose for this antivenom is typically two or less vials. However increasing the dose to six vials did result in a self-resolving and transient minor facial and cervical edema in 3/10 dogs and this reaction was attributed to early immune reaction and low bodyweight (9.1–18.2 kg) (Woods and Young 2011). An Australian retrospective study examined the records of 109 dogs and 85 cats treated with antivenom. This study attributed the deaths of one dog and three cats to 'anaphylactic' reaction to the antivenom, however no details of the antivenom product, clinical signs or clinical pathology associated with these events were described (Barr 1984).

The specificity of the antivenom required is dependent upon the venomous snake species found within each geographical region of Australia. In south eastern Australia the principal venomous snake species are the tiger snake (*Notechis scutatus*), eastern brown snake (*Pseudonaja textilis*), red bellied black snake (*Pseudechis porphyriacus*) and copperheads (*Austrelaps* sp.). A bivalent Tiger and Brown snake antivenom effectively neutralizes all of these venoms (Lewis 1978) and is likely to be the only antivenom held and used by veterinary practices in this region (Mirtschin et al. 1998). In Tasmania the two venomous snake species found are the copperhead and tiger snake; consequently due to the cross reactivity between these snake species (Sutherland 1976), and approximately similar venom quantity, only antivenom effective for tiger snake venom is required (Lewis 1978). In N.S.W. the red bellied black snake accounted for 44.6% of all snakebite cases reported by veterinary practices while the eastern brown snake accounted for 40.4% (Heller et al. 2005). Distinction of these two snake species based on

presenting clinical syndrome may allow veterinarians to be more selective in their use of tiger and, or, brown snake antivenom however a combined tiger and brown snake is often administered. The predominant snake species causing envenoming of animals in Queensland is the eastern brown snake responsible for 82% (549/667) of snakebites (Mirtschin et al. 1998). A similar situation applies in South Australia where veterinary practices reported that 94% (236/252) of snakebite cases were caused by the eastern brown snake (Mirtschin et al. 1998). In Western Australia, despite the potential presence of multiple venomous snake species the brown snakes accounted for 77% (108/140) and tiger snakes 22% (31/140) of cases reported to be treated by veterinary practices (Mirtschin et al. 1998). Monovalent death adder antivenom responsive animal snakebite cases have been described in Western Australia (Swindells et al. 2006) although recent work has shown that due to production methods used by bioCSL the 'monovalent' has polyvalent activity (O'Leary and Isbister 2009). In many regions of Australia, a wide variety of venomous snake species may be present and without venom identification testing, the most prudent choice of antivenom for treatment would be a five immunotype polyvalent antivenom (Polyvalent Antivenom, bioCSL, Parkville, Australia). Notwithstanding this there are very few confirmed reports of envenomation in animals that are caused by species other than the eastern brown snake, the tiger snake and the red bellied black snake (Mirtschin et al. 1998; Swindells et al. 2006). The greatly increased cost of polyvalent antivenom may not be justified by all veterinarians. Veterinarians commonly treat snake envenomation with monovalent or bivalent antivenom based on knowledge of the common local snake species and the presenting clinical syndromes of their patients.

Antivenom dosage in Australia is typically described using a unit system. One unit is defined as the amount of antivenom required to neutralize 0.01 mg of venom in a mouse bioassay (Sutherland 1987a). The total units of antivenom formulated into a vial of antivenom is typically sufficient to neutralize the average quantity of venom harvested from each venomous snake species. For tiger and brown snakes this is typically 3,000 U (30 mg) and 1,000 U (10 mg) respectively (Lewis 1978). Veterinarians vary in their approach to the initial dose of antivenom administered (Jacoby-Alner et al. 2011). The clinical outcome benefit of administering multiple vials of antivenom compared to one vial has not been investigated in animals. Titration of antivenom dosing in dogs and cats by serial testing of urine presence of venom has been described however there is no evidence to support that this is a rational method of antivenom dosing (Indrawirawan et al. 2014). Antivenom is expensive to administer to animals and this remains one understudied area where more cost effective protocols, particularly for repeated dosing, deserve further science based study.

Systemic neuromuscular paralysis caused by pre-synaptic acting neurotoxic venoms (particularly tiger, brown and taipan) are poorly reversed by antivenom. Administration of antivenom to humans envenomed by the Papuan taipan venom whom exhibited signs of generalized flaccid paralysis, and were more than 6 h post-snakebite resulted in little to no detectable clinical improvement (Campbell 1964). It is likely a similar scenario occurs in animals experiencing pre-synaptic neurotoxicity

and that the benefit of antivenom is probably greatly reduced at this time compare to within 0–6 h of snakebite.

In summary, antivenom is the principle treatment used in envenomed animals. Purified whole IgG or enzyme digested IgG antivenom appears to be well tolerated. The early administration of specific antivenom to envenomed animals, before generalized neuromuscular paralysis and other complications ensue, is recommended. Cost of antivenom therapy and lack of knowledge about the most appropriate dosage and titration regime remain significant issues for veterinarians in Australia.

Management of Paralysis

Snakebite in animals can result in generalized paralysis, thus supportive care is as important as definitive treatment in the management of this condition. The complications of paralysis include hypoventilation, respiratory arrest, aspiration pneumonia, pulmonary atelectasis, ileus, urine retention, urinary tract infections, decubital ulcers, corneal ulceration and peripheral edema. It is important to monitor for and implement treatment protocols to prevent or minimize the severity of these complications in snake envenomed animals.

It is imperative to assess the patient's respiratory pattern and function during the course of treatment. Serial clinical examinations should be performed and oxygenation and ventilation monitored. Blood oxygenation can be assessed using pulse oximetry or arterial blood gas analysis. Carbon dioxide partial pressure may be monitored via venous or arterial blood gas analysis or end tidal measurement (Pang et al. 2007). Supplemental oxygen should be provided if hypoxemia is suspected or confirmed. The patient should ideally be maintained in sternal recumbency which has been shown to improve pulmonary oxygen uptake (McMillan et al. 2009). In some instances, animals will require mechanical ventilation. Recumbency predisposes animals to the development of pressure sores. This can be prevented or minimized by frequent turning of the patient and the use of appropriate bedding. Increased skin moisture, as caused by hypersalivation, urine or feces, can lead to irritation of the skin. Patients should therefore be kept dry and clean.

Some patients may develop bladder dysfunction leading to urinary retention. These patients will require frequent bladder expression to prevent over-distension of the bladder which is painful and predisposes to urinary tract infections. To aid bladder management, a urinary catheter can be passed intermittently or left indwelling.

Additionally, patients with absent or reduced palpebral reflexes should have frequent eye lubrication to prevent or minimize corneal exposure ulceration. If the gag reflex is absent, the patient should not be offered food or water orally. If the animal is unable to eat safely for 3–5 days, nutrition can be delivered via an esophageal or gastrotomy tube. Studies have shown a positive correlation between energy intake and hospital discharge, and between early nutritional support and clinical outcome (Brunetto et al. 2010; Liu et al. 2012; Mohr et al. 2003). In general, enteral nutrition is preferred to parenteral nutrition because it is more physiologic, safer and cheaper (Prittie and Barton 2004). In snake envenomed patients with

megaesophagus, the placement of a gastrotomy tube is preferable to an oesophageal tube.

Mechanical Ventilation (MV)

A small percentage of elapid snake envenomed animal patients will require MV as part of treatment. In a study examining the clinical features of elapid snake envenomation in dogs and cats in Melbourne, 13% of the population required MV (Indrawirawan et al. 2014). However, due to the prohibitive cost of MV, only 45% of these animals ultimately received treatment; the remainder of the animals were euthanized (Indrawirawan et al. 2014). MV is indicated if there is: (a) severe hypoxemia in spite of oxygen supplementation, (b) severe hemodynamic compromise that is refractory to therapy, (c) severe hypoventilation (i.e., an arterial PCO_2 greater than 60 mmHg) and/or (d) excessive respiratory effort with imminent respiratory fatigue or failure (Hopper and Powell 2013). The latter two causes are the most common reasons for MV in elapid snake envenomation. (Ong et al. 2016; Trigg et al. 2014). Severe hypoxemia is defined as a partial pressure of arterial oxygen (PaO_2) of less than 60 mmHg or an arterial oxygen saturation of hemoglobin as measured by pulse oximetry (SpO_2) of less than 90% (Hopper and Powell 2013). Severe hypoxemia independent of that caused by hypoventilation may occur if there is concurrent pulmonary parenchymal disease (e.g., aspiration pneumonia) (Fig. 5).

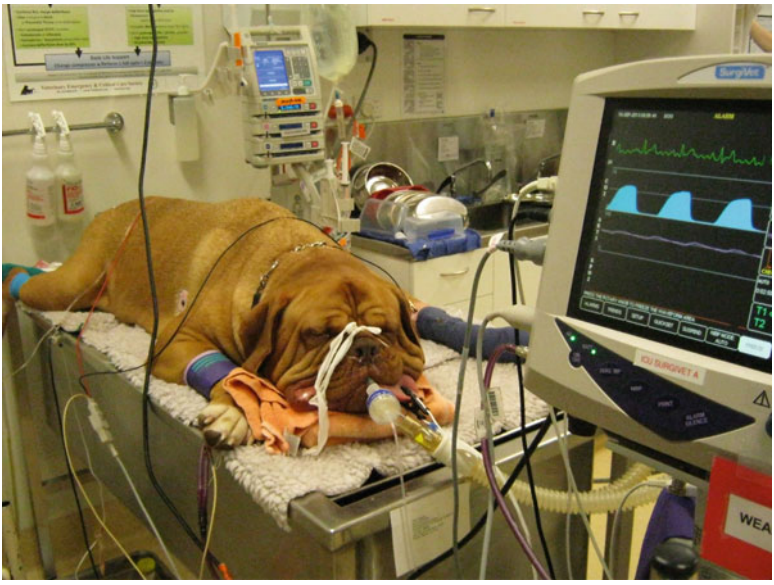

Fig. 5 Dog undergoing mechanical ventilation to support breathing following respiratory paralysis resulting from envenomation by a tiger snake (*Notechis scutatus*) (Photo credit K. Kelers)

Management of Coagulopathy

Australian elapid snake envenomation may result in severe prolongation of blood clotting times in animals. This is particularly so when procoagulant toxins are involved (Holloway and Parry 1989; Indrawirawan et al. 2014; Judge 2015; Lewis 1994b, Kelers 2005). Antivenom is effective for neutralization of unbound procoagulant toxins (Madaras et al. 2005; Bassett 2002) however the coagulopathic effect of procoagulant toxins in clinically affected animals is extremely rapid (Holloway and Parry 1989, Kelers 2005). Two studies measured the clotting times, of confirmed snake envenomed dogs and cats, on presentation to an Australian veterinary clinic. All cases that presented acutely (range 15 min to 4 h), had markedly prolonged clotting times (Holloway and Parry 1989; Kelers 2005). These studies indicate that although antivenom may be effective in binding procoagulant components of antivenom, in practice VICC often occurs prior to the opportunity for antivenom administration. Once procoagulant venom components are bound, antivenom is not able to remove them from the prothrombinase complex that leads VICC (Bassett 2002; Isbister et al. 2009). Due to the time lag required for resynthesis of clotting factors and increased levels of circulating fibrin degradation products, clotting times take many hours to return to normal in animals that have suffered from VICC. Even so, clinically significant hemorrhage in animals with VICC is uncommon (Indrawirawan et al. 2014).

The use of fresh frozen plasma (FFP) to treat VICC is controversial. Early use of FFP after antivenom administration has not consistently resulted in improved clotting times or fibrinogen levels in dogs or humans with VICC (Isbister et al. 2013b; Jelinek et al. 2005). A randomized trial in humans with VICC showed that treatment with FFP at least 6 h after antivenom administration was likely to normalize clotting times. However, despite improved clotting times in the FFP treatment group, there was no reduction in the duration of hospitalization (Isbister et al. 2013b). There are no studies comparing the outcome in animals with VICC treated with FFP.

The anticoagulant effects of snake venom that occur after envenomation from black snakes, have been shown to be reversible with appropriate use of antivenom (Lane et al. 2011). Venom induced coagulopathy due to both procoagulant and anticoagulant venom components resolve spontaneously given time (Churchman et al. 2010; Isbister et al. 2010).

Management of Myopathy

It is uncommon for human snakebite victims to present with myopathy (Churchman et al. 2010; Isbister et al. 2012). Tiger snake envenomed dogs and cats however commonly present with myopathy (Indrawirawan et al. 2014). Generalized rhabdomyolysis leads to weakness, pain and pigmenturia. Severe weakness of the respiratory muscles leads to hypoventilation and may progress to respiratory arrest. Management of dogs and cats with myopathy involves cage confinement, nursing care, analgesia and intravenous fluid therapy. Oxygen supplementation and mechanical ventilation are used to support patients with hypoventilation.

Dogs are unique in that they may suffer from megaesophagus secondary to rhabdomyolysis (Hopper et al. 2001). Recovery from myolysis is slow and thus

the megaesophagus may last for several days (Hopper et al. 2001). The management of megaesophagus in dogs has been described elsewhere and is outside the scope of this review.

A single case of acute renal failure has been described in a dog that suffered from pigmenturia secondary to red bellied black snake envenomation (Heller et al. 2006). The authors state that in this case the patient may have been predisposed to kidney injury due to prior renal insufficiency of the affected animal. Dogs and cats with tiger snake envenomation may suffer extensive damage to their muscles as reflected by the very high CK levels measured in these patients (Hopper et al. 2001; Indrawirawan et al. 2014; Ong et al. 2016; Padula and Winkel 2016a). Despite the extensive myolysis and subsequent pigmenturia caused by tiger snake envenomation in dogs and cats, acute kidney injury in this cohort of patients has not been described and appears to be a rare occurrence. Even so it is prudent to treat all patients with rhabdomyolysis with intravenous fluid therapy until their CK concentration starts to come down and their urine normalizes in colour. Urine output should be maintained at more than 1 mL/kg/h and renal function should be monitored.

Whilst antivenom will neutralize circulating myotoxin, once bound, resultant myotoxicity is irreversible (Churchman et al. 2010). For this reason, treatment with antivenom may lead to minimal clinical improvement in those patients that suffer from myopathy but no longer have circulating free venom.

Patient Outcomes

While there are very few deaths a year amongst human snakebite victims in Australia (Sutherland and Leonard 1995); death is still a common scenario following snakebite in animals (Heller et al. 2005). Reasons for this are likely to include an increased time between snakebite and treatment, lack of first aid application, inadequate or inappropriate antivenom administration, inadequate supportive care, misdiagnosis, and elective euthanasia (Fig. 6).

Fig. 6 Necrotic bite site marks on the face of a dog 48 h after envenomation by red-bellied black snake (*Pseudechis porphyriacus*) (Photo credit A. Padula)

Table 4 Summary of reported survival rates of snakebite animals from two studies. Survival of animals treated with antivenom compared to survival without antivenom

Animal Species	No. survived without antivenom/ No. that did not receive antivenom	Survival without antivenom (%)	No. survived with antivenom/ No. that received antivenom	Survival with antivenom (%)
Cat	209/319[a] 21/30[b]	66[a] 70[b]	447/491[a] 76/85[b]	91[a] 90[b]
Dog	46/149[a] 12/18[b]	31[a] 67[b]	401/546[a] 89/107[b]	75[a] 83[b]
Horse[a]	1/7	14	8/20	40
Cattle [a]	10/23	43	7/13	54

Table adapted from [a]Mitschin et al. (1998) and [b]Barr et al. (1984)

Survival with Antivenom

A small number of veterinary studies have shown that the successful treatment of snake envenomation requires administration of the appropriate antivenom (Barr 1984; Mirtschin et al. 1998). This is in agreement with the medical literature (Hawdon and Winkel 1997; Sutherland 1987b, 1992; Sutherland and Best 1991; White 1998). A survey conducted by Mirtschin et al. (1998) showed that a lower percentage of animals survived if they did not receive antivenom compared to animals that did (Table 4). A similar observation was made by Barr (1984). Other available data estimates the survival of antivenom-treated snake-envenomed dogs to range from 87% to 95%, and that of snake-envenomed cats to range from 89% to 97% (Hill 1979; Hill and Campbell 1978; Indrawirawan et al. 2014). It is important to note that across these studies, although there is much heterogeneity regarding the species of snake involved, study design (e.g., criteria used to determine snakebite, whether outcome was censored for euthanasia) and treatment (e.g., whether mechanical ventilation was employed), it is clear that the use of antivenom surpasses the lack of antivenom.

Survival with Ventilation

While snake envenomation can be treated successfully with antivenom and supportive care, severely affected animals may succumb to respiratory failure subsequent to severe neuromuscular weakness unless MV is instituted. There is a lack of information regarding elapid snake envenomed animals requiring MV. Published information consists of case reports or subgroups within larger studies that examine snake envenomation or MV (Indrawirawan et al. 2014; Judge 2015; Ong et al. 2015; Swindells et al. 2006; Trigg et al. 2014). Available data suggests that the likelihood of survival to discharge (STD) for dogs and cats undergoing MV is favorable, with STD rates ranging from 82% to 91.7% after censoring for cost-based euthanasia (Ong et al. 2016; Trigg et al. 2014). There are two possible reasons for the positive outcome. First, the nature of the neuromuscular disease is partially reversible. The prompt neutralization of the neurotoxin with antivenom results in

rapid improvement of paralysis and short ventilation times (Ong et al. 2016; Trigg et al. 2014). This is not unlike the scenario encountered in Australian tick paralysis and in human cases of neurotoxic snake envenomation (Webster et al. 2013) (Agrawal et al. 2001; Bhattacharya and Chakraborty 2007). Second, as alluded to previously, elapid snake envenomed patients are more commonly ventilated for hypoventilation and unsustainable respiratory effort rather than for hypoxemia due to pulmonary pathology (Ong et al. 2016; Trigg et al. 2014). Cats and dogs ventilated for neurological causes tend to have more favorable STD rates (39–69%) compared to those ventilated for pulmonary causes (20–42%) (Hopper et al. 2007; King and Hendricks 1994; Trigg et al. 2014). Irrespective of the cause, a protracted period of MV increases the likelihood of developing complications such as ventilator associated pneumonia, which negatively impacts length of hospitalization, weaning success rates and STD, and adds to the cost of treatment. (Anon 2011; Berton 2014).

Future Directions

Vaccination of Animals Against Venom

The development of protective immunity within animal populations at risk of snakebite is an appealing but largely unexplored domain. Protective antibodies were successfully induced in a human against tiger snake venom by repeated injection of low doses of the venom (Wiener 1960). The immunized human recipient ultimately produced serum with a neutralizing titer of up to 5.2 U/mL over 15 month period; based on this it was estimated his total neutralizing capacity was up to 170 mg of tiger snake venom, and that a serum titre of 1 U/mL would be protective against natural envenomation (Wiener 1960). However, the recipient's serum neutralizing titres rapidly declined when booster immunization was ceased and pain was often experienced with each booster dose.

A study was undertaken in dogs in Australia to explore the protective immunity generated by a novel anti-tiger snake venom vaccine (Lewis 1984). Dogs were immunized with a modified tiger snake venom immunogen using an aluminium adjuvant, immune responses were monitored by ELISA and protective immunity assed by lethal challenge with venom. Only limited protection against lethal effects of whole tiger snake venom was generated by the vaccine and there was no commercialization of the product (Lewis 1984).

A rattlesnake venom vaccine is available in the USA for use in animals; however, the level of protection provided has not been subjected to experimental challenge. Field data suggested that no measureable benefit could be attributed to this vaccine in a clinical study of 272 cases of rattlesnake envenomation in dogs and death occurred in a vaccinated dog following snakebite (Witsil et al. 2015). Nevertheless, the development of a non-toxic, effective and affordable snake venom vaccine would likely be appealing to animal owners in high risk circumstances.

Improved Diagnostics

Snakebite diagnosis can be challenging for veterinarians and improved diagnostic test methods are required. Highly sensitive, specific, inexpensive and rapid immunological methods to detect venom in urine, serum and body fluids would greatly facilitate veterinary diagnosis of snakebite. A promising new test approach to measuring snake venom associated phospholipase A2 activity in serum of human patients could also have application in animals (Maduwage et al. 2014). Some snake venoms such as that from *Pseudechis porphyriacus* contain very active phospholipase A2 enzymes, whilst brown snake venoms have much lower activity and smaller quantity of venom making this approach less reliable for envenomation by these species. Additionally other disease processes such as pancreatitis may interfere with this diagnostic approach (Maduwage et al. 2014).

Rational Antivenom Dosing

Antivenom is the mainstay of treatment for most veterinary presentations of snake envenomation. Methods to more accurately titrate or understand antivenom dosing regimens could lead to more cost effective treatments. Domestic animals vary markedly in bodyweight (1–500 kg) and this leads to wide variations in serum antivenom levels following administration. Although not documented with Australian antivenom, administration of a large quantity of $F(ab')_2$ antivenom has been shown to lead to infrequent and mild adverse acute hypersensitivity reactions in only a small percentage of normal healthy dogs (Woods and Young 2011). Due to its large size, the IgG molecule (commonly present in Australian manufactured antivenom), is retained in circulation substantially longer than pepsin digested $F(ab')_2$ antivenom products making the need for repeated IgG administration questionable. The benefit of serial administration of multiple vials of antivenom in close time proximity requires further exploration as to its efficacy, particularly when cost is a constraint.

Conclusions

Snakebite remains a significant reason for presentation of animals to veterinary practices during the warm weather seasons throughout Australia. Although precise numbers of cases are not known a survey estimated that more than 6,000 cases of snakebite may occur each year in animals across Australia (Mirtschin et al. 1998). Cases of snake envenomation can be difficult for veterinarians to diagnosis in some instances, particularly when the snakebite was not observed. Antivenom remains the most important treatment administered by veterinarians but unsolved issues remain regarding what is the most appropriate and cost effective dosing regimen. Antivenom is not the only treatment for snake envenomation and supportive care measures such as intravenous fluid therapy, oxygen supplementation and mechanical

ventilation are considered essential. The eastern brown snake, tiger snake and red bellied black snakes account for the majority of venomous snakebites in domestic animals in Australia. The majority of animals treated for snakebite are reported to survive however case presentation varies widely and access to critical care such as mechanical ventilation is not always available due to facility and financial limitations.

Cross-References

► Tick Paralysis of Animals in Australia

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Part VII

Clinical Toxinology in Australia, Europe, and Americas: Special Topics

Animal Venoms and Nephrotoxic Effects

21

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Abstract

Animal venoms are truly mortal cocktails of various toxins with the mere purpose of evolution for the survival of the organism it endogenously possesses. Due to the significant and unavoidable venomous animal-human interactions, human envenomation is an important public health concern. Animal venom toxins are capable of affecting human physiology resulting in nonlethal allergy, erythema, and pain at the site of sting or bite that subsides without any medical interventions to a more serious anaphylaxis and organ injuries which require prompt and continued medical treatment. Venom toxins are capable of imparting its effect to almost all the tissues it interacts with and through various mechanisms. In this review, it is intended to limit to the nephrotoxic effects of venom toxins from few medically detrimental species, namely, hymenoptera (bees, wasps, and ants), spiders, scorpions, caterpillars, centipedes, jellyfish, and snakes. The pathophysiology of venom-induced nephrotoxicity under the two broad categories of altered renal hemodynamics and direct renal toxicity is discussed, which incorporates various mechanisms such as anaphylaxis, coagulation cascade, the kinins, and heme toxicity that ultimately lead to kidney injury.

Introduction

In the Earth, humans share their living environment appreciably with other organisms and animals. Each living organism has developed its own unique way of survival. An arsenal of chemicals endogenously produced and stored is inevitable for some organisms for survival – helping them in predation and defense. Such chemical compounds that support the organisms that produced them and are lethal to at least some other organisms are called toxins, for the toxic effects it imparts in the tissues. Venomous animals are capable of injecting such toxins from their store via bites or stings for predation and defense. Human interactions with such venomous animals impart a significant medical condition in human health – the clinical sequelae ranging from a simple skin allergy, pain, and erythema at the site of sting/bite to anaphylaxis and multiple organ injuries leading to death. This review is concerned with the small number of medically detrimental species which possess venom that causes morbidity and mortality and in particular toxic effects on the kidneys – the organ which is of utmost significance in getting rid of the toxins in the body, which have been produced as metabolic by-products or exogenous chemicals and toxins that are ingested or injected.

Venom, Poison, and Toxins

Animals have developed different strategies to capture the prey on which they feed and various means to defend themselves from predators (Jungo et al. 2010). One of the strategies is the use of chemicals endogenously produced by the animals which are toxic (causing adverse effects) in at least some other living organisms (White et al. 2003). Such toxic biochemicals are called toxins, and in composition, they are mainly peptides, proteins, and amines. They have extremely diverse mode of actions such as affecting ion channels; stimulating transmitter release; disrupting host cell membrane leading to hemolysis, cytolysis, and antibacterial effects; stimulating the immune system resulting in hypersensitivity reactions; and many more (Jungo et al. 2010).

Animals that produce toxins are classified as either venomous or poisonous (Dongol et al. 2013). Venomous animals have a venom gland and ductwork for producing the toxin and a hardened apparatus for injecting a subdermal load of venom by a stinging or biting act (Vetter 2013). Though the terms "bite" and "sting" are interchangeably used in human envenomation, each term describes a different mode of venom delivery. "Bite" describes the venom injection via structures associated with the mouth such as fangs or mandibles, whereas "sting" connotes the injection of venom via a tapered, posterior structure most accurately called a sting or colloquially, a stinger (Vetter and Visscher 1998). Venomous animals (e.g., snakes, scorpions, hymenopterans, spiders, jellyfish, etc.) use their venom in predation as well as in defense (Jungo et al. 2010). Poisonous animals (e.g., poison-arrow frogs) possess a poison gland that secretes the toxic substances, but not through ducting, and are used only for defensive purposes. Poison generally just exudes from the gland or covers a body part and is effective when ingested or delivered topically (Jungo et al. 2010; Vetter 2013).

Since time immemorable, the interaction between human and animals has been the significant etiology of morbidity and mortality. Such interactions are one of the major causes of human envenomation by reptiles, arachnids, and insects, worldwide. Tens of thousands of venomous animal bites and stings occur each year, with most victims seeking treatment in emergency rooms. From the common bee sting to snake bite, envenomations have potential to inflict harm to humans. However, envenomations are spontaneous and usually occur without warning (Friday and Depenbrock 2014). Such human-venomous animal interactions increase in outdoor activities such as walking through the forest area or athletic activities in the forest area, picnics, and collecting fire woods or when these animals such as spiders and ants are hidden in the domestic living spaces such as garages or in the clothings, gloves, and boots.

Animal venom is a cocktail of complex compounds or toxins consisting of peptides, enzymes, proteins, and chemicals that are used for predation, digestion, and protection. These venom toxins impart wide range of physiological effects, leading to a broad spectrum of clinical manifestations. Reactions to animal envenomation vary from simple skin manifestations, mild local pain, and swelling to severe systemic symptoms such as anaphylaxis and multiple organ injuries, which depend upon the hypersensitivity of the person toward the venom toxin and upon the amount of venom injected. As a highly vascularized and excretory organ, kidneys are particularly vulnerable to venom

toxins (Dongol et al. 2013; Sitprija and Sitprija 2012). In this chapter, the nephrotoxic effects posed by various animal venoms are discussed.

The Kidneys and Venom

The kidneys play a central role in the homeostatic mechanisms of the human body, and reduced renal function strongly correlates with increasing morbidity and mortality. They form a paired organ system located in the retroperitoneal space, extended from the lower part of the 11th thoracic vertebra to the upper portion of the 3rd lumbar vertebra. The adult kidney is about 11–12 cm long, 5–7.5 cm wide, and 2.5–3 cm thick and weighs about 125–170 g in adult male and 115–155 g in adult female. In the majority of cases, each kidney receives its blood supply from a single renal artery derived from the abdominal aorta, with the venous return along a renal vein that emerges into the vena cava (Delaney et al. 2006; Nielson et al. 2012). In adults, the kidneys receive approximately 20–25% of the cardiac output, about 90% of which supplies the renal cortex to maintain the highly active tubular cells. From the enormous rate of blood flow to kidney (1–1.2 L/min), only a small quantity of urine is formed (1 mL/min) (Delaney et al. 2006; Munger et al. 2012). Kidney, therefore, is a highly vascularized organ with the filtration facility via glomerulus and concentrating (reabsorption and secretion) capacity via tubules. It is, therefore, physiologically obvious that not everything filtered is excreted and not all the quantity of particular analyte filtered is excreted. The venom toxins that are filtered through the glomerulus are, as well, reabsorbed by the renal tubules resulting in its redistribution in the body or are concentrated in the tubular lumen injuring the tubular epithelial cells, thereby delaying its removal from the body. Secondarily, pigments or casts filtered through glomerulus as a result of hemolysis or rhabdomyolysis after envenomation result in tubular obstruction or acute tubular necrosis. These factors are important determinants of rapid redistribution of the venom, upon venomous sting or bite, from the blood with slow removal from the kidney, resulting in the largest concentration of venom in the kidneys (Viswanathan and Prabhu 2011). The excretory function of the kidneys serves to rid the body of many end products of metabolism that are toxic to health upon accumulation and the exogenous chemicals and toxins that are ingested or injected. The maintenance of renal blood flow, thus, is essential to kidney function (Delaney et al. 2006; Munger et al. 2012).

Animal venom toxins contribute significantly toward the impairment of renal blood flow through various mechanisms ultimately leading to acute kidney injury (AKI). Besides, the venom toxins also have direct nephrotoxic effects. The nephrotoxicity observed during animal envenomation is generally acute and represents the entire spectrum of acute renal dysfunction from its earliest and mildest form to the need of renal replacement therapy. The term AKI, previously referred to as acute renal failure (ARF), defines either abrupt increase in serum creatinine (to denote a reduction in glomerular filtration rate (GFR)) or an abrupt decline in urine output. The important renal pathological changes leading to AKI after envenomation include acute tubular necrosis (ATN), acute cortical necrosis (ACN), acute interstitial nephritis

(AIN), thrombotic microangiopathy, vasculitis, glomerulonephritis (GN), mesangiolysis, and renal infarction. Besides the major renal manifestation of AKI, the rare manifestations observed include nephrotic syndrome (NS), distal renal tubular acidosis (dRTA), and proximal renal tubular acidosis (pRTA) (Dongol et al. 2013; Sitprija 2008). The pathophysiological basis of venom-induced AKI is attributable to hemodynamic alterations, ischemic assaults, direct toxicity of venom toxins, inflammatory reactions, and immunological mechanisms (Dongol et al. 2013).

Venomous Animal Distribution

Venomous animals are widespread throughout the animal kingdom, comprising more than 100,000 species distributed in all major phyla and virtually in all ecosystems on the Earth (Cunha 2013). They are a significant health problem for rural populations in many parts of the world, especially in the tropics (Junghanss and Bodio 2006). The terrestrial environment is a host to many venomous creatures which commonly includes snakes, spiders, scorpions, and insects such as Hymenoptera (bees, wasps, and ants) and Lepidoptera (moths and butterflies). Similarly, the marine environment is dominated by animals, many of which are largely sedentary, at least as adults, and there is frequent intense competition for living space. This has favored the evolution of many species utilizing toxins in either defense of offense. Jellyfish sting represents the most important and frequent marine envenomation in humans (White et al. 2003). Hereby, in this chapter discussion on the human envenomation by snakes, scorpions, spiders, hymenopterans, caterpillars, centipedes, and jellyfish is presented.

Venomous Animals and Their Venom Components

As a truly mortal cocktail which is naturally tailored by natural selection to operate in defense or offense (Cunha 2013), venoms are species-, subspecies-, or even geographic-variant-specific substances that are pharmacologically highly active and can cause a wide range of clinical signs and symptoms in humans which are grouped into 7 classes: local, autopharmacological, hemostatic and hemolytic, neurological, muscular, cardiac, and renal effects (Junghanss and Bodio 2006). Venom components can generally be categorized as (a) high molecular weight proteins such as enzymes and antigens, (b) low molecular weight peptides, and (c) bioactive molecules such as biogenic amines and lipids (Dongol et al. 2014).

Hymenoptera (Bees, Wasps, and Ants)

Hymenoptera is an insect order under phylum Arthropoda, which comprises approximately 115,000 described species such as wasps, bees, ants, ichneumons, calchids, sawflies, etc. It is the third largest of all insect order and perhaps the most beneficial

to humans as a pollinator, as a parasite of pests, and as a maker of honey and bees wax. However, the order also poses significant public health concern. The three medically important groups of stinging insect of the order Hymenoptera belong to the families of Apidae (bees), Vespidae (paper wasps, hornets, and yellow jackets, commonly referred to as *wasps*), and Formicidae (ants) (Dongol et al. 2014). The sting becomes clinically significant if the patient has an allergy to Hymenoptera venom or if the patient is exposed to a large quantity of the venom due to massive or multiple stings. Most deaths related to Hymenoptera stings are the result of immediate hypersensitivity reactions causing anaphylaxis, wherein a single sting is sufficient. Massive envenomation can cause death in nonallergic individuals, due to the toxic effects of venom leading to severe systemic reactions and multiple organ injuries (Dongol et al. 2013, 2014).

Wasp venom components are phospholipase A_1B, hyaluronidase, serine proteases, dipeptidylpeptidase IV, vitellogenin, antigen 5, mast cell-degranulating (MCD) peptides such as mastoparan and protonectin, wasp kinins and chemotactic peptides, and bioactive molecules such as histamine, serotonin, catecholamines, acetylcholine, and tyramine (Dongol et al. 2014).

Bee venom composition includes phospholipases, hyaluronidase, acid phosphatase, dipeptidylpeptidase IV, serine protease, carboxylesterase, melittin, apamin, icarapin, vitellogenin, mast cell-degranulating peptide (peptide 401), histamine, and catecholamines (Dongol et al. 2013).

The major components of ant venoms are alkaloids (2-methyl-6-alkylpiperidines), unlike the protein-rich venom of bees and wasps. These simple piperidines are tissue destructive, hemolytic, bactericidal, and fungistatic and are the causative factor for the burning sensation after a sting (Rhoades 2001). The venom also contains phospholipases A_1B, phospholipase A_2, hyaluronidase, lipase, esterases, and acid phosphatase (Vetter and Visscher 1998; Hoffman 2010). Formic acid is an important constituent of fire ant venom. The presence of formic acid is responsible for the local pain at the sting bite and is also the reason for the use of the term "Formicidae" for the fire ant superfamily (Koya et al. 2007). Alkaloid composition varies among the species. Other components identified in ant venoms include member of the antigen 5 (denoted as Sol i 3) family, vasoactive peptides related to kinins, bombesins, hemolysins, anti-inflammatory myrmexins, histamine, antimicrobials, and ion channel modifiers (Hoffman 2010).

Spiders

There are approximately 42,000 different types of spiders that live in every terrestrial environment, but only few species pose a great risk to humans such as Latrodectus (black widow spider) and Loxosceles (brown recluse spider). Spider venoms are made up of neurotoxic and proteolytic peptides, proteins, and biogenic amines (Friday and Depenbrock 2014).

Venoms from spiders are heterogeneous, not only between species but also within species. The major constituents of spider venom are phospholipase D, astacins, proteases such as serine protease and metalloproteases, hyaluronidase, serine-protease inhibitors, 5′-nucleotidases, venom allergens, neurotoxins such as latrotoxins, latrodectins, and atracotoxins and monoamines (Gremski et al. 2014).

Scorpions

Scorpions are venomous arthropods belonging to the order Scorpionidae and class Arachnida, represented by 18 families and approximately 1500 species, out of which two families, namely, Buthidae and Hemiscorpiidae, and about 30 species pose potential hazard to humans (Viswanathan and Prabhu 2011; Angsanakul and Sitprija 2013). Distributed in all continents, except in Antarctica, venom composition varies among the genus and species depending upon the region they inhabit (Viswanathan and Prabhu 2011). The major constituent includes neurotoxins such as α-toxins and β-toxins, bradykinin-potentiating peptides, low molecular weight peptides, and amines. The ion channel toxins are categorized as 1) sodium channel toxins (NaTx), potassium channel toxins (KTx), chloride channel toxins (ClTx), and calcium channel toxins (CaTx) (Isbister and Bawaskar 2014; Viswanathan and Prabhu 2011). Unlike most spider and snake venoms, many scorpion venoms generally lack enzymes or possess only very low levels of enzyme activity. However, the enzymes identified in spider venom include acid phosphatase, ribonuclease, 5′-nucleotidase, hyaluronidase, acetylcholinesterase, and phospholipase A_2 (Viswanathan and Prabhu 2011).

Caterpillar

Caterpillars are the larval stage of moths and butterflies and are found in a variety of ecosystems worldwide. Caterpillar species that are a potential hazard for humans are found in about 12 families in the Lepidoptera order (Carrijo-Carvalho and Chudzinski-Tavassi 2007). Caterpillar envenomation occurs when the victim comes in contact with the caterpillars' bristles, which are hard and spiny evaginations of the cuticle underneath which the toxins are stored. Upon contact, the caterpillar's chitinous bristles are broken, and the venomous secretions penetrate the human skin and enter the blood circulation (Berger et al. 2010).

The major venom components include histamine-liberating toxin known as thaumetopoein; ester hydrolases; phospholipase A_2; hyaluronidase; fibrinogenase and serine proteases such as lonomin II, lonomin V, Lopap (prothrombin activator protease) and Losac (Stuart-factor activator) which acts on coagulation cascade; cysteine proteinases; protease inhibitors such as serpins, Kazal-type etc.; lectins and lipocalins (Carrijo-Carvalho and Chudzinski-Tavassi, 2007; Veiga et al. 2005; Pinto et al. 2010).

Centipedes

Centipedes are terrestrial arthropods belonging to the class Chilopoda. About 2800 species are known in the world and are distributed in all continents except in Antarctica. The order Scolopendromorpha contains the largest centipedes in the world with several being medically important (Malta et al. 2008). The reported components in centipede venoms are serine proteases, endopeptidases, carboxypeptidases, esterases, acid or basic phosphatases, phospholipase A_2, hyaluronidase, hemolysins, polysaccharides and lipids, and biogenic amines such as serotonin and histamine (Gonzalez-Morales et al. 2014; Malta et al. 2008).

Jellyfish

Jellyfish belong to the phylum of Cnidaria which contains about 10,000 named species with 100 of them being dangerous to humans. These animals have highly specialized defense cells called cnidocytes, which contain three categories of organelles called cnidae. Nematocysts, one of the cnidae, contain a hollow, sharply pointed, coiled thread tube which is about 200–400 μm long and immersed in the venom. Thousands of these nematocysts are distributed along the tentacles and are discharged onto the victim's skin upon contact which may remain intact without releasing its content. Intradermal injection of venom from nematocyst occurs when the skin is penetrated (Cegolon et al. 2013; Balhara and Stolbach 2014).

Jellyfish venoms are composed of potent proteinaceous porins (cellular membrane pore-forming toxins), neurotoxic peptides, hemolysin, c-type lectins, phospholipase A_2, potassium channel inhibitor, protease inhibitor (e.g., serpin), metalloprotease, bioactive lipids, amines such as histamine and serotonin, and the nematocyst tubule components such as chitins and collagens that pose potent immunological reactions (Tibballs et al. 2011; Cegolon et al. 2013; Li et al. 2014).

Snakes

Snakes are the best known venomous reptiles which normally inject venom into their prey through hollow fangs. Snakebite is an important medical emergency in many parts of the world, particularly in tropical and subtropical regions (Cunha 2013). There are more than 3000 species of snakes in the world with about 350 species being venomous and only few posing significant hazards in humans. All the venomous species of medical importance inhabit most parts of the world and are distributed in mere four families: Colubridae, Elapidae, Viperidae, and Atractaspididae (White 2000; Warrell 2012). The chemical makeups of snake venom vary by species, subspecies, and geographic distribution of the species (Junghanss and Bodio 2006).

Important venom components reported include polypeptide toxins, enzymes such as phospholipases, hyaluronidases, proteases (e.g., metalloproteases), hydrolases,

oxidases and esterases, bradykinin-related peptides, natriuretic peptides, metalloproteins, glycoproteins, lipids, cytokines, and amines (histamine, serotonin, acetylcholine) (Adukauskiene et al. 2011; Lameu et al. 2013). Snake venom can be classified as neurotoxic, cardiotoxic, hemotoxic, necrotoxic, and nephrotoxic (Adukauskiene et al. 2011; White 2000).

Effects of Venom: Mechanism of Action and Consequences

Animal venom toxins have extremely diverse mode of actions, affecting almost every cells, tissues, and organs it interacts with. Their effects on ion channels, neurotransmission, release of transmitters and cytokines, disruption of host cell membranes, alterations in coagulation cascades, and modulation of immune system have been well reported and the consequences of which range from the very simple and common manifestation of pain, erythema, and allergy at the site of venom inoculation to the life-threatening systemic complications such as anaphylaxis and multiple organ injury and failure. Kidney, being the most vascularized organ, is highly susceptible to the toxic effects of the animal venoms of all origin.

Pathophysiology of Venom-Induced Nephrotoxicity

The maintenance of continuous renal blood flow is the utmost to the normal functioning of the kidney. The identified factors that impair the renal blood flow resulting in renal ischemia are hemodynamic changes, inflammatory reactions, and nephrotoxicity associated with insults such as hemolysis, rhabdomyolysis, or disseminated intravascular coagulation (Sitprija and Sitprija 2012). Next to the alteration in the renal blood flow, venom-induced nephrotoxicity is as well the result of immune-mediated reactions initiated by the venom toxins such as the inflammatory cell (T lymphocytes, monocytes, eosinophils) infiltration in the interstitium of the kidney resulting in allergic interstitial nephritis (AIN), T lymphocytes and their cytokines influencing the permeability of glomerular basement membrane resulting in proteinuria, and lymphocytic tubulointerstitial infiltrate resulting in renal tubular acidosis. Finally, the venom toxins also pose certain direct effects on the renal cells, especially tubular cells, resulting in tubular necrosis and thus renal injury (Dongol et al. 2013). In this section, the pathophysiology of venom-induced nephrotoxicity under the two broad categories of renal hemodynamics and direct renal toxicity is discussed.

Anaphylaxis: Hypersensitivity and Inflammatory Reactions

Literally, all four types of hypersensitivity reactions (types I, II, III, and IV) are possible to occur in venomous animal stings and bites, provided the venom contains the allergens. However, anaphylactic reactions are IgE-mediated type I reactions,

and life-threatening anaphylaxis involves hypovolemic shock and respiratory failure (Dongol et al. 2014).

In severe anaphylaxis, the cardiovascular system is frequently involved with symptoms like arterial hypotension and cardiovascular collapse. These symptoms are partly due to vasodilation and increased vascular permeability (Mueller 2007) which as well lead to hypovolemia and are responsible for renal hypoperfusion resulting in renal ischemia and injury. IgG- and IgM-mediated type III hypersensitivity reactions are involved in vasculitides, hemolytic events, and kidney injuries (Viswanathan et al. 2011). The potent allergens in animal venoms capable of inducing hypersensitivity reactions include phospholipases, hyaluronidase, antigen 5 (in wasp venom), odorant-binding proteins and pheromone-binding proteins (denoted as Sol i 2) in ant venom, collagen and chitin from the jellyfish stinger, etc., to name a few. These allergens bind to its specific IgE and are presented to mast cells and initiate the release of inflammatory mediators from mast cells.

Mast cells are potent drivers of inflammation, releasing biogenic amines such as histamine and other substances, including platelet-activating factor, prostaglandins, leukotrienes, proteases, and cytokines, into their tissue environment when stimulated. Animal venom includes mast cell degranulation peptides as one of its major components such as peptide 401 in bee venom and mastoparan in wasp venom. Mast cells may respond to different types of stimulus. Firstly, primary envenomation may activate mast cells directly through the introduction of porins, secretagogues (bioactive lipids, amines) in the venom itself. Secondly, components of the venom may activate innate or pattern recognition receptors on mast cells. Thirdly, physical changes (acidification, ROS) at the sting site may activate mast cells directly. In the case of repeated stings, classical IgE-dependent allergy to toxin may play a role in mast cell activation and mediator release in response to specific IgE bound to their surfaces (Tibballs et al. 2011).

Venom causes release of both pro- and anti-inflammatory cytokines – imbalances between which result in organ damage. Vasodilation due to inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α) and NO causes renal hypovolemia. TNF-α, IL-1β, and IL-6 have all been postulated to play a role in leukocyte activation and leukocytosis. Leukocyte activation may lead to production of reactive oxygen species and subsequent multi-organ injury (Viswanathan and Prabhu 2011).

Coagulation Cascade: The Hemorrhage and Hemostasis

Several venom toxins could be directly or indirectly involved in hemostatic disturbances illustrated by various laboratory findings such as severe prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT); decrease in plasma levels of fibrinogen, factors V and XIII, pre-kallikrein, plasminogen, protein C, and α2-antiplasmin; and increase in the levels of thrombin-antithrombin complex and D-dimers. The activation of blood coagulation and fibrinolysis in caterpillar envenomation causes the consumption of plasma factors, leading to a consumption coagulopathy (imbalance in the coagulation and fibrinolytic system,

resulting in a hemorrhagic syndrome) (Pinto et al. 2010). Serine-protease Lopap from caterpillar is capable of activating thrombin, causing the formation of micro-coagules that effectively consume coagulation factors. The resultant disseminated intravascular coagulopathy with massive deposition of fibrin could damage the endothelial glomerulus, resulting in hematuria and kidney injury (Gamborgi et al. 2006). Caterpillar venom possesses high proteolytic, procoagulant, and fibrin(ogen) olytic activities (Pinto et al. 2010). The prothrombin and factor X activators generate significant amounts of intravascular thrombin, which leads to the activation of coagulation system and to consumption of fibrinogen and other coagulation factors. The fibrin(ogen)olytic enzymes degrade both fibrinogen and fibrin, contributing to the reduction of fibrinogen levels and to blood incoagulability. Further, fibrin(ogen) olytic enzymes can also participate in the generation of FfDP, which are probably involved in the platelet aggregation disturbances leading to hemostatic disturbances (Pinto et al. 2010; Suntravat et al. 2011). Phospholipases (e.g., snake venom PLA_2) and lectins are also capable of modulating the hemostatic system. PLA_2 directly modulates platelet aggregation and destabilizes the coagulation complexes by degradation of phospholipids, whereas lectins interact with coagulation factors and/or platelet receptors. Protease inhibitors, such as serine-protease inhibitors (serpins), are also the key effectors in hemostasis, by inhibition of coagulation factors (Pinto et al. 2010). Caterpillar venom toxins such as lonomin II and lonomin V have anticoagulant activity – lonomin II has direct fibrinolytic activity, and lonomin V degrades coagulation factor XIII (Carrijo-Carvalho and Chudzinski-Tavassi 2007). PLA_2 promotes hydrolysis of phospholipids with generation of free fatty acids and lysophospholipids, thus producing indirect hemolysis. PLA_2 may inhibit blood coagulation by direct interaction with coagulation factors or through degradation of phospholipids involved in coagulation complexes, in addition to antiplatelet effects, neurotoxicity, cardiotoxicity, and myotoxicity. Lipocalins inhibit platelet aggregation and reduce blood coagulation as well as promote vasodilation. Lectins bind to coagulation factors and to platelet receptors and inhibit fibrinogen clotting (Veiga et al. 2005).

There, probably, is a relation between renal ischemia and systemic hypotension and/or fibrin deposition in glomerular capillaries and direct toxicity to kidney in caterpillar envenomation. Caterpillar venom is rich in several toxins that have procoagulant and fibrinolytic activities which can significantly affect the blood coagulation process. For example, the enzyme lonofibrase is able to trigger a hemorrhagic syndrome similar to DIC by increasing fibrinogen degradation products and decreasing plasminogen, fibrinogen, and factor XIII. The venom contains several lipocalins, such as Lopap, which are involved in the increase of expression of adhesion molecules on the cellular surface (Schmitberger et al. 2013). Lopap stimulates IL-8, ICAM-1, and E-selectin. It also increases the secretion of nitric oxide (NO) and prostacyclin (PGI_2), both potent vasodilators and inhibitors of platelet activation. Losac is a Stuart-factor (factor X) activator purified from caterpillar venom (Chudzinski-Tavassi and Alvarez-Flores 2005).

Caterpillar envenomation triggers intense inflammatory response and activates kallikrein-kinin system consequently releasing vasoactive mediators (mainly

bradykinin, histamine, and prostaglandins) which play an essential role in edematogenic, nociceptive, and vascular effects. Neutrophilic leukocytosis observed in histological sections indicates systemic inflammatory response (Berger et al. 2013).

The Venom Kinins

Venom kinins are bradykinin (BK), and its related peptides, e.g., the bradykinin-related peptides (BRPs), are widely found in venomous animals such as snakes and wasps. BRPs include bradykinin-potentiating peptides (BPPs) and bradykinin inhibitor proteins (BIPs). BPPs are proline-rich oligopeptides that inhibit the angiotensin-converting enzyme (ACE) and are responsible for the hypotensive effect. BPPs are found in several snake venoms and in wasp venom. These molecules are able to enhance some pharmacological activities of BK. BPPs include several sequences, either showing only one single amino acid substitution compared to BK or, in some cases, presenting just a frugal sequence similarity, but with unquestionable biological/functional correlation, for instance, acting on the same pathway or even the same target protein. BK, and BPPs, is an inflammatory mediator involved in the nociceptive process due to their ability to excite and/or sensitize nociceptors (Lameu et al. 2013).

BPPs Interfere in Renin-Angiotensin and Kallikrein-Kinin System

The angiotensin-converting enzyme (ACE) is mainly expressed in vascular endothelium and in epithelial cells of the proximal tubules of the kidney, brain, and intestinal cells. This enzyme is responsible for the conversion of angiotensin I to angiotensin II and for the degradation of BK. Therefore, this enzyme has roles in both renin-angiotensin and kallikrein-kinin system. Angiotensin II triggers several cellular processes such as vasoconstriction, regulation of renal function, and electrolyte balance. The kallikrein-kinin system is a metabolic cascade in which the tissue and plasma kallikrein release vasoactive kinins from both high and low molecular weight kininogens. The nonapeptide BK, derived from the cleavage of the high molecular kininogen by kallikrein, is the major plasma kinin. Kinins are involved in various physiological and pathological processes such as vasodilation, increased vascular permeability, release of plasminogen activator of tissue type (t-PA), and nitric oxide (NO) and arachidonic acid metabolism. Kinins, thus, participate in the regulation of blood pressure and cardiac and renal function and in the pathological processes of inflammation. The pharmacological effects observed include vasodilation and hypotension, angiogenesis, inflammation, and septic shock. ACE inhibitors such as BPPs not only inhibit the generation of angiotensin II but also potentiate the effects of BK and related peptides by inhibiting its degradation (Lameu et al. 2013; Moreau et al. 2005). Therefore, BPPs in venom can be expected to have stronger and prolonged vasodilatory effect leading to hypotension.

Renal hypoperfusion (as observed in use of ACE inhibitors), hypotension, and edematous states are some principal prerenal causes that lead to acute kidney injury (Hilton 2006). Among the various factors such as bleeding diathesis, systemic vasodilation is a considerable factor that leads to hypotension.

BK and BPPs or venom kinins are important vasoactive mediators which have a strong vasodilatory effect and increase vascular permeability. The increased vascular permeability is responsible for the edematous states and the inflammatory response. Similarly, the inhibitory effect of BPPs on ACE further aggravates the effect of BPPs such as vasodilation and edema, consequently resulting in hypotension and renal hypoperfusion (Lameu et al. 2013).

Despite of the renoprotective role of endogenous BK, the higher load of BPPs (venom kinins) inoculated during envenomation can be of higher suspect for hypotension-induced AKI. Such a hypotension-induced AKI could be an aftermath effect of massive envenomation (e.g., in swarm attack of hornets) (Xuan et al. 2010) or usage of ACE inhibitor (Hilton 2006) which results from a strong and prolonged vasodilation and consecutive angioedema. However, the theory of independent role of bradykinin and related peptides (BPPs) in causing kidney injury is yet to be established; it could be an additional component that has a synergistic effect to the nephrotoxic insults along with other venom toxins.

Therefore, the effects of venom kinins (BPPs) on manifestation of kidney injury in relation to vasodilation and ACE inhibition need further experimental elucidation.

Heme Toxicity: The Consequence of Hemolysis and Rhabdomyolysis

The phospholipid composition of the erythrocyte membrane structure is important for the membrane fluidity, integrity, and erythrocyte function, whereas the transmembrane proteins such as spectrin, band 3, and glycophorins (A, B, C, D) are essential to the integrity of erythrocyte shape, cell signaling, and ion transport across the lipid bilayer. Hemolytic toxins can act on these structures resulting in cell lysis. For example, mellitin from bee venom integrates itself into erythrocyte lipid bilayer and disorganizes it or the activity of venom phospholipase A_2 on membrane phospholipids causing hemolysis, whereas sphingomyelinase D from spider venom activates an endogenous cell surface enzyme, which in turn cleaves the sialic acid-rich external portions of glycophorins A, B, C, and D of erythrocyte membranes. This cleavage allows the autologous activation of the alternative pathway of complement system and consequently cell lysis (Seibert et al. 2010). Spider venom is thought to act on metalloproteinases in the red blood cells causing lysis. The volume of spider venom is not capable of causing such extensive damage on its own; therefore, the development of hemolytic anemia is thought to be triggered by mediators and other agents such as cytokines and interleukins; the local hemolysis spontaneously activates the complement system leading to massive hemolysis and potential renal failure (Vetter 2013). Hemolytic toxins can, thus, act on glycoproteins or on lipids.

The cytotoxins such as mellitin (bees), mastoparan (wasps), formic acid (ants), and myotoxins of various animal venom origins are responsible for rhabdomyolysis observed after envenomation. Besides, the inflammatory local reactions induced by venoms also result in tissue damage, which can provoke muscular injury (Malta et al. 2008).

One of the mechanisms involved in cytolysis is the oxidative stress posed by the venom. The oxidative stress includes ROS overproduction, lipid peroxidation, and mitochondrial dysfunction which may play important roles in the process of cell injury and death. Mitochondria are critically involved in cell death pathway. Jellyfish toxin has been shown to induce apoptosis-mediated cytotoxicity (Wang et al. 2013). Formic acid in larger doses acts as an inhibitor of the mitochondrial cytochrome oxidase complex, causing tissue suffocation and consequently cell death (Koya et al. 2007).

The cortex, especially the renal proximal tubules, possesses a greater heme-synthesizing capacity, and the pathophysiologic processes such as ischemic and nephrotoxic insults may destabilize intracellular proteins, thereby contributing to increased intracellular levels of heme in the kidney. Besides, the increased renal content of heme is attributable to the heme proteins that are extrinsic to the kidney, such as myoglobin from injured muscles and hemoglobin from lysed erythrocytes, which are delivered to and incorporated by the kidney via megalin/cubilin receptors (Tracz et al. 2007; Zager 1996). Higher amounts of heme are toxic: in plasma and intracellular membranes, heme can oxidize lipid, denature proteins, perturb the integrity of the attached cytoskeleton, impair the activity of cytosolic enzymes, and activate cell-damaging enzymes such as caspases and cathepsins. Heme in its pathophysiologically relevant amount is lethal to mitochondria. In addition to its direct cytotoxicity, heme can induce renal injury by its proinflammatory effects, for example, by inducing chemokines such as monocyte chemoattractant protein-1 (MCP-1) (Tracz et al. 2007). Myoglobin and hemoglobin are filtered through the glomeruli and reabsorbed in the proximal tubules by endocytosis. In acidic environment of lysosomes, the globin chain dissociates from the iron-containing ferrihemate, and free iron is converted to ferritin. However, in rhabdomyolysis and massive hemolysis, the amount of myoglobin and hemoglobin delivered to the proximal tubule cells overwhelms their ability to convert iron to ferritin, resulting in intracellular ferrihemate accumulation. Iron as a metal has the ability to donate and accept electron as well as the capability to generate oxygen free radicals. This leads to oxidative stress and injury of the renal cell. Besides, myoglobin cannot be reabsorbed when in excessive amounts in tubules resulting in formations of casts that obstruct the renal tubules leading to tubular ischemia and ultimately renal injury (Efstratiadis et al. 2007; Zager 1996).

Direct Nephrotoxicity

Kidneys are one of the major routes of venom elimination from the body. Being a highly vascularized organ with a concentrating capacity in tubules, it is obvious that renal tissues have higher concentration of the inoculated venom toxins. Accumulation of these venom toxins in renal tissue may cause morphological damage and

renal dysfunction leading to AKI without any clinical manifestation of hypotension and other associated insults including intravascular hemolysis, hemorrhage, rhabdomyolysis, DIC, or renal insults of non-immunologic origin. Such a category of renal impairment includes glomerular, tubular, and vascular injury and is categorized under direct nephrotoxicity. The renal manifestations observed include glomerulonephritis, mesangiolysis, vasculitis, endothelial damage, and tubular degeneration and necrosis (de Morais et al. 2013; Sitprija and Sitprija 2012; Silva et al. 2012).

Direct nephrotoxicity refers to the renal cell injury caused by the interaction of venom toxins and renal cells leading to morphological changes in the cell and triggering intracellular pathways that ultimately result in cell degeneration or death. Such an effect is attributable to the enzymes, such as phospholipase A2, phospholipase D, metalloprotease, etc., or cell-penetrating peptides such as mellitin, mastoparan, etc. (Kusma et al. 2008; Sitprija and Sitprija 2012; Vinhote et al. 2011).

Various changes in the renal structures have been documented on the renal culture cells upon treatment with venom or venom toxins such as degenerative changes in tubular cells and tubular necrosis, decreased cell viability and membrane integrity, toxic effects on glomerulus leading to deposition of proteinaceous material in urinary and tubular spaces, increased perfusion pressure and vascular resistance, increase in cytoplasmic vacuoles and electron-dense vesicles, altered cell spreading and adherence to each other, and decreased cellular viability (Kusma et al. 2008; Silva et al. 2012; Vinhote et al. 2011). Proteases, such as metalloprotease, can cause proteolysis of the extracellular matrix and disrupt cell-matrix and cellular adhesion, whereas phospholipases and cell-penetrating peptides (also known as polycationic peptides) interact with biological membranes, leading to membrane destabilization and loss of permeability which eventually result in cellular necrosis (Sitprija and Sitprija 2012; dos Reis et al. 1998). Treatment of Madin-Darby canine kidney cells with *Bothrops leucurus* venom led to an increase in expression of cell death genes (increased expression of caspase 3 and caspase 8) suggesting apoptotic mechanism for nephrotoxicity (de Morais et al. 2013).

Conclusion and Future Directions

Owing to the significant human-animal interactions, stings and bites from venomous animals are a significant health problem worldwide, especially in tropical and subtropical climates. A complete set of arsenal designed for predation and defense, animal venom toxins pose a lethal threat to mankind at times – if the person is hypersensitive to venom toxin or if the high/significant amount of venom is inoculated during bite or sting. The envenomation syndrome ranges from simple manifestation of allergy or pain and erythema at the sting site to fatal systemic anaphylaxis and multiple organ failure. Kidney being the most vascularized organ is more susceptible to venom intoxication with the pathophysiological basis of altered hemodynamics, immunological reactions, and inflammatory mediators, affecting the renal function and toxic injury mediated by venom itself and heme from hemoglobin and myoglobin.

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Translational Toxinology: Venom to Antivenom

22

Daniel E. Keyler

Contents

Abstract

A great natural diversity exists across animal taxa and species that produce venom, and although venoms are primarily used in the acquisition of prey and secondarily for defense, it is their adverse effects on humans that have driven scientific and medical research. The spectrum of venom-producing organisms and

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the venom components and toxins across organism species is highly variable. Venom components function in concert and selectively in their actions producing pathophysiological effects for subduing prey. In contrast, when non-prey species such as humans are encountered, the biting or stinging as a result of a defensive or fear response may result in envenomation. Following envenomation a myriad of venom-/toxin-induced adverse and toxicological insults to various physiological systems may result. What creatures are venomous, how they envenomate, their venom composition, how their venom components/toxins work mechanistically, and the pathophysiological effects of venom in envenomated humans led to the quest for remedies and antidotes. Prominently, among therapeutic advancements was the development of passive antisera therapy in the late1800s for cobra envenomation (Calmette 1894). Today's formulations of snake venom immunotherapies (antivenom) are relatively unchanged with respect to general antibody structure and mechanism of action. However, recent technological advances in antibody preparation, purification, and product formulation and combined venomic and antivenomic technologies are leading to novel, refined toxin-targeted antivenoms (Calvete et al. 2009). Toxinology has been translational over time, across biological systems, across scientific disciplines, and technologically, leading to our improved understanding of venom-producing animals, venoms, and the design and development of more efficacious antivenoms.

Introduction

Translational toxinology and the trail to a therapeutic antivenom may be considered the bridging between the fundamental biological traits of an animal progressing to scientific research, leading to applied immunological science and targeting the application of the derived biological product in clinical medicine (Fig. 1). It is a process typically driven by the medically important consequences that result from envenomation to humans by venomous organisms. From a historical perspective, the early beginning of translational toxinology began over 2,500 years ago with an Egyptian specialist known as the "Controller of Selqet" who documented toxicity and treated individuals who suffered scorpion stings and bites from venomous snakes (Ritner 2001). Although terminology and treatments may have been different in early times, the concept of a venomous animal causing adverse and life-threatening effects following an envenomating bite or sting was realized, as was the consequent need for a cure or some form of medical remediation (Calvete 2013).

Multiple species of terrestrial and aquatic/marine animals (snakes, frogs, scorpions, spiders, insects, and caterpillars, octopus, snails, and fish) are venomous (Table 1), producing a myriad of venom toxins, and as such animal toxinology is translational across a wide range of taxa. Venom functions primarily for acquiring prey for survival with venom introduced into the prey via a venom delivering anatomical adaptation. Secondarily, venom may be used in defense against predation and also serves as a survival mechanism. Again, toxinology becomes translational from predator to prey or from the venom-producing animal to the offending victim.

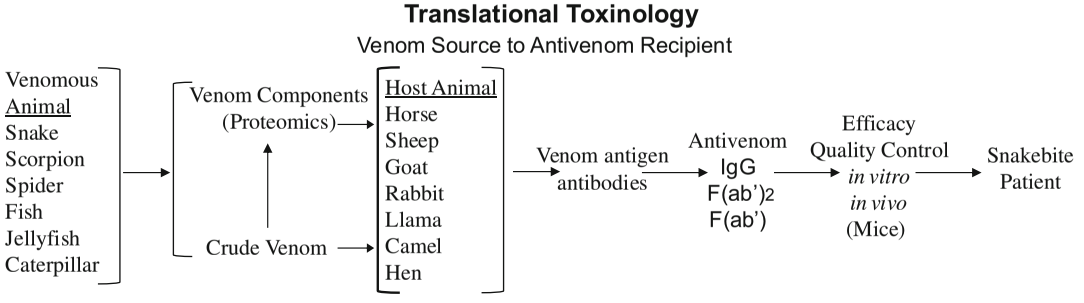

Fig. 1 Schematic illustration of translational toxinology showing the stepwise flow across four distinct taxonomic groups, from a venom-/toxin-producing animal transitioning to the development of a venom-/toxin-immunogenerated antivenom and its administration to an envenomated patient

Table 1 Representative venom-producing animals of medical importance: their geo-eco characteristics, venom-induced effects and available antivenoms

Animal (genus)	Geographic regions	Ecosystem	Principal venom/toxin-induced effects	Antivenom types (available)
Snake – Viperidae – Subfamily – Crotalinae – Pit vipers (*Calloselasma*, *Crotalus, Bothrops, Trimeresurus*)	Asia, North/ Central/South America, Mexico	Terrestrial	Coagulopathic, hemorrhagic, myotoxic, neurotoxic	Chicken IgY Ovine Fab, Equine F $(ab')_2$, Equine IgG
Snake – Viperidae – Subfamily Viperinae – True vipers (*Bitis, Daboia, Echis, Vipera*)	Africa, Asia, Europe, Middle East	Terrestrial	Coagulopathic, hemorrhagic, myotoxic, neurotoxic	Equine F $(ab')_2$, Equine IgG
Snake – Elapidae – Elapinae (*Bungarus*, *Dendroaspis, Micrurus, Naja, Oxyuranus*)	Africa, Asia, Middle East, Australia, Mexico, North/Central/ South America	Terrestrial	Coagulopathic, hemorrhagic, myotoxic, neurotoxic	Equine F $(ab')_2$, Equine IgG
Snake – Elapidae – Hydrophinae (*Pelamis, Laticauda,* multiple genera)	Indo-Pacific, Pacific Oceans	Oceanic	Myotoxic, neurotoxic	Equine F $(ab')_2$
Snake -Colubroidea (*Dispholidus, Rhabdophis*)	Africa, SE Asia	Arboreal, terrestrial	Coagulopathic, hemorrhagic	Equine F $(ab')_2$ Goat F $(ab')_2$
Lizard (*Heloderma*)	Southwestern North America/ Mexico	Arid desert	Hypotension, hypothermic	No antivenom
Scorpion *(Androctonus, Buthus, Centuroides, Tityus*)	Southwestern North America, Europe, Middle East, Africa	Arid desert	Neurotoxic	Equine F $(ab')_2$
Spider (*Latrodectus*)	Global		Neurotoxic	Equine IgG
Fish (*Synanceia*)	Indo-Pacific	Marine	Cardiotoxic, cytotoxic, myotoxic, neurotoxic	Equine IgG
Snail (*Conus*)	Global	Marine	Neurotoxic	No antivenom
Jellyfish (*Chironex*)	Tropical Australian Waters	Marine	Cardiotoxic, respiratory depression	Ovine IgG
Caterpillar (*Lonomia*)	South America	Rainforest	Coagulopathic, hemorrhagic	Equine F $(ab')_2$

The dynamics of these venom uses has led to the study of venoms and their components and, consequently, the quest for drug discovery and antidotes to be used in human and veterinary medicine (Harvey et al. 1998).

The study of venoms is a specialized science in its own right, and the methodologies and processes of studying venoms have evolved over time, from simply observing the effects of venom when introduced into a nonbiological medium, or into a living animal or human, to today's technologically advanced methods for characterizing and profiling venoms. Studying venom components or specific animal toxin effects on defined physiological systems and resultant pathophysiological effects are paramount to the discipline of toxinology. Venom was historically thought of as a homogeneous substance and today is known to be a highly complex mixture of bioactive substances (León et al. 2011).

Of all the venomous creatures potentially dangerous to humans, venomous snakes have posed, and continue to pose, the greatest medical problem, and it has been snake envenomation that has led to the greatest demand for antivenom development. The snakebite burden globally is an uncertain number despite attempts at quantification; however, the magnitude of the problem is staggering as there are reportedly 5–5.5 million snakebites annually, resulting in 20,000–125,000 deaths (Chippaux 2008; Kasturiratne et al. 2008). Further complicating the treatment of snakebite victims has been the decrease in antivenom production and availability in Africa as well as other geographic regions (Schiermeier 2015). Hence, the need for efficacious antivenoms with an absence of associated adverse side effects is significant, and new toxinological methodologies will continue to improve the design of antivenoms and their development processes.

Venom

Venom-Producing Animals

Venomous animals are present on all continents, occur in most countries, and are represented by multiple taxa and species of invertebrates (caterpillars, snails, jellyfish, scorpions, spiders, and a wide variety of insects) and vertebrates (fish, snakes, lizards, frogs, mammals, birds) that inhabit a broad range of environments that interface with human behaviors and human habitat (Table 1) (Calvete et al. 2009). Although many of these animals produce venom with a wide range of toxins capable of causing a variety of pathophysiological effects, not all are dangerous medically to humans. Despite the range of taxa that contain species with venoms capable of inflicting serious health problems to humans, there is no available targeted immunotherapeutic treatment for many due to a small number of serious envenomation cases or, in contrast, a high occurrence of envenomations in developing or impoverished countries, making the development of therapeutic antibodies prohibitive due to economic considerations and lack of technological resources (Gutiérrez 2012, 2016; Gutiérrez et al. 2014b; Chippaux 2013). Of the many venomous creatures known to harm humans, venomous snakes, scorpions, and spiders account for the large majority of envenomations (Espino-Solis et al. 2009), and it is venomous snakes and associated envenomations from snakebites that represent the greatest global burden with respect to severity of medical consequences and economic costs

(Chippaux 2013). The primary definitive therapeutic antidote for treating envenomated patients has historically involved, and continues to involve, passive immunization with species-specific or polyspecific therapeutic antibodies (León et al. 2011; Alvarenga et al. 2014). The ability to generate therapeutic antibodies for treating individuals suffering the medical consequences of envenomation requires venom and venom antigens harvested from the venomous animal. This transition from the venom-producing creature to the studying of venom and venom-derived antigens is an initial phase in translational toxinology toward the development of an immunotherapeutic treatment (antivenom) (Fig. 1).

Venom Properties and Actions

Venoms are biological fluids comprised of natural organism-synthesized bioactive substances that exhibit a high degree of complexity and diversity with respect to their biochemical composition, physiochemical properties, and pharmacological actions (Vetter et al. 2011; Chan et al. 2016) (Table 2). The natural venom functions are primarily for acquiring prey, facilitating the digestive process, and as a predator defensive action, all of which are required for species survival. Clinical manifestations of envenoming vary depending on the species inflicting the bite or sting and the protein and toxin profile constituting the venom of the offending animal.

The most medically important and complex venoms are produced by snakes of the Atractaspididae, Colubridae, Elapidae, and Viperidae families, which are important to human medicine, both in bioscience and medical health. Snakes in these families produce multiple venom components represented by several major protein families with significant pharmaco-toxicological actions (León et al. 2011) (Tables 1 and 2). While differences in snake venom properties would be expected to exist between different genera, there is increased complexity with the studying of venoms due to relative inconsistencies in the qualitative and quantitative properties for any given species as a result of geographical variation, intraspecific differences, sex differences, diet consumed, and even climatic seasonal changes (Chippaux et al. 1991).

Biochemically, snake venoms are complex mixtures of bioactive polypeptides and proteins (enzymatic and nonenzymatic) and nonproteins (amino acids, carbohydrates, lipids, nucleotides, biogenic amines) that exhibit a spectrum of pharmacological actions that can induce serious pathophysiological effects following envenomation (Meier and Stocker 1995; León et al. 2011). The individual venom components in the crude venom of any given species are present in varying ratios. The significance of this is demonstrated in the feeding ecology of a given species where the optimization of selective venom component ratios results in venom component interactions toward prey preference and pharmacological and toxicological actions for most effectively subduing specific prey within the geographic home range of a single species.

Venom polypeptides and proteins primarily exist as monomers; however, they can form complexes resulting in greater mass and conformational alterations that

Table 2 Snake venom components of medically important snake families: their mass properties and actions

Venom components (antigenically/ immunogenically important)	Snake family (predominantly present in)	Molecular mass (approximate)	Pharmaco-toxicologic action	Pathophysiological effects
C-type lectins – lectin-type proteins (CTLs)	Colubridae, Elapidae, Viperidae	135 kDa	Clotting factor binding, platelet function alterations	Coagulopathic
Disintegrins	Viperidae	4–14 kDa + (variable-multimeric)	Bind cell membrane integrins, platelet function alteration	Coagulopathic, hemorrhagic
L-amino acid oxidase (LAAO)	Elapidae, Viperidae	110–150 kDa	Oxidative deamination of L-amino acid to alpha-keto acid, ammonia, and hydrogen peroxide	Apoptotic, cytotoxic, edema, coagulopathic
Metalloproteinases (snake venom – SVMPs)	Atractaspididae, Colubridae, Elapidae, Viperidae	20–100 kDa	Proteolytic capillary basement membrane degradation, fibrinolysis, prothrombin activation, factor X activation, platelet function alterations	Coagulopathic, hemorrhagic, inflammatory
Phospholipases (PLA2)	Elapidae, Viperidae	14–18 kDa + (multimeric forms)	Hydrolyze phospholipids, bind plasma membrane receptors	Coagulopathic, hemolytic, myotoxic, neurotoxic, hypotension
Serine proteinases	Elapidae, Viperidae	25–70 kDa	Thrombin-like fibrinopeptide cleavage, Kallikrein-like bradykinin generation, Platelet function alterations, plasminogen and prothrombin activation	Hemostatic

(*continued*)

Table 2 (continued)

Venom components (antigenically/ immunogenically important)	Snake family (predominantly present in)	Molecular mass (approximate)	Pharmaco-toxicologic action	Pathophysiological effects
Three-finger toxins TFTs)	Elapidae	6–9 kDa	Post-synaptic receptor blockade at neuromuscular junction	Neurotoxic/ paralytic

modify their active-site functionality, leading to increased pharmacological action and consequential pathophysiological effects such as escalated lethal potency. Larger binding regions characterize these larger complexes with increased interaction affinities for ion channels and target receptors (Doley and Kini 2009). These larger complexes may be more antigenic and immunogenic in terms of inducing specific antibody responses beneficial in antivenom development. Venom components/toxins can function independently but can also work in concert and synergistically on ion channels and receptors to cause profound pathophysiological effects on cardiovascular, homeostasis, neuromuscular, and peripheral nervous systems (Calvete et al. 2009). An example is the potential interaction between presynaptic PLA_2 toxins with three-finger toxins, synergistically enhancing the venom's neurotoxic action toward prey immobilization and death (Doley and Kini 2009).

The significance of many venom toxins and components of multiple genera and species is not completely known. The low molecular masses of cardiotoxin and neurotoxin in equatorial spitting cobra (*Naja sumatrana*) venom, cardiotoxic sarafotoxins of the mole viper (*Atractaspis engaddensis*), and neurotoxins such as dendrotoxins of the black mamba (*Dendroaspis polylepis*) and fasciculins of the eastern green mamba (*Dendroaspis angusticeps*) are all pharmacologically active venom components whose relevance and specific function with respect to envenomation to humans are not clearly defined (León et al. 2011; Yap et al. 2014). The immunogenicity and importance of these small-mass toxins for generating toxin-specific antibodies of therapeutic relevance and their significance in the design and development of antivenoms require further investigation.

In addition to the major protein families, a variety of less completely understood venom components, which may or may not exhibit toxic effects and whose biological functions are not clear, are present in snake venom. The pharmacological actions of acetylcholinesterase, phosphodiesterases, nucleotidases, bradykinin-potentiating peptides, cysteine-rich secretory proteins (CRISPs), and vascular and nerve growth factors may (or may not) contribute to venom toxicity via various mechanisms. Acetylcholinesterase is a well-known elapid venom enzyme that induces neurotoxic effects via activity that regulates neuro-signal transmission across the cholinergic synaptic cleft, is located on either pre- or postsynaptic cleft sites, and alters neurotransmission via hydrolysis of acetylcholine preventing receptor desensitization (Fry

et al. 2015). The pharmacological action of hyaluronidase, frequently referred to as "spreading factor," although not directly toxic, contributes to venom-induced toxic effects by altering local tissue integrity and promoting the spread of venom from the bite site, resulting in increased venom penetration into local tissues and thereby providing venom access to the systemic circulation (Fox 2013).

The toxicologically active venom components responsible for inducing significant pathophysiological manifestations following envenomation are also those important for development of optimally therapeutic antivenoms. However, the diversity of venom components and their physiochemical properties also make the process of generating venom antigen-specific antibodies for the design and development of antivenom challenging due to their varying degrees of antigenicity and immunogenicity.

Venom Pharmacokinetics/Toxicokinetics

Pharmacokinetic properties of venoms are multiple, yet singular, in that each individual venom antigen possesses its own individual pharmacokinetic properties as observed by different individual rates of absorption, distribution, elimination, and clearance. The interaction of venom components in a specific physiological system may result in altered pharmacokinetic properties different than those of a single toxin alone, making venom pharmacokinetics multifactorial and complicated to study (Yap et al. 2014).

Lower mass neurotoxins found in elapid venoms have a more rapid distribution phase than the larger mass enzymatic toxins associated with viperid venoms and can distribute with less difficulty into the systemic circulation, while larger venom enzymatic proteins may not readily distribute out of the vascular compartment resulting in a limited volume of distribution, affecting their ability to reach target tissues and receptors (Ismail et al. 1998). As such they may require facilitation from other venom components for increased distribution to target organs (Fox 2013).

Variability in different venom toxin pharmacokinetics is illustrated by the variable pharmacokinetic parameters of three toxins isolated from crude *Naja sumatrana* venom. Following the intramuscular administration to rabbits of a cardiotoxin, neurotoxin, and PLA_2, the mean bioavailabilities (F %) were 45.6%, 81.5%, and 68.6%, respectively; the mean elimination half-lives ($T_{1/2}$ β h) were 8.2 h, 8.6 h, and 10.2 h, respectively; and the mean clearances (CL ml/h) were 173.7 ml/h, 164.1 ml/h, and 95.8 ml/h, respectively (Yap et al. 2014). These differences in toxin-specific pharmacokinetic properties contribute to the complexity of venom pharmacodynamic effects and also represent a challenge in the development of an array of antivenom antibodies capable of favorably altering individual venom antigen pharmacokinetics. Additionally, there is the possibility that the immune response to specific venom proteins may be influenced by each individual protein toxicokinetic profile.

Absorption and distribution of a venom antigen are determined by its stereochemical configuration, mass, and affinity for a given target tissue or receptor.

Table 3 Antibody/antivenom: forms, physiochemical and pharmacokinetic properties following intravenous administration

Hyperimmunized animal	Passively immunized species	Antibodies/ antivenom administered	Antibody (derived form)	Molecular mass (kDa)	VDss (mL/kg)	t 1/2β (h)	Clearance (mL/h/kg)
Equine IgG (Ho et al. 1990)	Human	Anti-C. rhodostoma	IgG	150	90	82	0.6
Goat IgG (Ho et al. 1990)	Human	Anti-C. rhodostoma	IgG	150	93	46	1.3
Equine $F(ab')_2$ (Ho et al. 1990)	Human	Anti-C. rhodostoma	$F(ab')_2$	100		36	
Ovine (Seifert-Boyer 2001)	Human	Anti-Crotalidae	F(ab)	50	110	18	
Chicken IgY (Diaz et al. 2014)	Rabbit	Preimmune eggs	IgY	180	58	40	1.4

VDss = steady state volume of distribution; t 1/2B = elimination half-life

Elimination may be determined via metabolism and renal clearance. Both of these may be affected by the redistribution of venom components via passive mechanisms or, in the presence of antivenom, due to the presence of venom antigen antibodies that provide binding sites of greater affinity than those of target tissues or receptors and/or the increased number of circulating antibodies providing an increased number of toxin-binding sites. The redistribution of toxins from target tissues and receptors, due to their binding to antibodies, not only results in neutralization of toxin effect but may also promote the elimination of antibody-bound venom components from the body. The specific antibody configuration is an important determinant with respect to venom distribution and elimination (Table 3) (Gutierrez et al. 2003).

The pharmacokinetic properties of venom antigens can differ from those of antivenom antibodies, creating a pharmacokinetic mismatch that can compromise antivenom pharmacotherapeutic efficacy. This pharmacokinetic mismatch affects the duration of interaction time for the binding of antibodies with venom antigen/toxins, potentially reducing the degree of their neutralization (therapeutic effect) and increasing the potential for the further development of consequent pathophysiological effects. Clinically, this mismatch has been observed in human cases of Crotalinae envenomation exhibiting coagulopathy. Patients treated with F (ab) antivenom to the level of symptom resolution later developed recurrent coagulopathy (Seifert and Boyer 2001). This reflects that the individual venom components responsible for the coagulopathy had a longer elimination half-life than that of the F(ab) antivenom.

Collectively, these highly variable venom component properties, due to different chemical compositions and different mechanisms of action, result in variable individual venom component pharmacokinetics and yet cause similar pathophysiological effects on a given physiological system; demonstrate the translational aspect of toxinology across the disciplines of biology, chemistry, pharmacology, pathology, and animal species; and represent the challenge in developing efficacious antivenoms (Table 2).

Venom: Immunogenic Properties

Following the delivery of venom into prey or victim, in addition to the pathotoxic effects, there is an immune response induced against the venom proteins toward the reduction and abolishment of venom antigen-induced toxic effects via antibody/venom antigen binding. The degree to which this response occurs represents the immunogenicity to any given antigen, and frequently the immune response following envenomation is overwhelmed by venom antigen adverse pharmacological actions. This results in the pronounced pathophysiological effects observed in cases of significant envenomation.

The immune response leading to antibody production primarily results from the interaction of venom antigens with antigen-processing cells and immune system lymphocytes (Delves and Riott 2000). The magnitude of the response is determined by multiple factors relating to the different properties of venom antigens that represent the majority of venom components (Table 2). Venom antigens (protein and nonprotein) of greater molecular mass and protein glycosylation tend to be more immunogenic. In general venom antigens with a mass >70 kDa such as LAO and P-III SVMPs in viperid venoms are more antigenic, while antigens such as the alpha-neurotoxins of elapids with molecular masses 5–30 kDa are less antigenic (León et al. 2011). Additionally, conformational properties (tertiary and quaternary configurations), protein amino acid sequential properties, and increased concentration of a given venom antigen are all venom antigen factors that enhance their immunogenicity (Leon et al. 2011). In contrast, a small percentage of snake venom components cause no apparent toxicity and are also nonimmunogenic (Mackessy 2009).

Omics

The complexity of snake venoms has made the detailed and systematic unraveling of their components and related toxinology equally as complex. To fully appreciate and understand a venom and its complexity, the analytical methodologies of *-omics* have emerged which includes proteomics (venom protein profiling), toxiconomics (functional assessment of toxic venom components), and antivenomics (immunorecognition of venom components by antivenom antibodies) (Calvete and Lomonte 2015).

Venomics

In a single venom extraction, there will be an array of proteins, a venom proteome, that is determined by *proteomic* analysis. Many venom proteins may all be within the same protein family, yet each single protein in that family may have its own unique pharmacological action and dynamic properties (Calvete et al. 2009). Individual protein differences within the venom of a single species, and within a single protein

family of the venom of that species, frequently vary over its geographic range. From a species survival perspective, it is beneficial for a particular venomous species to have multiple proteins in its venom from a single protein family. An array of snake venom metalloproteinases (SVMPs) or phospholipase $A2_s$ may be present in a snake venom such as that of the western diamondback rattlesnake (*Crotalus atrox*), where one protein from the family array will be selectively more effective toward the acquisition of prey associated with a specific region of the venomous animal geographic range, while a separate protein in the snakes' same protein family array will be effective for acquiring prey in a different region of the species geographic range.

To completely resolve an individual venom protein's characteristics and significance requires the correlation of the specific identified peptide/protein with a specific biological activity or function. Characterization of the venom proteome is achieved due to technological and analytical advances involving venom fractionation using reverse-phase liquid chromatographic separation of crude venom followed by the characterization of each protein fraction via N-terminal sequencing, gel analysis, and tandem mass spectroscopy followed by database searching (Calvete 2013). Importantly, this process has provided for the identification of specific venom proteins and allows for assessing specific pharmacological actions and properties, and the proteome of numerous medically important snake species (*Bitis* spp., *Bothrops* spp., *Bungarus* spp., *Crotalus* spp., *Daboia* spp., *Echis* spp., *Micrurus* spp., *Naja* spp., and *Vipera* spp.) has been determined by multiple investigators (Calvete 2013). However, the current proteomic methodologies do not provide an objective measure for correlation of the peptide/protein with a specific biological activity, yet the pathophysiological consequences of major venom protein classes have been characterized (Table 2). In terms of medical importance, these differences in the venom proteome may potentially translate into observed differences in envenomation symptoms in humans following snakebite (Calvete et al. 2011; Gutierrez et al. 2013; Warrell 1997).

The significant value derived from proteomic studies relates to the identification of clinically relevant venom antigens, which may be represented in venoms across multiple venomous species, using these relevant venom antigens as immunogens for generating antivenom antibodies. This would reduce the presence of multiple clinically irrelevant antibodies, which result when crude venoms are used as the antivenom immunogen. Venom proteomics reveals the translational aspects of toxinology as the venom proteome and isolation of specific toxins and toxicologically active venom antigens are used in the hyperimmunization of animals to elicit venom antigen-specific antibodies that comprise antivenom.

Antivenomics

Immunorecognition of important venom antigens (revealed via venomic analysis) by antivenom antigen antibodies that comprise an antivenom represents the complementary discipline of antivenomics (Calvete et al. 2009). This proteomics-based

process may be thought of as a methodology to measure the degree and correlation of fit between venom antigens of importance and the degree of representation of venom antigen-specific antibodies present in the entire antibody mix that comprise an antivenom. Immunoaffinity chromatography of venom proteins passed through an antivenom IgG or $F(ab')_2$ matrix column is the methodology of preference for varying the degree of venom protein binding that allows the separation of specific venom antigen proteins based on their differing affinities for their respective specific antibodies, characterizing the antivenom (Sintiprungrat et al. 2016). Thus, immunoaffinity-based antivenomics yields qualitative and quantitative information regarding the immunorecognition of toxins lacking immunoreactivity and toxins exhibiting antivenom-toxin-epitope recognition.

Ideally, the complementary merging of selected venom antigens of medical importance (those responsible for the relevant toxicological effects) via proteomic analysis with their parallel venom antigen-specific antibodies identified via antivenomic studies should result in an optimally pharmacotherapeutic antivenom. This process is useful in determining the preclinical efficacy of antivenoms using a series of in vitro experiments designed to measure the neutralizing ability of antivenom antibodies on several pathological parameters (Gutiérrez et al. 2014a). A detailed antivenomic study using the homologous and heterologous venoms from multiple medically important Costa Rica Crotalinae genera (*Atropoides*, *Bothriechis*, *Bothrops*, *Crotalus*, *Lachesis*, *Porthidium*) profiled their immunoreactivity with a Costa Rican polyvalent antivenom. Immunorecognition of a snake venom metalloproteinase (class I and III) and L-amino acid oxidase across all genera was observed, with varying relative abundances. In addition, and of lesser relative abundance, C-type lectin-like proteins, cysteine-rich secretory proteins, disintegrins, and PLA_2 venom proteins of some species within a genera such as *Bothrops asper* and *Lachesis stenophrys* exhibited immunorecognition (Gutiérrez et al. 2014b). Importantly, these antivenomic studies are significant for qualitatively identifying and quantifying toxicologically relevant venom proteins of medically important snakes and can demonstrate the need for amplification of these specific venom proteins for making more effective immunogens to be used in the hyperimmunization of animals for the production of more efficacious antibodies used in the preparation of antivenom.

Antivenom

The most cost-effective and pharmacotherapeutic effective intervention for the treatment of snake venom-induced pathologies following snakebite is the intravenous administration of antivenom (Brown and Landon 2010; Warrell 2010). Conventional antivenoms have representative manufacturing facilities located on every continent, and a wide range in the quality of antivenoms produced exists due to technological limitations and varying adherence to good manufacturing practices (Gutierrez et al. 2011). Antivenom formulations may be lyophilized (freeze-dried) or liquid preparations. Lyophilized formulations are composed of

immunoglobulin proteins, and the formation of aggregates has been a concern. However, recent studies have demonstrated that properly formulated and reconstituted lyophilized preparations using good manufacturing practices should be void of aggregations (Herrera et al. 2017). In order to surmount significant venom antigen-induced toxic effects following envenomation, the limited envenomated individual's endogenous immune antibody response can be supplemented via the parenteral administration of antivenom, a *passive immunization* process using venom antigen antibodies harvested from another animal species that has been previously immunized with specific venom antigens of the offending species.

Antivenom: Immunogens

Venom antigens are the essential components that are required to elicit an immunogenic response in a host animal and as such function as immunogens used in the development and design of antivenom. An initial consideration in antivenom design is determining which venom antigens will serve as immunogens. Since venom composition can vary across the geographic range of a single species, it is important that venom be collected from the species across its entire geographic range in order to insure a more comprehensive representation of the varying venom antigens/components that make up the venom profile for the species (Chippaux et al. 1991). The range of different venom samples can be pooled to comprise the venom antigen/component formulation mixture used in the immunization of animals for the generation of venom antigen antibodies. If the desired antivenom product is to be directed against a single species and is to be *monospecific*, the varying geographic venom samples from a single species are pooled, and if the final product is to be *polyspecific*, the venoms of several different species, across their geographic range, are pooled for use in the immunization process. Additionally, it is essential that venom antigens of toxicological significance and responsible for medical complications, such as venom antigen-induced procoagulant and anticoagulant toxicity and neurotoxicity via receptor antagonists, be present in adequate concentration to function as good immunogens.

Detoxified or inactivated venoms may be used as immunogens to avoid the pathophysiological effects elicited by active venom antigens yet maintain their antigenic properties. Technologies involving the use of a variety of chemicals, chelation, and photooxidation along with different physical forms of irradiation have been employed (León et al. 2011). These procedures, however, may reduce immunogenicity and alter venom antigen epitopes resulting in the lack of immunorecognition of circulating venom antigens by the generated antibodies in antivenom administered to patients.

In general, the venoms or venom antigens used for optimal antivenom design require the collective consideration and evaluation of clinical, epidemiological, immunological, and toxicological data.

Antivenom Antibodies: Sources and Immunization

Conventional antivenom antibodies are animal-derived immunoglobulins from a given animal species that has been immunized with venom antigens. The choice of appropriate animal species to generate antivenom antibodies should involve consideration of the resultant antivenom immunoglobulin properties in addition to the desired antibody functions as there are significant species differences in immunoglobulin characteristics. Since these animal-derived antivenom antibodies will be administered to humans, they will be recognized as foreign proteins by the immune system and potentially trigger an anticomplement and counter-immune response, compromising their efficacy and safety. These potential complications may be reduced if an antivenom was developed using specific clinically relevant venom protein antigens that were identified via proteomics and then isolated and mixed to make a tailored venom antigen immunogen. This would exclude multiple irrelevant venom proteins, allowing a focused immune response to relevant venom antigens, with the resultant antivenom product having more efficacious neutralization capacity and reduced adverse reactions due to a reduced irrelevant protein load administered to envenomated patients.

Historically, the animal of choice for immunization has been the horse as it is a large animal, and a considerable volume of antibody-containing blood can be harvested and plasma fractionated (León et al. 2011). Equine immunoglobulins have a unique heavily glycosylated 160 kDa immunoglobulin (IgG(T), composed of IgG_3 and IgG_5 subclasses, which is the primary venom-neutralizing antibody. Despite the favorable neutralizing effects of IgG(T), the high degree of glycosylation makes it more immunogenic and it has increased potential for inducing adverse reactions when administered to patients (León et al. 2011).

In recent years sheep have been used in the production of anti-crotalid antivenom as the ovine antibodies have been reported to be of reduced reactogenicity (Herrera et al. 2005). The reported reduction in this adverse effect is likely more attributable to the ovine IgG having been enzymatically digested to a smaller 50 kDa antibody fragment, F(ab), that is administered to patients. Other species that have been used for the generation of venom antigen antibodies are chicken hens, camels, donkeys, llamas, and rabbits. Of these, camels and hens have been the most investigated.

Camels are unique in that they exist comfortably in hot arid regions of the world, providing an antibody source where other species could not tolerate the climatic conditions and environment. They are also unique in the fact that their immune response to venom antigens generates both heterotetrameric (160 kDa) and homodimeric (100 kDa) immunoglobulin configurations, represented by three IgG subclasses, all of which have venom antigen neutralization properties (Cook et al. 2010a). Additionally, camel immunoglobulins are less immunogenic than horse or sheep immunoglobulins, and the heavy chain subclasses possess significant thermostability (Herrera et al. 2005). These unique immunoglobulin features make camels a desirable animal for antivenom development, and limited studies involving their use

in scorpion and snake antivenom development have been promising; however, further investigations are warranted (Cook et al. 2010b; Meddeb-Mouelhi et al. 2003).

Hens are egg layers, and by immunizing a hen with venom antigens, antibodies develop and will be present in egg yolk. The immunoglobulin derived from egg yolk is termed IgY, and it is a larger immunoglobulin than those of other species used for antibody production, having a molecular mass of 180 kDa (Warr et al. 1995). It was thought to have lesser immunoreactive properties than mammalian IgGs; however, when IgY immunogenicity was compared to anti-bovine and anti-equine in humans, higher anti-IgY antibody titers resulted suggesting that mammalian-derived antibodies may be less immunogenic (Sevcik et al. 2008).

Immunization schedules and methodologies leading to the generation of venom antigen antibodies in sufficient quantity, with specific venom antigen recognition for neutralizing those toxins of pathophysiological importance, can vary significantly and require a systematic developmental process. The venom used for immunization must be representative of that from a species throughout its geographic range as there is considerable intraspecies variability in the concentration and representation of venom antigens (Chippaux et al. 1991). Thus, the *immunization venom* is a pool of multiple different venom samples. Venom pools consisting of venom from multiple individuals of a single species or multiple individuals of multiple species result in the generation of *monospecific* or *polyspecific* venom antigen antibodies, respectively.

Crude venom that is diluted, or detoxified venom, is used for immunization via various routes of injection (intradermal, intramuscular, or subcutaneous) to multiple anatomical sites associated with lymph nodes. Venom diluted in saline is frequently used as the immunogen. However, enhancement of the immune response with adjuvants such as aluminum, bentonite, Freund's, and incomplete Freund's may be added to the immunization mixture (León et al. 2011). The dissolution of venom in an aqueous medium is added into the adjuvant with an oil-based vehicle to make the injectable immunogen. A standard schedule of immunization involves a primary immunization followed by boosts at varying intervals (usually weeks) until adequate venom antigen antibody titers are achieved (usually months) (León et al. 2011). Of utmost importance is that the health of the host animal is not compromised from using excessive venom immunization doses or an immunization schedule that is too intense.

Future immunization methodologies could involve the implantation of antigen-release devices with biocompatible or biodegradable coatings. This strategy has been used in cows to generate specific serum IgGs, which are also present in milk from their selective transfer via neonatal Fc epithelial mammary gland receptors. Whether this process would be applicable for use in the venom antigen delivery to traditional species requires investigation (Liu et al. 2009).

The use and characterization of venoms from their animal source to their introduction into a separate host species to elicit a biologically induced immune response represent a second major transitional phase in translational toxinology across species and biological systems.

Immune Response: Venom Antigen Antibodies

The perfect antivenom would be comprised of antibodies directed only at venom antigens, specifically those responsible for significant toxicity; however, the large majority of antibodies present in antivenom are non-venom antigen specific and of no value for neutralizing venom antigens/toxins.

Initial immune response following hyperimmunization of the host animal with venom antigens triggers a cascade of inflammatory mediators and is characterized by pain and edema due to cell infiltrates at the sites of injection. This process involves complement and kinins, cytokines, eicosanoides, matrix metalloproteinases, and nitric oxide. Additionally, there is a chemotactic response with the recruiting of macrophages and neutrophils, stimulation of leukocytosis, and the expression of adhesion molecules from endothelial cells at the sites of immunogen injection (Escalante et al. 2009).

The physiochemical properties of the venom from species used as an immunogen can vary significantly, affecting the rate and distribution of venom antigens. The venom antigens comprising the immunogen must reach the lymphatic and systemic circulation to interact with antigen-presenting cells to elicit an immune response or diffuse via lymphatic routes where they can interact with lymph nodal B-cell-specific immunoglobulin receptors via the interactions between antigen-presenting cells and T helper cells, resulting in the generation of an initial phase immunoglobulin response in the form of IgM antibodies. Low molecular mass elapid toxins may be rapidly taken up into the systemic circulation, while larger viperid enzymatic proteins may be taken up more slowly (Ismail et al. 1998). Thus, the toxicokinetic profile of venom antigens may also affect the immune response.

IgG antibodies are the primary antibody isotype desired in antivenom. Subsequent to a primary immunization with venom antigens, early immune antivenom IgM antibodies develop, followed by antivenom IgG antibodies with higher plasma concentrations that remain elevated for lengthy periods. Following a repeat venom antigen boost, IgG concentrations increase more rapidly and to a greater degree as a result of B-cell activation. IgG titers will continue to increase with each subsequent boost. Ideally, the IgGs with increased concentrations will be composed of toxicologically important venom antigen-specific IgG antibodies.

Antivenom: Antibody Types: Formulations

To harvest venom antigen IgG antibodies requires the fractionation of plasma collected from the venom-hyperimmunized animal using ammonium sulfate or caprylic acid precipitation for removing non-immunoglobulin proteins. Fractionation using caprylic acid typically results in greater antivenom yields and purity. Once fractionated the IgG proteins may be purified to isolate venom-specific antibodies, but this step involving affinity or ion-exchange chromatography is not always performed. As such there can be a large quantity of nonspecific antibodies

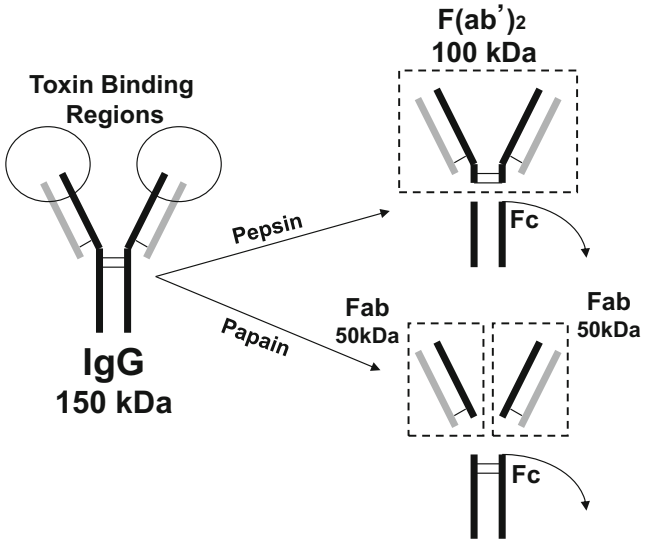

Fig. 2 Schematic representation of the three major antibody forms that comprise various antivenoms. Immunoglobulin G (IgG) and its two enzymatically cleaved binding forms all have toxin-binding properties

in the final antivenom product, which results in an unnecessary increased protein load administered to an envenomated patient.

In addition to traditional whole IgG antivenom products, there are methodologies for producing smaller IgG fragments that still maintain venom antigen-binding properties for neutralization. These antigen-binding fragments (Fabs) are of two main forms. If IgG is enzymatically digested with pepsin, a 100 kDa fragment, F $(ab')_2$, is produced. When IgG is enzymatically digested with papain, a 50 kDa fragment, F(ab), is generated (Fig. 2). Thus, the resultant antibody fragments are 66% and 33% the size of the parent IgG molecule and exhibit different pharmacokinetic and stoichiometric properties (Table 3). IgGs have a long elimination half-life of roughly 4–7 days and limited volume of distribution that is slightly greater than the central compartment blood volume. In contrast, F(ab) has a greater volume of distribution and much shorter elimination half-life, typically less than 24 h depending on the species it has been administered to, while the pharmacokinetic properties of $F(ab')_2$ are intermediate to those of IgG and F(ab) (Gutierrez et al. 2003). Toxin-binding valency is divalent for both IgG and $F(ab')_2$ and is monovalent for F(ab) (Fig. 2). An important feature relating to the generation of F(ab) and $F(ab')_2$ is the absence of the complement-binding fragment (Fc). The removal of the Fc portion of the IgG is intended to reduce adverse immunogenicity effects such as complement activation (Herrera et al. 2005).

Given the different properties of these three antivenom antibody forms, they each have advantages and disadvantages in relation to their pharmacotherapeutic

effectiveness. IgG, although it possesses greater potential for adverse reactions, is present in the systemic circulation for an extended time providing a greater time period for its binding interaction with venom toxins and the continued binding of toxins as they redistribute from tissues. Thus, its capability for venom toxin neutralization in the body is of greater duration. F(ab), in addition to its reduced adverse effect potential, resides in the body for a shorter period of time allowing limited interaction time for binding with venom toxins but, with its more rapid distribution and increased ability to reach deep tissue compartments, may bind damaging venom toxins more rapidly and those that IgG may not have access to. $F(ab')_2$ again is intermediary with respect to these IgG and Fab properties.

Antivenoms may be designed to provide venom neutralization for a single venom-producing species or for several species. If the antivenom is derived by the immunization from a single venom-producing species, it is monospecific, and if multiple different species have been used in the immunization process, it is a polyspecific antivenom.

Prior to the manufacturing of antivenoms, it is necessary to scrutinize the ability of the antivenom antibodies to adequately neutralize relevant toxic venom components and assess adverse effects from the antibodies themselves. Pending the results of this process, a clinical trial follows to evaluate both antivenom efficacy and safety in envenomated patients.

Antivenom Efficacy and Safety

The initial or preclinical phase for assessing antivenom is to insure its efficacy and safety prior to pharmacotherapeutic use, which involves the use of in vitro and in vivo methodologies. The World Health Organization (WHO) has developed guidelines toward the standardization of methodologies for appropriate quality control in antivenom manufacturing. Antivenom protein concentration, purity, excipient and preservative concentrations, residual moisture in lyophilized products, and immunochemical identity testing are all factors affecting antivenom quality and pharmaceutical purity (WHO 2010).

In vivo testing for efficacy of venom neutralization involves performing median effective dose (ED_{50}; the antivenom/venom ratio that results in 50% protection) or median lethal dose (LD_{50}); the venom dose resulting in 50% deaths within 24–48 h) studies in mice, in which a fixed venom dose is mixed with increasing serial dilutions of antivenom for 30 min at 37 °C. This compound mixture is administered in escalating doses either intraperitoneally or intravenously, and deaths occurring within a defined time period typically 24–48 h are recorded. In addition to these in vivo tests involving the neutralization of lethality, there are preclinical in vitro tests of medical relevance for assessing antivenom efficacy. Neutralization of the enzymatic activities of viperid venoms can be assessed for coagulant, defibrinogenating, hemorrhagic, myotoxic, PLA_2, and proteolytic actions (Gutiérrez et al. 2014a).

Assessing antivenom safety involves testing for pyrogenicity, the presence of bacterial endotoxins in the antivenom, and is accomplished using in vivo and in vitro methodologies. In vivo studies involve rabbits in which a small quantity of antivenom is injected intravenously and the subsequent monitoring body temperature response. Alternatively, an in vitro method using Limulus amoebocyte lysate (LAL), an aqueous extract from horseshoe crab (*Limulus polyphemus*) blood cells, reacts with the lipopolysaccharide membrane component of endotoxin-producing Gram-negative bacteria, allowing the quantification of bacterial endotoxins (Park et al. 2005).

The true measure of antivenom efficacy and safety is revealed when envenomated patients are administered a final manufactured therapeutic antivenom in a controlled medical setting. Efficacy becomes apparent with the timely halting and resolution of venom-induced pathophysiological effects as observed with the correction of coagulopathy, systemic hemorrhage, the reversing of neurological toxicity, and other venom-related toxicities. Adverse reactions in patients without a prior sensitization to the immunoglobulins from the host animal species, from which the antivenom is developed, are not uncommon. Early adverse reactions may be frequent, occurring during the first hours following antivenom administration, exhibiting a range of clinical manifestations (angioedema, bronchospasm, colic, hypotension, itching, nausea, and urticaria), and are attributed to complement activation (Gutierrez et al. 2011; Stone et al. 2013). Verifiable IgE-mediated, type I, anaphylactic reactions are uncommon and can occur from acute immune response to antivenom immunoglobulin proteins and may also result from immune response to proteins in the offending animals venom (Isbister et al. 2012). Serum sickness is a late adverse reaction (type III immune reaction), typically occurring 5–14 days post-antivenom administration (Gutierrez et al. 2011).

The testing of antivenom safety and efficacy in animals, followed by human clinical trials, represents a final phase in translational toxinology with respect to the transition from venom to antivenom.

Alternative: Novel Antivenom Forms

The ever-increasing progression of technological capabilities and methodologies and the rapidly growing database of venom and immunology knowledge provide for the development of alternative and nontraditional antivenom-type therapies. Nanotechnology has led to studies developing scorpion venom, toxin-specific nanobodies, recombinant antibody fragments of low molecular mass (single-domain recombinant antibodies) with favorable pharmacokinetic distribution and elimination properties, and potentially low antigenicity (Hmila et al. 2012). Aptamers, synthetic RNA, and single-stranded DNA oligonucleotides, with high selective toxin affinity, have been shown to inhibit the biological and pharmacological activity of the three-fingered snake venom toxins of *Bungarus multicinctus* alpha-bungarotoxin and *Naja atra* cardiotoxins and the potential ability for general interaction with the structural scaffolds of three-fingered loop structures (Chen et al. 2016). Humanized-camel

single-domain antibodies have been produced that have been shown to neutralize the phospholipases present in *Naja kaouthia* venom. Thus, these small, humanized antibodies can more readily penetrate tissues, reducing the local dermonecrosis, and are relatively absent of immunoreactive properties (Chavanayarn et al. 2012).

Although all these strategies are achievable in the laboratory setting, their progressing and translating to practical larger-scale and clinically used therapies may potentially be hindered by economic constraints.

The Politics of Venom and Antivenom

Venom is like *liquid gold*; its true and perceived values contribute to the political complexity of venom acquisition for antivenom development. Many of the countries that were formerly colonized are often sensitive to outside interference from other nations, are geographically situated in climatic and habitat conditions that provide for the existence of numerous unique venomous species, and are often *biological hot spots*. Consequently, these countries are frequently exploited for their natural resources, and snake venoms are a form of "liquid gold" due to their value in biomedical research, pharmaceutical applications, and homeopathic preparations. Thus, there is an economically driven component for their exploitation. Confoundingly, in countries where no real monetary value for venom may exist, venom has a perceived economic value as a unique natural resource.

A final consideration in translational toxinology with respect to the process of venom to antivenom stems from globally related complexities involving governments and private enterprises. Various political perspectives of different countries, highly variable manufacturing practices, and the questionable legitimacy for economic gain all compromise the optimal therapeutic benefit of toxinological venom and antivenom research and development. Antivenoms and venom-related immunotherapies should be manufactured using standardized regulatory methods, be pharmaceutically pure, undergo standardized testing for efficacy and safety, and be economically affordable (Theakston et al. 2003; WHO 2010). Antivenom pharmacotherapies should be available to all countries and patients that require their medical need (Gutierrez 2016).

Conclusion and Future Directions

The translational toxinology of venom to antivenom begins with the venom-source animal, initially transitioning to the venom hyperimmunization of a host animal for the generation of venom antigen antibodies, followed with transitioning to antivenom safety and efficacy studies in laboratory animals, transitioning to the final phase of the derived antivenom being administered to envenomated patients. Thus, translational toxinology as it relates to antivenom development bridges across four distinctly different biological taxa with significantly different bio-physiological systems.

The future of translational toxinology with respect to venoms and antivenoms will reflect that existing complications associated with antivenom therapy are resolvable. Newer developments in the antivenom production process relating antivenom to antibody design and improved antivenom antibody profiles as a result of molecular technology advancements such as proteomics and antivenomics, in conjunction with creative scientific intelligence, will lead to the production of more efficacious and safe immunologically derived antivenoms and antivenom-like pharmacotherapeutic products. Consequently, the humanitarian and therapeutic benefit of pharmaceutically pure therapeutic antibodies (antivenom) or antibody-related derivatives will justify their continued use as the predominant pharmacotherapy for the treatment of envenomated patients.

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Keeping Them Safe: The Management of Venomous Species in Captivity

23

Jessi Krebs and Victoria Krupp

Contents

Abstract

Institutions housing venomous animals must consider every risk to both humans and specimina. From the construction of exhibits and holding areas to emergency procedures in case of envenomation, facilities must strive for the highest standard of safety by having a rigid compilation of requirements. Due to the absence of universal standards or regulations regarding the housing and handling of venomous species, the protocol followed by establishments is institution-specific. An institution is responsible for creating and implementing a set of nonnegotiable standards that focus on the housing of venomous species; acquisition, transport, and maintenance of antivenom; handling expectations and training; and emergency procedures in case of accidental envenomation. It is the institution's responsibility and duty to compose a detailed plan to decrease the risk of

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envenomation and to minimize complications if this event should occur. This plan includes institutions outside of the housing facility; ideally a medical facility and the local center of poison control. It is of the utmost importance to maintain a strong collaboration and relationship among these institutions to avoid confusion and promote fluidity in the execution of preventative and emergency protocol.

Introduction

In order for any institution to house venomous animals, it is the institution's responsibility to devise and establish procedures that ensure the safety of the caretakers, visitors, and animals. It is essential to regulate rigid expectations in the following areas: exhibit safety for the animal, caretakers, and visitors; correct antivenom acquisition, transport, and storage at the housing location; proper training for individuals who will have daily contact; and emergency plans that involve zoo staff, poison control, local hospitals, and knowledgeable physicians who know how to treat envenomation. Many zoos and similar institutions created standards that have proven to be extremely effective in preventing escape and envenomation. These standards also promote the wellbeing of the specimen, both physically and mentally.

Considerations for Primary, Secondary, and Tertiary Containment Areas

Primary, secondary, and tertiary containment areas should be meticulously engineered. From the design and construction of the exhibit, to the employees' workspaces, each should be treated with the same degree of gravity. In primary areas, exhibits and enclosures, the design must not only promote the animal's physical and mental wellbeing, but needs to allow the qualified keepers and handlers a safe situation to work in. The working environment, secondary and tertiary areas, should also maintain the highest standard of safety for handlers and keepers, but one that also ensures the caretakers can effectively and safely carry out their duties.

In order to ensure that enclosures adequately fulfill the specimen's needs, certain important features are required. First and foremost are the dimensions and design of the enclosure. It should provide adequate space for the specimen. The animal's size, mobility needs, and natural habitat features should be the basis for the enclosure's design, e.g., if the animal is arboreal versus terrestrial (Figs. 1 and 2).

Although the size of the enclosure should be appropriate and proportionate for the needs of the animal, it should not be species-specific that it would not be able to house another species with similar space needs. Permanent furniture that is to that species should be avoided so if a new animal needs to be moved into that enclosure, less time and money has to be spent to renovate the space to accommodate the new

resident. Along with no permanent accessories, the furniture placed in the exhibit should not give the animal a place to hide from the handlers and keepers, especially near the access points. Shelves, caves, plants, etc. should located away from where a keeper would be entering. Lighting and heating sources should be placed in areas away from water that provide the warmth and light needed without injuring the animal.

All enclosures need to be equipped with adequate heating systems. Depending on the size of the enclosure and temperature required for the health of the animal, different methods of heat delivery need to promote sufficient thermoregulatory behaviors. Different methods of temperature regulation include: HVAC reheaters, window air-conditioners, space heaters, under tank heat tape, heat lamps, and radiant heat panels (Figs. 3, 4, and 5).

Fig. 1 Terrestrial specimen exhibit

Fig. 2 Arboreal specimen exhibit

Fig. 3 HVAC reheater

Fig. 4 Wall air-condition unit

Lighting is also very important because it not only provides heat for the animal, but also delivers the necessary light wavelengths to support the animal's vitamin needs; the most important being UV-A and UV-B rays. These can be emitted by full-spectrum bulbs and by ceramic-based mercury vapor bulbs. The placement of these light sources needs to be very strategic. The light source must be out of the reach of the animals to reduce the risk of burns. The lights also need to be away from water sources. The placement of heat and light sources must also allow for a variance in temperature within the appropriate range respective to the species. Examples include a basking area with a direct light beam and also areas so the animal can cool off. To ensure the correct temperatures, each enclosure or exhibit should have a thermostat with alarms so the temperatures can be constantly monitored and documented. If there should be a drastic fluctuation in temperature the alarm will sound to notify personnel. Facilities need to also be equipped with backup generators in case of a power outage (Fig. 6).

It is recommended that enclosures have some type of clear viewing port or window; this allows the handlers to have an uninhibited view of the enclosure

Fig. 5 Radiant heat panel

Fig. 6 Examples of temperature monitoring

furniture and specimen so keepers can assess the space and animal. When maintenance is required, keepers need free access into the enclosure through the points of entry. The access ports should be located at the top and/or adjacent sides of the enclosure. The doors on the enclosure should be in proportion to the size of the enclosure to allow ease of remove animals and other items within (Fig. 7).

For many species small access points can be connected to shift boxes or small secondary holding enclosure in case the animal needs to be moved out of the primary exhibit. In the past situations of venomous care, handlers played a game of "wait and see." The port was locked opened and the animal then had the choice of moving which could be very time consuming. Today many animals have been either trained using conditioned response to move to another space by using positive

Fig. 7 Door and access point sizes and placement

Fig. 8 Shift boxes located on the enclosure

reinforcement, usually food. This method of animal movement limits stress to the animal and is more time efficient (Fig. 8).

In order to provide proper ventilation for the animal, there must be sufficient airflow. The ideal placement for ventilation is across the top of the enclosure and the side to cross ventilation. To maintain safety, a two-layer boundary of thick mesh or

Fig. 9 Examples of mesh barriers on the top of enclosures

small diameter ventilation louvers has proven to be ideal. Keepers must be vigilant, however, because of the risk of envenomation from this access point. An example of this is when working with the characteristically long-fanged *viperidae*, keepers must be cognizant of their surroundings and rely on self-awareness to avoid placing their hand or body parts against these ventilation ports (Fig. 9).

Not only do the enclosures and exhibits have to be secure and accessible, but the workspaces behind the scenes need to be as well. Even though the enclosures are extremely secure, every precaution should be taken in case of accidental handler error which results in escape. Secondary containment areas should be established to prevent animals from getting into locations that can cause harm to the keeper and the kept; this could be a two-door entry system with an appropriately sized vestibule separating them. Each door should have a suitable window so anyone entering the room has a clear view of the floor area and room furniture in case a specimen has found its way out of the enclosure. First aid and emergency equipment should be located outside of the secondary area and be highly visible and easily accessed by staff and first responders in case of envenomation or other injury. Have the equipment in this location allows escaped animals to be secured within the room and have no chance of future envenomation. Systems for alerting other staff as to an emergency such as alarm systems, two-way radios, and phones should be implemented in case of an emergency. When utilizing alarm systems, if possible, an indicator panel should be placed in appropriate areas that can guide personnel to the area of emergency. A map of the facility should neighbor these panels for ease to directing (Fig. 10).

Room furniture and items that must be maintained on the floors of the venomous holding rooms should be kept to a minimum to reduce clutter so if there is an escape, the animal has very few options for hiding. This minimalistic approach also reduces the risk of other injuries and provides obstacle-free access for emergency crews if needed. The equipment that keepers and handlers use should be located in a designated area. The tools can be hung on the wall in plain view, as to keep the floor clear. Any possible animal escape routes need to have a way to be closed off. Ventilation and plumbing systems that run by the venomous rooms should have a

Fig. 10 Emergency phone, panel, and facility map

three-layer thick barrier of mesh over openings and vents to prevent an animal from leaving the area. All doors need to have door sweeps.

Each venomous room should be supplied with equipment for the handlers and keepers to carry out their jobs. Many different types of tools can be used when working with these species. Most of the tools are also utilized with nonvenomous species. Snake hooks of varying sizes, clear tubes to immobilize animals, and long-handled metal grab sticks are types of tools that all qualified employees will need to use on a daily basis.

A tertiary containment area consists of the previously discussed vestibules that separate the venomous rooms from the rest of the structure and the main building of containment. The most important feature that should be throughout the building is an alarm system. As mentioned earlier, when an alarm is triggered in a venomous area, all facility staff is notified. Ideally, there should also be panels that indicate where the emergency occurred so identified individuals can respond as quickly as possible (Fig. 11).

The staff must also be provided with safe and quick evacuation routes in case of an emergency. These routes must be communicated with the staff and displayed throughout the building.

Acquiring Antivenom and Proper Storage

It is pertinent that each primary facility take responsibility for the acquisition of antivenom and storage on location. It is also essential that it is stored onsite because it expedites the emergency procedures if envenomation occurs. There needs to be a well-defined process of the acquisition, storage and disposal of antivenom.

Fig. 11 Door sweeps and blocked escape routes

The best resource for antivenom information is the Antivenom Index (AI), an interactive, continuously updatable, collaborative product of the Association of Zoos and Aquariums (AZA), and the American Association of Poison Control Centers (AAPCC). The AI was developed by snake and envenomation experts and access is restricted to AZA zoos and to AAPCC poison centers. The AI includes information resources regarding selection of antivenom(s) for venomous snakebites, manufacturer product insets, the current location of antivenoms at AZA institutions, contact information, monographs on antivenom acquisition, position statements on housing venomous animals and emergency procedures, envenomation management, and a list of available medical consultants.

Because antivenoms to nonnative snakes are not approved for use in the USA by the Food and Drug Administration (FDA), any institution wishing to purchase and store these antivenoms must import them under an Investigational New Drug (IND) Application. Compliance with the procedures and reporting requirements governing IND medications is a requirement for the primary storage site, other institutions working with the primary facility, as well as those facilities which receive and use these antivenoms to treat an envenomation. The location housing the antivenom is also held accountable for sharing updated antivenom inventory information with the designated medical control officer. It is recommended that local healthcare facilities which may be expected to receive envenomated patients and nonnative (IND) antivenoms, as well as regional poison centers, should be aware of nonnative venomous species in their service areas and provided with or help to develop management protocols.

Antivenom should be purchased from well-known respected distributors. The antivenom comes in two forms, liquid or lyophilized. Each manufacturer provides a package insert written instructions on proper storage and handling. Failure to adhere to manufacturer guidelines may result in accelerated loss of potency and the possible loss of product sterility, with potentially catastrophic consequences.

When the antivenom is delivered, it needs to be promptly labeled, coded according to species and stored appropriately. We recommend a large, easily read, color- as well as text-based coding system to allow for rapid identification of the proper antivenom for a biting snake. This allows for a quick and correct response in

case of an emergency. The labels will correspond to file folders that contain information about each specimen. The folders provide information about the species, the venom, manifestation and timeline of symptoms, and antivenom administration. This folder accompanies the antivenom to the partnering medical facility and should be located directly next to the temperature-controlled storage unit and bite victim medical packets.

Antivenom storage protocols should mirror medical pharmacy storage requirements, as well as follow manufacturer's guidelines. The storage unit should have the capabilities to continuously monitor and document temperature. A reliable and consistent way to document the temperature is to use a sensor connected to a computer and have the information immediately stored electronically. Times and dates can also be recorded and archived in respect to when the temperature was assessed using this system. To protect against the possibility of an interruption of power, backup generators are necessary to immediately engage and provide adequate power to limit temperature changes and guarantee continued recordings. The temperature requirements are set by the manufacturer and have to be maintained during storage and handling. To minimize temperature changes, antivenom should to be transported in a sufficiently insulated container (Fig. 12).

Each antivenom has a different shelf life and "expiration" dates need to be monitored on a consistent basis by the supervisor. This date often corresponds to a point where more than 10% of potency has been lost. Ideally, zoos should maintain "in-date" antivenoms for potential envenomations of their personnel. However, even if an antivenom has passed the manufacturer-applied "expiration" date, it may retain significant and sufficient potency for many additional years. Unlike FDA-approved drugs, these "expiration" dates do not carry the force of law and the antivenoms may be used, if needed, as long as sterility has not been lost, although higher doses may be required. They should be retained and stored as with in-date products. The efficient way to dispose of antivenom that has lost sterility, or is deemed likely ineffective, is through incineration. Any change in the available antivenom supply, whether it is additions or disposals should be updated in the AI in a timely manner.

Fig. 12 Alarm system and backup generator

Interactions Between the Animal and Their Caretakers

A facility's plan to decrease the probability of envenomation is essential in the day-to-day interactions between personnel and the animal. Individuals working with these creatures face dangers on a regular basis, so the institution housing venomous specimina need to create a system to guarantee a high degree of safety for both keepers and animals. Applicants for a keeping position in a venomous area have to be well trained and educated before any interactions and handling take place.

Training

Before individuals can have interactions with these animals or be around them in any way, they must go through extensive training. This training should not only includes the basics of handling of animals and enclosure maintenance, but also encompasses requirements of the storage and information relating to antivenom, details about each specific animal, emergency procedures, and important locations within the building. It is imperative that any individual taking on this responsibility realize the gravity of it. Staff members working in the venomous animal areas are a team. They need to work together and must be able to depend on one another to correctly and safely complete tasks. Failure to perfect work habits could result in harm to both humans and animals. For this reason, it is essential to implement a rigorous training program that includes reading material, observation, shared and guided experiences, and evaluations by supervisors. The training should be individualized for each future handler.

First and foremost, potential venomous animal handlers must familiarize themselves with information regarding general zoology, venomous species housed at the institution, venom, antivenom, basic and injury-specific first aid procedures, and short- and long-term management of envenomated individuals. Applicants should be required to read inhouse documentation and literature to prepare them for working with these animals. Examples of these texts include: handouts with information from the most recent Venomous Animal Safety and Husbandry Training Seminar and *Venomous Snakes of the World: A Manual for Use by U.S. Amphibious* (Shupe 2013). The trainee must also memorize emergency plans, procedures, and important locations at the establishment. It is essential to require that every individual who interacts with venomous species have a sound understanding of the location and access points of exhibits and enclosures, location of the antivenom and its information, first aid and medical supplies, required daily procedures, and the emergency plan if there is an envenomation.

The Gradual Release Process, most often referred to in academia, allows for the individual to observe procedures modeled by expert keepers, assist with procedures guided by a trained handler, then carry out duties as a trained member of the staff. The individual first begins by shadowing trained zookeepers as they perform their daily tasks. The trainee is able to see how each procedure is carried out, e.g., proper equipment and use, two people working at the same time, use of the color-coded

system, etc. The applicant is also given the opportunity to practice and learn appropriate handling techniques by working with smaller, nonvenomous species. The trained zookeepers observe and give constructive feedback so the trainee can perfect his or her skills. From this point, the applicant can move forward to interactions with venomous animals. This process should go from "small to big." This simply means the trainee should interact with smaller creatures, such as a baby rattlesnake. Throughout the training, each animal interaction is commenced and ended with a reflective discussion. The applicant self-evaluates his/her performance, noting what went well and what could be improved on. The expert keeper joins in on this discussion to provide positive feedback but also constructive criticism.

The applicant can then request evaluation when they feel confident in their abilities. Even if the veteran keepers training the individual feel they are not ready, the person applying can still request observation. The supervisor questions the applicant over antivenom information, facts about each species, correct animal handling and interactions, emergency procedures and protocol. The trainee then demonstrates their knowledge of the actual handling of the animal. There is no formal written exam; however, the supervisor completes a detailed checklist during the evaluation process. The supervisor assesses the applicant's abilities and makes the final decision.

Requirements and Procedures for Animal Handling and Interactions

The trained zookeepers and handlers have requirements they must adhere to while interacting with and managing venomous species. All handlers should have completed a rigorous training course and given approval by the supervisor. Zookeepers are held responsible for applying what they learned in their training and must demonstrate this everyday with consistency and fidelity.

The keepers have a set of procedures they must adhere to. It is essential that at least two approved individuals are working simultaneously in case of an emergency. If one keeper is accidentally envenomated or injured, the other keeper can trigger the alarm, contain the animal if it has escaped, and immobilize the victim. A very effective and easy way for handlers and other zoo personnel to maintain safety and communication is by adopting a color-coding system. How this system works is that each venomous animal is assigned a color that corresponds with the antivenom used to treat envenomation. Every enclosure, exhibit, piece of equipment used, information packet, and identification tab is coded depending on the species. If envenomation were to occur, first responders and staff can quickly identify the species responsible and which antivenom is needed. This is a simple yet extremely effective way of maintaining safety standards as well as strengthening communication during an emergency situation.

When working with an animal, a keeper must use the appropriate tools needed for the job. These tools can be found in the designated area. After retrieving what they need, each keeper must remove a colored tag that is located on the identification card and affix it to their shirt. This small step not only easily communicates to others

which specimen the keeper is working with, but it also gets the keeper into the mindset of what they are about to do. Handlers must be completely focused during every interaction. If an envenomation does occur, first responders and emergency crews can quickly gather the necessary antivenom, specimen-specific information, and employee health information card to send with the individual. When finished with an animal and its enclosure, the tag is then removed from the keeper's shirt and then placed back onto the identification card located on the enclosure.

Restrictions also need to be set into place about whom, when, and where interactions with venomous animals occur. Unauthorized personnel should never be allowed to enter venomous animal holding areas without the permission of the reptile supervisor or curator. A qualified keeper or handler must be on facility grounds during operating hours. Qualified keepers and handlers need to interact with venomous species during normal working hours. If items need to be attended to outside of this time, the supervisor needs to give approval. Not only are keepers and handlers not supposed to work outside of their schedules, interactions should never occur during a situation where power or telephone outages might occur. These situations include, but are not limited to: severe thunderstorms, possible tornadic activity, ice storms, and dangerous wind advisories. If an event like this does occur, all emergency telephones in the areas need to be tested before anyone can continue working with the venomous species.

Interactions between approved keepers and handlers also affect other staff working in the main housing facility. If a venomous species needs to be moved offsite, typically to an animal medical site, the personnel transporting the specimen are responsible for bringing the following: animal identification card, first aid and emergency treatment protocol for that species, employee medical packets for each attending keeper, appropriate antivenom, emergency bite kit, and all necessary handling equipment. The animal being transported needs to be placed in an appropriate-sized insulated carrier that is heavy duty and fully locking. After arriving at the location, the reptile supervisor or curator needs to advise and brief all individuals involved on the procedure that needs to be done, what each of their duties will be if there is an envenomation. All staff present at the institution needs to be notified of this change of location. Signs signaling the presence of the venomous animal need to be placed on all doors of the facility where the procedure is being performed.

A plan for handling deceased venomous species also needs to be created. If a handler or keeper suspects an animal has died, they need to immediately notify the supervisor or curator. The specimen should be removed with a snake hook or other appropriate tool. The animal's head should be restrained and have its mouth securely shut so no teeth are showing. Envenomation can still occur even if the animal has died. The specimen is placed in a clear plastic bag and is labeled warning others that it is venomous. The veterinarian who will be responsible for performing the necropsy must be notified to make arrangements for the procedure. All necropsies involving a venomous animal need to be carried out by veterinarians who are employed by the institution; a qualified keeper needs to transport the animal and all emergency envenomation information, including health cards for all staff present, antivenom if needed, and emergency bit kit. He or she must remain onsite during the procedure.

Emergency Plan Preparation and Execution

Although many institutions housing venomous animals are extremely proactive in minimizing the chances of envenomation, there is always a possibility that it may occur; no plan is infallible. An emergency plan must include collaboration and input from all institutions involved in the execution of it. This plan must detail the responses and responsibilities of each institution.

It is up to the institution to establish a way of communicating an emergency. Depending on the size of the collection, institutions need to adopt a mode of communication that is appropriate for their needs. Systems can be as simple as alerting staff by two-way radio or can be as complex as a nurses' station, with trained individuals on site to immediately respond with medical care. When an accident happens, and alarm goes off alerting every staff member present that assistance is needed. An alarm is not nearly enough, so panels should be placed in all keeper areas that light up showing where the incident occurred. Panels should be placed in most rooms of the main building where the venomous animals are located. The room in which the incident happened is indicated on the panel so everyone knows where to go. Regardless if other keepers in the building work with venomous species or not, they are required to assist in the emergency plan. These individuals include: all keepers, supervisors, and custodial staff. As changes in the plan are made, every employee in the building needs to be notified immediately. Emergency drills need to be unannounced and routinely carried out. The plan should be evaluated approximately every 6 months (Fig. 13).

If an individual is bitten, the staff in the building or area needs to be notified via the chosen method of emergency communication. The first workers on the scene have three jobs: secure the area, immobilize the victim, and the individual. It is of the utmost importance that the envenomated individual is immobilized quickly to

Fig. 13 Alarm system mounted on a wall

decelerate the venom's progression through the body. The injured person, health information card, antivenom, and species informational folder are then all transported to the partnering medical center. All employees that might come into contact with a venomous animal need to be required to provide a complete history of past and present medical information. In an event of an emergency, the envenomated individual will not always be conscious or coherent enough to relay information about allergies or pre-existing conditions that would signal a change in the initial plan of treatment. The medical facility needs to be contacted immediately so they can begin to prepare for the arrival of the patient and to also contact physicians that have already agreed to take on such cases.

It is imperative to identify, before the fact, a partnering medical facility and providers who are comfortable with caring for a patient with an envenomation by a nonnative snake. Issues of acceptance of patients at a facility, the importation, pharmacy preparation, and provider administration of IND antivenoms, perhaps past their "expiration" date, pre-existing management protocols or referrals, as well as other aspects that occur following a bite should, as much as possible, be anticipated and planned for ahead of time.

Conclusion

Housing and caring for venomous animals is a huge undertaking that carries a heavy responsibility for any institution. Many considerations have to be taken into account regarding enclosures, training, handling, proper antivenom acquisition and storage, and emergency procedures. It is incumbent that any institution wishing to add a venomous animal display or enclosure to their property creates a detailed and well-thought out plan in each of the areas discussed. As mentioned, each establishment has the flexibility in engineering their own guidelines of standards and procedures. The plan must coincide with the needs of the institution and their collaborating partners. The considerations and examples above have proven to be extremely effective in keeping both animal care staff and venomous species safe and healthy. Regardless of the variations in an institution's adopted protocol, the top priority should always focus on safety of the animals and their caretakers.

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