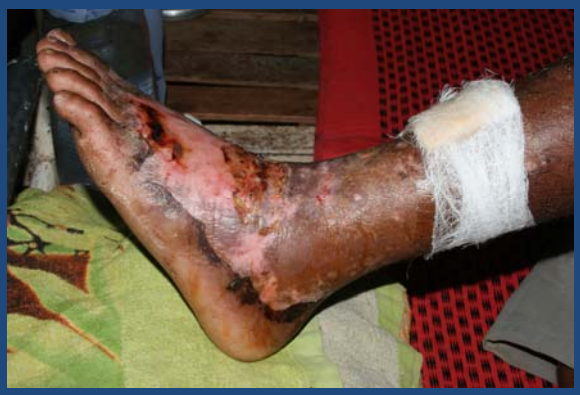
Snake bite management in Cambodia:

towards improved prevention, clinical treatment and rehabilitation



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We also wish to generously thank all of the people who met with DW in Cambodia during the consultation process. In particular we thank H.E. Dr Ung Phyrun MD (Secretary of State) and H.E. Dr Te Kuyseang MD (Secretary of State) from the Cambodian Ministry of Health; Dr Jean-Louis Sarthou, Director, Institut Pasteur du Cambodge; Professor Chhuoy Meng, Head, Poison Unit, Calmette Hospital; Mr Chheang Dany, Deputy Director, Wildlife Protection Office, Forestry Department; Ms Mary Scott, Country Director, The Cambodia Trust; Dr Socheata Sann, Program Manager, Handicap International; Mr Sok Long, Director, Health Department, Cambodian Red Cross; Dr Ung Sophal, Emergency Department, National Paediatric Hospital; Dr Hem Sokumthir, Neurosurgery Unit, Preaah Kossamak Hospital; Dr Chham Sary, Technical Officer, Kontha Bopha Children's Hospital; Mr William Mfuko, Technical Officer, EDM-WHO; Dr Sok Bunso, Pharmaceutical Center, DDF; Dr Mam Dathara, Pharmaceutical Center, DDF; Ms Sorya Chan, Health Officer, Child Survival Programme, UNICEF Cambodia, Prey Veng Province; Dr Sabrina de Rosa, Medical Coordinator, Emergency Trauma Hospital, Battambang; Dr William Housworth, Executive Director, Angkor Hospital for Children in Siem Reap; Mr Meas Meanith and colleagues from Chup Rubber Plantation, Tbaung Khmom District, Kampong Cham; and the many medical officers, administrators, pharmacists and nursing staff from Provincial Health Departments and Hospitals in Pursat, Battambang, Banteay Meanchey, Siem Reap, Kampong Thom, Kampong Chhnang, Kampong Som, Prey Veng and Kampong Cham Provinces.

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Finally we would like to thank Dr Wolfgang Wüster, University of Wales, Bangor, UK for reviewing sections of this report, making constructive comments, and providing the photographs of Indo-Chinese spitting cobras (*Naja siamensis*); and Professor David Warrell, University of Oxford, Oxford, UK for constructive criticism, access to references, and his generosity in sharing with us his lifetime of experience in the care and treatment of snake bite patients.

TERMS OF REFERENCE

1. To prepare a report on the distribution, behaviour and biting habits of dangerous snakes in Cambodia and make recommendations for future improvements in monitoring and reporting of snake distribution and behaviours;
2. To develop a pilot project to investigate methodologies for collecting and documenting the epidemiology, incidence, morbidity and mortality associated with snake bites in Cambodia;
3. To prepare a report on the suitability, safety and potential efficacy for use in Cambodia of various currently available antivenom products, and make recommendations for future improvements;
4. To prepare a report on the current system for identifying, procuring, storing and distributing appropriate antivenoms, and make recommendations for future improvements; and
5. To prepare a collaborative protocol for the investigation of clinical syndromes of envenoming in Cambodia to improve core knowledge and provide a clinical evidence base to guide the development of rational snake bite management protocols in Cambodia that are relevant at all levels of health service delivery.
6. To consult with Cambodian health professionals and draft national guidelines for snake bite management.
7. To develop and test a standard curriculum and teaching materials for training health staff in snake bite management at the various levels of the health care system.
8. To conduct a training course for master trainers and test their teaching skills by monitoring pilot training courses in selected provinces.
9. Review and make recommendations for the revision of currently available public information materials and develop new materials on prevention, first aid and management of snake bite in Cambodia, targeted to a range of audiences including the general public and health care providers.

INTRODUCTION

This report has been prepared at the request of the Cambodian Ministry of Health through the Cambodian Country Office of the World Health Organisation. Concern about the burden of envenoming by snake bite in Cambodia was prompted by a perception that cases increase during the "rainy season" (late May to August), particularly in Provinces along the Mekong and Tong le Sap floodplains[1]. Despite this perception a lack of accurate data within the Ministry of Health has made it difficult for health managers to make informed decisions on the extent of resourcing required to deal with snake bite accidents. Snake bite is not currently a notifiable injury, and as a consequence centralised reporting of incidence, disability and mortality does not occur. It is perceived by health authorities that a majority of snake bite injuries are not treated in a health facility, and while there is some direct evidence that supports this from at least one Province, there is no specific data that would enable calculation of the proportion of non-presenting cases at provincial or national level. In 2007 WHO-WPRO engaged consultants from the SEAMEO TropMed Network to conduct a preliminary investigation of the snake bite problem in Cambodia, and as a result a report was presented to the Cambodia Ministry of Health identifying several priority issues[1]:

1) Need for epidemiological data;
2) Requirement for comprehensive information on the types and distributions of venomous snake species;
3) Development of national guidelines for the management and prevention of snake bites;
4) Review of current and future antivenom procurement and distribution policies;
5) Development of a relevant clinical management training programme;
6) Increased effort to prevent snake bites;
7) Overseas study visit by a technical working group to either Thailand or Viet Nam to examine commercial antivenom production and distribution methods.

[1] SEAMEO TropMed Network. (2007) *Review of snake bites in emergencies in Cambodia*. Report to Cambodian Ministry of Health & WHO Country Office. The report states that snake bites are prevalent in Kandal, Kampong Cham, Kampong Chhnang, Pursat, Prey Veng, Rattanakiri, Kampong Thom, Kampot, Kratie and Strung Treng Provinces during the rainy season, but no direct data is provided to support this due to a number of reasons.

In response to this initial report, the Ministry of Health requested further assistance through WHO-WPRO to action some of these identified priority issues. One of us (DW), was engaged to visit Cambodia in October and December 2008 to carry out an initial assessment and collect information to enable us to address specific terms of reference (*page 4*). The major priorities during these two visits were to:

a) Develop an understanding of the capacity of the Cambodian health system (and in particular, its hospitals and personnel) to treat and manage envenomed snake bite patients;
b) Collate and examine any snake bite data available from Cambodian hospitals or other sources;
c) Obtain an appreciation for the practical issues that may influence the outcome of any proposed intervention strategies;
d) Determine which antivenom products are available, and to investigate the current supply and distribution of antivenoms throughout Cambodia;
e) To seek information on the distributions of various medically important snake species.

We have now used this information to develop a proposed action plan for improving several aspects of the management of snake bite in Cambodia, focusing on:

I). Improved reporting and surveillance;
II). Community awareness and education;
III). Specific paramedical and medical education and training.

This report presents the findings of the initial assessments carried out in Cambodia, and outlines the proposed approach along with specific comments and recommendations aimed at assisting the Ministry of Health to make significant improvements in the treatment of snake bite in Cambodia.

EXECUTIVE SUMMARY

Despite the near complete devastation of its infrastructure and human resources during the period from 1975-1979, and the further challenges that beset the nation during the following two decades as it sought to divest itself of the Khmer Rouge insurgency and reopen doors to international engagement and development, the Cambodian health system is recovering rapidly, and while numerous challenges remain, Cambodians are fortunate to have a proactive reformist Health Ministry[2,3,4]. While it was clear that many hospitals still lack adequate equipment, trained personnel and basic capacities, both the management of the Ministry of Health, and the management and staff of health facilities themselves appear to share a commitment to improving services and expanding service delivery capacity. There are currently 8 national and 69 referral hospitals functioning in 77 Operational Districts that each service a population of between 100-200K people[5]. Within this framework are a further 972 health centres and 72 health posts. Numerous NGO's also operate special purpose health facilities (i.e.: trauma centres, rehabilitation clinics or specialist paediatric hospitals) throughout the country.

Cambodia's dangerous venomous snakes

Although there are at least 86 snake species, including 17 that are known to be venomous, there are six species which are likely to account for the majority of cases of serious envenoming:

Malayan pit viper (*Calloselasma rhodostoma*)

- Extremely dangerous: causes severe local pain, oedema, ecchymoses, bullae formation, and necrosis (sometimes leading to gangrene); systemic effects dominated by consumption coagulopathy.
- Most common in forested areas but also in oil palm, rubber, tea and other plantations.
- Bites are most common during the wet season and many occur at night.
- Common in southern and eastern Provinces such as Kratie, Kampong Cham, Kampong Som, Koh Kong and Kampot.

White-lipped pit viper (*Cryptelytrops albolabris*)

- Causes many non-lethal bites: majority result in pain and swelling without systemic sequelae, but a proportion also develop consumption coagulopathy; deaths are rare, but do occur.
- Widespread in many different habitats.
- Usually arboreal, but moves to ground at night.
- Possibly the most common cause of snake bite throughout Cambodia, and well known by people from all Provinces visited.

Indo-Chinese Russell's viper (*Daboia siamensis*)

- Extremely dangerous: clinical effects include local pain, swelling, intravascular haemolysis, rhabdomyolysis, consumption coagulopathy, shock and renal failure
- Only venomous Cambodia snake likely to cause oliguric acute renal failure
- Typically inhabits rice paddies and nearby areas
- Recognised by local people in Pursat, Battambang, Banteay Meanchey, Siem Reap and Kampong Thom
- Exact distribution needs to be determined by herpetologists

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[2] Barber S., *et al* (2002) Takeo Provincial Referral Hospital: pioneering a health financing scheme. MoH, Swiss Red Cross, WHO joint report.
[3] Grundy JJ., (2001) The impact of health system reform in remote health in Cambodia and the Philippines. *Rural and remote health 1* (online); no 84. http://www.rrh.org.au/publishedarticles/article_print_84.pdf
[4] Hill PS, Mao TE. (2007) Resistance and renewal: health sector reform and Cambodia's national tuberculosis programme. Bulletin of the WHO. 85: 631-636.
[5] Veasnakiry L., *et al*. (2006) Cambodia Health Information System: Review and Assessment. *Department of Planning and Health Information*, Cambodian Ministry of Health.

Monocellate cobra (*Naja kaouthia*)

- Dangerous: local effects include pain, some swelling and necrosis (which typically only involves the skin and subcutaneous tissues and not deeper muscle); systemic neurotoxicity (i.e.: cranial and bulbar nerve palsies).
- Common in rice paddy and other farming areas, rarely in deep forests, but also occurs in oil palm and rubber plantations.
- Widespread throughout open and semi-open habitats in Cambodia.

Indo-Chinese spitting cobra (*Naja siamensis*)

- Dangerous: clinical effects of bites are very similar to those of Monocellate cobra envenoming; the only cobra in Cambodia that can spit venom defensively; severe corneal injury can result from entry of spat venom into eyes.
- Tends to be a smaller and more agile species.
- Widely distributed in non-forest habitats and farmland across Cambodia.

Malayan, or blue krait (*Bungarus candidus*)

- Dangerous: bites cause neurotoxicity that results in nerve terminal destruction and irreversible paralysis that is poorly reversed by antivenom.
- Widespread species in a variety of habitats ranging from open farmland to rubber plantations and primary forests.
- Common in many Provinces but often less frequently seen than the black and yellow banded krait (*Bungarus fasciatus*).
- Nocturnal and may enter homes at night when searching for food.

At least three other venomous species (Banded krait *Bungarus fasciatus;* Red-headed krait *Bungarus flaviceps;* King cobra *Ophiophagus hannah*) may be the cause of occasional cases of severe envenoming including fatalities:

Epidemiological and clinical data acquisition

Dealing with cases of snake bite is just one of many problems facing Cambodian health professionals working in these hospitals and health centres. While there are clear perceptions within communities that snake bite is a common event, objective data is not readily available, and there are currently no recent published accounts of the burden of snake bite in Cambodia. It is also evident that there is a perception in both the general community and the medical fraternity that only a relatively small proportion of snake bite patients currently attend hospital for primary treatment. If this assumption is indeed correct, then any available hospital data significantly understates the extent of the problem, and will be insufficient to enable accurate assessment of the overall burden.

These data deficiencies are unlikely to be resolved in the short to medium term. Although this information is a fundamental requirement to appropriate resourcing, there are currently no mechanisms in place which specifically enable extraction of snake bite incidence or mortality data. Conducting a comprehensive national survey of snake bite injury would require significant resource mobilization at all levels of government, along with collaboration with experts in the field and ancillary (i.e.: NGO) organisations that could potentially facilitate data collection at community level. Significant planning would be required before this could be attempted.

This report recommends that the Ministry of Health support three strategies to improve the current standard of snake bite surveillance data in Cambodia:

1) Nominate snake bite as a reportable medical condition and request that all hospitals and health centres include specific snake bite case information in monthly reports. At a minimum each facility should report the total cases; the number of cases given antivenom; the number of ‘fit’ discharges, ‘disability’ discharges and deaths;

2) The design and trial of village-based community survey protocols to investigate snake bite epidemiology in a number of representative locations across the country as a strategy towards enabling accurate estimation of the true burden of snake bite;
3) The design and trial of a collaborative, multi-centre prospective clinical study of snake bite at National and Provincial Referral Hospitals throughout Cambodia as a means of significantly advancing our understanding of the clinical syndromes caused by various species, and as a means to further improve the clinical management of snake bite in a resource-relevant manner.

Antivenoms

None of the antivenoms currently supplied to health facilities by the Ministry of Health are known to be effective against the venoms of Cambodian snake species. In particular, no antivenoms made in India have specificity against venoms of Cambodian snake species.

We recommend that the following Indian-made antivenoms be withdrawn from use in Cambodia immediately:

- **Snake Antivenin Polyvalent I.P.** manufactured by Biological E Limited of India;
- **Snake Antivenom Serum I.P. SII Anti-snake Venom Serum** manufactured by Serum Institute of India/Haffkine Biopharmaceutical Corporation of India;
- **ASNA Antivenom C (Snake Venom Antiserum Africa)** manufactured by Bharat Serums & Vaccines Limited of India

The two polyvalent antivenom products manufactured by (a) Serum Institute of India/Haffkine and (b) Biological E Limited have no specific activity against snakes that occur in Cambodia. The potency of both these products is low, even against the venoms of the Indian species of snakes for which they are made. Starting doses in India and Sri Lanka where these products have been used for many years, range from 10-40 vials per patient, and published accounts of patients receiving more than 100 vials without significant clinical improvement exist in the literature. Adverse reaction rates of up to 80% have been reported, with half of these being anaphylactic reactions.

The situation with regard to Bharat Serums & Vaccines Limited's ASNA Antivenom C is more serious, since this antivenom is clearly labelled and represented by the manufacturer to be made specifically to treat bites by several **AFRICAN** snake species. Not only does the product have no demonstrated specificity against venoms from any Cambodian snake species, but even against the African species for which it is raised, the maximum neutralising potency is equal only to the amount of venom needed for 25 times the MOUSE 50% lethal dose (LD50). A recent study found this product inefficacious against one of the species it is marketed for in Africa, and reported that 6 times more patients died following use of this antivenom compared to those who died after receiving a competitor's product. Based on the snake species which occur in Cambodia, and their occurence in neighbouring Thailand, we recommend that the Ministry of Health deal directly with the Queen Saovabha Memorial Institute (QSMI) at the Thai Red Cross (TRC) in Bangkok to negotiate the purchase of antivenoms with direct specificity against the six main medically important snake species present in Cambodia.

The QSMI manufactures two polyvalent snake antivenoms that would be appropriate for use in the treatment of snake bite envenoming in Cambodia:

TRC Haemato Polyvalent Snake Antivenom

- Raised from venom of Malayan pit viper, white-lipped pit viper and Indo-Chinese Russell's viper

TRC Neuro Polyvalent Snake Antivenom

- Raised against venom from Malayan krait, Monocellate cobra, banded krait and king cobra
- Monocellate cobra antivenom has been shown to neutralise Indo-Chinese spitting cobra venom

The likely cost of each of these products would be approximately US$40 per vial, with two vials being the recommended initial dose as per the product insert.

Cambodia currently distributes antivenoms in an ad-hoc manner through the Central Medical Stores in Phnom Penh. The available data suggests that distribution is user driven, and is based on requests received since the data necessary to model a distribution strategy on actual need is, as discussed above, currently absent. In 2007-2008 a total of 848 vials were issued to health facilities with the largest quantities being supplied to Pursat (85), Kampong Cham (67), Kampot (55), Banteay Meanchey (50) and Kampong Chhnang (47) Provinces, while the lowest allocations were made to Kep (5), Svey Rieng (5), Kampong Som (10), Mondulkiri (10) and Oddar Meanchey (10) Provinces. In late 2008 there were 60 vials issued to the Ministry of National Defence in Phnom Penh. Some Provinces which received very small amounts of antivenom experienced snake bite commonly, and there were hospitals that received large amounts of antivenom that they did not use to treat patients for fear of adverse reactions or other reasons.

With regards to purchasing, specific transaction details were not obtained, however records show that Cambodia has purchased 2600 vials of Indian-made antivenoms between 200-2008. Purchases are made through a wholesaler and the unit cost of antivenom (US$60-148) in Cambodia is 9-15 times higher than the Indian retail price (US$7-9). We would recommend that in the future the Cambodia Ministry of Health purchase antivenoms direct from the appropriate manufacturers as a means of ensuring the lowest possible price.

The need for National Snake Bite Treatment Guidelines

The consultation process revealed that knowledge of specific snake bite treatment among doctors and other health professionals is currently poor. In particular, deficiencies exist with regard to:

Clinical assessment and diagnosis of snake bite

- Specific determination of blood coagulation status was not assessed;
  - 20 Minute Whole Blood Clotting Test (20WBCT) was rarely used anywhere.
  - PT , APTT and fibrinogen or FDP measurements not available;
  - Only some hospitals recognised the importance of platelet counts;
- Local effects rarely objectively assessed, and clinical assessment of ischaemic risk was not made;
- Standard neurological assessment techniques are not used in any of the hospitals visited;
- Lack of proper clinical assessment often leads to incorrect management decisions.

Primary medical treatment

- Limited capacity to deal with the consequences of very severe pit viper bites or with severe neurotoxicity, and there is a clear need to adequately resource emergency rooms;
- Many doctors expressed uncertainty with regard to the correct use of antivenom, and some refused to use antivenom due to lack of confidence and/or distrust of the products themselves;
- Antivenoms were often given to patients who did not need it;
- Most patients are not objectively assessed after antivenom is used, and this lack of the use of specific clinical endpoints for treatment needs to be corrected;
- Adjunctive treatments were not used by the majority of doctors.

Subsequent ongoing inpatient care

- Fluid balance and hydration state were rarely monitored in the wards;
- Patients in some hospitals were subjected to attempted fasciotomies without prior assessment of ischaemic risk using appropriate clinical tests for increased compartment pressure, or confirmation of the correction of coagulopathy;
- Provincial hospitals in areas where Indo-Chinese Russell's vipers are believed to occur, do not have renal dialysis equipment and must rely on other treatment modalities;
- Most hospitals lacked the equipment to be able to manage patients with neurotoxic paralysis.

Rehabilitation and the management of disability

- Most hospitals do not currently offer rehabilitation services to patients with disability after snake bite;
- Amputations occur in some patients bitten by snakes, notably after bites by Malayan pit vipers;
- Few snake bite patients appear to be accessing disability services after discharge, although a small number have received prostheses from The Cambodia Trust;
- Doctors need to be educated to provide counselling and referral to disabled patients;
- Disability service providers need to be engaged with to improve knowledge of the extent of disability incidence after snake bite, and to broaden patient access to services.

Despite these problems, it was encouraging that many medical personnel expressed strong interest in having access to specific training, protocols and other educational resources and clinical tools. We believe there is clear need for a standardised National Snake Bite Protocol, and recommend that the Ministry of Health establish a small working group of experts to collaborate with us in developing a working draft of such a document.

Snake bite education and training

We propose a three-pronged approach to snake bite education and training:

Basic community education

There is a clear need to improve community awareness of the public health risks presented by venomous snakes in Cambodia. This needs to focus on increasing awareness of snake bite dangers, providing information on prevention strategies, first aid interventions and appropriate health care seeking behaviours, and should also address areas of occupational risk (i.e.: oil palm and rubber plantations, rice cultivation, fishing and timber gathering). We believe it is also important to engage with traditional healing practitioners through specific education aimed at encouraging them to refer patients with severe snake bites to hospitals early. The broad aims of community education are:

- Heighten community awareness of snake bite dangers;
- Encourage communities to seek conventional medical treatment of snake bite;
- Teach safe, beneficial first aid for snake bite;
- Reduce snake bites by encouraging prevention and risk avoidance.

Rural health centre education

Early commencement of appropriate treatment is crucial if snake bite outcomes are to be improved, so the involvement of basic health centres and health posts in primary patient assessment, resuscitation, referral and transport is particularly important. We recommend involving staff from these smaller centres in a simplified one day training course that teaches basic concepts and skills, including:

- Awareness of local venomous snake species;
- Principles of effective first aid;
- Community outreach and education;

- Basic resuscitation: airway, breathing and circulation including fluid resuscitation and the emergency treatment of shock;
- Primary assessment of clinical signs and symptoms, and principles of triage;
- Preparation and conduct of emergency transport.

The aim of this training initiative would be to help primary health workers to accurately assess cases of possible snake bite, to properly identify those patients with serious clinical effects and/or major life-threatening complications (i.e.: bleeding, shock, neurotoxicity, renal failure), and to stabilise and transport them safely to a referral hospital for more specific treatment.

Advanced snake bite management training

A standardised National Snake Bite Management Course is proposed for medical personnel working in National Hospitals, Provincial Referral Hospitals and major NGO-run Hospitals. The course will address the current deficiencies in clinical toxinology expertise, by seeking to provide specialised training in the assessment, diagnosis, primary and secondary care of snake bite patients in Cambodia. This will be a three day intensive course designed to be taught to doctors and nurses and supplemented with written treatment and ongoing patient management protocols, teaching resources and ancillary materials.

The emphasis will be on teaching sound clinical skills, using training materials presented in both Khmer and English language formats. Course materials will be prepared by the WHO consultants, David Williams and Simon Jensen, and the material will then be taught to selected local clinicians with training roles in their institutions. The aim is for the course to then be taught throughout Cambodia by local trainers with support from the WHO consultants.

The course curriculum will include lectures and resources relevant to:

- Developing an understanding of Cambodia's venomous snakes and the effects of their venoms;
- Understanding the principles of first aid for snake bite in a Cambodian setting;
- Improving resuscitation skills (DRABC, shock, fluid resuscitation, etc.);
- Recognising the symptoms and signs of snake bite;
- Patient assessment and diagnosis (includes laboratory and bedside testing);
- Principles of immunotherapy with snake antivenoms;
- Treatment of coagulopathy (bleeding);
- Treatment of neurotoxicity (airway and breathing);
- Treatment of local injuries (including surgical/assessment and treatment of compartment syndrome/grafting);
- Treatment of oliguria and renal failure;
- Patient recovery, physiotherapy and rehabilitation;
- Patient transport and referral;
- Special considerations relevant to treating snake bite in children.

The course objectives are to increase the capacity of medical personnel to effectively assess, diagnose and treat snake bites. In particular the course will promote the rational use of antivenom on the basis of clearly defined clinical indications, with the aim of eliminating unnecessary use and wastage of antivenoms, and improving overall clinical outcomes from antivenom use.

Key recommendations

The most important recommendations of this report are:

1. Epidemiology

A. Introduction of mandatory reporting of snake bite injuries in monthly surveillance reports to the Ministry of Health.
B. Design and trial of village-based community survey protocols to investigate snake bite epidemiology in representative locations to improve accurate estimation of burden.
C. Design and trial of a collaborative, multi-centre prospective clinical study of snake bite at National and Provincial Referral Hospitals to significantly improve current clinical knowledge.

2. Antivenoms

A. Immediate withdrawal of the following Indian-made antivenoms from use in Cambodia:
   i. **Snake Antivenin Polyvalent I.P.** manufactured by Biological E Limited of India;
   ii. **Snake Antivenom Serum I.P. SII Anti-snake Venom Serum** manufactured by Serum Institute of India/Haffkine Biopharmaceutical Corporation of India;
   iii. **ASNA Antivenom C (Snake Venom Antiserum Africa)** manufactured by Bharat Serums & Vaccines Limited of India
B. Replacement purchasing of the following two polyvalent antivenoms manufactured by the Queen Saovabha Memorial Institute with mandatory reporting of clinical outcomes:
   i. **TRC Neuro-specific Polyvalent Antivenom** active against venoms from *Naja kaouthia*, *Naja siamensis*, *Bungarus candidus*, *Bungarus fasciatus* and *Ophiophagus hannah*
   ii. **TRC Haemato-specific Polyvalent Antivenom** active against venoms from *Calloselasma rhodostoma*, *Cryptelytrops albolabris* and *Daboia siamensis.*

3. Education and training

A. Basic community education with a clear focus on increasing awareness of snake bite dangers, prevention strategies, first aid interventions and appropriate health care seeking behaviours.
B. Rural health centre education to increase standards of patient triage, resuscitation, emergency treatment of shock, patient assessment and emergency transport.
C. Advanced snake bite management training for major hospitals, including Phnom Penh and Provincial capital referral hospitals, based around an intensive course design to be taught to selected candidates and supplemented with written treatment and ongoing patient management protocols, teaching resources and ancillary materials.

Conclusions

There are currently significant shortcomings in the knowledge of the Cambodian medical and public health communities with respect to the treatment of snake bite. These problems are compounded by a lack of formal policy direction from the Ministry of Health, deficient epidemiological data, and an absence of formal treatment protocols, training resources or sources of qualified clinical advice. This lack of local expertise has led to a situation in which inappropriate, inefficacious and potentially unsafe foreign-made antivenoms are being used for the treatment of snake bite. A range of public access and confidence issues appears to have resulted in the majority of snake bite patients seeking treatment outside the health system, sometimes with disastrous results for patients. We propose a series of steps designed to improve the knowledge base within the general, medical and public health communities, address data deficiencies, provide access to efficacious antivenoms, and promote clinical toxinology skills development that will improve outcomes by reducing delays in the commencement of specific antivenom treatment; reduce morbidity and disability by reducing unnecessary surgical intervention, while promoting evidence-based procedures that functionally benefit patients; and reduce mortality.

THE CONSULTATION PHASE

An initial phase of consultation and preliminary investigation was undertaken by David Williams accompanied by Dr Phok Chansorphea from the MoH-PMD between 20-31 October, 2008 and 7-17 December 2008 was facilitated by the WHO's Cambodian Country Office and the Cambodian Ministry of Health. This consultation phase involved:

1. Meetings with key MoH personnel in Phnom Penh including:
   a. H.E. Dr Ung Phyrun MD (Secretary of State)
   b. H.E. Dr Te Kuyseang MD (Secretary of State)
   c. Dr Prak Piseth Raingsey (Director, PMD-MoH)
   d. Dr Khuon Eng Mony MD (PMD-MoH)
   e. Dr Phok Chansorphea MD (PMD-MoH)
2. Meetings with Provincial MoH personnel in Pursat, Battambang, Banteay Meanchey, Siem Reap, Kampong Thom, Kampong Chhnang, Kampong Som, Prey Veng and Kampong Cham, including inspections of hospitals and health centres and interviews with doctors, pharmacists and other health workers;
3. Meetings with personnel from Hospitals and other Units in Phnom Penh, including:
   a. Professor Chhuoy Meng MD (Head, Poison Unit, Calmette Hospital)
   b. Dr Ung Sophal MD (Emergency Department, National Paediatric Hospital)
   c. Dr Hem Sokumthir (Neurosurgery Unit, Preaah Kossamak Hospital)
   d. Dr Chan Sary (Technical Officer, Kontha Bopha)
   e. Dr William Mfuko (Technical Officer, EDM-WHO)
   f. Dr Sok Bunso (Pharmaceutical Center, DDF)
   g. Dr Mam Dathara (Pharmaceutical Center, DDF)
4. Meeting with Dr Jean-Louis Sarthou, Director, Institut Pasteur du Cambodge;
5. Meeting with Mr Chheang Dany, Deputy Director, Wildlife Protection Office, Forestry Department
6. Meeting with General Directorate of Rubber Plantations;
7. Meeting with Ms Sorya Chan, Health Officer, Child Survival Programme, UNICEF Cambodia, Prey Veng Province;
8. Meetings with Ms Mary Scott, Country Director, The Cambodia Trust, and with Ms Socheata Sann, Program Manager, Handicap International;
9. Meeting with Mr Sok Long, Director, Health Department, Cambodian Red Cross;
10. Meeting with Dr Sabrina de Rosa, Medical Coordinator from Italian NGO, Emergency at their hospital in Battambang;
11. Meeting with Dr William Housworth, Executive Director of the Angkor Hospital for Children in Siem Reap Province;
12. Meeting with Mr Meas Meanith and colleagues from Chup Rubber Plantation, Tbaung Khmom District, Kampong Cham and an initial collection of venomous snakes (in this case Malayan kraits *Bungarus candidus*) from within the plantation;
13. Meetings with Mr Nikolai Doroshenko, Mr Martin Biener and colleagues from the Snake House in Sihanoukville.

The purpose of these consultations was to endeavour to collect a range of perspectives on the snake bite issue in Cambodia from a cross-section of stakeholders in a variety of fields. The objective was to identify priorities and in particular key problems and issues that need to be addressed if real improvements are to follow.

A summary of the key findings of this consultative process are provided in the following pages. A more comprehensive report of specific discussions will be included in the final report when it is tendered to the WHO and Ministry of Health.

VENOMOUS SNAKE FAUNA OF CAMBODIA

Cambodia shares many aspects of its venomous snake fauna with neighbouring Thailand, Viet Nam, and Laos. There are approximately 86 species of snakes in Cambodia, with a possibility that more species will be discovered by biologists working in remote areas, such as the Cardamom Mountains. Of these 17 are known to venomous, and potentially dangerous to humans. Six of these venomous snakes have been classified as Category One taxa by the World Health Organisation[6], and are considered to be the species responsible for the majority of severe and potentially fatal snake bites.

Malayan pit viper (*Calloselasma rhodostoma*)

This extremely dangerous snake is a major occupational hazard of plantation workers in the oil palm and rubber industries throughout many parts of its range in South-East Asia. It is considered to be the most medically important venomous snake in Cambodia (on the basis of distribution, habits, venom activity and clinical outcomes).

Cambodian distribution: Common throughout most of Cambodia but less common on the easily flooded central plains. *Extralimital*: western Laos; southern Vietnam; throughout Thailand; north-west peninsular Malaysia (despite its common name this species exhibits a very limited Malaysian distribution); possibly southern peninsular Burma; disjunct population in Indonesia (Java).

Description: This is a moderately sized (0.5-1.2m adult length) ground-dwelling pit viper with a broad triangular head covered by nine large regular scales, a pointed snout and eyes with vertically elliptical pupils, body covered with smooth scales, and a short tail. It is grey to reddish- or dark-brown above with 19-31 characteristic dark brown triangular markings broken by lighter (reddish brown, orange or tan) scales along the middle of the back. The triangular head bears broad dark stripes that extend backwards from the eye to the upper lips. The belly is usually unmarked and creamish to pinkish-grey in colour. *Scale counts*: dorsals at midbody in 21 rows (sometimes in 19); ventrals 138-166; subcaudals 33-55 (paired).

Habitat: Dry lowland forests, but occurring up to 2,000m, especially common in plantations (oil palm, rubber, coffee, tea, etc) and along timbered verges around farms and villages, densely vegetated

[6] WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. Approved by ECBS in October 2008; currently in press.
See http://www.who.int/bloodproducts/animal_sera/en/
The Guidelines define Category One taxa as "*Highly venomous snakes which are common or widespread and cause numerous snake bites, resulting in high levels of morbidity, disability or mortality*"

stream banks and bamboo thickets. This is a species that adapts well to habitat disturbance with potentially higher densities occurring in man-made monocultures compared to pristine forest.

Habits: Typically nocturnal, and most active on nights with high relative humidity, especially after rain. During the day this snake is secretive and sedentary and can lie still, cryptically camouflaged among the undergrowth, for very long periods. This ambush-hunting behaviour increases the risk of snake bite because this species typically makes little attempt to move away at the approach of humans, but will bite with provocation if it feels threatened. Adult Malayan pit vipers exhibit catholic diets, including amphibians, other snakes, birds and rodents, but some populations appear to have distinct preferences. Juvenile snakes are more likely to feed on arthropods and insect-eating vertebrates such as small frogs or lizards. Juveniles use their contrastingly coloured tail to lure prey within strike range. Females lay clutches of from 3-40 eggs, with an average size of 13-19 eggs. Incubation time is 45-62 days and female snakes actively incubate and protect the eggs. Oviparity is an unusual trait in vipers.


Malayan pit viper (*Calloselasma rhodostoma*)

Venom: The venom of the Malayan pit viper is particularly rich in toxins, such as haemorrhagic metalloproteinases, prothrombin activators, thrombin-like enzymes and Factor X activators which destroy vascular epithelium, produce necrosis or affect the normal function of key coagulation factors[7,8,9,10]. Venom C-type lectins that bind to platelet glycoproteins and lead to thrombocytopenia are also present[11]. Potent cytotoxic, haemorrhagic, oedema-producing and myolytic toxins contribute to the devastating local effects of envenoming[12]. Malayan pit viper venom also contains cytotoxic toxins with phospholipase A_2 activity that cause destructive changes in developing muscle cells producing myonecrosis and oedema[13,14].

Clinical effects: Bites by Malayan pit vipers may result in both local and systemic effects. Local pain, swelling, oedema, blistering, bullae formation and ecchymoses can be very severe and may be followed by deep tissue necrosis and gangrene, necessitating amputation. Swelling may appear very rapidly after a bite from this snake[15]. Shock may be a feature of severe envenoming. The incidence of local tissue damage has been reported to be as high as 94-95%, with severe local necrosis in 15.9%[16]. The major systemic effects of envenoming are bleeding disorders that result in fibrinolysis and thrombocytopenia. Profuse bleeding from the mouth, nose, lungs, GI tract, reproductive organs and from venepuncture sites or local wounds is common and can be catastrophic, leading to intracerebral haemorrhage, spontaneous abortion and other complications.

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[7] Yamada D *et al*. (1997) Prothrombin and factor X activators in the venoms of Viperidae snakes. *Toxicon*. 35(11): 1581-1589.
[8] Moura-da Silva AM., *et al*. (1996) Processing of pro-tumour necrosis factor-α by venom metalloproteinases: a hypothesis explaining local tissue damage following snake bite. European *Journal of Immunology*. 26: 2000-2005.
[9] Tseng YL, *et al.* (2004) Rhodostomin, a disintegrin, inhibits adhesion of neutrophils to fibrinogen and attenuates superoxide production. *Journal of Biomedical Science*. 11(5): 683-691.
[10] Hsu CC, *et al.* (2007) Antithrombotic effect of a protein-type 1 class venom metalloproteinase, kistomin, is mediated by affecting glycoprotein Ib-von Willibrand factor interaction. *Molecular Pharmacology*. 72(4): 984-992.
[11] Navdeav A., *et al.* (2001) Aggretin, a heterodimeric C-type lectin from *Calloselasma rhodostoma* (Malayan pit viper), stimulates platelets by binding to $\alpha_2\beta_1$ integrin and glycoprotein 1b, activating Syk and phospholipase $C\gamma_2$ but does not involve the glycoprotein VI/Fc receptor γ chain collagen receptor. *Journal of Biological Chemistry*. 276: 20882-889.
[12] Ponnudurai G, *et al.* (1993) Isolation and characterisation of a hemorrhagin from the venom of *Calloselasma rhodostoma* (Malayan pit viper). *Toxicon*. 31(8): 997-1005.
[13] Bonfim VL, *et al.* (2008) Structural and functional characterisation of myotoxic Cr-IV 1, a phospholipase A_2 D49 from the venom of the snake *Calloselasma rhodostoma*. *Biologicals*. 36(3): 168-176.
[14] Bonfim VL, *et al.* (2006) Structural and functional properties of Cr5, a new Lys49 phospholipase A_2 homologue isolated from the venom of the snake *Calloselasma rhodostoma. Protein Journal*. 25(7-8): 492-502.
[15] Reid HA. (1968) Snakebite in the tropics. *British Medical Journal*. 3:359-362
[16] Wongtomkam N., *et al*. (2005) A study of 225 Malayan pit viper bites in Thailand. *Military Medicine*. 170(4): 342-349.

White-lipped pit viper (*Cryptelytrops albolabris*)

There are other confusing green pit vipers in South-East Asia but this moderately sized arboreal pit viper is a very common cause of sub-lethal envenomation in Thailand, and the same is possibly also true in some areas of Cambodia and Viet Nam.

Cambodian distribution: Countrywide. *Extralimital*: Entire Indo-Chinese region from southern Himalaya to central peninsular Thailand, disjunct populations in Indonesia (southeast Sumatra, west Java), and Nicobars.

Description: This is a medium sized (0.5-1.0m adult length) arboreal pit viper with a broad triangular head covered by numerous small, keeled scales, a pointed snout, and small eyes with vertically elliptical pupils, body covered with keeled scales, and a long prehensile tail. Bright green to blue-green, paler green to yellow on the lower flanks, a white stripe being present in males on the first dorsal scale row, yellowish under the chin and along the lips with a fine white stripe passing from the snout, under the red to orange eye, with vertical pupil, to the angle of the jaw, the tail has a broad red-brown stripe down the dorsal surface. The undersides are white to pale yellow. *Scale counts*: dorsals at midbody 21 (occasionally 19); ventrals 149-173; subcaudals 44-78 (paired). Sometimes confused with big-eyed (*C. macrops*) or Vogel's (*Viridovipera vogeli*) pit vipers.


White-lipped pit viper (*Cryptelytrops albolabris*)

Habitat: Preferring open country below 400m, these vipers often enter urban areas, gardens, hedges and small trees.

Habits: Shelters in shrubs and trees, including tea or coffee crops, rubber and banana trees. Hunts at night, and forages for mice, birds, lizards or frogs, both in vegetation and on the ground. Females give birth to 7-16 neonates, which feed on frogs and small lizards.

Venom: Green pit viper venom is rich in a number of toxins which affect blood coagulation and normal haemostasis. Several toxins which activate platelet aggregation through interactions with glycoprotein VI (GPVI) or glycoprotein Ib (GPIb) receptors, including alboaggregin-A, -B and –C[17,18], alborhagin[19], and alboluxin[20] have been described. There are also toxins which inhibit platelet aggregation, including the metalloproteinase disintegrins albolabrin[21,] and albolatin[22]. GPV-TL1 and GPV-TL2 (albofibrases), are α-fibrinogenases that converts fibrinogen to fibrin and also activates plasminogen[23,24,25]. A non-catalytic, cytotoxic Lys49 phospholipase A_2 may be a cause of local oedema following bites by this snake, and GPV-PA a trypsin-like serine protease activates plasminogen[24].

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[17] Peng M., *et al*. (1991) Alboaggregin-B: a new platelet agonist that binds to platelet membrane glycoprotein Ib. *Biochemistry*. 30(49): 11529-11536.

[18] Peng M., *et al*. (1992) Characterisation of three alboaggregins purified from *Trimeresurus albolabris* venom. *Thrombosis and Haemostasis*. 67(6): 702-707.

[19] Andrews RK., *et al*. (2001) A novel pit viper venom metalloproteinase, albohagin, is an agonist at the platelet collagen receptor GPVI. *Journal of Biological Chemistry*. 276(30): 28092-28097

[20] Du Xiao Yan, *et al*. (2002) Alboluxin, a snake C-type lectin from *Trimeresurus albolabris* venom is a potent platelet agonist acting via GPIb and GPVI. *Thrombosis and Haemostasis*. 87(4): 692-698.

[21] Calvete JJ., *et al* (1991) Identification of the disulfide bond pattern in albolabrin, an RGD-containing peptide from the venom of *Trimeresurus albolabris*: Significance for the expression of platelet aggregation inhibitory activity. *Biochemistry*. 30(21): 5225-5229.

[22] Singhamatr P. and Rojnuckarin P. (2007) Molecular cloning of albolatin, a novel snake venom metalloproteinase from green pit viper (*Trimeresurus albolabris*), and expression of its disintegrin domain. *Toxicon*. 50(8): 1192-1200.

[23] Rojnuckarin P., *et al* (1999) The effects of green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*) venom on the fibrinolytic system in humans. *Toxicon*. 37(5): 743-755.

[24] Rojnuckarin P., *et al*. (2006) Molecular cloning of novel serine proteases and phospholipases A_2 from green pit viper (*Trimeresurus albolabris*) venom gland cDNA library. *Toxicon*. 47(3): 279-287.

[25] Muanpasitporn C. and Rojnuckarin P. (2007) Expression and characterisation of a recombinant fibrinogenolytic serine protease from green pit viper (*Trimeresurus albolabris*) venom. *Toxicon*. 49(8): 1083-1089.

Clinical Effects: The majority of bites by white-lipped pit vipers result in non-life-threatening injuries where local pain, swelling and oedema are the only apparent clinical problems. Mild coagulation disturbances occurred in 70% of children bitten by this species in Hong Kong[26], a finding consistent with an earlier study of bites by this species in the island State[27]. In a study in Thailand, ecchymoses, petechial haemorrhage, gingivial bleeding and menorrhagia were among signs of coagulopathy observed in patients[28]. Two patients were suffering from shock and two others developed digital necrosis. Increases of up to 28.3% in limb circumference were recorded, with limb swelling grading scores among non-coagulopathic patients (n=11; mean=2.8) less severe than in patients with coagulopathy (n=13; mean=3.5). In another Thai study nearly 55% of patients bitten by either *C. albolabris* or its relative *C. macrops* had thrombocytopenia, and 97.4% had elevated FDPs. Other elevated haemostatic values included thrombin time (35%), prothrombin time (28%) and activated partial thromboplastin time (17%) while hypofibrinogenaemia was seen in 39% of patients[23]. Fibrinopeptide A levels were 24 times higher in snake bite patients than in a control group, most probably due to plasminogen activation by fibrin deposition. Despite these abnormalities, only 8.7% of patients had mild clinical bleeding. Electron microscopy of erythrocytes and platelets exposed to *C. albolabris* venom has shown spheroechinocytosis of the red cells and shrinkage and malformation of platelets[29]. A paper from Thailand which wrongly attributes bites by "dark green pit vipers" in the Bangkok area to *Popeia popeiorum* (previously *Trimeresurus popeiorum*), rather than to *C. macrops* or *C. albolabris*, notes that 52% (12/23) patients bitten on the fingers of toes developed haemorrhagic blebs, with 4 of these progressing to superficial gangrene[30,31].

Indo-Chinese Russell's viper (*Daboia siamensis*)

On the basis of current knowledge it has long been believed that the Indo-Chinese Russell's viper (*Daboia siamensis*) is limited to the north-western Provinces of Cambodia.

Accurate information about the exact distribution of this snake in Cambodia is currently lacking, but the consequences of its bite are such that it must be considered extremely dangerous and care should be taken when within, or close to, it possible geographical range.

Cambodian distribution: Although considered by many western experts to be rare, this snake was well-known to villagers in Pursat, Battambang, Banteay Meanchey, Siem Reap and southern Kampong Thom who uniformly call it '*Srarkarchas*', and describe it as a common and very dangerous snake in rice paddies. *Extralimital*: Thailand's Central Plain (Kampaeng Phet south to Bangkok and eastwards); disjunct populations in central and southern Burma; southern China (Fujian, Guangdong, Guangzi), Taiwan; Indonesia (eastern Java, and Flores). Formerly treated as a subspecies of the South Asian Russell's viper (*D. russelii*) of Pakistan, India, Nepal, Sri Lanka and Bangladesh (see below).

Description: This is a moderate to large (1.0-1.5m adult length) ground-dwelling, true viper (it lacks the heat-sensitive facial pits of other Cambodian vipers) with a broad triangular head covered by numerous small, strongly keeled scales, a pointed snout with enlarged nostrils, and eyes with vertically elliptical pupils, body covered with strongly keeled scales, and a short tail. It is reddish-brown to tan above with one dorsal and two lateral rows of dark brown elongate blotches or lozenges with black and white edges, the blotches of the dorsal row sometimes linking to form a broken stripe. *Daboia siamensis* is distinguishable from *D. russelii* by the presence of an extra row of small dark spots between the dorsal and lateral rows of blotches. The triangular head bears broad a

[26] Hon KL., *et al.* (2004) Snakebites in children in the densely populated city of Hong Kong: a 10 year survey. *Acta Paediatrica*. 93(2): 270-272.
[27] Chan TY., *et al*. (1993) Clinical features and hospital management of bites by white-lipped green pit viper (*Trimeresurus albolabris*). *Southeast Asian Journal of Tropical Medicine and Public Health*. 24(2): 772-775.
[28] Hutton RA., *et al*. (1990) Arboreal green pit vipers (genus *Trimeresurus*) of south-east Asia: bites by *T. albolabris* and *T. macrops* in Thailand and a review of the literature. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 84: 866-874.
[29] Soogarun S., *et al* (2006) The effect of green pit viper (*Trimeresurus albolabris*) venom on platelet morphology by electron microscopy. *Southeast Asian Journal of Tropical Medicine and Public Health*. 37(5): 937-939.
[30] Visudhiphan S., *et al*. (1989) Dark green pit viper (*Trimeresurus popeiorum*) bite: clinical and serial coagulation profiles in 51 cases. *American Journal of Tropical Medicine and Hygiene*. 41(5): 570-575.
[31] Warrell DA. (1990) Letter. American Journal of Tropical Medicine and Hygiene. 42(6): 623.

large brown arrow marking which runs from a point forward of the eyes, back to above the angle of the jaws. The tail is striped. The undersides are white with small black spots. *Scale counts*: dorsals at midbody 27-33; ventrals 153-180; subcaudals 41-46 (paired).

Habitat: Showing a preference for open country up to 2,000m, these vipers often frequent paddy-fields, grasslands and grassy hills, sheltering near rocks and sometimes close to human habitations.

Habits: Primarily nocturnal, this is a sedentary viper that does not move away at signs of disturbance, but it does hiss noisily, producing a prolonged warning which should not be ignored. Strikes are rapid and far reaching. Prey consists primarily of rodents, hence the frequency of this viper in rice-producing areas. Females give birth to 30-63 neonates which may initially feed on lizards.

Venom: There is considerable variation in the venom composition and activity of Russell's viper's (*Daboia russelii* and *Daboia siamensis*) from different geographical regions[32], and this appears to be independent of taxonomy. Venom from *Daboia siamensis* populations in Thailand, Myanmar and Taiwan have been shown to differ from one another[33,34], and while venom from western Cambodian specimens might be assumed to be similar to that of Thai specimens, specific investigations would be necessary to confirm this. *Daboia siamensis* venom contains RVV-X, a metalloproteinase activator of coagulation Factor X and to a lesser degree Factor IX[35], as well as RVV-V, a Factor V activator[36] that mimics thrombin by cleaving FV at the Arg1545-Ser1546 bond[37] Two phospholipase A_2 enzymes (PLA2S1 and PLA2S2) in the venom of *Daboia siamensis* from Thailand are homologues of the subunits of viperotoxin F isolated from Taiwanese *Daboia siamensis*[33]. Viperotoxin F is a homologue of a neurotoxic, myotoxic, oedema-producing, indirectly haemolytic, and cytotoxic PLA_2, daboiatoxin, characterised from the venom of *Daboia siamensis* from Myanmar[38,39]. The venom of Thai Russell's vipers has also been reported to contain a disintegrin, daboistatin that weakly inhibits platelet aggregation[40], as well as hyaluronidase[41], proteases[34], zinc-fingered ptoteins[34], protein kinase C-binding protein[34], a galectin[34], dehydrogenases[34] and L-amino acid oxidase[42]. It is likely that venom from Thai and Cambodian Russell's vipers contains toxins similar to those described in specimens


Russell's viper (*Daboia siamensis*)

[32] Warrell DA. (1989) Snake venoms in science and clinical medicine. 1. Russell's viper: biology, venom and treatment of bites. *Transactions of the Royal Society for Tropical Medicine and Hygiene*.83: 732-740.
[33] Tsai I-H., *et al*. (1996) Two types of Russell's viper revealed by variation in phospholipases A_2 from venom of the subspecies. *Toxicon.* 34(1): 99-109.
[34] Nawarak J., *et al*. (2003) Proteomics of snake venoms from Elapidae and Viperidae families by multidimensional chromatographic methods. *Electrophoresis.* 24: 2838-2854.
[35] Chen HS., *et al*. (2008) New insights into the functions and N-Glycan structures of factor X activator from Russell's viper venom. *Febbs Journal*. 275(15):3944-58.
[36] Tokunaga F., *et al*. (1988) The factor V-activating enzyme (RVV-V) from Russell's viper venom. Identification of isoproteins RVV-V alpha, -V beta and –V gamma and their complete amino acid sequences. *Journal of Biological Chemistry*. 263(33): 17471-17481.
[37] Segers K., *et al*. (2006) Structural models of the snake venom Factor V activators from *Daboia russelli* and *Daboia lebetina*. *Proteins: Structure, function and bioinformatics*. 64: 968-984.
[38] Maung-Maung-Thwin *et al*. (1995) A major lethal factor of Burmese Russell's viper (*Daboia russelli siamensis*): isolation, N-terminal sequencing and biological activities of daboiatoxin. *Toxicon*. 33(1) 63-76.
[39] Gopalan G., *et al*. (2007) Structural and pharmacological comparison of daboiatoxin from *Daboia russelli siamensis* with viperotoxin F and vipoxin from other vipers. *Acta Crystallographica D: Biological Crystallography*. 63(6): 722-729.
[40] Ondee T and Nuchprayoon I. (2008) Molecular cloning and expression of Jerdostatin homolog, a novel RTS-disintegrin from Russell's viper venom (*Daboia russellii siamensis*). *Proceedings of 8th Asia-Pacific Meeting on Animal, Plant and Microbial Toxins*. Hanoi and Halong Bay, Vietnam, December 2008.
[41] Pukrittayakamee S., *et al*. (1988) The hyaluronidase activities of some Southeast Asian snake venoms. *Toxicon*. 26: 629-637.
[42] Tsai I-H., *et al*. (2008) Geographic variations, glycoprotein structure and kinetic specificities of L-amino acid oxidase of Russell's viper venom. *Proceedings of 8th Asia-Pacific Meeting on Animal, Plant and Microbial Toxins*. Hanoi and Halong Bay, Vietnam, December 2008.

from elsewhere in the range, such as the nephrotoxin RVV-7[43], the basic procoagulant metalloproteinase RVBCMP[44], and dabocetin, a recently described C-type lectin-like platelet agglutination inhibitor[45]. Research to determine the composition of the venom in Cambodia should be encouraged.

Clinical Effects: The extremely variable nature of Russell's viper venoms throughout the range of the two species, translates into different clinical presentations, depending on the origin of the snake involved. In Thailand, it has been reported that bites may involve less severe local swelling than in other countries[46], but significant intravascular haemolysis[47], and coagulopathy are common effects, with many deaths due to shock, oliguric renal failure and in some cases, cerebral haemorrhage[48]. Bleeding and renal failure[46,47] are seen as the most important clinical effects, since both may lead to fatal outcomes. The effects on haemostasis include severe hypofibrinogenaemia with depletion of Factors V and X via venom-induced activation, and depletion of Factor XIIIa, activated protein C, antithrombin III, plasminogen and antiplasmin[32,49]. FDP and XDP levels may both be very high. Platelet aggregation is inhibited, while significant platelet activation occurs and thrombocytopenia is a common laboratory finding[32]. Haematocrit may rise initially and in Sri Lanka a subsequent fall in the haematocrit was found to be associated with intravascular haemolysis[50]. Bleeding from bite wounds, venepuncture sites, gingivial sulci, gastro-intestinal and urinary tracts, lungs and central nervous system are common in all populations[32,49]. Primary hypotensive shock, increased capillary permeability and pituitary gland infarction are features of *Daboia siamensis* bite in Myanmar, while neurotoxicity and myolysis are clinical outcome of bites by *Daboia russelii* in Sri Lanka and to a lesser extent India, none of these effects have been reported in Thailand[32], but secondary shock was a major cause of fatality in one study[48]. Numerous mechanisms have been implicated in the acute renal failure that is the hallmark of severe Russell's viper envenoming; a direct nephrotoxin has been described[43], studies suggest ischaemia and disseminated intravascular coagulopathy along with direct tubular toxicity are involved[51,52,53].

Typical Russell's viper habitat: rice fields in Banteay Meanchey Province.

[43] Mandal S., *et al*. (2007) Ability of a small, basic peptide from Russell's viper venom (*Daboia russelli russelli*) to induce renal tubular necrosis in mice. *Toxicon*. 50: 236-250.
[44] Mukherjee AK, (2008) Characterisation of a novel pro-coagulant metalloproteinase (RVBCMP) possessing alpha-fibrinogenase and tissue haemorrhagic activity from the venom of *Daboia russelli russelli* (Russell's viper): evidence of distinct coagulant and haemorrhagic sites in RVBCMP. *Toxicon*. 51(5): 923-933.
[45] Zhong SR., *et al*. (2006) Characterisation and molecular cloning of dabocetin, a potent antiplatelet C-type lectin-like protein from *Daboia russellii siamensis* venom. *Toxicon*. 47(1): 104-112.
[46] Mahasandana S., *et al*. (1980) Clinical manifestations of bleeding following Russell's viper and Green pit viper bites in adults. *Southeast Asian Journal of Tropical Medicine and Public Health*. 11(2): 285-293.
[47] Sitprija V., *et al*. (1974) Further observations of renal insufficiency in snakebite. *Nephron*. 13(5): 396-403.
[48] Looareesuwan S., *et al*. (1988) Factors contributing to fatal snake bite in the rural tropics: analysis of 46 cases in Thailand. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 82(6): 930-934.
[49] Than-Than *et al*. (1988) Haemostatic disturbances in patients bitten by Russell's viper (*Vipera russelli siamensis*) in Burma. *British Journal of Haematology*. 69:513-520.
[50] Phillips RE., *et al*. (1988) Paralysis, rhabdomyolysis and hemolysis caused by bites of Russell's viper (*Vipera russelli pulchella*) in Sri Lanka: failure of Indian (Haffkine) antivenom. *Quarterly Journal of Medicine*. 68(257): 691-716.
[51] Soe-Soe., *et al*. (1990) The nephrotoxic action of Russell's viper (*Vipera russelli*) venom. *Toxicon*. 28(5): 461-467.
[52] Willinger CC., *et al*. (1995) In vitro nephrotoxicity of Russell's viper venom. *Kidney International*. 47(2): 518-528.
[53] Swe TN., *et al*. (1997) Russell's viper venom fractions and nephrotoxicity. *Southeast Asian Journal of Tropical Medicine and Public Health*. 28(3): 657-663.

Monocellate cobra (*Naja kaouthia*)

The common non-spitting cobra of South East Asia, this species is often encountered around human dwellings and in farmland. It is a major cause of snake bite.

Cambodian distribution: Countrywide except possibly the extreme north. *Extralimital*: western Laos, Southern Vietnam, Thailand and northern peninsular Malaysia, Bangladesh, Burma and eastern and northern India.

Description: This is a moderately large (1.0-2.0m adult length) cobra that may be light brown, dark brown or almost black above and greyish below with darker mottling, white under the throat and chin. Some Thai (Suphan) populations are cream coloured, mottled grey specimens sometimes occur and wild albinos are not unknown. The classic hood marking is a single monocle eye-spot but sometimes this mark may trail onto the back as a 'tear', or consist of simply a pale band, or be absent altogether in some specimens. *Scale counts*: dorsals at midbody 21 (occasionally 23); ventrals 170-196; subcaudals 43-58 (paired).

Habitat: An inhabitant of open country, such as plains and upland areas to 950m altitude, but especially common around human habitations, and in paddy-fields. Generally prefers wetter, more frequently flooded habitats than the smaller, Indo-Chinese spitting cobra (*Naja siamensis*).

Habits: Although primarily nocturnal, this cobra may be encountered at any time, often emerging to defend itself if disturbed in its retreat i.e. a termite mound or rodent burrow. It hunts rodents, toads and sometimes other snakes. Females lay up to 45 eggs and will sometimes remain close to the nest. This cobra does not spit venom.

Venom: Monocellate cobra venom is rich in postsynaptic neurotoxins[54], phospholipases A2[55], cardiotoxins, cobra venom factor, cysteine-rich secretory proteins (CRISPs)[56], cytotoxins[57,58] and platelet aggregation-inhibiting metalloproteinases[59]. Several postsynaptic neurotoxins, such as α-cobrotoxin have been described, including toxins with affinities for both nicotinic and muscarinic acetylcholine receptors[54,60]. Kaouthiagin, a zinc metalloproteinase with two disintegrin domains cleaves von Willebrand factor (vWF) and disrupts agonist-induced platelet aggregation[61]. Another

[54] Kulkeaw K., *et al* (2007) Proteome and immunome of the venom of the Thai cobra, *Naja kaouthia*. *Toxicon*. 49(7): 1026-1041.
[55] Mukherjee AK. (2007) Correlation between the phospholipids domains of the target cell membrane and the extent of *Naja kaouthia* PLA(2)-induced membrane damage: evidence of distinct catalytic and cytotoxic sites in PLA(2) molecules. *Biochimica et Biophysica Acta*. 1770(2): 187-195.
[56] Osipov AV., *et al*. (2005) Cobra venom contains a pool of cysteine-rich secretory proteins. *Biochemical and Biophysical Research Communications*. 328(1): 177-182.
[57] Osipov AV., *et al*. (2004) The first representative of glycosylated three-fingered toxins. Cytotoxin from the *Naja kaouthia* cobra venom. *European Journal of Biochemistry*. 271(10): 2018-2027.
[58] Feofanov AV., *et al*. (2004) Comparative study of structure and activity of cytotoxins from venom of the cobras *Naja oxiana, Naja kaouthia*, and *Naja haje*. *Biochemistry (Moscow)*. 69(10): 1148-1157.
[59] Wijeyewickrema LC., *et al*. (2007) Fractionation of snake venom metalloproteinase by metal ion affinity: a purified cobra metalloproteinase, Nk, from *Naja kaouthia* binds Ni^{++}-agarose. *Toxicon.* 50(8): 1064-1072.
[60] Kukhtina VV., *et al*. (2000) Muscarinic toxin-like proteins from cobra venom. *European Journal of Biochemistry*. 267(23): 6784-6789.
[61] Ito M., et al. (2001) Complete amino acid sequence of Kaouthiagin, a novel cobra venom metalloproteinase with two disintegrin-like sequences. *Biochemistry*. 40(14): 4503-4511.

Naja kaouthia metalloproteinase with sequence homology to Mocarhagin 1 (from the venom of the African spitting cobra Naja mossambica), also disrupts platelet adhesion by cleaving vWF[62]. Cardiotoxins from *Naja kaouthia* venom, activate tissue phospholipase C, liberate Ca^{2+}, increase capillary permeability[63], cause haemolysis[64], muscle contracture[65], myolysis and cytolysis[66]. Cobra venom factor (CVF) inhibits complement activity and has been used to improve the survival of xenographic transplants in experimental models[67,68]. Phospholipases A_2 in *Naja kaouthia* venom with wide-ranging activities including intercellular membrane phospholipid hydrolysis[69], myotoxicity and neurotoxicity[70], anticoagulant, indirect haemolytic activity and cytotoxicity towards heart and liver tissues have been described[71].

Hood markings on the neck of a Monocellate cobra (*Naja kaouthia*)

Clinical effects: Typical effects of *Naja kaouthia* envenoming include local pain, swelling, blistering and necrosis, with or without neurotoxicity, depending on the quantity of injected venom[72]. In a Thai study where the diagnosis of N. kaouthia bite could be confirmed immunologically, 50% of patients had only local effects and another 8% had no clinical signs at all. The case fatality rate was 12.5%[72]. A study in northern Peninsula Malaysia reported no clinical signs in 45%, local effects in 43% and neurotoxicity in 13% with a case fatality rate of 4%[73]. Eight out of sixteen (50%) patients who brought dead Naja kaouthia with them to hospital in Thailand had only local envenoming, and only one patient had neurotoxicity[74].

Another review of snake bite in Thailand reported pain, pruritus, paraesthesia, oedema and bite site necrosis as effects of cobra bite within the first hour of injury, but the authors do not discriminate between bites by *Naja kaouthia* and *Naja siamensis*[75]. Anoxia was reported as an early systemic sign, and was seen as a complication in 35% of patients who received no antivenom as well as in 29% of those who were treated. Necrosis was present in 45% of patients who had no antivenom, and in 54% of those who did receive antivenom. A recent study reported that necrosis occurred in 65.0-91.1% of patients, and 12.5-31.11% had neurotoxicity, including respiratory failure[76].

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[62] Ward CM., *et al*. (1996) Mocarhagin, a novel cobra venom metalloproteinase, cleaves the platelet von Willebrand factor receptor glycoprotein Ibalpha. Identification of the sulphated tyrosine/anionic sequence Tyr-276-Glu-282 of glycoprotein Ibalpha as a binding site for von Willebrand factor and alpha-thrombin. *Biochemistry*. 35(15): 4929-4938.

[63] Miller RA and Tu AT. (1989) Factors in snake venom that increase capillary permeability. *Journal of Pharmacy and Pharmacology*. 41(11): 792-794.

[64] Jiang MS., *et al*. (1989) Factors influencing the hemolysis of human erythrocytes by cardiotoxins from *Naja naja kaouthia* and *Naja naja atra* venoms and a phospholipase A_2 with cardiotoxin-like activities from *Bungarus fasciatus* venom. *Toxicon*. 27(2): 247-257.

[65] Fletcher JE and Lizzo FH. (1987) Contracture induction by snake venom cardiotoxin in skeletal muscle from humans and rats. *Toxicon*. 25(9): 1003-1010.

[66] Fletcher JE., *et al*. (1995) Effects of three myotoxins on membrane phospholipid hydrolysis in cell culture systems. *Toxicon*. 33(3): 301

[67] Sun Q-Y., *et al*. (2003) Prolonged cardiac xenograft survival in guinea pig-to-rat model by a highly active cobra venom factor. *Toxicon*. 42(3): 257-262.

[68] Chen G. (2004) Improved suppression of circulating complement does not block acute vascular rejection of pig-to-rhesus monkey cardiac transplants. *Xenotransplantation*. 11(2): 123-132.

[69] Doley R., *et al*. (2004) Differential hydrolysis of erythrocyte and mitochondrial membrane phospholipids by two phospholipase A_2 isoenzymes (NK-PLA2-I and NK-PLA2-II) from the venom of the Indian monocled cobra *Naja kaouthia*. *Archives of Biochemistry and Biophysics*. 425: 1–13.

[70] Reali M., *et al*. (2003) Neurotoxic and myotoxic actions of *Naja naja kaouthia* venom on skeletal muscle in vitro. *Toxicon*. 41(6): 657-665.

[71] Doley R and Mukherjee AK. (2003) Purification and characterisation of an anticoagulant phospholipase A(2) from Indian monocled cobra (*Naja kaouthia*) venom. *Toxicon*. 41(1): 81-91.

[72] Viravan C., *et al*. (1986) ELISA confirmation of acute and past envenoming by the Monocellate cobra (*Naja kaouthia). American Journal of Tropical Medicine and Hygiene*. 35(1):173-181.

[73] Reid HA. (1964) Cobra bites. *British Medical Journal*. 2: 540-545.

[74] Silamut K., *et al*. (1987) Detection of venom by enzyme linked immunosorbent assay (ELISA) in patients bitten by snakes in Thailand. *British Medical Journal*. 294(6569): 402-404.

[75] Pochanugool C., *et al*. (1998) Venomous snakebite in Thailand II: Clinical experience. *Military Medicine*. 163(5): 318-323.

[76] Wongtongkam N., *et al*. (2005) A study of Thai cobra (*Naja kaouthia*) bites in Thailand. *Military Medicine*. 170(4):336-341.

Indo-Chinese spitting cobra (*Naja siamensis*)

Indo-Chinese spitting cobra (*Naja siamensis*)

The common spitting cobra of South East Asia, this species is common around human dwellings and in farmland. It is a major cause of snake bite and occasionally, ophthalmic injuries.

Cambodian distribution: Western and southern Cambodia, possibly not present in the northeast but may be present in isolated pockets. *Extralimital*: Vietnam (particularly Phan Tiet Province, north of Ho Chi Minh City), throughout Thailand (except the Peninsula), scattered records in southern and central Laos and possibly into south-eastern Burma.

Description: This is a moderately large (1.0-1.6m adult length) cobra that is highly variable in patterning, being unicolour olive-brown, or black and white, the black pigment forming a broad vertebral stripe or broad bands around the body that may partially or completely obscure the paler pigmentation. The hood markings may consist of a U-shape, V-shape, H-shape, a spectacle shape like that of the Indian cobra (*Naja naja*) or no marking at all. *Scale counts*: dorsal scales in 25-31 scale rows around hood, 19-21 just ahead of midbody; 153-174 ventrals and 45-54 subcaudals. Typically distinguished from *Naja kaouthia* by having less than 170 ventral scales on average.

Habitat: An inhabitant of open country, such as plains and hill country, this cobra may occur around human habitations and in paddy-fields. It also inhabits scrubland and regrowth areas.

Habits: Although primarily nocturnal, spitting cobras may be encountered during the day as well as at night, often emerging to defend themselves if disturbed in a retreat i.e. a termite mound or rodent burrow. They hunts rodents, toads and sometimes other snakes. Females lay up to 19 eggs. This cobra does spit venom defensively, a shot-gun splatter in the direction of the face over 1.0-2.0m.

Venom: Specific, reliable data on the composition of *Naja siamensis* venom is currently lacking, mainly because the majority of studies over the last 30-40 years have used venoms of indeterminate origin. Taxonomic confusion has resulting in this species being confused with other cobra species, including *Naja kaouthia*, *Naja atra*, *Naja naja* and *Naja sputatrix*. While *Naja siamensis* venom is less toxic than that of *Naja kaouthia*, it also contains many of the same components, including both long

and short postsynaptic neurotoxins[77], metalloproteinases, powerful cardiotoxins with cytolytic activity[78] and phospholipases A_2 with a diversity of activities.


Spectacle-shaped markings on neck of *N. siamensis*

Clinical effects: Cranial palsy and respiratory depression are reported to be more common after bites by *Naja siamensis* than by *Naja kaouthia*[79]. Local swelling and necrosis were reported as being common following bites by either species. In one case of envenoming, the bite was accompanied by immediate pain, with swelling and a small area of necrosis around the bite site that worsened between two and four days after the bite[80]. Indo-Chinese spitting cobras will use their venom for self-defence with minimal provocation, and as the name implies, are capable of spitting venom when alarmed, often at the face and eyes of the animal or human threatening them. A case report in the literature describes pain and irritation of the eyes, bilateral redness, excessive tear production and whitish discharge, with superficial corneal opacity but normal acuity[80].

Malayan or blue krait (*Bungarus candidus*)

The Malayan krait is a serious snake bite risk in South East Asia. It is easily mistaken for a harmless species such as the wolf snakes of genus *Lycodon* or *Dinodon* and since bites cause little or no pain they may be ignored, yet they are seriously life threatening.

Cambodian distribution: Countrywide. *Extralimital*: Vietnam, Laos, most of Thailand, peninsular Malaysia and Indonesia (Sumatra, Java, Bali).


Malayan krait (*Bungarus candidus*)

Description: This is a slender snake of moderate length (1.0-1.5m adult length) with a rounded head covered by large smooth scales, small dark eyes, and a cylindrical body with smooth glossy scales throughout, the vertebral row being much larger than the other dorsal scales. Colouration consists of 19-30 alternating broad black bands or saddles, the intervening white bands being immaculate or speckled with darker pigment. The lips and the underside are also immaculate white. The tail is moderately long and banded like the body. *Scale counts*: dorsals at midbody 15 (occasionally 17); ventrals 194-237; subcaudals 40-50 (single).

Habitat: This krait prefers forested habitats up to 1,500m but is common around human dwellings and farm buildings. In Kampong Cham this snake was very common in rubber plantations and could be found crossing plantation roads and foraging under rubber trees at night.

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[77] Namiranian S and Hider RC. (1992) Use of HPLC to demonstrate variation of venom toxin composition in the Thailand cobra venoms *Naja naja kaouthia* and *Naja naja siamensis*. *Toxicon*. 30(1): 47-61.

[78] Hinman CL., *et al*. (1990) Selective cytolysis by a protein toxin as a consequence of direct interaction with the lymphocyte plasma membrane. *Toxicology and Applied Pharmacology*. 104(2): 290-300.

[79] Viravan C., *et al* (1992) A national hospital-based survey of snakes responsible for bites in Thailand. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 86(1): 100-106.

[80] Wüster W., *et al*. (1997) Redescription of *Naja siamensis* (Serpentes: Elapidae), a widely overlooked spitting cobra from S. E. Asia: geographic variation, medical importance and designation of a neotype. *Journal of Zoology (London)*. 243: 771-788.

Habits: This nocturnal snake preys on other snakes and often enters human habitations at night in search of its prey, and this is when most snake bites occur. During the day kraits are inoffensive, refusing to defend themselves, hiding their heads under their coils, but at night they become much more defensive and will bite with minimal provocation. Females lay 4-10 eggs.


Malayan krait (*Bungarus candidus*)

Venom: Krait venoms are dominated by strong neurotoxins and venom from Malayan kraits contains several presynaptic and postsynaptic neurotoxins. The most important of the presynaptic neurotoxins are homologues of β-bungarotoxin, a lethal presynaptic toxin[81], comprised of a 119 amino acid phospholipase A_2 A-chain, and a 59 amino acid kunitz protease inhibitor B-chain, that together acetylcholine (ACh) depletion from nerve end-plates, and obliteration of miniature-end-plate potentials (MEPPs)[81,82]. Bucandin, a presynaptic neurotoxin that has a unique structural modification in one of its β-sheets, enhances ACh release and contributes to neurotransmitter exhaustion[83,84]. Postsynaptic neurotoxins include the α-bungarotoxins that bind to muscle tissue nicotinic acetylcholine receptors (nAChR)[85] and the neuronal nAChR specific κ-bungarotoxins[86]. The κ-bungarotoxins exist as heterodimers, and this coupling enables binding to both neuronal α7-nAChR and α3β2-nAChR[84]. Candoxin, a postsynaptic neurotoxin active against neuromuscular and neural nAChR and α7-nAChR has been shown to cause hippocampal, frontal cortex and temporal lobe damage[87]. Although candoxin is rapidly reversible with anticholinesterases (i.e.: neostigmine methylsulfate) when bound at the neuromuscular junction[88,89], reversal is poor once bound to neuronal α7-nAChR[87]. Bucain, a cardiotoxic, 3-fingered postsynaptic neurotoxin is specific to muscarinic AChR[90], while candiduxins[91], αδ-bungarotoxins[92] and bungatoxin[93] are neuromuscular junction nAChR antagonists. Non-neurotoxic components of Malayan krait venom include abundant L-amino acid oxidase, large amounts of hyaluronidase and acetylcholinesterase (AChE)[94], the latter contributing to neuromuscular blockade by degrading native ACh before it can bind to receptors.

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[81] Yanoshita R., *et al*. (2006) Molecular cloning of the major lethal toxins from two kraits (*Bungarus flaviceps* and *Bungarus candidus*) *Toxicon*. 47(4): 416-424.

[82] Khow D., *et al*. (2003) Isolation, toxicity and amino terminal sequences of three major neurotoxins in the venom of Malayan krait (*Bungarus candidus*) from Thailand. *Journal of Biochemistry*. 134(6): 799-804.

[83] Kuhn P., *et al*. (2000) The atomic resolution structure of bucandin, a novel toxin isolated from the Malayan krait, determined by direct methods. *Acta Crystallographica D: Biological Crystallography*. 56(11): 1401-1407.

[84] Torres AM., *et al*. (2001) NMR structure of bucandin, a neurotoxin from the venom of the Malayan krait (*Bungarus candidus*) *Biochemical Journal*. 360(3):539-548.

[85] Kuch U., *et al*. (2003) Identification of alpha-bungarotoxin (A31) as the major postsynaptic neurotoxin, and complete nucleotide identity of a genomic DNA of Bungarus candidus from Java with exons of the *Bungarus multicinctus* alpha-bungarotoxin (A31) gene. *Toxicon*. 42(4):381-390.

[86] Osipov AV., *et al*. (2008) Naturally occurring disulfide-bound dimmers of three-fingered toxins: a paradigm for biological activity diversification. *Journal of Biological Chemistry*. 283(21): 14571-14580.

[87] Pachiappan A., *et al*. (2005) Glial inflammation and neurodegeneration induced by Candoxin, a novel neurotoxin from *Bungarus candidus* venom: global gene expression analysis using microarray. *Toxicon*. 46(8) 883-899.

[88] Paaventhan P., *et al*. (2003) Crystallization and preliminary X-ray analysis of candoxin, a novel reversible neurotoxin from the Malayan krait *Bungarus candidus*. *Acta Crystallographica D: Biological Crystallography*. 59(3): 584-586.

[89] Nirthanan S., *et al*. (2003) Neuromuscular effects of candoxin, a novel toxin from the venom of the Malayan krait (*Bungarus candidus*). *British Journal of Pharmacology*. 139(4): 832-844.

[90] Watanabe L., *et al*. (2002) Crystallization and preliminary X-ray analysis of Bucain, a novel toxin from the Malayan krait *Bungarus candidus*. *Acta Crystallographica D: Biological Crystallography*. 58(10-2): 1879-1881.

[91] Tsai, I.H., et al. (2001) Structural and functional genomics of *Bungarus candidus*. NCBI Protein database (GI:24459180; GI:24459182) http://www.ncbi.nlm.nih.gov

[92] Kuch, U., *et al*. (2006) Alpha-delta-bungarotoxin, a novel reversible long-chain neurotoxin from Malayan Krait (*Bungarus candidus*) venom with high toxicity and site-selective binding at the muscle nicotinic acetylcholine receptor. NCBI Protein database (GI:121489568; GI:121489570; GI:121489566) http://www.ncbi.nlm.nih.gov

[93] Vivekanandan,S., *et al*. (2007) NMR solution structure of Bungatoxin from *Bungarus candidus* (Malayan Krait) venom. NCBI Protein database (GI:150261321) http://www.ncbi.nlm.nih.gov

[94] Pukrittayakamee S., *et al*. (1988) The hyaluronidase activities of some Southeast Asian snake venoms. *Toxicon*. 26(7): 629-37.

Clinical effects: Bites by Malayan kraits lack the local tissue destruction seen in bites by pit vipers and Asian cobras, and are devoid of haemostatic disorders. As can be anticipated from the abundance of neurotoxins in the venom, the clinical effects are almost entirely paralytic. Descending paralysis involving the cranial, bulbar, thoracic and peripheral nerves is typical. Bite sites are unremarkable with occasional redness, paraesthesia and slight swelling[95,48]. Non-specific symptoms of headache and abdominal pain often precede development of ptosis, ophthalmoplegia, dysarthria, dysphagia, dyspnoea, progressive airway compromise and respiratory failure[95,96]. Loss of deep tendon reflexes has been reported. Thirteen of 46 snake bite deaths reported in Thailand in the 1980's were attributed to Malayan kraits, with death due either to respiratory failure or complication of prolonged ventilation[48]. In Ho Chi Minh city, Vietnam, the case fatality rate after bites by Malayan kraits was 15.5%, and complications of ventilation contributed to some of these cases[97].

Neurotoxicity may develop rapidly; generalised weakness with nausea, vomiting and Myalgia within 30 minutes of being bitten, with ptosis, ophthalmoplegia and tightness in the chest at 1 hour have been reported[98]. Respiratory failure occurred within 8 hours necessitating 96 hours of ventilator support. Others have reported development of ptosis, ophthalmoplegia, dysarthria, dysphagia and thoracic paralysis within from 2-6 hours of being bitten[99]. Malayan kraits are also reported to cause persistent parasympathetic neuropathy with hypertension lasting from 6-60 days and mydriasis, difficult micturition and constipation which were present two years after envenoming[96].

Other highly venomous species

While the six species described above are the most common highly venomous snakes, and those most likely to be implicated in severe envenoming, there are at least three other species that should be regarded as dangerous:

Banded krait (*Bungarus fasciatus*)

Banded krait (*Bungarus fasciatus*)

Unlike the Malayan krait (*Bungarus candidus*) which prefers wet, forested habitats, the larger banded krait (*Bungarus fasciatus*) favours drier, open country, including dry forests, farmland and rice paddies. Typically marked with black and yellow bands of equal width, this common snake is often encountered by people, and is frequently blamed for snake bites. In reality it is shy and remarkably docile, rarely biting even when handled. In Thailand and Myanmar it has been shown to be a relatively rare cause of bites but its abundance has resulted in antivenoms being produced in both Thailand and Indonesia.

Venom from banded kraits is highly toxic and contains many of the same types of neurotoxins that have been documented in the venoms of other species of *Bungarus*. As a consequence, although the frequency of bites is low, the consequences of envenoming can be catastrophic, and this species should be regarded as a potentially dangerous snake.

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[95] Warrell DA., *et al.* (1983) Severe neurotoxic envenoming by the Malayan krait *Bungarus candidus* (Linnaeus): response to antivenom and anticholinesterase. *British Medical Journal*. 286: 678-680.

[96] Laothong C and Sitprija V (2001) Decreased parasympathetic activities in Malayan krait (*Bungarus candidus*) envenoming. *Toxicon*. 39(9): 1353-1357.

[97] Le KQ., *et al*. (2008) Clinical features in patients bitten by Malayan krait. *Proceedings of 8th Asia-Pacific Meeting on Animal, Plant and Microbial Toxins*. Hanoi and Halong Bay, Vietnam, December 2008

[98] Pochanugool C., *et al*. (1997) Spontaneous recovery from severe neurotoxic envenoming by a Malayan krait, *Bungarus candidus* (Linnaeus) in Thailand. *Wilderness & Environmental Medicine*. 8(4): 223-225.

[99] Kanchanapongkul J. (2002) Neurotoxic envenoming following bites by the Malayan krait (*Bungarus candidus*) *Journal of the Medical Association of Thailand*. 85(8): 945-948.

Red-headed krait (*Bungarus flaviceps*)


Red-headed krait (*Bungarus flaviceps*)

This seldom-seen species of krait inhabits the lowland and hill forests of Cambodia and can be easily recognised by the spectacular bright red head, neck and tail colouration. Like all kraits it is primarily nocturnal, and hunts during the evening. Diet consists largely of other snakes, but lizards are sometimes eaten.

Bites by red-headed kraits are rare, and hunters or snake collectors are often the most likely to be bitten, although red-headed kraits can enter dwellings close to forest. The venom has many of the neurotoxins found in other krait species.

King cobra (*Ophiophagus hannah*)

Contrary to the previously reported perception that the majority of snake bites in Cambodia are caused by king cobras (*Ophiophagus hannah*)[1], the reality is that bites by this large, formidable snake are rare.


King cobra (*Ophiophagus hannah*)

Growing to more than 5.5 metres in length, this giant snake inhabits wet forests, plantations, bamboo groves and thickets, especially near creeks, drains, rivers and lakes. Despite their large size king cobras are equally comfortable both on the ground and high in foliage. Juvenile snakes have been found high in trees, but size does not limit their ability to climb. If they are confronted king cobras will attempt to escape, but are capable of ferocious defence. They are one of the most vocal of snakes, and have a loud growl produced by structural modification of the trachea. The long body of the king cobra is an adaption to feeding on other snakes.

King cobras produce very large amounts of venom that contains neurotoxins, cytotoxins and a range of other components including an endogenous β-adrenergic receptor blocker[100], platelet aggregation inhibitors[101, 102] and a fibrinogenolytic toxin[103]. Bites by king cobras can lead to severe local swelling and occasionally necrosis, with neurotoxicity being the dominant systemic effect[104,105,106]. The only specific king cobra antivenom is manufactured by the Queen Saovabha Memorial Institute in Bangkok, Thailand.

[100] Rajagopalan N., *et al*. (2007) Beta-cardiotoxin, a new three-finger toxin from *Ophiophagus hannah* (king cobra) venom with beta-blocker activity. *FASEB Journal*. 21(13): 3685-3695.
[101] Oyama E and Takahashi H. (2007) Distribution of low molecular weight platelet aggregation inhibitors from snake venoms. *Toxicon*. 49(3): 293-298.
[102] Jin Y., *et al*. (2007) Molecular characterisation of L-amino acid oxidase from king cobra venom. *Toxicon*. 50(4): 479-489.
[103] Guo XX., *et al*. (2007) Isolation and cloning of a metalloproteinase from king cobra venom. *Toxicon*. 49(7): 954-965.
[104] Gold BS and Pyle P. (1998) Successful treatment of neurotoxic king cobra envenomation in Myrtle Beach, South Carolina. *Annals of Emergency Medicine*. 32(6): 736-738.
[105] Karnchanachetanee C. (1994) King cobra bite. *Journal of the Medical Association of Thailand*. 77(12): 646-651.
[106] Tin-Myint *et al*. (1991) Bites by the king cobra (*Ophiophagus hannah*) in Myanmar: successful treatment of severe neurotoxic envenoming. *Quarterly Journal of Medicine*. 80(293): 751-762.

EPIDEMIOLOGY OF SNAKE BITE IN CAMBODIA

There is currently very little reliable data available on the burden of snake bite envenoming, disability and death in Cambodia. The recent SEAMEO TropMed Network report provides scant new information on snake bite incidence, morbidity or mortality. Data was provided for only two Provincial, and two Phnom-Penh Hospitals. Estimates provided by the Forestry Department indicating 600 snake bites, 50-80 deaths and 50-150 amputations were provided without any explanation of a means of calculation[1], and require clarification.

Few data on snake bite epidemiology in Cambodia exist in the available scientific literature. An estimate of between 794 and 21,482 cases with mortality ranging from 80 to 722 persons per year provided by Kasturiratne *et al* (2008) lacks credibility given the broad ranges, lack of any specific citations, and apparent extrapolation from other SE Asian data[107]. An estimate of 800 snake bites/yr among 35,000 rubber tappers in Kampong Cham Province, of which 10% of bites were said to be fatal[108] could not be confirmed by initial inquiries in that Province, but as no timeline for the data was provided it is difficult to determine whether this is refers to a recent or historical data. Considering the recent history of Cambodia it is unlikely that any accurate records of snake bite incidence or mortality exist from the Khmer Rouge years and period of recovery that has been taking place since Prime Minister Hun Sen came to power.

Relatively little specific data on snake bite incidence, disability or mortality was available from any of the hospitals visited during the initial consultative phase. Staff at Hospitals and Provincial Health Departments were asked about the numbers of patients presenting with real or suspected snake bite, and while some data was available, it was generally limited to estimates of monthly or annual presentations. Many hospitals retain the medical records of patients who have presented for the treatment of snake bite, and while it would be feasible to extract these individual files, and conduct a more thorough retrospective analysis of the clinical syndromes of envenoming in each region, this was beyond the scope of the visits made during the consultative process, and was not attempted.

Data with sex, age and date of injury information was provided for Battambang Referral Hospital, Pursat Provincial Hospital, Kantha Bopha Children's Hospital (Phnom Penh) and for Sihanoukville's "Snake House" clinic, run by Nikolai Doroshenko. Some hospitals were also able to provided valuable summaries of the clinical course of envenoming, treatments provided, and outcomes observed. This data provides useful information on population groups that may be risk, and highlights some of the issues and challenges currently being experienced.

The data provided were, with a few exceptions, estimates, and as such they should not be used to extrapolate either Provincial or National case rates. A frequent comment during the consultation phase was that the majority of snake bite patients do not attend hospitals, preferring instead to

[107] Kasturiratne A, Wickremasinghe A.R, de Silva N, Gunawardena N.K, Pathmeswaran A, Premaratna R, Savioloi L, Lalloo D.G, de Silva H.J. (2008)
[108] Personal communication from Nikolai Doroshenko to Professor David A Warrell, Oxford University.

consult traditional healers for treatment. This view tends to be supported by the data from Kampong Som where nearly four times more patients (47-80 vs. 12-24) receive treatment from staff at the "Snake House" clinic compared to those attending Sihanoukville Provincial Referral Hospital itself.

The following data was collected in relation to annual estimates of snake bite at various institutions:

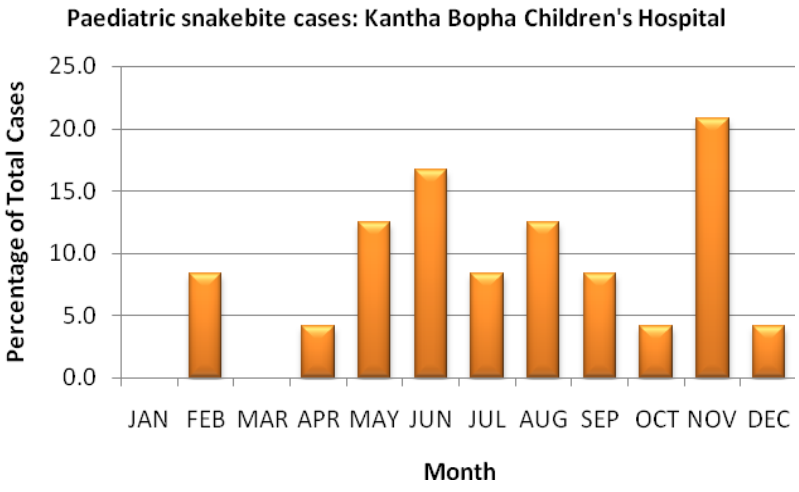
Province	Hospital	Cases	Deaths
Phnom Penh	Calmette Hospital	50-55	1-2
	Kantha Bopha Children's Hospital	20	1
Kampong Chhnang	Kampong Chhnang Provincial Hospital	30-36	1-2
	Pongley Health Centre (CPA-1)	2-3	-
Kampong Cham	Kampong Cham Provincial Hospital	15-20	2
	Memot Referral Hospital	5	1
Pursat	Pursat Provincial Hospital	11-32	1-3
Kampong Som	Sihanoukville Provincial Hospital	12-24	2
	Snake House clinic	47-80	7-8
Prey Veng	Neak Loeung Referral Hospital (CPA-2)	<6	0
	Prey Veng Provincial Hospital	4-5	0
	Kampong Leav Referral Hospital	0	0
Battambang	Battambang Referral Hospital	50-120	1
	Emergency Trauma Center	6	0
Banteay Meanchey	Banteay Meanchey Provincial Hospital	10	1
Siem Reap	Siem Reap Provincial Hospital	12-24	1
Kampong Thom	Kampong Thom Provincial Hospital	10-12	2

Specific data from some of these hospitals provided further insight into some of the issues pertaining to snake bite in Cambodia:

Kantha Bopha Children's Hospital

Dr Chham Sary from Kantha Bopha provided a considerable amount of information based on the retrospective analysis of 51 cases of snake bite treated between January 2006 and June 2008, all involving children between the ages of 3-15 years. Of these 29 (56.8%) were males and 22 (43.2%) females, with 38 (74.5%) referred from Provinces and the remaining 13 (25.5%) having been bitten within Phnom Penh itself.

The majority had bites to lower legs (n=30; 58.8%) or arms (n=18; 35.3%). Only 10 (19.6%) arrived at hospital within 4 hours of having been bitten. The majority came to hospital late, typically after having been seen first at either rural hospital or by a traditional healer in the village. Over half the patients that an admission time given for (n=22; 55.0%), took more than 2 days to reach hospital.



Only 6 (11.7%) patients had received conventional medical treatment prior to being brought to the Kantha Bopha Children's Hospital. More than half (n=28; 54.9%) had been seen by traditional healers. The following chart shows the pre-hospital care that had been provided:

Bites were graded as either mild, moderate or severe, based on a combined assessment of local injury, generalised symptoms and presence or absence of coagulopathy. Only 12 (23.5%) patients were considered to have 'mild' envenoming, while 16 (31.4%) were considered 'moderate' and 23 (45.1%) were graded as 'severely' envenomed. Thrombocytopenia was a clinical feature in 30 (58.8%) cases, and 20 (39.2%) were said to have coagulopathy. Haemolytic anaemia was reported in 22 (43.1%) and 2 (3.9%) patients had renal dysfunction. Two patients developed gangrene and two patients required skin grafts to repair tissue injuries. Bacteria at wound sites was identified in culture samples from 8 (15.7%) patients with *Acinetobacter* spp. found in 4 patients, *Klebsiella pneumoniae* in 2 others, and *Staphylococcus* spp. isolated in the last 2 children. *Acinetobacter* spp. were also identified in blood cultures of two septicaemic patients. More than half of the children had prolonged admissions, with 35 (68.6%) patients having hospital stays of more than 14 days, and the average stay reported as 21 days. There were 14 (27.5%) patients whose admissions lasted 60 days or longer. Two patients died; one from renal failure and the other from shock.

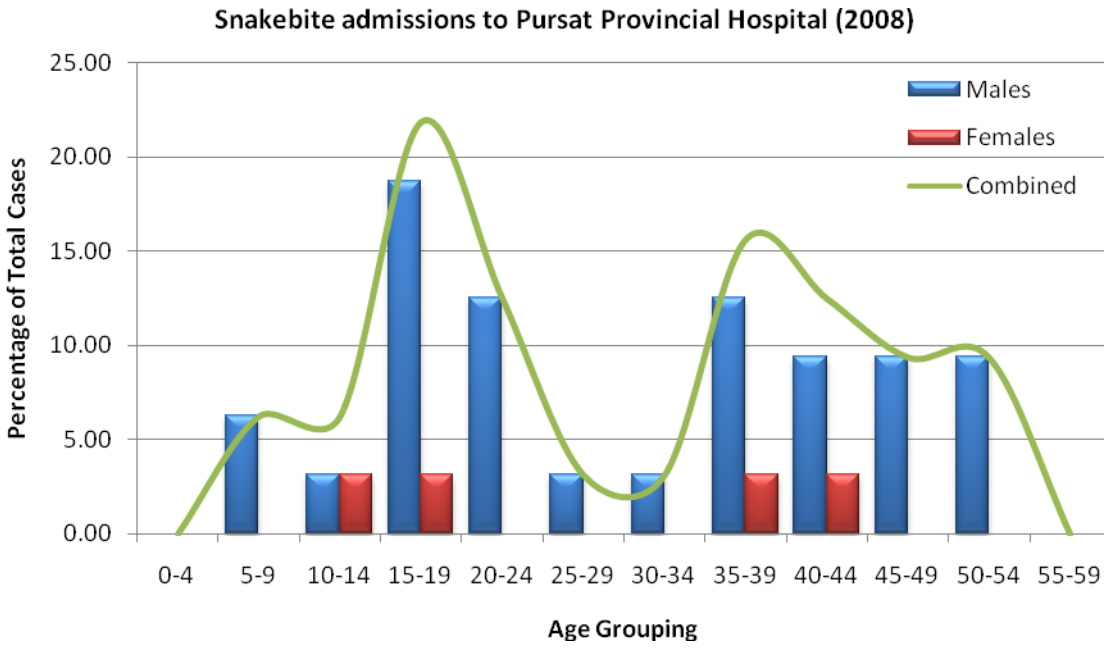
Kampong Thom Provincial Hospital

Dr Nget Bochum, director of the Emergency Unit at Kampong Thom Provincial Hospital reported that the hospital had seen 10 patients in 2007 and 12 in 2008. Of these 13 (59.0%) were said to have improved after treatment, while 7 (31.8%) others discharged themselves to return to traditional healers because they believed improvement had no occurred. There were 2 fatal snake bites in 2008.

Symptoms and signs among patients treated in Kampong Thom included bite site pain, local swelling and oedema, nausea, purpura, muscle "contracture", bullae, ecchymoses, necrosis, gangrene, myoglobinuria, shock and respiratory failure. None of the patients required any surgical interventions, and fasciotomies and amputations were not performed. Both of the patients who died, presented late.

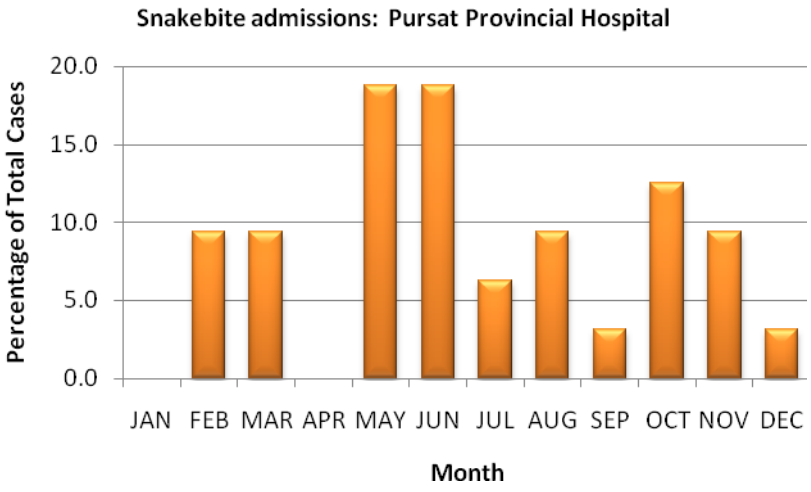
Pursat Provincial Hospital

There were eleven admissions for snake bite in 2007, ten of them involving males. Bites occurred between February and July. In 2008 there were 32 admissions for which age, sex and date of injury data was available. The majority of patients (n=28; 87.5%) were males who had a median age of 29.0 years (range=5-54 years). The four female patients were aged 13, 15, 37 and 40 years respectively. Three patients died, including two boys aged 6 and 15, and a girl aged 13 years.



Two of the fatalities reported in 2008 were the result of late-presenting patients who were admitted with anuria and acute renal failure.

Just over two-thirds (68.75%) of snake bites occurred during the monsoon season (May-October) with most of these taking place during May and June. Local doctors indicated that many people seek help from traditional healers as the first choice before hospital.



Siem Reap Provincial Hospital

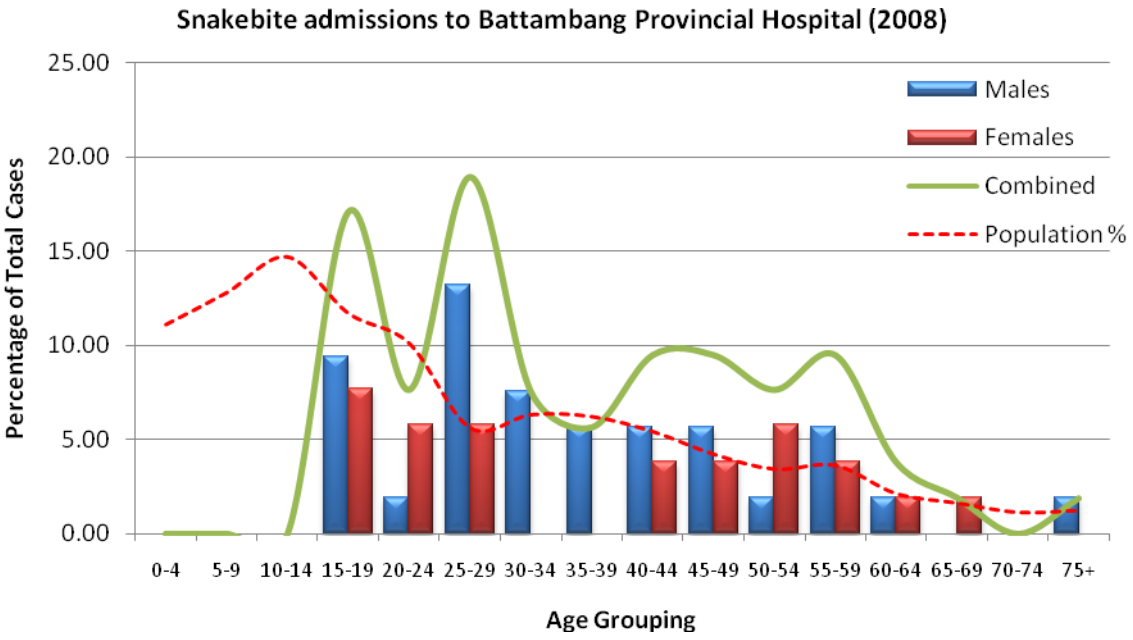
Dr Uy Chanthol from Siem Reap Provincial Hospital, reported that 9 cases of snake bite involving 6 male and 3 female patients were treated at the hospital in 2007. Five of these cases were considered to be severely envenomed, and two patients subsequently died as a result of shock, and acute renal failure. In 2008 there were 11 admissions, involving 8 males and 3 females, with 4 cases of severe envenoming, and one death, attributed to acute shock.

Severely envenomed patients were reported to have presented with local pain, bleeding, oedema and local necrosis, combined with symptoms of chest tightness, chills and palpitations, and signs of tachycardia, shock, oliguria or anuria. The hospital reported having had no supplies of antivenom.

Battambang Provincial Hospital

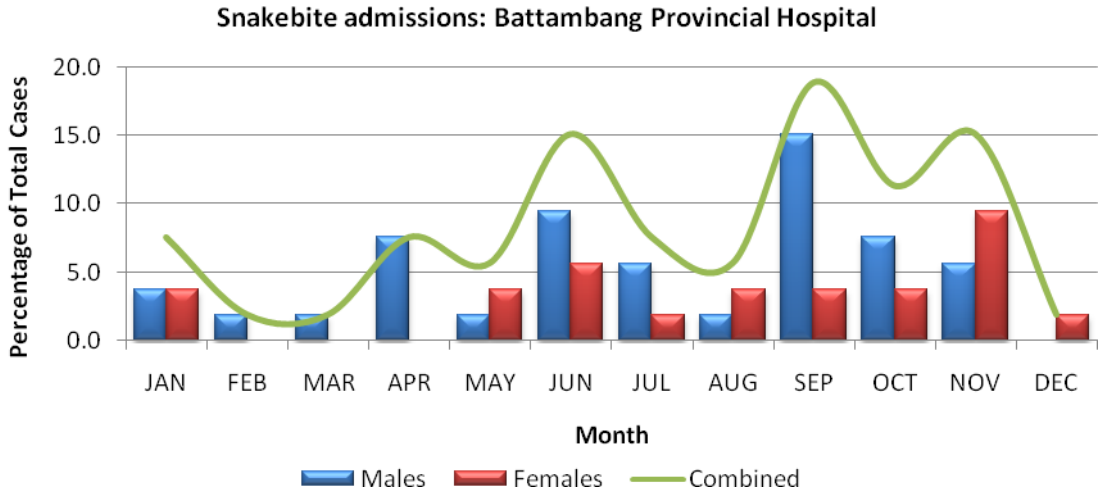
Medical personnel from Battambang Provincial Hospital reported that approximately 50-120 people a year sought treatment for snake bite. Many patients sought initial treatment from traditional healers, and as a result some presented late at hospital. A death at the hospital in 2006 was attributed to sepsis. Typical clinical presentation included swelling, skin blisters and bullae, necrosis, haemorrhage and haemolysis. Decreased urine output was often noted, as was haemoglobinuria. It was estimated that 20% of patients developed renal failure, which was treated with fluid challenge and peritoneal dialysis.

Data for 53 patients who presented with real or suspected snake bite in 2008 were provided. There were 32 males with a median age of 31.0 years (range=18-79 years) and 21 females whose median age was 42.0 years (range=16-65 years). No explanation was available for the absence of paediatric records in this series. Age ranges proportions are shown in the figure below:



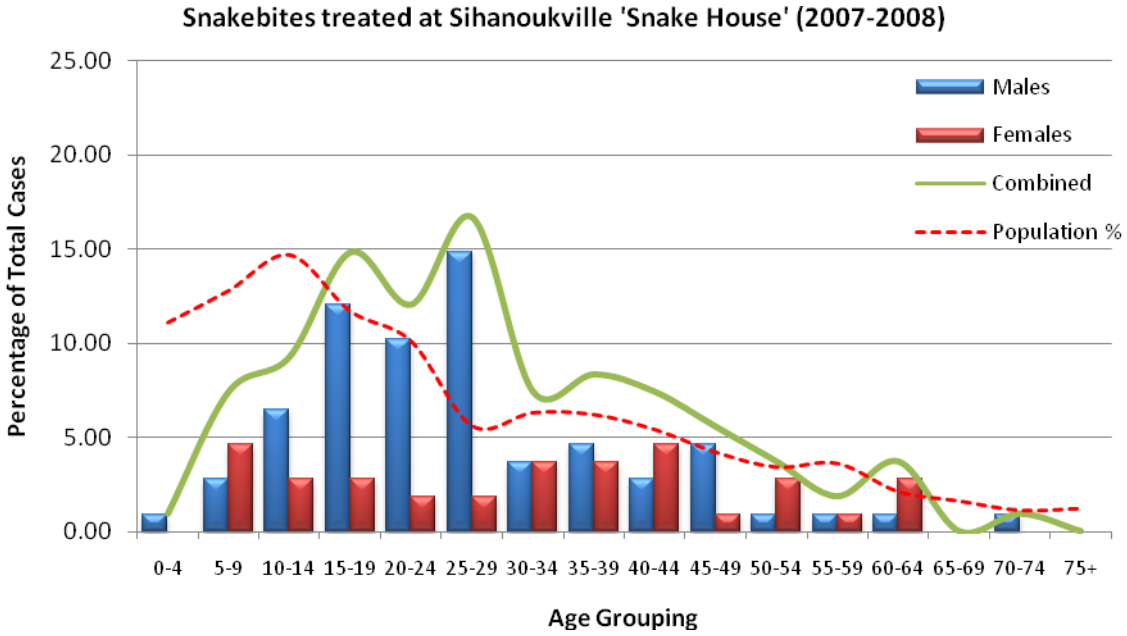
The heavy green line shows the combined (male + female) proportion in each age group, while the dashed red line shows the combined 2004 Cambodian population percentile for each age group (Source: UNFPA, 2005). The absence of data for 0-15 year olds makes it impossible to draw any inferences on the possibility of any particular age group being over-represented at this time.

The seasonal distribution of snake bite cases for 2008 is shown in the following diagram, in contrast to nearby Pursat Province, more cases were reported late in the rainy season than at the start:



Sihanoukville 'Snake House' Clinic

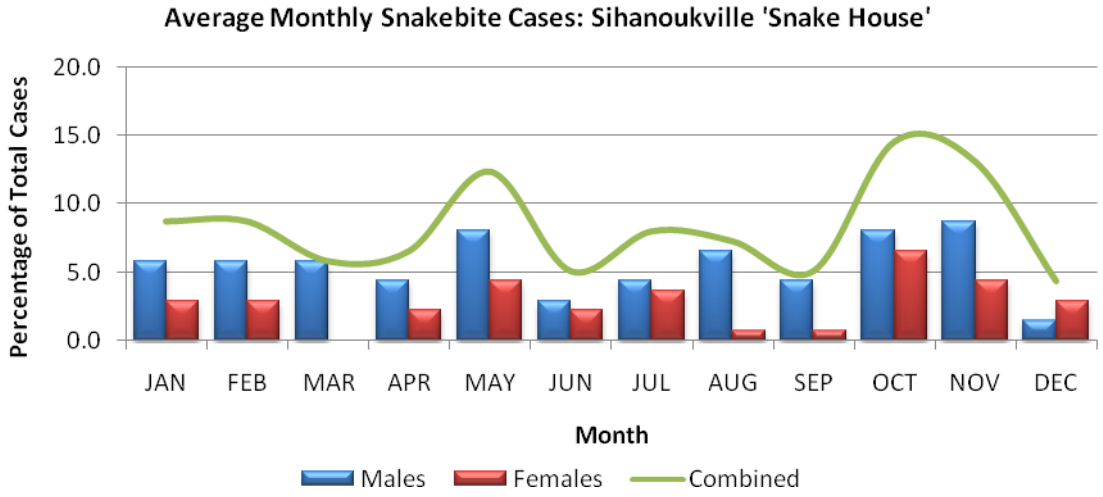
The significant contribution of basic case data from 112 patients seen in the 18 months from 27 April 2007 to 20 October, 2008 by Mr Nikolai Doroshenko and staff at the Snake House Zoo in Sihanoukville enables some analysis of snake bite epidemiology in the Sihanoukville area. Age and sex data was available for 109 (97.3%) cases, comprising 72 men (median 25.0 years; range = 3-72 years) and 36 women (median 37.5 years; range = 5-67 years). A graphical representation of the age and sex data shows that these accidents are skewed towards highest incidence involving young males (11-30 years), and that there exists a statistically significant (p=0.008) dichotomy in age-related male vs. female snake bite frequency. A prospective study of snake bite cases presenting for treatment in Sihanoukville would provide precise information on the demographic profiles of patients, the activities that lead to snake bite, and give insight into which potential interventions might prevent many of these injuries.



Age profiles of snake bite patients presenting for treatment at the Snake House Zoo in Sihanoukville between 27 April 2007 and 20 October 2008 where $N_{(total)}$=108, $N_{(males)}$=72 and $N_{(females)}$=36. Age class data are represented as percentages of the total number of cases. Statistical analysis reveals that male patients are significantly younger (p=0.008) than female patients. The dashed red line shows the combined 2004 Cambodian population percentile for each age group (Source: UNFPA, 2005). There is over-representation of largely male 15-29 year olds, and particularly 25-29 year old men.

Written data on the clinical syndromes of envenoming in Sihanoukville were not available, however information on local snakes and photographs of patients treated at the Snake House, suggest that bites by Malayan pit vipers (*Calloselasma rhodostoma*), white-lipped pit vipers (*Cryptelytrops albolabris*) and cobras (*Naja kaouthia* and *Naja siamensis*) are relatively common, with most possibly being the result of bites by Malayan pit vipers. Patients were reported to typically present with severe local pain, swelling, oedema, blisters, bullae and necrosis, often with clinical bleeding from the mouth, nose or bite wounds, and sometimes with petechial haemorrhage and ecchymoses. Photographs of patients support these anecdotal reports. There were two deaths acknowledged in the preliminary data provided, however on subsequent discussion it was suggested that at least 8-9 people had died of snake bite in the Sihanoukville district during the preceding 18 months.

Allowing for the limitations of single value data for the months of November to February (compared to March-October for which 2 years of data are available) it appears that in the Sihanoukville area snake bite was recorded most in the months of May (10.9%), October (12.8%) and November (11.5%). Snake bite presentations were lowest in December (3.8%). It is likely that this correlates to either seasonal conditions or agricultural activities in the region such as oil palm harvesting.



Improving epidemiological and clinical data

Apart from the very basic data presented here, it is not presently possible to make any statements regarding the demographics and epidemiology of snake bite in Cambodia. As a consequence of these deficiencies we are currently unable to provide an accurate assessment of the previous burden of snake bite injuries and deaths. Without significant additional data it will be difficult to provide the Ministry of Health with clear evidence upon which it may make key decisions with regards to snake bite resource planning, funding requirements and mitigation strategies.

Improving the quality of the data available to the Ministry of Health in relation to snake bite would improve capacity to adequately resource health facilities and to determine actual antivenom needs throughout Cambodia. Developing the capacity to map snake bite incidence using basic data obtained from hospitals and health centres across the country, and having the ability to examine basic clinical records to define envenomation syndromes in different Provinces would be valuable contributions to improving and significantly expanding the current knowledge base, and putting Cambodia in a position to be able to capably manage this public health problem.


Cambodian woman who survived a snake bite near Sitnikom in Siem Reap Province.

There are a number of steps which could be taken to improve the quality of epidemiological data available to the Ministry of Health with regard to this issue, including:

<u>Nominating snake bite as a reportable injury.</u>

The institution of mandatory reporting of snake bite injury has been an effective tool in enabling the mapping of snake bite incidence severity in Brazil, where antivenom dispensing is linked to submission of incidence and epidemiological data to a central surveillance section in their Health Ministry.

While Cambodia may not yet be in a position to enforce a reporting program with this degree of rigor, a more practical initial approach might be to include reporting of snake bite statistics in current monthly disease reporting by Provincial Referral Hospitals. This will provide simple numerical data on the number of snake bite cases presenting to various Provincial Health facilities, and will go some way to improving the current

knowledge base with regards to determining where the greatest burden of injury lies, and hence where the most resources need to be allocated.

Limitations to this approach exist and include a lack of precise attribution of biting species, although use of some simple clinical indications (such as spontaneous bleeding, paralysis, skin blistering, necrosis or renal failure) in the reporting of cases would enable differentiation between the clinical syndromes of some snake species, and hence presumptive attribution. The major limitation however is the lack of completeness offered by sole reliance upon hospital based data in a setting where few snake bite patients are believed to actually access conventional treatment. For this reason, we recommend that data collection from hospitals be combined with a community-based approach to data acquisition.

Community-based surveys of snake bite

The use of community-based survey data to inform health authorities about the incidence of snake bite injury, disability or death is a technique that has been employed by a number of researcher and health administrations.

In Mexico for example, sentinel sites in Guerrero Province were used to provide information on scorpion envenoming[109], while in the African Republic of Guinea, tri-level (community, health centres and traditional healers) surveys proved to be extremely valuable in identifying the true burden of snake bite in a situation where very few patients actually presented to conventional health care facilities[110]. In this study only 2 of 175 cases were seen at a health centre, with 80% having used traditional healers, and 9 cases ending fatally. This later study has strong similarity to the current situation in Cambodia. Similar community-based studies in Nepal using robust sampling techniques and trained interviewers also succeeded in providing an accurate assessment of the snake bite burden where reliance on hospital data had proven to be deficient[111].

As with hospital-acquired data, a limitation of this approach is the lack of reliable snake species attribution. One advantage of a community-based approach would be the potential to combine collection of snake bite data with a broader community survey of health conditions, such as childhood injury, malaria, tuberculosis or disability. Coupling the data collection objective in this way is also likely to facilitate greater cooperation in addressing the snake bite issue from non-governmental organisations such as UNICEF, Handicap International or The Cambodia Trust, all of whom have existing community contact programs in place.

Trial of a prospective clinical research protocol

The current information base could be significantly improved through the prospective collection of clinical data and blood serum and plasma samples from snake bite patients presenting to a series of collaborating Provincial and Phnom Penh hospitals. Using this approach, clinicians in each hospital would be required to complete a clinical data reporting form for each patient who presents with snake bite, and to collect serial plasma and serum samples for later laboratory analysis. This project could be carried out as a collaboration between the clinicians involved and the WHO consultants with additional involvement by the Institut Pasteur du Cambodge in Phnom Penh.

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[109] Andersson N, *et al* (1989) Epidemiologic monitoring and decentralized planification: the use of sentinel sites in Guerrero. *Salud Publica de Mexico*. 31(4): 493-502.

[110] Balde C, *et al* (2005) Impact of snakebites in rural environment: community survey in the rural development community (DRC) of Frilguiagbe, Republic of Guinea. *Bulletin de la Societe de Pathologie Exotique*. 98(4): 283-284.

[111] Sharma S, *et al* (2004) Impact of snake bites and determinants of fatal outcomes in southeastern Nepal. *The American Journal of Tropical Medicine and Hygiene.* 71(2): 234-238.

Data from completed forms would then be entered into a database for analysis of epidemiological and clinical data, and the places where the bites occurred plotted using GIS to produce maps of snake bite occurence. Clinical plasma and serum samples would then be used to assemble data on the haematological, liver, renal and cardiac function of patients during the course of their illness. Serum would also be used in a sandwich ELISA to identify the species of snake responsible in each case.

Minimal resources are required to facilitate the collection of the clinical/epidemiological data, and biological sample collection can also be accomplished with a small investment of time and attention to sample processing and storage. The data that can be extracted is however significant. Using this strategy we can precisely define the syndromes of envenomation of specific snake species, as well as mapping the origins of bites by each type of snake in each Province that participates, the epidemiological characteristics of snake bite patients, the human activities in which snake bite is a common risk, the seasonal burden of injury, and base rates of incidence, disability and mortality.

Such a research project conducted over 12-24 months would yield significant benefit since the information can be used to improve the design of snake bite treatment protocols, and to inform the medical management of snake bite by providing a clear understanding of the effects of snake bite by individual species of snake in Cambodia.

VISITS TO PROVINCIAL HEALTH FACILITIES

Interviews were conducted with administrators, doctors, pharmacists and other health workers from hospitals and health centres in Phnom Penh, Kampong Chhnang, Pursat, Battambang, Banteay Meanchey, Siem Reap, Kampong Thom, Kampong Cham, Kampong Som and Prey Veng Provinces. Hospital facilities were inspected and some snake bite patients were seen at Pursat, Kampong Chhnang and Sihanoukville hospitals, and a number of recovered patients were encountered during random community visits to interview villagers about their knowledge of local snakes.

The aims of this component of the consultation process were to:

A. Assess the general availability of basic medical equipment, particularly in trauma or emergency rooms. This is because it is crucial that training resources and treatment protocols reflect the current functional realities of health facilities. For example, there is little benefit in teaching protocols for machine-based renal dialysis if such facilities are not available.

B. Gather information about the current skills and competencies of provincial medical personnel when it comes to treating snake bite patients. Doctors were asked questions regarding both initial and ongoing clinical assessment; primary and subsequent treatment of snake bite; availability of antivenoms; laboratory tests and other specific investigations; general wound care; and the use of surgical interventions.

C. To provide provincial medical personnel with an opportunity to communicate their own specific concerns with regard to treating and managing snake bite cases in their hospitals or health centres. Our experience has been that training modules that address the particular issues that are important to the personnel who have responsibility for patient care, are better received by participants, and with higher retention on subsequent assessment.

Medical infrastructure

The overall impression was that basic hospital infrastructure (buildings) was superior to that seen in some other developing economies within the Asia-Pacific region. Donors (such as ADB) were involved in a considerable amount of this sort of infrastructure development, but this appears to some extent to have ignored the additional infrastructure needs of facilities in terms of basic medical equipment.

Many of the hospitals visited had emergency rooms that were significantly lacking in their capacity to deliver the services for which they are intended. Some had no specific medical equipment and were little more than specifically designated rooms for initial examination of acute cases. Others had very basic equipment including infusion pumps, suction machines, superseded cardio-respiratory function monitors, and in some cases ventilators.

Provincial referral hospitals were able to perform basic laboratory investigations including haemograms, but had no capacity to measure specific blood factors or conduct specific coagulation tests such as the prothrombin time (PT) activated partial thromboplastin time (APTT), fibrinogen levels or fibrin/cross-linked fibrin degradation product (FDP/XDP) tests. Most hospitals also reported being unable to conduct investigations of urine electrolytes, liver function (LFT), creatine kinase (CK) or lactate dehydrogenase (LDH). Although most had surgical, radiology and ultrasound facilities, none were able to offer expert management of renal failure other than by peritoneal dialysis.

Basic monitoring, suction and infusion equipment in the Emergency Room at Sihanoukville Provincial Hospital.

There was evidence that these problems may be being addressed in some settings, and that some hospitals are better equipped than others. For example, Fondation Mérieux has provided assistance to rehabilitate parts of Sihanoukville Provincial Hospital, and is has developed new laboratory facilities in other centres. USAID has been active in assisting with the improvement of hospital facilities in a number of the provinces visited including, Sihanoukville, Battambang and Pursat. Groups such as Clear Path International (CPI) have also been active in assisting some hospitals with donations of medical equipment from overseas sources.

As currently resourced, many emergency rooms would be unable to appropriately manage a very severely envenomed snake bite patient, particularly one with significant neurotoxicity, such as might occur following envenoming by the Malayan krait (*Bungarus candidus*), or a patient suffering from hypovolaemic shock, venom-induced consumption coagulopathy (VICC), and deep tissue necrosis or gangrene after a bite from a Malayan pit viper (*Calloselasma rhodostoma*). The lack of automated renal dialysis equipment, relevant consumables and appropriately trained personnel complicates the management of acute renal failure which can be a consequence of envenoming by Indo-Chinese Russell's vipers (*Daboia siamensis*). This is borne out by the reports from medical personnel that shock and renal failure are common causes of death after snake bite.

Hospital ward at Kampong Chhnang Provincial Hospital

A fundamental requirement to improving the clinical care of snake bite patients (and other Emergency or Trauma Department patients generally) is the need for appropriate, adequate basic resourcing. Even with the benefit of situationally relevant clinical training, medical personnel will still face significant challenges in providing basic care and ongoing treatment unless a parallel commitment to functional step-wise improvements to Emergency and Intensive Care Unit equipment inventories, Laboratory services and access to supplies of consumables are also undertaken. Where possible equipment should be standardised to reduce overall training, operating, and maintenance costs.

Advanced intensive care facilities are absent at Provincial hospitals. Ventilation is generally only available via bag/valve/mask equipment with supplemental oxygen. Equipment is generally limited to suction pumps, cardiac monitors (including some defibrillators), infusion pumps and blood pressure gauges. The Calmette Hospital in Phnom Penh is relatively well equipped and has ventilators in the intensive care unit. Primary Health Centres have little if any specific trauma or life support equipment.

Current situation with regard to clinical management

In addition to limited medical facilities and equipment, there are significant issues that need to be addressed with respect to the medical treatment of snake bite patients in Cambodia. The overall impression is that medical personnel lack specific skills when it comes to clinical assessment and diagnosis of snake bite, primary medical treatment and subsequent patient care, rehabilitation and management of disability. Many doctors expressed the view that they did not feel adequately trained to administer treatment for snake bite, particularly antivenoms. Although the local signs of snake bite were recognised, the lack of reporting of neurotoxic, haemotoxic and other sometimes discrete signs reinforces a view that current clinical assessment skills are poor. Encouragingly, many of the doctors interviewed were eager to become better informed, and made specific requests for better training and information resources, such as posters, diagnostic algorithms, treatment protocols and practice guidelines.

Initial clinical assessment

It was apparent that both initial and ongoing clinical assessment skills were lacking. Two hospitals reported use of a published guideline, but in the majority there were no formalised protocols or procedures specific to snake bite in place at any hospital. Several particular issues were noted:

1. Despite the importance of haemostatic disturbances in the syndromes of envenoming caused by some species, such as Malayan pit vipers (*Calloselasma rhodostoma*), white-lipped pit vipers (*Cryptelytrops albolabris*) and Indo-Chinese Russell's vipers (Daboia siamensis), specific determination of blood coagulation status was not assessed. Some hospitals measured haemoglobin levels and undertook platelet counts, but the most important measurement – clotting ability – was not investigated. None of the Provincial medical personnel spoken with reported the use of the 20 Minute Whole Blood Clotting Test (20WBCT), a widely accepted, standard bedside test for blood coagulation after snake bite, as part of their process of assessing the need for antivenom therapy, nor was the 20WBCT used to evaluate success or failure of any specific treatment. Many doctors had no knowledge of the test.
2. Because laboratory services at Provincial hospitals are limited, few specific investigations are carried out. Although hospitals generally appeared able to perform full blood counts (FBC) the capacity to perform haemostatic assays (PT, APTT, fibrinogen, FDP, XDP, factors levels), enzyme assays (LFT, CK, LDH, troponin), routine microbiological cultures or urine electrolyte studies is absent. High costs prohibit outsourcing to private laboratories in Phnom Penh. Several hospitals did report monitoring haemoglobin and platelet levels.
3. Local effects of snake bite are not assessed objectively using any of the standard approaches that can be applied, such as serial measurement of limb circumference or the extension of local swelling. Two hospitals in Phnom Penh made reference to using an informal system of snake bite severity scoring. At the Calmette Hospital, a four-level grading system based on that of a French hospital was in use, while at the Kantha-Bopha Children's Hospital, patients were graded as mild, moderate or severe on the basis of combinations and severity of local, systemic and haemostatic problems.
4. It was not apparent from interviews that critical objective assessment of limb viability was undertaken. The information provided indicated very strongly that

specific risk of ischaemia in snake bite patients is currently poorly assessed and not based on standard, well recognised criteria, such as peripheral pulse pressure, assessed intra-compartmental muscle weakness, relative hypoaesthesia or invasive, specific measurement of the compartment pressure.

5. No doctors reported the use of standard neurological examination techniques to investigate possible paralysis in snake bite patients. Many simply stated that paralysis was never seen, but in the absence of specific assessment, and with the knowledge that neurotoxicity arises following bites by Monocellate cobras, Indo-Chinese spitting cobras, Malayan and banded kraits, as well as other species, these statements are difficult to accept at face value. Several included '*asphyxia*' in their descriptions of clinical signs, without associating this with neurotoxicity.
6. Reliable attribution of snake bite to a specific species of snake is rare, and patients almost never present with the biting snake. There was a general perception expressed by a number of medical personnel that snake bites were caused by '*king cobras*', an observation reported previously by the SEAMEO consultants[1]. The only location where attribution was being attempted was at the Snake House facility run by Nikolai Doroshenko in Sihanoukville, Kampong Som Province. Here, patients with bleeding and local effects were assumed first to have been bitten by Malayan pit vipers (*Calloselasma rhodostoma*), and treated with TRC Malayan pit viper antivenom.

Primary Treatment

In the majority of Provincial hospitals, there were no formal protocols for the treatment of snake bite being followed, and care was provided on a symptomatic basis. Doctors at the Sihanoukville Provincial Hospital in Kampong Som did however have translated copies of the World Health Organisation monograph "*WHO/SEARO Guidelines for the clinical management of snake bites in the Southeast Asian region*"[112] which were used in the hospital as a guide to treatment. This guideline had also been used at the Calmette Hospital in Phnom Penh to guide their treatment of snake bite.

The majority of hospitals focused upon symptomatic management of obvious symptoms and signs, with all prescribing routine pain medications (including paracetamol, tramadol or morphine), anti-inflammatory agents, corticosteroids (hydrocortisone and/or dexamethasone), antibiotics and wound cleaning/dressing. One provincial hospital reported using local infiltration with 2-3 cc xylocaine for pain relief. At Kantha Bopha Children's Hospital in Phnom Penh pain management was achieved with either paracetamol (15mg/kg) or morphine (0.1mg/kg). Betadine was often used to clean local wounds, although some used normal saline. There was variable use of dressings. Some hospitals left wounds uncovered, while others used Betadine soaked sterile compresses.

Although Provincial hospitals reported being unable to send wound aspirates or other samples for microbiological culture, all reported using antibiotics routinely, including both orally and intravenous administered drugs. Kantha Bopha Children's Hospital reported some wound and blood culture results and had used both cefamandole (100mg/kg) and metronidazole (30mg/kg/day x 10 days) to treat infection. A number of provincial hospitals reported using either penicillin or amphicillin. Tetanus toxoid was given to all children with snake bite at Kantha Bopha Children's Hospital. Some provincial hospitals reported giving routine tetanus prophylaxis, while others reported supply problems as one of the reasons why use of prophylaxis was not consistent.

The use of antivenoms was an area of contention and sometimes confusion in almost all settings. A number of hospitals reported having had no supplies of antivenom, and consequently chose either to manage patients symptomatically or to refer them to other hospitals for treatment. In one Province, District Referral Hospitals with no antivenom were referring patients to the Provincial Hospital (which had supplies of antivenom, but did not use them), who in turn referred the patients to hospitals in Phnom Penh. The common complaint from all hospitals was that medical staff lacked specific training in the use of antivenoms, and this was a major reason why they felt their treatment

[112] Warrell DA. (1999) WHO/Searo Guidelines for the clinical management of snake bite in the Southeast Asian region. *Southeast Asian Journal of Tropical Medicine and Public Health*. 30 Suppl. 1: 1-85.

of snake bite was suboptimal. Many doctors voiced concerns about the safety of antivenom. There was a widespread belief that antivenoms were potentially harmful drugs, and in at least one case this had lead to a situation where antivenom was actively withheld from patients, for fear that its use might lead to more severe consequences than the actual snake bite itself. All of the antivenoms seen in hospitals were Indian-made products, and none of them had specificity for any of the species of snakes found in Cambodia. None of the medical personnel spoken to appeared to be aware that the available antivenoms lacked specificity to the venoms of Cambodian snakes, and although vials of one antivenom were labelled as being for treatment of bites by African snakes, the appropriateness of the product was not questioned.

In Pursat, Chinese-made cobra antivenom had at one time been available, and was given to all patients regardless of the type of snake that may have been responsible, despite doctors considering that it caused little improvement. Several hospitals said that they had previously been supplied with Thai Red Cross (TRC) king cobra antivenom, and this had been used for any case of snake bite requiring treatment regardless of what the species of snake may have been. The only location visited which was using antivenoms specific to snake species found in Cambodia was the 'Snake House' in Sihanoukville. From March 2007 to October 2008, the owner, Mr Nikolai Doroshenko had supplied Thai Red Cross antivenoms to 112 patients (for whom records were kept[113]) at no cost to the patients themselves. The suitability of the currently supplied antivenoms is discussed separately in the next chapter of this report.

Where antivenom was used, dosage was typically limited to a single vial due to issues of cost and availability. One hospital reported using 1 vial per day for three days as treatment for severe snake bites, and a single vial for those patients whose bites were regarded as mild. They complained however that on an occasion when the supplied antivenom had originated in China, even doses of 3-5 vials were ineffective. Antivenom was administered either as a bolus injection via either the intravenous or intramuscular routes, or by infusion in from 250-1000 mL of intravenous fluids. A number of doctors said that they used either test doses of antivenom, or performed skin tests, but most admitted that these practices did not reliably discriminate patients at risk of adverse antivenom reactions from those non-reacting patients. The most commonly enunciated concern of doctors spoken to during consultative meetings was their common concern that insufficient information and training in the use of antivenoms was available. Issues such as how the need for antivenom could be determined, the route, dose and management of adverse reactions were raised in all of the meetings held. As mentioned previously a number of doctors had elected not to use antivenom because of their concerns about these issues. A further concern was expressed that antivenom package inserts were not in translated into Khmer from English, Thai, or Chinese language, and that this provided an additional barrier to the use of the products.

20 year old man with local envenoming after a suspected bite by a white-lipped pit viper (*Cryptelytrops albolabris*)

A common problem reported in many interviews was the absence of any objective determination of the efficacy of antivenom treatment. In Provincial hospitals systematic patient review was rarely carried out post-antivenom to assess the success or failure of the treatment given, and any belief in

[113] Nikolai Doroshenko's assistant, Mr Bean Ra only keeps records of patients actually treated, rather than records of all patients who present for assistance.

the success of treatment was usually based more on personal perception or patient opinion, than on any specific test for a relevant endpoint (i.e.: such as restoration of coagulation measured by 20WBCT or laboratory means).

Patients with coagulation disturbances were often treated with transfused blood, either from blood bank stocks or from blood donated by relatives. Monitoring of haemoglobin levels and platelet counts was reported, but as has been discussed elsewhere specific laboratory tests of coagulation status were not available in the majority of clinical settings. The 20 Minute Whole Blood Clotting Test was not used by any of the Provincial hospitals, but was well known to medical staff from the Calmette Hospital in Phnom Penh. It was common for medical personnel to report that bleeding persisted even after antivenom administration. In Banteay Meanchey and Battambang bleeding patients were regularly treated with up to 20 mg Vitamin K (i.m.) as an adjunctive treatment. Supplementary calcium was also administered.

Other Clinical Issues

A consistent theme in most descriptions of the symptoms and clinical signs of snake bite, was the inclusion of palpitations, coldness, dizziness, tachycardia and hypotension particularly in patients with severe local envenoming (significant swelling and/or oedema, blistering, bullae) and bleeding. Anuria was also said to have been present in some of these patients. Some patients were described as 'comatose', and some were specifically said to be suffering from shock. Medical staff often mentioned shock as a cause of death after snake bite, both in patients who died after delayed admission, and in patients who died a number of days post-admission. Patients with hypovolaemic shock were typically resuscitated with intravenous crystalloid or occasionally with blood or blood products. Few reported the use of colloids such as Haemaccel. Oxygen was administered, but airways were rarely secured, and oxygen was typically delivered either by mask or nasal prongs.

In Battambang, Pursat, Banteay Meanchey, Siem Reap, Kampong Thom and Kampong Chhnang a number of patients were reported as having either presented late with oliguric acute renal failure (ARF), or having developed ARF in hospital. In these provinces, shock and renal failure were the two main reported causes of death. As discussed in a previous chapter bites by Indo-Chinese Russell's vipers (*Daboia siamensis*) are known to cause ARF, and villagers spoken to in five of these six Provinces recognised photographs of Russell's viper and referred to it by the local name '*Srarkarchas*'. None of the Provincial hospitals visited had facilities available to provide dialysis, other than by peritoneal dialysis. Patients with oliguria or anuria were treated with fluid bolus, furosemide or dopamine. The use of mannitol was not reported. In Battambang peritoneal dialysis had been used in the treatment of ARF successfully. In one case, urine output improved to normal after three days of peritoneal dialysis. Routine monitoring of fluid balance was not reported as part of the standard management of snake bite patients, nor was specific assessment of hydration state upon admission. It was said that monitoring fluid balance was problematic since relatives are typically charged with responsibility for the care of the patient, including provision of food and liquids. This presents some clear problems for the treatment of both hypovolaemic shock and renal failure, in particular the lack of reliable monitoring makes it difficult to objectively detect the development of oliguria among inpatients, while there is also a risk of water intoxication developing in patients with electrolyte disturbances.

The administration of tetanus toxoid was mentioned by some hospital staff, as was the routine use of antibiotics, such as single doses of intravenous penicillin. The use of antibiotics after snake bite is often unnecessary, although some snakes do have abundant oral flora, in which case penicillin alone might be inadequate. A study of the oral flora of Malayan pit vipers has found *Enterobacter, Pseudomonas, Staphylococci* and *Clostridia* species, and recommended combination treatment with gentamicin and benzyl penicillin to prevent or treat infection after bites by this snake[114]. In countries where tetanus vaccination is not universally available, it MUST be administered to every snake bite

[114] Theakston RD., *et al*. (1990) Bacteriological studies of the venom and mouth cavities of wild Malayan pit vipers (*Calloselasma rhodostoma*) in southern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 84(6): 875-879.

victim, unless they have had a full initial course of 3 doses over a year or less, and completed less than 10 years beforehand. If the patient has never before been vaccinated against tetanus, they should be encouraged to re-present after 6 weeks, and again after 6 months, for review of the results of their snake bite and completion of their vaccination course. Since vaccination is always given intramuscularly in the deltoid muscle, i.e. in the lateral aspect of the shoulder, it should not be given to a patient with a snake venom-induced consumption coagulopathy (VICC) or significant myotoxicity until those conditions have been reversed with an appropriate dose of the correct antivenom. A delay of up to a day, for this reason, will not be of clinical significance.

If a patient presents with a significantly tetanus-prone wound, such as ruptured bullae; scarification wounds inflicted with non-sterile blades in a non-vaccinated individual; or a wound contaminated by a "treatment" such as a cow dung poultice; then tetanus immunoglobulin should be administered as soon as possible, half injected around the wound and the rest given intramuscularly in the opposite shoulder to the tetanus vaccination, which must also be administered at this time. It is not usually given intravenously. These treatments should be accompanied by regular administration of intravenous penicillin and/or other suitable antibiotic, according to an agreed protocol, after thorough wound cleaning. Where there is no wound swab culture result, broad spectrum antibiotics should be used, covering gram positive, gram negative and anaerobic bacteria, but with a change to a narrower spectrum if the result of a wound swab culture does become available.

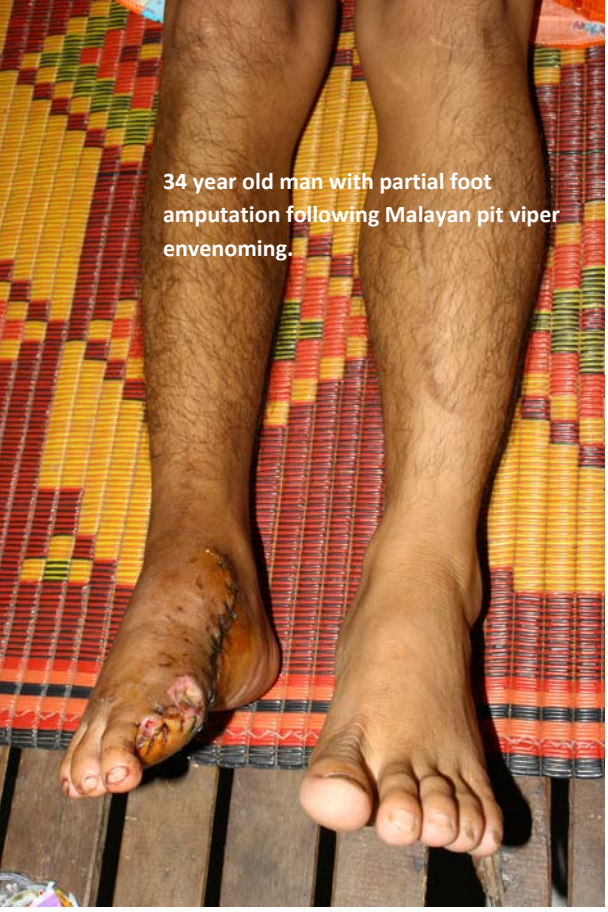
The management of local tissue injury raised some specific concerns. To the greatest extent possible wounds should be carefully cleaned without causing rupture of blisters or bullae. Keeping these intact has a positive role in reducing infection risk, particularly in less than aseptic nursing conditions. Wounds should be carefully cleansed with copious irrigation, under gentle pressure, using sterile fluids, with or without a small amount of added Betadine or Chlorhexidine. It needs to be recognised that concentrated solutions of these substances are toxic to healthy tissue, as is hydrogen peroxide, and should not be used). Foreign objects in wounds should be carefully removed, if necessary with gentle scrubbing with a sterile instrument, such as sterile gauze, to ensure removal of microscopic contaminants. When large blisters are present, and there is concern about these rupturing or possible infection of the blister fluid, or a need to decompress them in order to administer other treatments effectively, then the skin at a site at the blister base should be sterilised with Betadine and a large sterile syringe with a new narrow gauge needle inserted, parallel to the limb surface, so that the fluid can be carefully aspirated.

Attempted fasciotomy on dorsum of right forearm of a 20 year old man: the incisions are too short and shallow, and incorrectly located to be beneficial

In some hospitals 'fasciotomy' was attempted routinely in patients with oedema, swelling and ecchymoses after snake bite, however there was no evidence of an objective assessment pathway prior to fasciotomy being attempted.

One patient seen during a hospital visit, had received superficial incisions to the dorsum of the right hand and forearm which although described as "fasciotomies", were of inadequate depth to have reached muscle tissue requiring decompression, and were incorrectly positioned relative to the dorsal muscle compartments. Although the patient had a moderately swollen arm following a bite from what was presumed to be a white-lipped pit viper (*Cryptelytrops albolabris*), there was little evidence to support the need for any surgical intervention, and it is very likely that swelling would have resolved over 3-5 days with slight elevation to promote drainage.

There are a number of dangers inherent to the performance of fasciotomy after snake bite, such as long-term neurological, muscular and vascular injury, but it is particularly important to recognise the dangers that may arise due to unresolved coagulopathy.



34 year old man with partial foot amputation following Malayan pit viper envenoming.

Fasciotomy should also only be considered where there is adequate assessment of the ischaemic risk. For example, signs of possible increased intracompartmental pressure such as disproportionately severe local pain in a cold, pulseless, and severely swollen limb should be investigated. Weakness in the muscles of the affected limb, obvious tenseness on palpation, pain on passive stretching and hypoaesthesia may indicate increased intracompartmental pressure. Measurement of the compartment pressure prior to consideration being given to surgical intervention is strongly recommended; pressures above 40 mmHg indicate increased ischaemic risk. When compartment syndrome is confirmed by appropriate investigation, consideration of fasciotomy to deflate the swollen compartments should be a decision made by the most experienced surgeon in the hospital, and in consultation with colleagues who have expertise in this field. This will help to ensure that the procedure is carried out correctly. Where facilities are not available in a hospital to provide nursing care to a patient post-fasciotomy, then careful consideration must also be given to medical evacuation to a properly equipped centre.

Rehabilitation

Envenoming by pit vipers and cobras can lead to significant local injury, sometimes resulting in lasting disability. Bites by pit vipers often involve the deep injection of cytotoxic venom that causes extensive necrosis of not just the skin and subcutaneous tissue, but also underlying muscle, tendons and other structures. Permanent scarring, contractures, tendon, nerve and muscle damage may result, and in the most severe cases, necrosis and gangrene may make amputation inevitable.

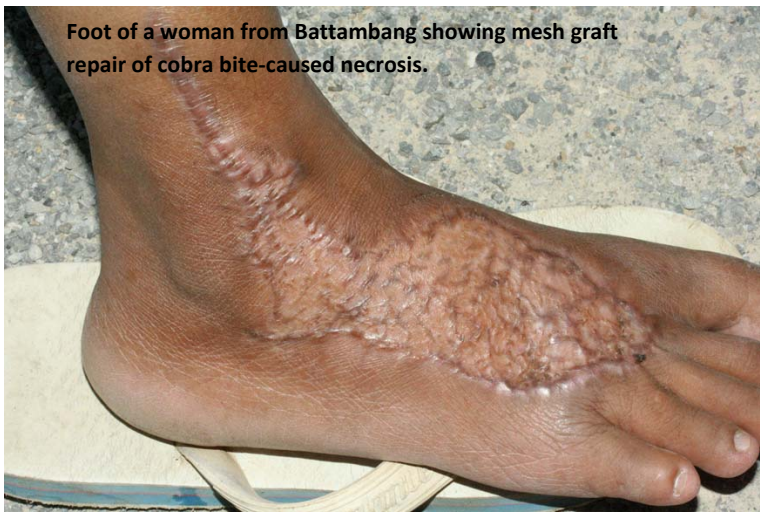
In Cambodia, bites by Malayan pit vipers (*Calloselasma rhodostoma*) in particular, often result in lasting sequelae, including loss of function and amputation. Bites by cobras which typically produce necrosis of the skin and subcutaneous tissue, rather than deeper structures, can still result in contractures and permanent scarring with loss of sensation, movement and function.

Very few hospitals reported providing physiotherapy or rehabilitation services to snake bite patients, although the Sihanoukville Provincial Hospital said that patients were sometimes referred to a local NGO-based rehabilitation service. There was no substantive information available from hospitals as to the proportions of patients who suffer disability as a consequence of snake bite. Data provided by the Cambodia Trust from clinics in Sihanoukville and Kampong Cham for 2008 indicated that relative to other disabilities including UXOs and motor vehicle accidents, only a handful of snake bite patients had sought assistance with prosthetic devices. Details are provided in the following table:

Location	Sex	Age	Type of Device
Sihanoukville	M	16	Right trans-femoral prosthesis
Sihanoukville	F	35	Right trans-radial prosthesis
Sihanoukville	M	35	Left partial foot prosthesis
Sihanoukville	F	19	Left trans-tibial prosthesis
Sihanoukville	M	15	Right trans-femoral prosthesis
Kampong Cham	M	61	Right trans-tibial prosthesis
Kampong Cham	M	23	Left trans-tibial prosthesis

In addition two patients presented to the Cambodia Trust's clinic in Phnom Penh and were provided with prostheses for 'animal bite' injuries, not specifically identified as snakes.

In Battambang, a plastic surgeon working with the Italian NGO group Emergency, which operates a very well resourced trauma clinic, reported that he had assisted approximately 5-6 patients who had ongoing disabilities after snake bites that had occurred sometime previously, including patients with significant contracture due to scar tissue. During a visit to a nearby community, DW met a woman who had been bitten on the dorsal aspect of the right foot some months prior by a cobra that came inside her home. The outcome was an area of necrosis that was managed with a mesh graft from the thigh, although the lady complained that there was still residual hypoaesthesia, and weakness.



Foot of a woman from Battambang showing mesh graft repair of cobra bite-caused necrosis.

The overall lack of information available on snake bite disability in Cambodia, mirrors that of the rest of the world. The WHO's Teach-VIP injury prevention and control curriculum states that up to 400,000 people a year may suffer ongoing disability as a result of snake bite[115], but published data is extremely scant. We would recommend that monitoring of snake bite-caused disability be a part of any reporting system for snake bite data that is established in Cambodia, and we would encourage close cooperation between the Ministry of Health and organisations such as The Cambodia Trust, Handicap International, UNICEF and the Cambodian Red Cross to collate and assemble information on the extent of the problem. Training in snake bite management for Cambodia will include a module on disability, and doctors will be encouraged to counsel patients on the availability of rehabilitation services prior to hospital discharge, and where possible provide direct referrals.

The need for National Snake Bite Treatment Guidelines

The findings of these consultative meetings reinforce the need for the development of sound, practical, evidence-based guidelines for the treatment of snake bite in Cambodia. The current extent of clinical toxinology expertise is very limited, and in particular it is clear from the preceding discussion that there are several notable deficiencies with respect to a number of fundamental areas of patient assessment, treatment and ongoing care:

Clinical assessment and diagnosis of snake bite

A. Specific determination of blood coagulation status was not assessed:
   i. 20 Whole Blood Clotting Test (20WBCT) was rarely used anywhere;
   ii. PT , APTT and fibrinogen or FDP measurements not available;
   iii. Only some hospitals recognised the importance of platelet counts.
B. Local effects rarely objectively assessed, and clinical assessment of ischaemic risk was not made;
C. Standard neurological assessment techniques are not used in any of the hospitals visited;
D. Lack of proper clinical assessment often leads to incorrect management decisions.

Primary medical treatment

A. Limited capacity to deal with the consequences of very severe pit viper bites or with severe neurotoxicity, and there is a clear need to adequately resource emergency rooms;
B. Many doctors expressed uncertainty with regard to the correct use of antivenom, and some refused to use antivenom due to lack of confidence and/or distrust of the products themselves;
C. Antivenoms were often given to patients who did not need it;

[115] http://www.who.int/violence_injury_prevention/capacitybuilding/teach_vip/en/

D. Most patients are not objectively assessed after antivenom is used, and this lack of the use of specific clinical endpoints for treatment needs to be corrected;
E. Adjunct treatments were not used by the majority of doctors.

Subsequent ongoing inpatient care

A. Fluid balance and hydration state were rarely monitored in the wards;
B. Patients in some hospitals were subjected to attempted fasciotomies without prior assessment of ischaemic risk using appropriate clinical tests for increased compartment pressure, or confirmation of the correction of coagulopathy;
C. Provincial hospitals in areas where Indo-Chinese Russell's vipers are believed to occur, do not have renal dialysis equipment and must rely on other treatment modalities;
D. Most hospitals lacked the equipment to be able to manage patients with neurotoxic paralysis.

Rehabilitation and the management of disability

A. Most hospitals do not currently offer rehabilitation services to patients with disability after snake bite;
B. Amputations occur in some patients bitten by snakes, notably after bites by Malayan pit vipers;
C. Few snake bite patients appear to be accessing disability services after discharge, although a small number have received prostheses from The Cambodia Trust;
D. Doctors need to be educated to provide counselling and referral to disabled patients;
E. Disability service providers need to be engaged with to improve knowledge of the extent of disability incidence after snake bite, and to broaden patient access to services.

It was very encouraging that so many medical personnel expressed strong interest in having access to specific training, protocols and other educational resources and clinical tools. The broad impression is that Cambodian doctors are self-aware when it comes to recognition of clinical limitations both in terms of infrastructure resources and personal skill sets. There was uniform interest in access to resource-relevant treatment guidelines or protocols for the management of snake bite, and we would strongly recommend that the development of a practical clinical guideline or treatment protocol be undertaken jointly with the implementation of snake bite treatment training courses, since the two have a parallel and complimentary function.

We propose therefore to draft a standardised National Snake Bite Protocol in collaboration with a small working group from the Ministry of Health and selected National Hospitals in Phnom Penh. This document can then be put forward to become part of the national policy on snake bite.

ANTIVENOMS

The Cambodian Ministry of Health currently purchases a variety of antivenom products for distribution to hospitals, however none of these products are appropriate for use in the treatment of snake bite envenoming caused by any of the medically important snake species that occur naturally in Cambodia.

This unfortunate situation arose in the wake of severe flooding along the Mekong River in 1997 as a result of incorrect advice being provided to the Cambodian Ministry of Health by the internet-based "*Global Health Disaster Network*" (GHDNet)[116]. A perception that flooding would lead to a snake bite emergency led the MoH to seek assistance from the WPRO Emergency and Humanitarian Action (EHA) office, and this request was in turn passed to a member of GHDNet who forwarded it to three online mailing lists.

Three of the inefficacious Indian-made polyvalent antivenoms currently in use by Cambodian hospitals

As a result, the WPRO EHA were put in contact with personnel from both the Japanese Snake Institute (JP) and the Colorado State University (US), and advised to purchase polyvalent snake antivenom from an Indian manufacturer, The Serum Institute of India. The reasons for this incorrect advice appear to have been an assumption by the Japanese and American advisors that Cambodia shared a common snake fauna with India. The consequence of this error is that the Ministry has wasted many hundreds of thousands of US dollars purchasing antivenoms which not only lack specificity and efficacy, but have poor safety profiles and a well documented history of high adverse reaction rates[117,118,119,120,121,122].

Indian-made polyvalent antivenoms have dominated supplies procured for the treatment of snake bite since 1997. Information obtained during Provincial hospital and PHD visits, and data supplies by the Central Medical Stores in Phnom Penh indicate that at least five different antivenoms have been, or are currently being used in Cambodia:

a. Snake Antivenin Polyvalent I.P. manufactured by Biological E Limited of India;
b. Snake Antivenom Serum I.P. SII Anti-snake Venom Serum manufactured by Serum Institute of India/Haffkine Biopharmaceutical Corporation of India;
c. ASNA Antivenom C (Snake Venom Antiserum Africa) manufactured by Bharat Serums & Vaccines Limited of India;
d. King Cobra Antivenom manufactured by Red Cross Society of Thailand;
e. Chinese Cobra Antiserum manufactured by Shanghai Institute of Biological Products from China.

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[116] Ochi G., *et al.* (1999) Information transmission through the internet for the preparedness against venomous snake as the aftermath of Cambodian flood in 1997. *Japanese Journal of Disaster Medicine*. 4: 47-50.
[117] The International Collaborative Study of Severe Anaphylaxis. (2003) Risk of anaphylaxis in a hospital population in relation to the use of various drugs: an international study. *Pharmacoepidemiology and Drug Safety*. 2003 12(3):195-202.
[118] Phillips RE., *et al.* (1988) Paralysis, rhabdomyolysis and haemolysis caused by bites of Russell's viper (*Vipera russelli pulchella*) in Sri Lanka: failure of Indian (Haffkine) antivenom. *Quarterly Journal of Medicine*. 68(257):691-715.
[119] Theakston RD., *et al.* (1990) Envenoming by the common krait (Bungarus caeruleus) and Sri Lankan cobra (*Naja naja naja*): efficacy and complications of therapy with Haffkine antivenom. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 84(2):301-8.
[120] Ariaratnam CA., *et al.* (2001) An open, randomized comparative trial of two antivenoms for the treatment of envenoming by Sri Lankan Russell's viper (*Daboia russelii russelii*). *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 95(1):74-80.
[121] Sharma SK., *et al.* (2002) Krait bite requiring high dose antivenom: a case report. *Southeast Asian Journal of Tropical Medicine and Public Health*. 33(1):170-1.
[122] Visser LE., *et al.* (2008) Failure of a new antivenom to treat *Echis ocellatus* snake bite in rural Ghana: the importance of quality surveillance. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. 102(5):445-50.

Appropriateness of Current Antivenoms

The first two antivenoms listed are manufactured in India using venom from four Indian species of venomous snakes: the spectacled cobra (*Naja naja*); the saw-scaled viper (*Echis carinatus*); Indian common krait (*Bungarus caerulus*); and Indian Russell's viper (*Daboia russelii*). While three of these genera (*Naja*, *Bungarus* and *Daboia*) have species that are represented in Cambodia, there are significant differences in the venoms of the different species within each genus[32,33,123,124,125]. Even within different populations of the same species of snake there may be considerable differences in the compositions of the venoms. The species of Russell's viper found in India (*Daboia russelii*) exemplifies this: venom from the same species from different regions within India have been found to contain different toxins, and as a result available Indian-made antivenoms have been reported as being ineffective against some populations of this species[126,127]. Similar variation in the venom of Indian spectacled cobras (*Naja naja*) from different regions of India has also been reported[128].

The potencies of both the Snake Antivenin Polyvalent I.P. manufactured by Biological E Limited, and the Snake Antivenom Serum I.P. SII Anti-snake Venom Serum manufactured by the Serum Institute of India/Haffkine Biopharmaceutical Corporation are comparable. Both claim to neutralise 0.6 mg *Naja naja*, 0.45 mg *Echis carinatus*, 0.45 mg *Bungarus caerulus* and 0.6 mg *Daboia russelii* venom per millilitre/antivenom. Notwithstanding the clear differences in venom composition that render these antivenoms unsuitable for use in Cambodia, these potencies are very low. The ranges of the venom yields for snakes in the genera *Naja* (58-742 mg[129]), *Bungarus* (8-60 mg[130]) and *Daboia* (21-268 mg[131]) suggest that large amounts of these antivenoms would be necessary to neutralise injected venom (despite this being typically less than the yields for milked venom shown here). In the case of *Daboia siamensis*, the average yield of venom obtained from Burmese specimens during laboratory extraction has been shown to be 127 ± 13 mg (range=21-268 mg), but the mass of injected yield was recorded as 63 ± 7 mg (range=6-147 mg)[127]. On the basis of an antivenom potency of 0.6 mg venom per millilitre, the hypothetical number of 10 mL antivenom vials needed to neutralise the average amount of injected Burmese *Daboia siamensis* venom would be 10.5 vials, with a hypothetical range of 1-24.5 vials. The literature however indicates that the actual requirement can be significantly higher. A study of snake bite in Chandigarh in northern India found that patients bitten by elapid snakes (*Naja* or *Bungarus*) required an average of 51.2 (range=5-190) vials of Indian-made polyvalent antivenom each. Patients bitten by vipers (*Daboia* or *Echis*) required an average of 32 (range=1-130) vials of the same polyvalent antivenom[132]. Another study in the Indian State of Kerala reports an average of 22 (range=3-62) vials per patient[133], and an earlier study of neurotoxic (elapid) snake bite in northern India used a median dose of 90 vials per patient[134].

As the situation currently stands, there is no published data that indicates that any antivenom raised in India against venoms from *Naja naja*, *Echis carinatus*, *Bungarus caerulus* or *Daboia russelii*, are able to adequately neutralise the venoms of any Cambodian snake species. Neither the Snake Antivenin Polyvalent I.P. manufactured by Biological E Limited, or the Snake Antivenom Serum I.P. SII

[123] Bon C. and Saliou B. (1982) Isolation of ceruleotoxin from *Bungarus fasciatus* venoms. *Toxicon*. 20(1): 111-114.
[124] Mukherjee AK. And Maity CR. (2002) Biochemical composition, lethality and pathophysiology of venom from two cobras – *Naja naja* and *Naja kaouthia*. *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology*. 131(2): 125-32).
[125] Tan NH. And Ponnudurai G. (1990) A comparative study of the biological properties of krait (genus Bungarus) venoms. Comparative Biochemistry and Physiology C: Comparative Pharmacology. 95(1): 105-109.
[126] Prasad NB., *et al*. (1999) Comparative characterisation of Russell's viper (*Daboia-Vipera russelli*) venoms from different regions of the Indian peninsula. *Biochimica Et Biophysica Acta*. 1428(2-3): 121-136.
[127] Kumar AV. And Gowda TV. (2006) Novel non-enzymatic toxic peptide of *Daboia russelii* (Eastern region) venom renders commercial polyvalent antivenom ineffective. *Toxicon*. 47(4): 398-408.
[128] Shashidharamurthy R., *et al*. (2002) Variations in biochemical and pharmacological properties of Indian cobra (*Naja naja naja*) venom due to geographical distribution. *Molecular and Cellular Biochemistry*. 229(1-2): 93-101.
[129] Mirtschin PJ., *et al*. (2006) Venom yields from Australian and some other species of snakes. *Ecotoxicology*. 15(6): 531-538.
[130] Prasarnpun S., *et al*. (2005) Envenoming bites by kraits: the biological basis of treatment resistant neuromuscular paralysis. *Brain*. 128(12): 2987-2996.
[131] Tun-Pe and Khin Aung Cho. (1986) Amount of venom injected by Russell's viper (*Vipera russelli*). *Toxicon*. 24(7): 730-733.
[132] Sharma N., *et al*. (2005) Snake envenomation in a north Indian hospital. *Emergency Medicine Journal*. 22: 118-120.
[133] Suchithra N., *et al*. (2008) Snakebite envenoming in Kerala, South India: clinical profile and factors involved in adverse outcomes. *Emergency Medicine Journal*. 25(4): 200-204.
[134] Agrawal P., *et al*. (2001) Management of respiratory failure in severe neuroparalytic snake envenomation. *Neurology India*. 49(1): 25-28.

Anti-snake Venom Serum manufactured by the Serum Institute of India/Haffkine Biopharmaceutical Corporation of India have been subjected either to preclinical assessment against venoms from Cambodian species to the standards contained in the WHO's '*Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins*', or to any clinical trial to establish safety and efficacy in the treatment of envenoming by any species of snake naturally occurring in Cambodia. Given that these antivenoms have been shown to perform poorly even within clinical settings where the species from which the antivenoms are raised occurs naturally[116,117,118,135], there can be no medical or scientific basis for their continued use in Cambodia.

The situation with regard to the marketing in Cambodia of the ASNA Antivenom C (Snake Venom Antiserum Africa) manufactured by Bharat Serums & Vaccines Limited (India) is disturbing, given that this product is clearly labelled as being an antivenom raised for the treatment of bites by African and not Southeast Asian venomous snakes. There should a full and thorough investigation by the Ministry of Health with the objective of determining how, and on what basis this particular product came to be approved, marketed and sold in Cambodia.

The manufacturer claims that ASNA Antivenom C (Snake Venom Antiserum Africa) is made using venom from several species of snakes endemic to the African continent, and is on record specifically stating that their product is only for use against the venoms of snakes listed both on the package and package insert materials. A recent study has shown that in Ghana, where this less expensive Indian-made antivenom was substituted for an antivenom made in Europe, the result was a six-fold increase in the case fatality rate among patients bitten by saw scaled vipers[136]. As with the other two Indian-made antivenoms marketed in Cambodia, there is no published data that indicates that the ASNA Antivenom C (Snake Venom Antiserum Africa) manufactured by Bharat Serums & Vaccines Limited is capable of adequately neutralising the venom of any Cambodia venomous snake species. This antivenom has not been subjected either to preclinical assessment against venoms from Cambodian species to the standards contained in the WHO's '*Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins*', nor has it been subject to any clinical trial to establish safety and efficacy in the treatment of envenoming by any species of snake naturally occurring in Cambodia. The use of this antivenom in Cambodia is very likely to have indirectly contributed to the deaths of snake bite patients.

The use of all three of these, and any other Indian-manufactured antivenom products, should be discontinued immediately. Alternative products are available, and these are discussed at the end of this chapter.

Of the two other antivenoms whose use was reported in Cambodia, the King Cobra Antivenom manufactured by Red Cross Society of Thailand using king cobra (*Ophiophagus hannah*) venom obtained from snakes captured in Thailand has very limited, and specific potential use in Cambodia. Although this antivenom has not been preclinically assessed for neutralising efficacy against venom from Cambodian king cobras, this product is potentially suitable for the medical treatment of specific cases of king cobra envenoming in Cambodia, provided the identity of the snake is certain. It should however be noted that there is common confusion in Cambodia regarding the identity of cobra species, and many of the people interviewed during this assessment assumed that the more common Monocellate cobra (*Naja kaouthia*) was a "king cobra". It was also clear that when it had been available, Thai Red Cross King Cobra Antivenom had been used to treat snake bites without any accurate identification of the biting snake, suggesting that use was inappropriate. The only situation in which the use of the antivenom would be proper, would be in the case of envenoming by either a cobra snake measuring more than 2.5 metres in length, or in the case of a smaller snake, one that was positively identified by an expert in snake identification as a juvenile or subadult king cobra (*Ophiophagus hannah*).

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[135] Shashidharamurthy R., and Kemparaju K. (2007) Region-specific neutralization of Indian cobra (*Naja naja*) venom by polyclonal antibody raised against the eastern regional venom: A comparative study of the venoms from three different geographical distributions. *International Immunopharmacology*. 7(1): 61-69.

[136] Visser LE., *et al*. (2008) Failure of a new antivenom to treat *Echis ocellatus* snake bite in rural Ghana: the importance of quality surveillance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102(5): 445-450.

The last of the antivenoms listed is manufactured by the Shanghai Institute of Biological Products in China, and raised against the venom of Chinese cobras (*Naja atra*). As is the case with variation in the venoms of Southeast Asian Monocellate cobra (*Naja kaouthia*) that occurs in Cambodia and the Indian spectacled cobra (*Naja naja*), there are a biochemical differences in the venoms of these two snakes in different areas of their geographical distribution. A study of their α-neurotoxins found that while there were similarities between the toxins in Chinese *Naja atra* from Zhejiang Province, and *Naja kaouthia* from Yunnan Province, there was a difference between both of these, and the toxins in venom from *Naja kaouthia* from Thailand[137]. Like the Thai king cobra antivenom, it is also important to recognise that this is a monospecific antivenom, and therefore offers protection only against the species whose venom was used to raise the antibodies. In a clinical setting where the accurate identification of the biting snake is either problematic or absent, use of monospecific antivenom is not appropriate. This is reinforced by the anecdotes from Pursat Provincial Hospital, where even doses of 3-5 vials of this monospecific antivenom (albeit used to treat bites from unidentified species of snakes) were said to be ineffective. The reality is that many of the patients were in all probability bitten by species other than cobras.

As with the three Indian-made antivenoms marketed in Cambodia, there is no published data that indicates that the Chinese cobra (*Naja atra*) antivenom manufactured by the Shanghai Institute of Biological Products in China is capable of adequately neutralising the venom of any Cambodia venomous snake species. Since this antivenom has also not been subject to preclinical assessment against venoms from Cambodian species to the standards contained in the WHO's '*Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins*', or to any clinical trial to establish safety and efficacy in the treatment of envenoming by any species of snake naturally occurring in Cambodia, it's use in Cambodia should be considered inappropriate at this time.

Antivenom supplies and distribution

Information about the quantities of antivenom purchased by the Ministry of Health for distribution to hospitals and other facilities in Cambodia was made available at a meeting with the Director of the Central Medical Stores in Phnom Penh. Although no data was available for the period 2002-2004, making it difficult to determine the actual total quantity of antivenom purchased between 2000-2008, information about the type and quantity of antivenom purchased during the periods 2000-2001 and 2006-2008 were available, and are shown in the following table:

Year	Product		Quantity
2008	Snake Antivenin Polyvalent I.P.	Biological E Limited (India)	500
2007	Snake Antivenom Serum I.P. SII	Serum Institute of India (India)	200
2006	ASNA Antivenom C (Snake Venom Antiserum Africa)	Bharat Serums & Vaccines Limited (India)	300
2005	Snake Antivenin Polyvalent I.P.	Biological E Limited (India)	800
2001	Snake Antivenom Serum I.P. SII	Serum Institute of India (India)	400
2000	Snake Antivenom Serum I.P. SII	Serum Institute of India (India)	400

Price data for the years 2007-2008 was also provided. The unit price for antivenom made by the Serum Institute of India and supplied to the Ministry of Health in 2007 was 611,300 Riel/ vial (approximately US$148.92). Given that the retail price in India of this particular antivenom is approximately 412 Indian Rupees/vial (approximately US$9.96 in 2007) it can be seen that significant price inflation took place, either on the part of the manufacturer or

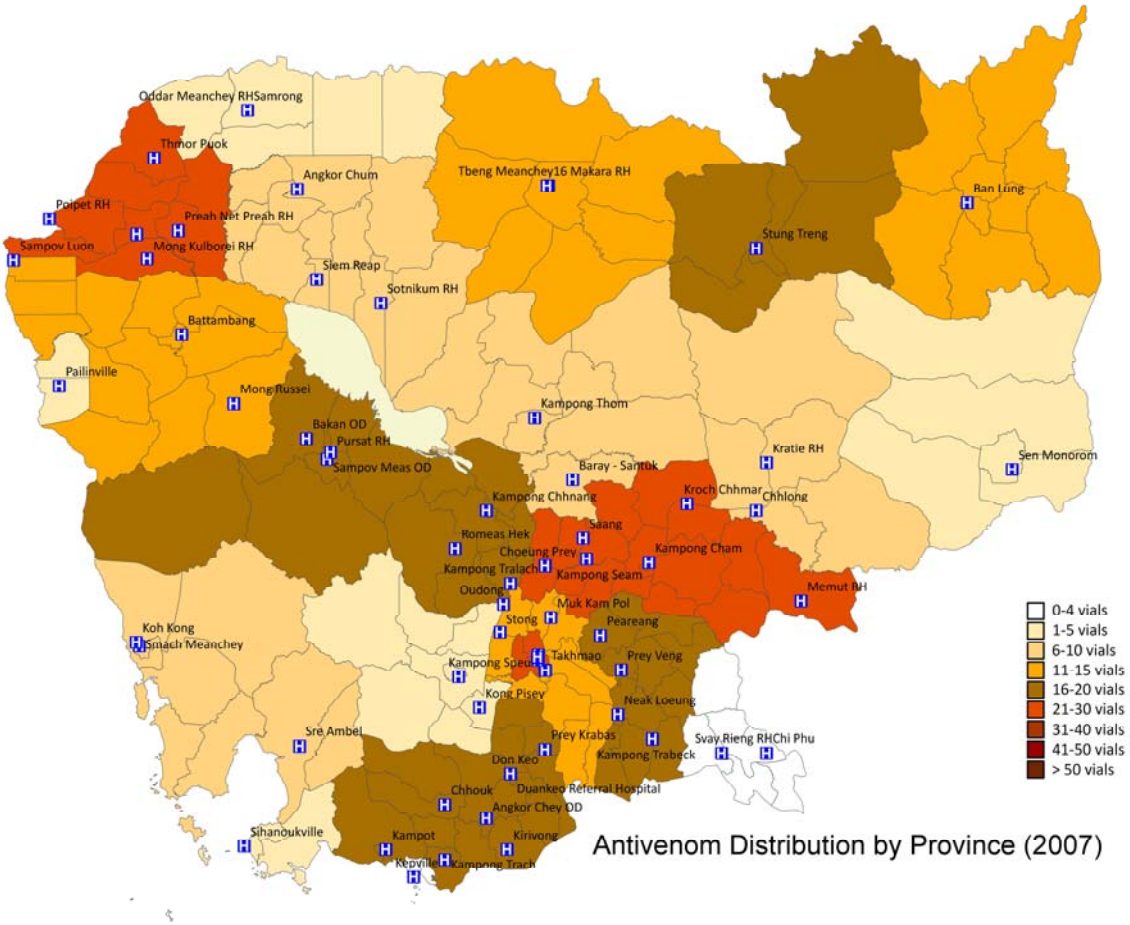
[137] Wei JF., *et al*. (2003) Alpha-neurotoxins of *Naja atra* and *Naja Kaouthia* snakes in different regions. *Acta Biochimica Et Biophysica Sinica*. 35(8): 683-688.

the wholesale importer. Towards the end of 2007, and continuing through 2008, the Ministry of Health has purchased antivenom made by Biological E Limited, another Indian company. The unit price for this product was initially 244,000 Riel/vial (US$60.50) until September 2008, and thereafter has been listed at a unit price of 273,000 Riel/vial (US$67.68). Again the price of this product in India is approximately 220 Indian Rupees (approximately US$4.50) and hence the Cambodian market price represents a mark-up of more than 14 times the manufacturers price. A detailed examination of the process by which the Ministry of Health procures antivenom supplies was not conducted during the consultative process, and individual wholesale suppliers have not been identified and asked for information on their own procurement processes. It appears however that there are factors inflating drug prices in Cambodia far and above those of the manufacturers.

Data provided, shows that in 2007 there were 327 vials of antivenom distributed to 45 health facilities throughout the country, with the five largest numbers of vials being provided to the following referral hospitals or Provincial Health Departments:

Code	Facility Name	Province	Quantity	Value (Riel)
1901	Stung Treng	Stung Treng	20	12,226,000.00
1501	Bakan	Pursat	20	12,226,000.00
0303	Kampong Sie	Kampong Cham	15	9,169,500.00
1301	Preah Vihear	Preah Vihear	15	9,169,500.00
1601	Rattanakiri	Rattanakiri	15	9,169,500.00

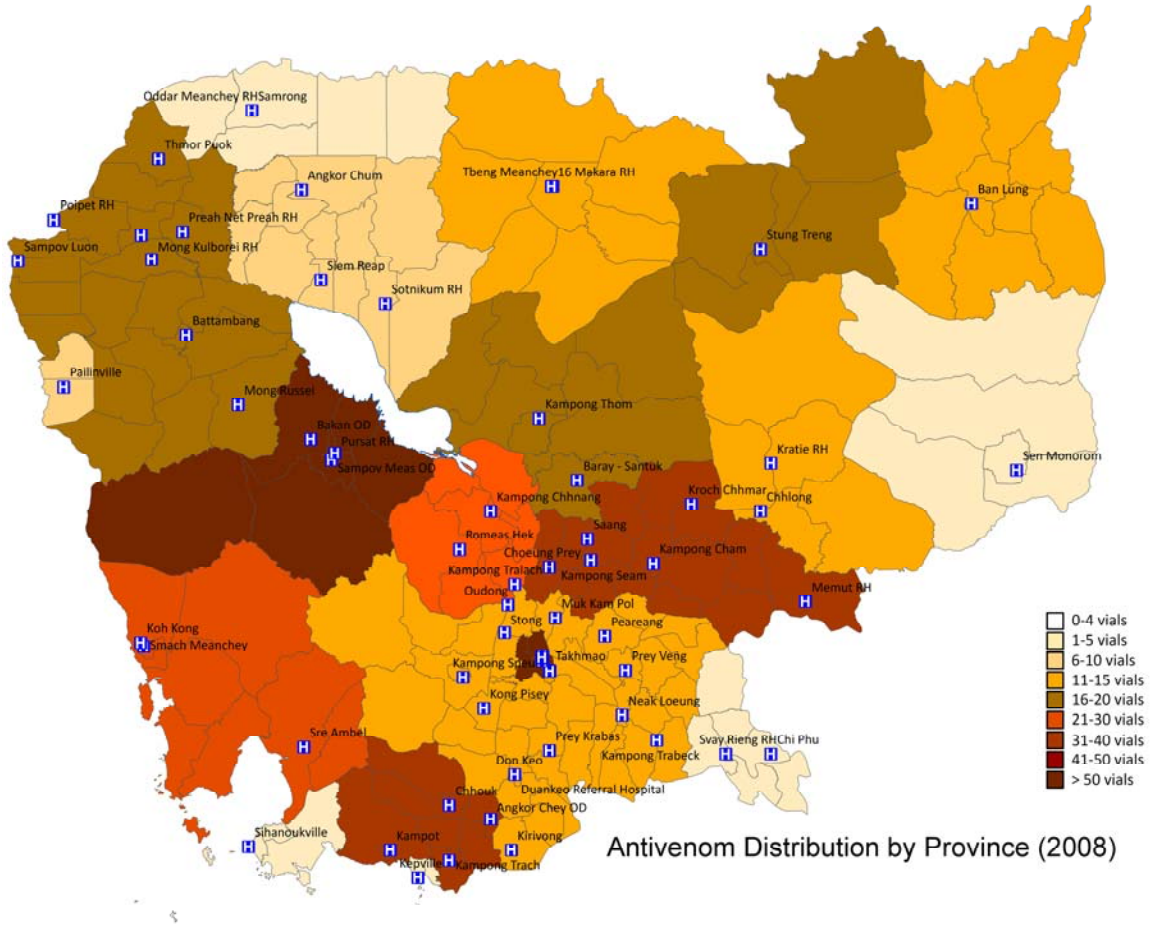
The following map shows antivenom vial distribution throughout Cambodia in 2007:



In 2008 there were 521 vials of antivenom distributed to 61 health facilities around the country, with the five largest numbers of vials being provided to hospitals, Provincial Health Departments or other organisations in:

Code	Facility Name	Province	Quantity	Value (Riel)
9902	Ministry of National Defence	Phnom Penh	60	16,380,000.00
1599	Pursat RH	Pursat	37	9,231,000.00
9702	Calmette Hospital	Phnom Penh	26	6,344,000.00
0999	Koh Kong RH	Koh Kong	23	5,757,000.00
1501	Bakan	Pursat	22	5,368,000.00
0399	Kampong Cham RH	Kampong Cham	22	5,513,000.00

A map shows antivenom vial distribution to Provinces throughout Cambodia in 2008:



It is interesting to observe that in Kampong Som Province where the Sihanoukville Referral Hospital only received 5 vials of antivenom each year, few people (1-2/month) attended hospital for the treatment of snake bite, and the explanation offered for this by local medical staff and others was that general knowledge that the hospital lacked antivenom drove people to seek assistance elsewhere. The nearby Snake House 'clinic' saw between 47-80 snake bites a year in 2007-2008, which suggests that the incidence of snake bite in the Province was less than trivial. At the same time, Kampong Cham Referral Hospital received 22 vials of antivenom in 2008, but doctors there reported rarely using antivenom for fear of the potential complications. This same concern was also reported at other hospitals, and as a consequence some antivenom issued to these facilities was left to expire and be thrown away, rather than being returned to the Central Medical Stores for reallocation to other locations. It is also noteworthy that the distribution of antivenoms in 2007 and 2008 appears to follow no consistent pattern This could be improved significantly by coupling distribution to evidence-based demand using epidemiological data from each operational district.

Antivenom Recommendations

There is an urgent need for the Cambodia Ministry of Health to replace the current Indian-made antivenoms which lack specificity and efficacy, with alternative antivenom products, that are specific to the species of snakes naturally occurring in Cambodia.

There is currently only one manufacturers producing commercial antivenoms that might be suitable for the treatment of snake bite in Cambodia. The Queen Saovabha Memorial Institute (QSMI), a division of the Red Cross Society of Thailand (TRC) has produced rabies vaccines since 1912 and snake antivenoms since the mid-1920's. Over the past decade the Institute has undergone a number of production improvements and GMP process upgrades, including the development of a captive breeding facility to produce venomous snakes from which venoms could be obtained, and a US$2.4 million revitalisation of immunoglobulin production facilities to meet GMP-compliance requirements.

The QSMI currently uses the venom from Thai specimens of *Naja kaouthia*, *Daboia siamensis*, *Calloselasma rhodostoma*, *Cryptelytrops albolabris, Bungarus candidus, Bungarus fasciatus* and *Ophiophagus hannah* to produce seven monospecific and two polyvalent antivenoms. Monovalent antivenoms are appropriate for the treatment of envenoming by identified species of snakes, while polyvalent antivenoms provide protection against the effects of several snake species. We would recommend that the Cambodian Ministry of Health replace the current Indian-made polyvalent antivenoms with the following two TRC/QSMI polyvalent products:

A. TRC Haemato Polyvalent Snake Antivenom

Raised against venom from Malayan pit vipers (*Calloselasma rhodostoma*), white-lipped pit vipers (*Cryptelytrops albolabris*) and Indo-Chinese Russell's vipers (*Daboia siamensis*). All these of these species of snakes occur naturally in Cambodia, and all three can produce a syndrome of envenoming that is easily recognised by clinicians – incoagulable blood and/or systemic bleeding, thrombocytopenia, often with severe local tissue injury. It would be appropriate to prescribe this antivenom preparation for treatment of all cases of snake bite in Cambodia in which clinical bleeding and/or incoagulable blood are demonstrable clinical signs.

B. TRC Neuro Polyvalent Snake Antivenom

Raised against venom from Malayan kraits (*Bungarus candidus*), banded kraits (*Bungarus fasciatus*), Monocellate cobras (*Naja kaouthia*), and king cobras (*Ophiophagus hannah*). A study has shown that antisera raised against Monocellate cobra venom also neutralises Indo-Chinese spitting cobra (*Naja siamensis*) venom[138]. TRC Neuro Polyvalent Snake Antivenom provides protection against the lethal neurotoxic effects of the cobras (*Naja kaouthia, Naja siamensis, Ophiophagus hannah*) and kraits (*Bungarus candidus, Bungarus fasciatus*) and would be suitable for the treatment in Cambodia of envenomation syndromes in which neurotoxicity can be demonstrated, with or without local tissue injury (dermal and subcutaneous necrosis may occur after bites by cobras).

Preclinical Assessment of Antivenoms

The recently approved WHO '*Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins*'[139] recommends that preclinical testing of antivenoms should be implemented when an existing antivenom is to be introduced for use in a new geographical region or country. The guidelines recommend that preclinical assessment should be a regulatory requirement enforced by local regulators as part of the process of licensing antivenoms in a particular setting. Since Thailand is immediately adjacent to Cambodia, and therefore in the same geographical area, it

[138] Khow O., *et al*. (1997) Cross-neutralization of Thai cobra (*Naja kaouthia*) and spitting cobra (*Naja siamensis*) venoms by Thai cobra antivenom. *Toxicon*. 35(11): 1649-1651.

[139] These guidelines were formally adopted by the WHO Expert Committee on Biological Standardization at the annual meeting of the Committee in Geneva on 17 October, 2008.

may be reasonable to excuse the requirement to subject TRC antivenoms to preclinical assessment prior to introduction into the Cambodian marketplace, given the fact that the currently available Indian-made antivenoms are products from a different geographical region with clearly questionable specificity and efficacy in Cambodia.

Our recommendation would be that the two TRC polyvalent antivenoms be introduced without undue delay in order to make an immediate improvement to the treatment of snake bite in Cambodia, but that both antivenoms be subjected to the two essential preclinical assessment assays proposed in the new WHO Guideline document at the earliest opportunity. Such testing should take place utilising venom samples obtained from snake specimens captured in Cambodia, and accurately identified by expert herpetologists. The tests in question are the Median Lethal Dose (LD_{50}) and the Median Effective Dose (ED_{50}), and both should be conducted in an independent laboratory, and in the antivenom manufacturer's laboratory. Results of both sets of assays should be published for transparency, and to make a contribution to the available research literature. The results of these tests will be useful in determining the appropriate dosages of these antivenoms in Cambodian hospitals.

EDUCATION AND TRAINING

It was clear from the discussions held during consultative meetings that there was a broad need for education about snakes and snake bite at a number of levels. From conversations with doctors and Provincial Health Department personnel, and a number of random informal interviews with people from communities in all of the Provinces visited, it is clear that community education is needed just as badly as improved medical education. We therefore recommend that the issue of education be addresses at the following three levels:

1. Basic community education with a clear focus on increasing awareness of snake bite dangers, prevention strategies, first aid interventions and appropriate health care seeking behaviours.
2. Rural health centre education based on increasing knowledge of patient triage, basic resuscitation skills (airway, breathing, circulation) including fluid resuscitation and emergency treatment of shock, basic patient assessment and preparation/conduct of emergency transport.
3. Advanced snake bite management training for major hospitals, including Phnom Penh and Provincial capital referral hospitals. Intensive course design to be taught to selected candidates and supplemented with written treatment and ongoing patient management protocols, teaching resources and ancillary materials.

Community Education

There was near unanimous agreement that for the majority of Cambodia's snake bite victims, hospital care is the last option. There are of course a variety of sometimes complex social issues that lead many snake bite patients to seek help from traditional healers rather than from conventional health providers. Cambodia is currently exiting from a long period of violent and disruptive social upheaval, with many of its citizens living well below the international poverty line. This situation has created a number of barriers to health care access, and none of these will be easily overcome. In addition to issues of cost, traditional beliefs run strong in many rural Cambodian communities, and while some people are not bound by strong reliance on local practitioners, many are. As a number of doctors reported, this unfortunately means that some snake bite patients insist on remaining in their communities after being bitten. During community visits we learned from traditional healers and members of the general public that patients often died in the care of these practitioners, while doctors in Provincial hospitals reported notable cases of patients presenting very late after snake bite, having first sought treatment from a traditional healer. A number of deaths reported by doctors involved patients who arrived at hospital under these circumstances. Several doctors expressed the view that education about snake bite needed to be extended to the broader community, and we strongly agree with this view.

Appropriately conceived and targeted community education has the potential to significantly reduce the burden of snake bite. As with many public health messages, an emphasis on prevention and identification of risky behaviours can lead to reduction in the incidence of the particular disease or injury. Community education messages about snake bite that (a) identify behaviours, activities or situations which increase risk, and (b) provide clear information about biting species, can help to prevent snake bites.

Traditional ideas about snake bite management, and the advice of traditional healers, differ dramatically from conventional medical treatments. The relative lack of access to hospitals carrying effective and safe snake antivenoms, and a lack of confidence in those hospitals, often leads to a significant dependence on non-conventional treatment, including potential dangerous practices such as wound scarification, both at the bite site and at other sites on the bitten limb. The consequences of such injuries include pain; bleeding (particularly when envenomation is associated with a coagulopathy, and life-threatening bleeding may result); infection (potentially including tendon sheath and joint infections, which can lead to chronic disability and pain); and scarring (causing reduced limb mobility and cosmetic deformity) and should, therefore, be discouraged. Given that Cambodians do still heavily rely upon traditional healing practices, we feel there is merit in actively engaging with healers and other non-conventional practitioners as part of community-based education initiatives. We would suggest that efforts be made to teach healers the dangers of practices such as tourniquet use, or wound scarification, and that they be actively encouraged to use and promote safe first aid, such as simple immobilisation techniques for patients with bleeding and swelling, and pressure bandaging for patients with 'sleepiness' or heavy eyelids. Traditional healers could also be taught to recognise that bleeding, 'sleepiness', heavy eyelids or inability to pass urine are critical signs of severe envenoming, and encouraged to persuade these patients to go to hospital.

Young girls in Siem Reap Province studying photographs of venomous snakes during a community interview.

Another important avenue for community-based education is the introduction of appropriate teaching materials, posters and even theatrical experiences in school environments. Children are naturally inquisitive, and this is often one of the reasons why they are disproportionately represented in snake bite statistics. Their smaller body mass often means that they deteriorate more rapidly after snake bite, and since the concentration of circulating venom will be higher as a result of their smaller volume paediatric envenoming is often more severe with higher morbidity and mortality. Educating children about the risks of contact with venomous snakes is a particularly effective way to reduce their own risk taking behaviours, and at the same time ensure that parents are actively engaged, since children will typically talk about the experiences they have at school when they return to the family home.

Posters with simple learning objectives; story books that introduce children to easily assimilated information about how to avoid snake bite; and role-playing class exercises that carry an underlying message about the importance of first aid and the need to attend hospital; could be designed and introduced through joint collaboration with the Ministries of Health and Education, as part of the normal curriculum. For older students, lessons on dangerous snakes could be designed and written into science curriculums.

One of the benefits of improved epidemiological surveillance is that data can be used to identify particular groups that are at higher risk of injury, disability or death following snake bite. For example, it is immediately clear from the age/sex data provided for the Sihanoukville by Mr Doroshenko, that young men between the ages of 15-29 years are at a significantly higher risk of snake bite that women in the same age groups, and members of both sexes from other ages groups. Using this information it would be possible to design public education programmes for this part of Cambodia that specifically target this high risk group, and in so doing potentially reduce this imbalance by identifying the activities that lead these men to be bitten more frequently than other people in their communities. This form of targeted approach will undoubtedly yield greater benefit from the money invested, than a broadly targeted large-scale general programme. Similar targeted

approaches could also be directed at particular high risk occupations. For example oil palm, coffee, tea and rubber plantation workers are at particular risk of envenoming by Malayan pit vipers, white-lipped pit vipers or Malayan kraits. Engagement with plantation owners which leads to provision of improved personal protective equipment (PPE) such as rubber gumboots, or thick gloves in conjunction with targeted information via posters or leaflets might help reduce the incidence of snake bite in this industries. Similarly rice paddy workers in north-western Provinces who are at particular risk of bites from cobras and Russell's vipers would benefit from education programmes that could be run in collaborative with major rice buying cooperatives.

Rural Health Centres

Small, basic rural health centres are often the first destination for the victims of snake bite. These health centres have a vital role to play in determining which patients require urgent medical care, and in stabilising and preparing these very ill people for transport to a larger hospital. This important role in primary triage, initial first aid, emergency care and transport/referral call for a particular skill set. Staff need to be able to accurately assess each new patient quickly, institute emergency treatment for conditions such as shock, haemorrhage or airway compromise, and make well-informed decisions on the need for, feasibility, and safety of referral.

Ward building at a small referral hospital in Banteay Meanchey Province

We therefore propose the establishment of specific short course in the primary care of snake bite patients at rural health centres and minor referral hospitals. The target audience would include medical officers, nursing staff and community volunteers employed at these smaller facilities, and the course would be taught as a one (1) day learning module comprised of lectures and practical demonstrations/workshops. The curriculum has been designed so that one or two trainers could present the material during visits to the participants in their own communities. With a strong emphasis on initial case management needs, the course would comprise of the following modules:

1. **Venomous snakes and envenomation syndromes in Cambodia**

   Overview: An introduction to the diversity of snake species that occur in Cambodia, with specific emphasis on the key syndromes of envenoming by different species, snake distributions and the clinical significance of medically important species. The aim is to provide information and to address several significant misconceptions that have major impacts upon clinical management and clinical outcomes.

2. **First aid for snake bite**

   Overview: Compares and contrasts the advantages and disadvantages of various common snake bite "first aid" or "home remedies" in use throughout Cambodia, and discusses the appropriateness of different first aid techniques, and teaches techniques which are safe and beneficial. Emphasis is on the high desirability of first aid techniques which present minimal risk of harm to the patient.

3. **Resuscitation**

   Overview: It is crucial that medical personnel at all levels are able to recognise the key features and causes of clinical emergencies such as hypovolaemic and septic shock, airway obstruction, respiratory failure and acute renal failure. The emergency treatment of these problems will be taught in this module.

4. **Signs and symptoms of snake bite in Cambodia**

   Overview: Introduces participants to recognising the symptoms and signs of envenoming. The aim of the lecture is to provide participants with a clear understanding of how to differentiate between non-envenomed and envenomed patients accurately and with confidence.

5. **Patient transport and referral**

   Overview: There is evidence in many countries around the world that many snake bite patients either fail to reach primary care, or die during referral transfer to secondary or tertiary care facilities, due to inadequate preparations for transport or to inadequate care during transport. This lecture deals with the requirements for successful patient transport and referral.

6. **Assessment**

   Overview: At the end of the course all participants would be required to sit a formal assessment examination to measure their individual learning success. Participants would also be sent a simple questionnaire one year after completion of the course to assess their skill retention over time, and to determine the relative need for periodic retraining.

This training course should ideally be taught by Cambodian doctors who have been specifically trained to deliver the key messages, and to teach relevant skills. Trainers would be resourced with all of the key training materials (lecture presentations in both English and Khmer; trainer's technical manuals comprising detailed background information, including relevant scientific literature; and training manikins, medical equipment and consumables for use in practical sessions). Local trainers could be supported by one of us during an initial training calendar to assist with course direction, assessment of participants and to provide additional technical support. One year reassessment would be carried out as a collaboration between the local trainers and the consultants.

Advanced training for major hospitals

Taking into account the issues that arose during the consultative interviews at Provincial Referral Hospitals and Provincial Health Departments, we have designed a comprehensive training course in the clinical management of snake bite that addresses all of the key concerns. If the overall treatment of snake bite is to improve it is essential that attending medical officers are adequately prepared and have skills that will facilitate correct diagnosis, early institution of appropriate medical treatment, with relevant and timely reassessment and follow-up care. Like the smaller, simplified course we have designed for rural health centres and district referral hospitals, the objective of this more advanced snake bite management training course is to concentrate on developing sound clinical skills, objective, evidence-based judgement, and to help medical personnel put in place a situationally relevant treatment protocol.

As with the other course, we will emphasize the importance of accurate primary triage, initial first aid, and the ability to recognise urgent conditions such as shock, haemorrhage or airway compromise and successfully resuscitate and stabilise critically ill snake bite patients. Extending beyond this, a key function of this course will be to teach the theory and practice of immunotherapy with antivenoms; the recognition of adverse antivenom events; and their treatment. Specific complications of envenoming by certain species of snakes will be discussed in detail, including the management of coagulopathy, neurotoxic paralysis, acute renal failure and local tissue injury.

The target audience for this Advanced Snake bite Management Course should include medical officers and nursing staff working in National and Provincial hospital Emergency Departments and Critical Care/Intensive Care Units. Doctors and nursing staff from other functional units or from non-governmental hospitals who are involved in the care of seriously envenomed snake bite patients would also benefit from this training. The course is designed to be taught as a three (3) day learning module comprised of lectures and practical demonstrations/workshops.

The curriculum for the Advanced Snake bite Management Course has been designed so that two or three trainers could present the material either during visits to the participants in their own hospitals or to a larger group at a regional training session that could be held for delegates from several adjoining Provinces. The course would comprise of the following modules:

1. **Snakes of Cambodia**

   Overview: An introduction to the diversity of snake species that occur in Cambodia, with specific emphasis on the key features, distributions and clinical significance of medically important species. The aim is to provide information and to address several significant misconceptions that have major impacts upon clinical management and clinical outcomes.

   Specific Focus: This lecture is aimed at improving knowledge of the diversity and distribution of snakes in Cambodia, and in particular, those species that are involved in the majority of envenoming snake bites:

   A. General biology and ecology of snakes
   B. Non-venomous snakes
   C. Venomous snakes
      i. Medically important terrestrial species
         - Malayan pit viper (*Calloselasma rhodostoma*)
         - White-lipped pit viper (*Cryptelytrops albolabris*)
         - Indo-Chinese Russell's viper (*Daboia siamensis*)
         - Monocellate cobra (*Naja kaouthia*)
         - Indo-Chinese spitting cobra (*Naja siamensis*)
         - Malayan or blue krait (*Bungarus candidus*)
         - Other medically important terrestrial species
      ii. Medically important aquatic species
         - True *Hydrophiinae* seasnakes

2. **Cambodian snake venoms and envenomation syndromes**

   Overview: This lecture is designed to provide a basic understanding of the major venom components of medically important Cambodian snakes and their mechanisms of action. The objective is to provide a fundamental explanation for the clinical presentations seen in snake bite patients and of the limitations of treatment. The syndromes of envenoming associated with bites by particular species are discussed.

   Specific Focus: The lecture focuses on major toxins responsible for the clinical effects seen in snake bite patients:

   A. The biological role of snake venoms – why do some snakes have venom?
   B. Snake venom evolution and diversity – short introduction to explain the concept that different snakes have different venoms (therefore effects and treatment varies)
   C. Components of Cambodian snake venoms
      i. Toxins affecting haemostasis
      ii. Cytotoxins, cardiotoxins and myotoxins
      iii. Neurotoxins
      iv. Minor toxins and other components
   D. Envenomation syndromes of Cambodian snake species

3. **First aid for snake bite**

   Overview: Compares and contrasts the advantages and disadvantages of various common snake bite "first aid" or "home remedies" in use throughout Cambodia, and discusses the appropriateness of different first aid techniques, and teaches techniques which are safe and beneficial. Emphasis is on the high desirability of first aid techniques which present minimal risk of harm to the patient.

Specific Focus: This lecture focuses on the use of immobilisation for bites where local swelling or tissue changes have occurred, and the use of pressure-immobilisation for bites dominated by neurotoxicity:

A. Traditional methods
   i. Traditional medicines and herbal applications
   ii. Imported 'traditional' treatments (i.e.: Chinese snake bite tablets)
   iii. Tourniquets and ligatures
   iv. Scarification
   v. Poultices
B. Medically acceptable methods
   i. Immobilisation (for pit viper and cobra bites)
   ii. Pressure Immobilisation Bandaging (for krait and seasnake bites)

4. Resuscitation

Overview: It is crucial that medical personnel at all levels are able to recognise the key features and causes of clinical emergencies such as hypovolaemic and septic shock, airway obstruction, respiratory failure and acute renal failure. The emergency treatment of these problems will be taught in this module.

Specific Focus: To recognise the symptoms, signs and causes of emergencies such as shock, respiratory failure, airway obstruction and acute renal failure, and how to treat them:

A. Immediate recognition of airway, breathing and circulation emergencies
   i. Symptoms, signs and causes of shock in snake bite
   ii. Airway and breathing emergencies
   iii. Acute renal failure
B. Emergency treatment
   i. Basic and advanced resuscitation techniques
   ii. Fluid resuscitation in hypovolaemic shock
   iii. Antibiotic treatment for sepsis
   iv. Acute renal failure
C. Referral and emergency transport considerations for critically ill patients

5. Signs and symptoms of snake bite in Cambodia

Overview: This lecture is designed to introduce the participants to the key features of snake bite that can be recognised by either detectable signs or emphasized symptoms. The aim of the lecture is to provide participants with a clear understanding of the typical presentation features of various snake bite syndromes in Cambodia.

Specific Focus: To teach clinicians how to recognise the symptoms and signs of envenoming:

A. Appearance of the bite site
B. Local symptoms and signs of snake bite
C. Systemic signs and symptoms
   i. Haematological
   ii. Rhabdomyolysis
   iii. Neurological and neurophysiological
   iv. Electrophysiological disturbances
D. Secondary complications of snake bite
   i. Infections and sepsis
   ii. Shock
   iii. Renal failure

6. Patient Assessment & Diagnosis

Overview: The objective of this lecture is to teach participants the essentials of both initial and ongoing patient assessment, diagnosis and monitoring. What we will present is a practical standard protocol that is intended to provide the best possible model for correct diagnosis and the implementation of appropriate early treatment.

Specific Focus: The lecture aims to teach a systematic process for assessing patients and reaching a rational, evidence-based diagnosis:

A. The importance of clear and complete patient histories
B. Patient examination
    i. Bite site presentation and local signs & symptoms of snake bite
    ii. General signs & symptoms of snake bite
    iii. Specific signs & symptoms of snake bite
C. Assessment of signs and symptoms
    i. Advantages and disadvantages of snake bite grading systems
    ii. Serial measurement of local swelling and oedema
D. Fundamental diagnostic assessment tests
    i. 20WBCT Test
    ii. Neurological examination
E. Laboratory Investigations
    i. Relevance and availability of laboratory investigations
    ii. Overview of basic investigations and the interpretation of results
F. Importance of reassessment and patient monitoring

7. Treatment Overview

Overview: The objective of this lecture is to introduce participants to the key issues associated with primary treatment and ongoing clinical management of snake bite patients, particularly in rural or Provincial settings with limited medical resources.

Specific Focus: The aim is to provide a step-wise approach to the treatment of envenoming:

A. Priorities and objectives
B. General treatment and nursing strategy
C. Antivenom
    i. Indications for use
    ii. Selection of the most appropriate antivenom
    iii. Premedication issues, antivenom dosage and administration
    iv. Advantages, disadvantages and limitations
D. Coagulation disturbances
    i. Hazards and complications
    ii. Adjunctive treatment
    iii. Role of FFP (fresh frozen plasma) and other blood products
E. Rhabdomyolysis and myoglobinuria
    i. Hydration, alkalinisation and renal function support
F. Paralysis and neurotoxicity
    i. Airway maintenance, respiratory support and ventilation strategies
    ii. Drug interventions in postsynaptic neurotoxicity
G. Local wound care, pain management and support
    i. Wound cleaning and the need for an aseptic environment
    ii. Blisters, bullae, necrosis and gangrene
    iii. Identification of compartment syndrome
H. Referral and transport of stable envenomed patients to other facilities
    i. Advantages & disadvantages
    ii. Clinical care and nursing during transportation

8. The role and use of antivenom in Cambodia

Overview: The objective is to teach participants the essentials of appropriate antivenom selection and administration. Key components of this module include issues such as antivenom selection, minimum therapeutic doses, administration protocols, recognition and treatment of adverse antivenom reactions, and determination of appropriate clinical endpoints.

Specific Focus: This lecture will address the following topics:

A. The history of antivenoms
B. Appropriate antivenoms for the treatment of snake bite in Cambodia
C. Antivenoms that should not be used
D. Principles of antivenom therapy
    i. Antivenom selection: monovalent vs. polyvalent
    ii. Dose and appropriate route of administration
    iii. Timing of administration
    iv. Premedication
    v. Titration of dose against effects
    vi. Delayed administration
E. Advantages and disadvantages of antivenoms
F. Complications and contraindications
    i. Anaphylactoid reactions
    ii. Pyrogenic reactions
    iii. Delayed Serum Sickness
G. Assessing the success or failure of antivenom
H. Repeat dosing
I. Costs and availability

9. Clinical Assessment and Treatment of Coagulopathy

Overview: Coagulation disturbances are often the major systemic effect of bites by pit vipers such as the Malayan pit viper (*Calloselasma rhodostoma*), white-lipped pit viper (*Cryptelytrops albolabris*) and the Indo-Chinese Russell's viper (*Daboia siamensis*). Clinical bleeding after snake bite in Cambodia can lead to fatal haemorrhages or precipitate hypovolaemic shock. Shock can also be the result of direct cardiotoxicity of some venoms, severe hypoxia, dehydration, or sepsis (in the patient who presents late).

Specific Focus: This lecture will address the following topics:

A. Mechanisms of snake venom coagulation disturbances
    i. Prothrombin activation
    ii. Thrombin-like enzymes
    iii. Platelet function alteration
B. Assessment and recognition of coagulopathy
    i. 20WBCT Test
    ii. Other tests of coagulation status
    iii. Clinical signs and symptoms
    iv. Non-laboratory monitoring of haemostasis
C. Reversal of coagulation disturbances with antivenom
D. Ancillary treatment strategies
    i. Fluid replacement and other adjunctive therapy
    ii. Use of FFP and other blood products

10. Treatment of Acute Renal Failure

Overview: Oliguric acute renal failure is an outcome after some bites by Indo-Chinese Russell's vipers (*Daboia siamensis*), and can also occasionally complicate the management of bites by other species as a result of either poor medical management, or secondary physiological disorders. This lecture deals with the early recognition of renal dysfunction and appropriate management strategies.

Specific Focus: The lecture summarises key causes of renal failure after snake bite, and treatment in Provincial settings:

A. Causes of oliguric acute renal failure after snake bite
   i. Direct nephrotoxicity
   ii. Secondary causes of oliguria in snake bite
   iii. Inadequate medical management
B. Signs, symptoms and diagnosis
C. Early treatment of acute renal failure
D. Peritoneal dialysis
E. Potential complications

11. Clinical Assessment and Treatment of Neurotoxicity

Overview: Neurotoxicity is the major clinical consequence of bites by kraits (*Bungarus* spp.) and marine seasnakes, and can also be present in some victims of cobra (*Naja kaouthia*, *Naja siamensis* or *Ophiophagus hannah*) envenoming. Poorly managed cases can be fatal. This lecture deals with the diagnosis and assessment of progressive neurotoxicity and its treatment.

Specific Focus: The lecture covers the following topics:

A. Mechanisms of neurotoxicity and implications for envenomed patients
   i. Presynaptic neurotoxicity
   ii. Postsynaptic neurotoxicity
   iii. Effects on smooth muscle
B. Assessment and recognition of neurotoxicity
   i. Neurological examination for signs of snake venom neurotoxicity
   ii. Assessment of airway patency and protection
   iii. Peripheral paralysis
C. General treatment strategy
D. Management of patients with respiratory difficulties
   i. Airway management principles
   ii. Supplementary oxygen
   iii. Assisted ventilation strategies in rural health centres
   iv. Assisted ventilation strategies in urban hospitals
   v. Complications associated with various interventions
E. Reversal of neurotoxicity with antivenom
F. Anticholinesterases
   i. Assessment of suitability: the "Tensilon Test"
   ii. Treatment Strategy – Benefits and limitations
   iii. Drug choice
      - Dose and route of administration
      - Concurrent atropine administration
      - Duration of use
   iv. Assessment of benefit
   v. Potential complications and contraindications
G. Urinary catheters

12. Respiratory Management Training Module

Overview: This three hour session is designed as a combination of theory teaching and practical skills instruction and/or demonstration. This lecture teaches practical techniques and strategies for providing airway protection and maintaining respiration.

Specific Focus: This is an intensive module dealing with airway and breathing management:

A. Theory and general principles of respiratory management
B. Basics of ABC (Airway, Breathing, Circulation)
C. Airway maintenance
    i. Use of suction
    ii. Posture
    iii. Basic airway adjuncts
D. Bag and mask ventilation
E. Endotracheal intubation
    i. Indications
    ii. Preparation
    iii. ETT (endotracheal tube) size and length selection
    iv. Intubation techniques
    v. Tube position and security
    vi. Complications
F. Alternatives to ETT
    i. Laryngeal mask airways
    ii. Other alternatives
G. Mechanical ventilation
    i. Equipment
    ii. Ventilation modes and strategies
    iii. Adjuncts (orogastric tubes)
    iv. Patient management: physiological parameters
    v. Complications
H. Nursing issues
    i. Patient positioning and prevention of lung collapse
    ii. Prevention of pressure ulcers
    iii. Physiotherapy (lung and limb)
I. Weaning from mechanical ventilation

13. Treating the Local Effects of Snake bite

Overview: Poor management of the local effects of snake bite can lead to delayed recovery, infection and permanent disability, including the loss of limbs. Appropriate management of the local effects of snake bite in Cambodia is crucial to reducing morbidity and disability. This lecture describes the local effects of envenoming and provides treatment guidelines.

Specific Focus: Treatment of local injury and early rehabilitation:

A. General wound care, functional positioning, cleaning and general nursing considerations
B. Infection risks and appropriate use of antibiotics
C. Compartment syndrome
    i. Clinical assessment of increased intracompartmental pressure
    ii. Techniques for measuring intracompartment pressure
    iii. Management options and surgical intervention
D. Necrosis and gangrene
    i. Surgical debridement
    ii. Alternatives to surgery
    iii. Plastic surgery referral for repair of necrotic injury
E. Rehabilitation and access to disability services

14. Patient transport and referral

Overview: There is evidence in many countries around the world that many snake bite patients either fail to reach primary care, or die during referral transfer to secondary or tertiary care facilities, due to inadequate preparations for transport or to inadequate care during transport. This lecture deals with the requirements for successful patient transport and referral.

Specific Focus: The lecture will focus upon:

A. Reasons for patient referral
B. Timing and appropriateness of referral
C. Patient transport requirements, preparation and escort needs
D. Referral letters

15. Snake bite in children

Overview: Children are particularly vulnerable to the effects of snake venoms and doctors and health workers must recognise this and treat envenomed children with particular thoroughness and diligence. This lecture deals with special considerations related to the treatment of snake bite in children.

Specific Focus: Emphasis is placed on the physiological differences between children and adults and the special considerations that arise because of this:

A. Physiological differences
B. Considerations in relation to the administration of antivenoms
C. Fluids
D. Other pharmacological considerations
E. Case examples

16. Management Plans for Snake bite Patients

Overview: This final topic deals with establishing clear management plans for the consistent assessment and treatment of snake bite patients. Inconsistencies in management protocols frequently contribute to poor outcomes. Participants will be taught how to use algorithms as aids in diagnosis and treatment, and how to develop consistent management plans that can be taught to staff as a means of introducing a minimum standard of care model for all snake bite patients that addresses key problem areas in current treatment methods.

Specific Focus: The focus is on setting functional, practical, understandable guidelines for the consistent assessment, diagnosis, treatment and ongoing management of snake bite:

A. The importance of consistency
B. Standard treatment algorithms
    i. Evidence-based snake identification algorithm for Cambodia
    ii. Evidence-based snake bite management algorithm for Cambodia
C. Standard patient administration
    i. Medical records, data reporting and epidemiological evaluation
    ii. Pathology samples
    iii. Importance of data collection for improving patient care in the future
D. Standard nursing protocols and guidelines

17. Assessment

Overview: At the end of the course all participants would be required to sit a formal assessment examination to measure their individual learning success. Participants would also be sent a simple questionnaire one year after completion of the course to assess their skill retention over time, and to determine the relative need for periodic retraining.

The content of this course will initially be taught to a small cadre of senior doctors from National hospitals in Phnom Penh, and medical officers representing a number of Provincial Referral Hospitals from around the country. Training will extend over five (5) days and will include extended sessions so that detailed discussion of the learning objectives, theory and presentation of the material can take place. The objective is to teach these participants the content of the course, and the basis for each component, so that they in turn can teach the material to their colleagues. Each of these prospective "trainers" will be individually assessed throughout the course, and at completion. Each participant will need to be graded with a content comprehension level of at least 95% in order to be considered suitable to become a future course lecturer. We expect that some participants will have greater aptitude for some components of the course than for others, and consequently some "trainers" should expect to be endorsed to teach specific course modules as 'specialities' while omitted from the future teaching roster of other modules. The final objective will be to produce several course instructor rosters made up of lecturers with the collective ability to teach the overall course to other medical personnel.

The course lectures, participant handouts, assessment materials and lecturers technical manuals will all need to produced in both English and Khmer languages. Detailed lists of the training accessories, including training manikins, medical equipment and consumables for the practical sessions, will be prepared and provided. We recommend that equipment be purchased specifically for the teaching of this course, and that it be packaged, inventoried and stored securely.

The roll-out of the training to the broader medical community should be structured around a series of decentralised courses staged in Provincial centres for carefully selected participants. Although the ultimate choice of trainees is the prerogative of the Ministry of Health, we strongly recommend that during the first year, at least four Provincial courses should be conducted with trainees drawn from the Emergency Departments and Critical Care/Intensive Care Units of Provincial Hospitals and to a lesser extent Operational District Referral Hospitals. During this first round of training courses it would be advantageous for one or more of the WHO consultants to participate in the courses for the purpose of refining the curriculum, evaluating the effectiveness of the local training faculty, and supporting the local faculty with primary assessment of trainees and additional technical support. One year reassessment would be carried out as a collaboration between the local trainers and the consultants.

CONCLUSIONS

The information that was obtained during the various consultative meetings and hospital visits could on the surface be interpreted as painting a particularly dire picture of the likely fate of snake bite patients in Cambodia. By western standards, and even by the standards of some adjacent Southeast Asian countries, the current capacity of the Cambodian health system to treat snake bite is extremely rudimentary, aggravated in particular by widespread lack of medical technology; well-trained clinicians and nurses; and the absence of local clinical toxinology expertise. This reality should however be balanced against the recent history of Cambodia and the very apparent rapid advances that are being made in restoring and revitalising both the health system and the country overall. There are a number of developing country settings which lack Cambodia's tumultuous past, and have ample resources, yet are languishing, or even going backwards in terms of a broad range of health, social and economic benchmarks. On balance while there are a number of fundamental problems with the current clinical management of snake bite, including some sector-wide issues which cannot be resolved easily, such as the need for widespread improvements to Emergency Department and Intensive/Critical Care Unit resourcing, there is almost unanimous recognition that this is the case, and a strong desire both within the Ministry of Health and the broader medical community to address the problem in a practical and pragmatic manner. We are firmly of the belief that commitment shown by the Ministry of Health in seeking to address the problem of snake bite is an extremely positive and encouraging indication that the long-term prognosis for Cambodia's snake bite patients is far better than that of many of its neighbours.

In concluding this report it is important to reiterate some of the key issues:

A. The Ministry of Health currently lacks a formal policy on the prevention and treatment of snake bite in Cambodia. This lack of direction is being addressed through the current project, and it is expected that the development of protocols, training courses, education programmes and basic research capacity proposed here will provide a basis for an appropriate national policy position that can be debated, refined and adopted.

B. Improving currently deficient epidemiological surveillance and clinical research data collection are key requirements to improving decision making capacity, particularly with regard to resource allocation, and forward contingency planning.

C. Cambodia's doctors and nurses require formal treatment protocols and guidelines and access to well-structured, appropriately resourced clinical toxinology training courses and supplementary materials, along with support in the form of qualified clinical advice. Improving the basic clinical skills of medical professionals is an additional advantage of formalised snake bite training, and will result in improved clinical skills in a number of areas, such as resuscitation, infection control, emergency and critical care, airway management and renal disease.

D. A major factor contributing to poor clinical outcomes for snake bite patients is the absence of safe, efficacious and immunologically appropriate antivenoms. Current Indian-made antivenoms are not suitable for the treatment of snakebite in Cambodia, and there is an urgent need to

remove these products from the current inventory and replace them with Thai-made antivenoms that are specific to the species of snakes present in Cambodia. Any failure to address this issue will completely negate any positive outcomes that might arise from improved medical training.

E. Improved clinical training, access to clinical resources, snake bite information and even specific antivenoms will significantly the capacity of hospitals and their staff to provide treatment to snake bite patients, but the benefits will be limited to some extent by a range of public access and confidence issues in the health sector that are beyond the scope of this document to address. Making access to health services affordable is a sector-wide problem, as are issues of public confidence. That being said, improved management of snake bite in hospital will result in some degree of restoration of public confidence in health services.

This report proposes a series of steps designed to address these broad issues by:

1. Providing both an administrative data collection pathway, and independent yet inter-related research pathways to improve epidemiological surveillance, clinical oversight and broad knowledge of envenomation syndromes, resource needs and system costs.
2. Designing and implementing education and training initiatives to improve the knowledge base within the general, medical and public health communities.
3. Identifying current antivenom supply problems and providing recommendations for the use of alternative antivenoms with appropriate specificity, efficacy and safety profiles.
4. Promoting specific clinical toxinology skills development that will improve outcomes by reducing delays in the commencement of specific antivenom treatment; reduce morbidity and disability by reducing unnecessary surgical intervention, while promoting evidence-based procedures that functionally benefit patients; and reduce mortality.