1 INTRODUCTION

Snake antivenom immunoglobulins (antivenoms) are the only therapeutic products for the treatment of envenomings due to snakebites. The unavailability of effective snake anti-venom immunoglobulins to treat the specific types of envenomings encountered in various regions of the world has become a critical health issue at global level. The crisis has reached its greatest intensity in sub-Saharan Africa, but other regions, such as South East Asia, are also suffering from a lack of effective and affordable products.

The complexity of the production of efficient antivenoms, in particular the importance of preparing appropriate snake venom mixtures for the production of hyperimmune plasma (source of antivenom immunoglobulins), the decreasing number of producers and the fragility of the production systems in developing countries further jeopardize the availability of efficient antivenoms in Asia, Africa, the Middle East, and South America. Most of the remaining current producers are located in countries where the application of quality and safety standards needs to be improved.

In October 2005, the WHO Expert Committee for Biological Standardization (ECBS) recognized the extent of the problem and asked the WHO secretariat to support and strengthen world capacity to ensure long-term and sufficient supply of safe and efficient antivenoms. In March 2007, antivenom immunoglobulins were included in the WHO Essential Medicines List (WHO, 2007a), acknowledging their role in a primary health care system.

Urgent measures are needed to support the design of immunizing snake venom mixtures that can be used to make the right polyspecific antivenoms products for various geographical areas of the world. Sustainable availability of effective and safe antivenom immunoglobulins should be ensured and production systems of these effective treatments should be strengthened at global level. Meaningful preclinical assessment of the neutralizing capacity of anti-snake venom immunoglobulins needs to be undertaken before products are used in any country and Medicines Regulatory Authorities should enforce the licensing of these products in all countries, before being used in the population.

The present "WHO Guidelines on Production, Control and Regulation of snake antivenoms immunoglobulins" were developed in response to the above mentioned needs. These Guidelines cover all the steps involved into the production, control and regulation of venoms and antivenoms. It is hoped that this document, by covering comprehensively the current existing experience in the manufacture, control, and preclinical and clinical assessment of these products will serve as a guide to national control authorities and manufacturers to support worldwide production of these essential medicines.

In addition to the need for appropriate antivenoms to be produced, other issues need to be addressed in ensuring both that antivenoms are appropriately used, and that outcomes for envenomed patients improve. These include availability of antivenoms and appropriate distribution policies, affordability of envenoming treatment and training of health workers to allow safe and effective use of antivenoms and effective management of snakebites envenoming. These important issues are outside the scope of this document and so will not be further addressed specifically herein, but should be considered as vital components in the care pathway for envenoming.

2 LIST OF ABBREVIATIONS AND DEFINITIONS USED

The definitions given below apply to the terms used in these Recommendations. They may have different meanings in other contexts.

Apheresis - Procedure whereby blood is removed from the donor, separated by physical means into components and one or more of them returned to the donor.

Antivenoms – A purified fraction of immunoglobulins or immunoglobulin fragments fractionated from the plasma of animals that have been immunized againsts a snake venom or a snake venom mixture (also called antivenins).

Batch - A defined quantity of starting material or product manufactured in a single process or series of processes so that it is expected to be homogeneous.

Batch records - All documents associated with the manufacture of a batch of bulk product or finished product. They provide a history of each batch of product and of all circumstances pertinent to the quality of the final product.

Blood collection - A procedure whereby a single donation of blood is collected in an anticoagulant and/or stabilizing solution, under conditions designed to minimise microbiological contamination of the resulting donation.

Bulk product - Any product that has completed all processing stages up to, but not including, aseptic filling and final packaging.

BVDV - Bovine virus diarrhea virus. An enveloped, single stranded RNA virus that can be used for viral validation studies.

CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora. It is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival.

Clean area - An area with defined environmental control of particulate and microbial contamination, constructed and used in such a way to reduce the introduction, generation, and retention of contaminants within the area.

Combined antivenoms – Antivenoms directed against several venoms prepared by mixing different monospecific plasma prior to the plasma fractionation process, or purified monospecific antivenom fractions prior to the aseptic filling stage.

Contamination - The undesired introduction of impurities of a microbiological or chemical nature, or of foreign matter, into or on to a starting material or intermediate during production, sampling, packaging, or repackaging, storage or transport.

CPV - Canine Parvovirus. A non-enveloped, single stranded DNA virus that can be used virus for viral validation studies.

Cross-contamination - Contamination of a starting material, intermediate product or finished product with another starting material or product during production.

Cross-reactivity – The ability of an antivenom raised against a venom, or a group of venoms, to react and neutralize the toxic effects of the venom of a related species not included in the immunizing mixture.

Dessication – A storage process where venoms are dehydrated under vaccum in the presence of calcium salts or phosphoric acid.

ED_{50} - Median Effective dose: quantity of antivenom that protects 50% of the mice injected with a number of LD_{50}s of venom.

EAV – Equine arteritis virus, an enveloped virus found in horses; this virus has been used in viral validation studies.

EIA – Enzyme immunoassay.

EMCV - Encephalomyocarditis virus. A non-enveloped, single stranded, RNA virus that can be used for viral validation studies.

Envenoming - Process by which venom is injected into a human by the bite of a poisonous snake, leading to pathological manifestations (also called envenomation).

Fab – A monovalent immunoglobulin fragment resulting from the proteolytic digestion of immunoglobulins by papain.

$F(ab')_2$ – A bivalent immunoglobulin fragment resulting from the proteolytic digestion of imunoglobulins by pepsin.

FCA – Freund complete adjuvant. It may be used in the immunization process of animals to enhance the immune response to venoms

FIA – Freund incomplete adjuvant.

FPLC – Fast-performance-liquid-chromatography

Fractionation - Large-scale process by which animal plasma is separated to isolate the immunoglobulin fraction, that is further processed for therapeutic use or may be subjected to digestion with pepsin or papain to generate immunoglobulin fragments. The term fractionation is usually used to describe a sequence of processes, generally including plasma protein precipitation and/or chromatography, ultrafiltration, and filtration steps.

Fractionator - A company or an organization performing plasma fractionation to manufacture antivenom immunoglobulins or fragments.

GMP - Good Manufacturing Practice- that part of Quality Assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization or product specification. It is concerned with both production and quality control.

HPLC – High-performance-liquid-chromatography

Immunization- a process by which an animal (typically horse or sheep) is injected with venom (s) to produce a long-lasting and high titer antibody response against the lethal and other deleterious components in the immunogen.

Immunoglobulin – Antibody molecule generated by immunizing an animal (most often horse) against a snake venom or a snake venom mixture. Immunoglobulin G (IgG) is the most abundant immunoglobulin fraction.

IgG – Immunoglobulin G, the lost abundant immunoglobulin fraction.

IgM – Immunoglobulin M.

In-process control - Checks performed during production to monitor and, if necessary, to adjust the process to ensure that the antivenom conforms to specifications. The control of the environment or equipment may also be regarded as part of in-process control.

LD_{50} - Lethal dose 50%: it is the amount of snake venoms, injected intravenously or intraperitonaly, that leads to the death of 50% of the animals of a group after an established period of time (usually 24 to 48 hrs)

Manual apheresis – A plasma collection procedure where whole blood is collected and erythrocytes are reinfused to the animal within 24 hours.

Manufacture - all operations of procurement of materials (including collection of plasma for fractionation) and products, production, quality control, release, storage, distribution, and quality assurance of plasma-derived medicinal products.

Milking - The process of collecting venom from live snakes.

Monospecific - defines antivenoms that are limited in use to a single species of venomous snake or to a few closely related species whose venoms show clinically effective cross neutralisation with the serum. The term "monovalent" is often used and has the same meaning.

Nanofilter - Filters, most typically with effective pore sizes of 50 nm or below, designed to remove viruses from protein solutions.

NRA - National Regulatory Authorities. WHO terminology to refer to national medicines regulatory authorities. Such authorities promulgate medicine regulations and enforce them.

Pasteurization- A process of heating protein in solution, typically at 60°C for 10 hours

Plasma- the liquid portion remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure.

Plasmapheresis- Procedure in which whole blood is removed from the donor, the plasma is separated from the cellular elements by sedimentation, filtration, or centrifugation, and at least the red blood cells are returned to the donor

Polyspecific - Defines antivenoms that are obtained by fractionating the plasma from animals immunized by a mixture of venoms from several species of venomous snakes. The term "polyvalent" is often used and has the same meaning.

Prion- A particle of protein that is thought to be able to self-replicate and to be the agent of infection in a variety of diseases of the nervous system, such as mad cow disease and other transmissible spongiform encephalopathies (TSE). It is generally believed not to contain nucleic acid.

PRV- Pseudorabies virus, a lipid-enveloped virus that can serve as a model for pathogenic equine herpesvirus.

Production- all operations involved in the preparation of anti-snakevenoms immunoglobulins, from preparation of venoms, immunization of animals, collection of blood or plasma, processing and packaging, to its completion as a finished product.

PRV- Pseudo Rabies virus. An enveloped, double stranded DNA virus that can be used as a model for viral validation studies.

Quarantine- A period of enforced isolation and observation typically to contain the spread of an infectious disease among animals. The same terminology applies to the period of isolation used to perform quality control of plasma prior to fractionation, or of antivenom immunoglobulins prior to release and distribution.

SDS-PAGE – Sodium dodecyl sulphate – polyacrylamide gel electrophoresis

Serum- A liquid portion remaining after clotting of the blood. Serum has a composition similar to plasma (including the immunoglobulins) apart from fibrinogen and other coagulation factors which constitute the fibrin clot.

Sindbis virus- An enveloped, single stranded, RNA virus that can be used as a model for viral validation studies.

SOP - Standard operating procedure. An authorized written procedure giving instructions for performing operations not necessarily specific to a given product or material (e.g. equipment operation, maintenance and cleaning; validation; cleaning of premices and environmental control; sampling and inspection). Certain SOPs may be used to supplement product-specific master and batch production documentation.

Toxin - A poisonous substance, especially a protein, which is produced by living cells or organisms and is capable of causing disease when introduced into the body tissues. It is often also capable of inducing neutralizing antibodies or antitoxins.

Traceability - Ability to trace each individual snake, venom, animal, or unit of blood or plasma used and the final fractionated antivenom immunoglobulin batch. The term is used to describe forward and reverse tracings.

TSE - Transmissible spongiform encephalopathy

Venom - The toxic secretion of a specialized venom gland which, in the case of snakes, is delivered through the fangs and provokes deleterious effects. Venoms usually comprise many different protein components of variable structure and toxicity.

Validation - Action of proving, in accordance with the principles of GMP, that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

Viral inactivation- A process of enhancing viral safety in which viruses are intentionally "killed".

Viral reduction- A process of enhancing viral safety in which viruses are inactivated and/or removed.

Viral removal- A process of enhancing viral safety by partitioning viruses from the protein(s) of interest.

VSV- Vesicular Stomatitis virus. An enveloped, single stranded RNA virus that can be present in horses, and that can be used for viral validation studies.

WFI – Water for injection.

WNV - West Nile virus. An enveloped, single stranded RNA virus that can be present in horses, and that can be used for viral validation studies.

3 GENERAL CONSIDERATIONS

Snake antivenom immunoglobulins (henceforth referred to as antivenoms) are the only specific treatment for envenoming by snakebites. They are produced by the fractionation of plasma obtained usually from large domestic animals hyper-immunized against relevant venoms. Important but low use antivenoms may be prepared in small animals. When injected into an envenomed human patient, antivenom will neutralize any of the venoms used in its production, and will also in some instances neutralize venoms from closely related species.

3.1 Historical background

Shortly after the identification of diphtheria and tetanus toxins, von Behring and Kitasato reported the antitoxic properties of the serum of animals immunized against diphtheria or tetanus toxins and suggested the use of antisera for the treatment of these diseases (von Behring and Kitasato, 1890). In 1894, von Behring diphtheria antitoxin was first successfully administered by Roux to save children suffering from severe diphtheria. Thus, serum therapy was born and the antitoxin was manufactured by Burroughs Wellcome in UK. The same year, Calmette (1894) and Physalix and Bertrand (1894) simultaneously, but independently, presented during the same session of the same meeting their observations on the antitoxic properties of the serum of rabbits and guinea pigs immunized against cobra and viper venoms, respectively. Immediately after his discovery of "antivenin serum-therapy", Albert Calmette was actively involved in proving its efficacy in the treatment of human envenoming. The first horse-derived antivenom sera that he prepared were already under clinical evaluation in 1895 by Hankin in India and by Lépinay in Viet Nam. The latter reported the first successful use of antivenin serum therapy in patients in 1896 (Calmette, 1897).

3.2 The use of serum versus plasma as source material

Historically, the pioneers Calmette, Vital Brazil and others, used serum separated from the blood of hyperimmunized horses for the preparation of antivenom ("antivenin serum-therapy"). Later, antibodies (immunoglobulins) were demonstrated to be the active molecules responsible for the therapeutic action of "antivenom serum". Subsequently, immunoglobulins, or immunoglobulin fragments ($F(ab')_2$), purified from serum were used instead of crude serum (Pope, 1939a; 1939b).

Nowadays, plasmapheresis, whereby erythrocytes are re-injected to the animal within 24 hours of blood collection, is commonly employed to reduce anaemia in the hyperimmunized animal that donates the plasma. Accordingly, it is, almost exclusively, plasma rather than serum that is used as the starting material for the extraction of the immunoglobulin or its fragments (WHO, 1981; Raw et al., 1991; Grandgeorge et al., 1996). Thus "antivenom immunoglobulin" is the preferred term, rather than "anti-snakebite serum" or "antiserum" which is no longer accurate.

3.3 Antivenom purification methods and product safety

Purification methods were introduced to reduce the frequency of antivenom reactions by removing the Fc fragment from IgG, thus preventing complement activation and perhaps reducing the intensity of immune-complex formation responsible for late antivenom reactions (serum sickness). For 60-70 years, IgG $F(ab')_2$ fragments have been widely used. However, antivenom protein aggregation, and not Fc-mediated complement activation, was increasingly identified as a major cause of antivenom reactions.

The recent use of intact IgG prepared by precipitation of non-IgG plasma proteins using caprylic acid, revealed that such IgG preparations could be less reactogenic than some conventional $F(ab')_2$ preparations (Otero-Patiño et al., 1998). Thus, a critical issue in antivenom safety likely lies in the physico-chemical characteristics of antivenoms and not exclusively in the type of neutralizing molecules constituting the active substance.

It is also important to ensure that the current methodologies to produce antivenoms provides a sufficient margin of safety with regards to the risk of transmission of zoonosis.

3.4 Pharmacokinetics and pharmacodynamics of antivenoms

Rapid elimination of some therapeutic antivenoms (e.g. when Fab fragments are used) has led to recurrence of envenoming in patients. However, the choice of preparing IgG or fragments appears to depend on the size and toxicokinetics of the principal toxin(s) of the venoms. Large M_r bivalent antibodies (IgG and $F(ab')_2$ fragments) may be effective for the complete and permanent neutralization of intravascular toxins (e.g. procoagulant enzymes) which have a long half-life in envenomed patients, whereas low M_r and monovalent IgG fragments such as Fab may be more appropriate against low molecular mass neurotoxins which are rapidly distributed to their tissue targets and are rapidly eliminated from the patient's body (Gutiérrez et al., 2003)

3.5 Need for National and Regional reference venom preparations

Antivenom production is technically demanding. The need to design appropriate polyspecific antivenoms is supported by the difference in venom composition among venomous animals, associated with the fact that (a) most countries are inhabited by several medically important species, and (b) that in many circumstances there is no distinctive clinical syndrome to direct the use of monospecific antivenoms. However, similarities in the venom toxins of closely related venomous species may result in para-specific neutralization thus reducing the number of venoms required for the preparation of polyspecific antivenoms. Para-specific neutralization should be tested in animal models and ideally by clinical studies in envenomed patients. Preclinical testing of antivenoms against medically important venoms present in each geographical region or

country is a pre-requisite for product licenses and batch approval, and should always preceed clinical use in envenomed patients. This requires efforts by manufacturers and/or regulators to establish regional or national reference venom preparations that can be used to test the neutralization capacity of antivenoms.

4 EPIDEMIOLOGY BACKGROUND

The incidence of snakebites in different parts of the world and the recognition of the particular species of greatest medical importance is fundamental to the appropriate design of monospecific and polyspecific antivenoms in countries and regions. Updated knowledge is therefore highly relevant to antivenoms producers and regulators, especially for the selection of the most appropriate venoms, or venom mixtures, to be utilised in the manufacture and quality control of antivenoms.

4.1 Global burden of snakebites

Envenoming and deaths resulting from snakebites are a particularly important public health problem in rural tropical areas of Asia, Africa, Latin America and Papua New Guinea (WHO, 2007b). Agricultural workers and children are the most affected groups. Epidemiological assessment of the true incidence of global snakebite mortality and morbidity has been hindered by several well recognised problems (Swaroop and Grabb, 1954; Chippaux 1998). Snakebites and associated mortality are under-reported because many victims (20-70% in some studies) do not seek treatment in government dispensaries or hospitals and hence are not recorded. This occurs because medical posts in regions of highest incidence are unable to keep accurate records and because death certification of snakebite is often imprecise, despite the increased precision of recent ICD indexing (Snow *et al.*, 1994; Fox *et al.*, 2006). Correctly designed population surveys, in which questionnaires are distributed to randomly selected households in well demographed areas, are the only reliable method for estimating the true burden of snakebites in rural areas. Results of the few such surveys that have been performed have produced surprisingly high rates of bites, deaths and permanent sequelae of envenoming (Hati et al., 1992; Pugh et al., 1980; Sharma et al., 2004; Snow et al., 1994; Trape et al., 2001). However, because of the heterogeneity of snakebite incidence within countries, results of surveys of local areas cannot be extrapolated to give total national values. Most of the available data suffer from these deficiencies and, in general, should be regarded as underestimates and approximations. Despite these limitations, the available information suggests that the total number of snakebite envenomings worldwide may be around 2.5 million cases per year, with more than 100,000 deaths (Chippaux, 1998). In addition, the number of people left with permanent sequelae as a result of these envenomings is likely to be higher than the number of fatalities (WHO, 2007b). As already identified, most of the estimated burden of snakebite is from South and Southeast Asia, Sub-Saharan Africa, and Central and South America.

The current literature on snakebite epidemiology highlights the inadequacy of available data on this neglected tropical disease. It reinforces the need to improve reporting and record keeping of venomous bites in health facilities, support financially high quality epidemiological studies of snakebite in different regions, and improve the training of medical personnel. Making venomous bites notifiable and fully implementing the use of the International Statistical Classification of Diseases and Related Health Problems 10th Revision (WHO 2007c) in official death certification (e.g. T 63.0 snake venom) would further help to determine the burden of snakebite more accurately (see available data on snakebites from countries in the different regions in Appendix).

4.2 Main recommendations

In most parts of the world, snakebites are under-reported and in some parts completely unreported. This deficiency in surveillance and the paucity of properly designed epidemiological studies explain why the impact of this important public health problem has remained for so long unrecognised and neglected.

National health authorities should be encouraged to improve the scope and precision of their epidemiological surveillance of this disease by:

- **Improving the training of all medical personnel so that they are more aware of the local causes, manifestations and treatment of venomous bites**
- **Making venomous bites notifiable diseases**
- **Setting up standardized and consistent epidemiological surveys**
- **Improving the reporting and record keeping of venomous bites by hospitals, clinics, dispensaries and primary health care posts, relating the bites to the causative snakes venomus species wherever possible**
- **Fully implementing the use of the International Statistical Classification of Diseases and Related Health Problems 10th Revision version for 2007 in official death certification (e.g. T 63.0 snake venom[1]).**

[1] http://www.who.int/classifications/apps/icd/icd10online/

5 MEDICALLY IMPORTANT VENOMOUS SNAKES

5.1 Essential medically important venoms

Based on current herpetological and medical literature, it is possible to partially prioritize the species of snakes that are of greatest medical importance in different regions. Detailed statistics on the species of snakes responsible for envenomings and fatalities throughout the world are lacking, except for a few epidemiological studies which include rigorous identification of the biting snake in a few scattered localities. Thus, establishing a list of medically important species for different countries relies, at least in part, on extrapolation from the few known studies, as well as on the biology of the snake species concerned: e.g. where species of a group of snakes are known to be of public health importance, based on epidemiological studies, it seems reasonable to deduce that closely related species with similar natural history occurring in hitherto unstudied regions are also likely to be medically important. Examples include Asian cobras (*Naja*) in several understudied regions of Asia, lowland *Bungarus* species in Asia, and spitting cobras (*Naja*) in Africa.

Tables 1-4 list the most medically important venomous snake species in each of four broad geographical regions. Species listed in these tables are either those which are common or widespread in areas with large human populations and which cause numerous snakebites, resulting in high levels of morbidity, disability or mortality amongst victims, or poorly known species that are strongly suspected of falling into this category, or species which cause major and life threatening envenoming responsive to antivenom, but are not common causes of bites. The venoms of these species should be considered a starting point for establishing the most important targets for antivenom production. The need for additional epidemiological and toxinological research to better define which venoms to include and exclude for antivenom production in various regions, territories and countries around the world is emphasised.

Detailed country-specific data on species believed to contribute most to the global burden of injury, and/or which pose the most significant risk of morbidity or mortality are provided in Appendix.

Table 1: Medically important venomous snakes: Africa and the Middle East

North Africa/Middle East
Atractaspis andersonii; Bitis arietans; Cerastes cerastes; Cerastes gasperettii; Daboia mauritanica; Daboia palaestinae; Echis carinatus; Echis coloratus; Echis omanensis; Echis pyramidum; Macrovipera lebetina; Montivipera xanthina; Naja haje; Naja nigricollis; Naja oxiana; Pseudocerastes persicus
Central Sub-Saharan Africa
Naja nigricollis; Bitis arietans; Bitis gabonica; Bitis nasicornis; Dendroaspis jamesoni; Dendroaspis polylepis; Echis leucogaster; Echis ocellatus; Echis pyramidum; Naja anchietae; Naja haje; Naja melanoleuca
Eastern Sub-Saharan Africa
Bitis arietans; Bitis gabonica; Bitis nasicornis; Dendroaspis angusticeps; Dendroaspis jamesoni; Dendroaspis polylepis; Naja nigricollis; Echis pyramidum; Naja anchietae; Naja annulifera; Naja ashei; Naja haje; Naja melanoleuca; Naja mossambica
Southern Sub-Saharan Africa
Bitis arietans; Dendroaspis angusticeps; Dendroaspis polylepis; Naja anchietae; Naja annulifera; Naja mossambica; Naja nigricincta; Naja nivea
Western Sub-Saharan Africa
Bitis arietans; Bitis gabonica; Bitis nasicornis; Bitis rhinoceros; Cerastes cerastes; Echis jogeri; Echis leucogaster; Echis ocellatus; Dendroaspis jamesoni; Dendroaspis viridis; Dendroaspis polylepis; Naja haje; Naja katiensis; Naja melanoleuca; Naja nigricollis

Table 2: Medically important venomous snakes: Asia and Australasia

Central Asia
Echis carinatus; Gloydius halys; Macrovipera lebetina; Naja oxiana
East Asia
Bungarus multicinctus; Cryptelytrops *albolabris; Daboia russelli; Deinagkistrodon acutus; Gloydius blomhoffii; Gloydius brevicaudus; Naja atra; Protobothrops flavoviridis; Protobothrops mucrosquamatus; Viridovipera stejnegeri*
South Asia
Bungarus caerulus; Bungarus ceylonicus; Bungarus niger; Bungarus sindanus; Bungarus walli; Cryptelytrops erythurus; Daboia russelii; Echis carinatus; Hypnale hypnale; Macrovipera lebetina; Naja naja; Naja oxiana; Naja kaouthia
South-East Asia *(Excluding Indonesian West Papua)*
Bungarus candidus; Bungarus magnimaculatus; Bungarus multicinctus; Bungarus slowinskii; Calloselasma rhodostoma; albolabris; Cryptelytrops erythurus; Cryptelytrops insularis; Daboia siamensis; Deinagkistrodon acutus; Naja atra; Naja kaouthia; Naja mandalayensis; Naja philippinensis; Naja samarensis; Naja siamensis; Naja sputatrix; Naja sumatrana
Australo-Papua *(Includes Indonesian West Papua)*
Acanthophis laevis; Notechis scutatus; Oxyuranus scutellatus; Pseudonaja affinis, Pseudonaja nuchalis, Pseudonaja textilis

Table 3: Medically important venomous snakes: Europe

Central Europe
Vipera ammodytes
Eastern Europe
Vipera berus
Western Europe
Vipera aspis, Vipera berus

Table 4: Medically important venomous snakes: the Americas

North America
Agkistrodon bilineatus, Agkistrodon contortrix, Agkistrodon piscivorus, Agkistrodon taylori Bothrops asper, Crotalus adamanteus; Crotalus atrox; Crotalus helleri, Crotalus horridus; Crotalus oreganus; Crotalus simus; Crotalus scutulatus; Crotalus totonacus; Crotalus viridis
Caribbean
Bothrops cf. *atrox* (Trinidad); *Bothrops caribbaeus* (St Lucia); *Bothrops lanceolatus* (Martinique); *Crotalus durissus* (Aruba)
Central America
Bothrops asper; Crotalus simus
South America
Bothrops alternatus; Bothrops asper; Bothrops atrox; Bothrops brazili; Bothrops bilineatus; Bothrops diporus; Bothrops jararaca; Bothrops jararacussu; Bothrops leucurus; Bothrops matogrossensis; Bothrops moojeni; Bothrops pictus; Bothrops venezuelensis; Crotalus durissus; Lachesis muta

5.2 Minor venomous species for which antivenoms may be needed

In many countries, there are species of snakes that rarely bite humans but are capable of causing severe or fatal envenoming. Their medical importance may not justify inclusion of their venoms in the immunizing mixture for production of polyspecific antivenoms but the need to prepare antivenoms against these species needs to becarefully analysed. .

5.2.1 Paraspecific coverage by existing antivenoms

In some cases, such as with some Central American pit vipers (genera *Agkistrodon, Porthidium, Bothriechis, Atropoides* etc.), there is clinically effective cross-neutralisation of venoms by standard national polyspecific antivenoms (Theakston & Warrell, 1991).

5.2.2 Need for specific antivenoms

In other cases, there is no effective paraspecific neutralisation and so national manufacturers may consider that the production of a small volume of monospecific antivenom is justifed for use in the rare but potentially fatal cases of envenoming provided that these can be identified. Such antivenoms are currently available for envenoming by the boomslang (*Dispholidus typus*), desert black snake (*Walterinnesia aegyptia*), Arabian burrowing asp (*Atractaspis andersoni*) (Ismail et al., 2007), king cobra (*Ophiophagus hannah*), Malayan krait (*Bungarus candidus*) (Chanhome et al., 2002) "yamakagashi" (*Rhabdophis tigrinus*) and red-necked keelback (*R. subminiatus*), Martinique's "Fer-de-lance" (*Bothrops lanceolatus*), St Lucia's *B. caribbaeus*, some species of American coral snake (*Micrurus*).

No antivenoms are currently available for envenoming by species such as African bush vipers (*Atheris, Proatheris* etc.), berg adder (*Bitis atropos*) and several other small southern African Bitis spp. (e.g. *B. peringueyi*), Sri Lankan and south-west Indian humpnosed vipers (*Hypnale* spp.) (Joseph et al., 2007, Ariaratnam et al., 2008), many Asian pit vipers ("*Trimeresurus*" sensu lato), some species of kraits (e.g. *B. niger*) and all but one species of burrowing asp (genus *Atractaspis*).

An alternative for antivenom production against species that induce few, but potentially severe accidents, is to manufacture polyspecific antivenoms for broadly distributed groups that have similar venom compositions (e.g. African *Dendroaspis and Atractaspis*; Asian *'green pit vipers'*; American *Micrurus*). This may result in antivenoms that offer broad protection against venoms from minor species within genera, or species whose bites are less frequent than those of others in the same taxonomic groups (i.e.: Genus, Sub-Family or Family).

5.2.3 Sea snake venoms

Although venomous marine sea snakes have not been included in the tables of medically important snakes from each region/country, it is important to recognise that there are a number

of species of marine snakes with potent venoms that can cause illness or death. Available evidence, particularly clinical experience, indicates that the current major sea snake antivenom commercially available, which uses venom of a single sea snake, *Enhydrina schistosa*, plus a terrestrial Elapid, *Notechis scutatus*, in the immunising venoms mixture, is effective against envenomings by other sea snakes for which there are clinical data. Further research would be needed to better define the full extent of para specific protection offered by this antivenom against other sea snake species.

5.3 Taxonomy of venomous snakes

Recognising the species causing the greatest public health burden, designing and manufacturing antivenoms and optimising patient treatment are all critically dependant on a correct understanding of the taxonomy of venomous snakes. Like other sciences, the field of taxonomy is constantly developing. New species are still being discovered regularly, and many formerly recognised widespread species have been found to comprise multiple separate species as scientists obtain better information, often with new technologies. As the understanding of the relationships among species is still developing, the classification of species into genera is also subject to change. The listings of venomous species used in this guideline follow the current taxonomic nomenclature at the time of writing (July, 2008). Some groups of venomous snakes remain understudied and poorly known. In those cases, the classification best supported by what evidence exists is presented with the limitation that new studies may result in new nomenclatural changes.

Clinicians, toxinologists, venom producers and antivenom manufacturers have therefore an absolute responsibility to remain abreast of these nomenclatural changes. These often reflect improved knowledge of the homogeneity of snake populations, and may have implications for venom producers, researchers and antivenom manufacturers. Although taxonomic changes do not necessarily indicate the presence of "new" venoms, they strongly suggest that toxinological and epidemiological research into these "new" taxa may be required to establish their medical relevance, if any.

Since some of the names of medically important species have changed in recent years, the following points are intended to allow readers to relate the current nomenclature to former literature information:

- The large group of Asian pit vipers that for many years were referred to a single genus (*Trimeresurus*), have been split into a number of new genera (e.g: *Cryptelytrops, Parias, Peltopelor, Himalayophis, Popeia, Viridovipera, Ovophis, Protobothrops*, with a few species retained in *Trimeresurus*) based on current views of the inter-relationships between these groups. There are divergent views on this approach to the taxonomy of these snakes, and interested parties should consult the literature. Medically important species formerly classified in *Trimeresurus* include *Cryptelytrops albolabris, C. erythrurus, C. insularis, Protobothrops flavoviridis, P. mucrosquamatus* and *Viridovipera stejnegeri.*

- It is likely that new species of cobra (*Naja* spp.) will be identified within existing taxa in both Africa and Asia; three new species (*N. ashei, N. mandalayensis* and *N. nubiae*) have been described and several subspecies elevated to specific status since 2000 (*Naja annulifera* and *N. anchietae*, from being subspecies of *N. haje*), in addition to the recent synonymisation of *Boulengerina* and *Paranaja* within *Naja*. Such changes may hold significance for antivenom manufacturers and should stimulate further research to test whether existing antivenoms cover all target snake populations.

- Several medically important vipers have been reclassified: *Daboia siamensis* has been recognised as a separate species from *Daboia russelii*; *Macrovipera mauritanica* and *M. deserti* have been transferred to *Daboia*, the Central American rattlesnakes formerly classfied with *Crotalus durissus* are now *Crotalus simus*, and *Bothrops neuwiedi* has been found to consist of a number of different species, three of which (*B. neuwiedi, B. diporus, B. mattogrossensis*) may be of public health importance.

5.4 Main recommendations

- **Identification of the medically important venomous snakes (those that cause the greatest burden of injury, disability and/or mortality) is a critical pre-requisite to meeting the need for efficacious antivenom.**
- **Improving the quality of the available data, correcting and amplifying the level of geographic detail and precision of attribution should be an important priority.**
- **Clinicians, toxinologists, poison centers, regulators, venom producers and antivenom manufacturers should be well-informed of current nomenclature and new taxonomy changes, so as to ensure the currency of information, correct identification of species in their countries, and correct selection and sourcing of venoms used in the manufacture of antivenoms. Potentially transient and sometimes conflicting nature of taxonomic nomenclature may occur.**

6 ANTIVENOMS DESIGN: SELECTION OF SNAKE VENOMS

An accurate selection of snake venoms is critical for the production of antivenoms that have the capacity to cover the majority of cases of envenoming in a given geographical region, territory or country. The composition of snake venoms is very complex and a high inter- and intra-species variation has been documented. Therefore, in contrast with other animal-derived immunoglobulins (e.g. tetanus or rabies immunoglobulins), the design of the antigenic mixture to be used in antivenom manufacture is a critical and delicate task that should be carefully considered.

The selection of the most appropriate snake venoms for the production of antivenoms needs to be carefully analyzed and should take into consideration:

1. The geographical region where the antivenom is going to be used.
2. The medically most relevant snakes from the geographical region where the antivenom is going to be used.
3. The variability of venom composition within the region of distribution of a snake species.
4. The information on paraspecific protection of antivenoms against the venoms of species not included in the mixture of venoms used to immunize animals for antivenom manufacture.

6.1 Selection and preparation of representative venom mixtures

The information provided in the Appendix presents an updated list of the most relevant species of snakes, from a medical standpoint, in the various regions and countries of the world. Manufacturers should consider, as a priority, the venoms of species included in the category 1 of this Appendix for the design of venom mixtures for immunization. Venoms to include in a venom pool used for animal immunization should be selected on the basis of the geographical region where an antivenom is intended to be distributed. On a case by case basis, venoms from species listed in category 2 of the Appendix could be included in an immunizing mixture.

There are variations in venom composition and antigenicity within the geographical range of a single taxonomic species as well as other causes of intra-species variation (such as changes according to the age of the specimens) (Warrell, 1997; Fry et al., 2003). Therefore, pooled representative samples of venoms should be prepared from snakes of different geographical origins and ages (see Section 7 on venom preparation). Paraspecific neutralization of venoms outside the range of venoms used for immunization may extend the range of therapeutic applications of some antivenoms. Results of preclinical potency testing may be used to identify a potential crossed-neutralization capacity of antivenoms, which should subsequently be confirmed by clinical testing in envenomed patients. *In vitro* immunological cross-reactivity should not be used as the single basis for recommending therapeutic use of an antivenom outside the range of venoms used in its production.

6.2 Manufacture of monospecific or polyspecific antivenoms

A major issue in designing antivenoms is to define whether they should have monospecific or polyspecific activity.

6.2.1 Monospecific antivenoms

Monospecific antivenoms are limited in use to a single species of venomous snake or to a few closely related species whose venoms show clinically effective cross-neutralisation with such monospecific antivenoms. These conditions apply in areas where:

1. there is only one medically important species (e.g. *Vipera berus* in the United Kingdom and Scandinavia),
2. a simple blood test, amenable for use even in peripheral health care centres, can define the biting species (e.g. detection of incoagulable blood by the 20 minute whole blood clotting test in the northern third of Africa where only *Echis* spp. cause coagulopathy),
3. a simple algorithmic approach allows the species to be inferred from the pattern of clinical features,
4. there is reliable and affordable rapid immunodiagnostic test readily available allowing to identify the toxins unambiguously

However, most countries are inhabited by several medically important species of snakes, where there may be no distinctive clinical syndrome to direct the use of a monospecific antivenom. In these cases, the manufacture of polyspecific antivenoms should be highly recommended.

6.2.2 Polyspecific antivenoms

Some clinicians are prejudiced against using polyspecific antivenoms, by fear of inherently lower potency than monospecific antivenoms. This is not necessarily the case. Polyspecific antivenoms may be obtained in two ways:

- They can be generated by immunizing animals with a mixture of venoms from various snake species. The resulting antivenom will then contain antibodies against venom components of various snake species. When a polyspecific antivenom is produced this way, by immunizing an animal with venoms from several taxonomically-related snakes (e.g. different vipers), the titre of neutralising antibodies against individual venoms may be higher than in a monospecific antivenom produced by immunizing an animal with only a single venom (Raweerith and Ratanabanangkoon, 2005). However, this synergistic immune response may not occur when the venoms are from taxonomically unrelated snakes (e.g. an elapid and a viper)