Investigation of the coagulant effects of Sri Lankan snake venoms and the efficacy of antivenoms

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*The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library**, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.*

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Maduwage Kalana Prasad

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Maduwage Kalana Prasad

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I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Maduwage Kalana Prasad

DEDICATION

I dedicate this thesis to

My beloved parents

Without whom, this could never occurred.

I also dedicate this thesis to

Rohan Pethiyagoda and his family

For their invaluable support throughout my higher studies

and constantly being there for me at time of needs.

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LIST OF PUBLICATIONS INCLUDED AS PART OF THIS THESIS

1. 2013. **Kalana Maduwage**, Fiona E. Scorgie, Seyed Shahmy, Fahim Mohamed, Chandana Abeysinghe, Harendra Karunathilake, Lisa F. Lincz, Christeine A. Gnanathasan, Geoffrey K. Isbister. Factor deficiencies in Hump-nosed pit viper (*Hypnale hypnale*) envenoming. *Clinical Toxicology* 51: 527-31.

2. 2015. Isbister GK, **Maduwage K**, Scorgie FE, Shahmy S, Mohamed F, Abeysinghe C, et al. Venom Concentrations and Clotting Factor Levels in a Prospective Cohort of Russell's Viper Bites with Coagulopathy. *PLoS Neglected Tropical Diseases* 9(8): e0003968. doi:10.1371/journal.pntd.0003968.

3. 2014. **Maduwage K**, O'Leary MA, Scorgie FE, Shahmy S, Mohamed F, et al. Detection of Venom after Antivenom Is Not Associated with Persistent Coagulopathy in a Prospective Cohort of Russell's Viper (*Daboia russelii*) Envenomings. *PLoS Neglected Tropical Diseases* 8(12): e3304. doi:10.1371/journal.pntd.0003304.

4. 2014. **Maduwage K**, O'Leary M, Isbister GK. Diagnosis of snake envenomation using a simple Phospholipase A_2 assay. *Nature Scientific Reports* 4 (4827): DOI:10.1038/srep04827 (2014).

5. 2015. **Maduwage KP**, Scrogie FE, Lincz LF, O'Leary MA, Isbister GK. Procoagulant snake venoms have differential effects in animal plasmas: Implications for antivenom testing in animal models, *Thrombosis Research*, 137; 174–177.

6. 2016. **Maduwage K,** Silva A, O'Leary M, Hodgson WC, Isbister GK. Efficacy of Indian polyvalent snake antivenoms against Sri Lankan snake venoms: lethality studies or clinically focussed *in vitro* studies. *Nature Scientific*. *Reports*. **6**, 26778; doi: 10.1038/srep26778 (2016).

7. 2014. **Maduwage K**, Buckley NA, de Silva HJ, Lalloo DG, Isbister G. Snake antivenom for snake venom induced consumption coagulopathy (Protocol). *Cochrane Database of Systematic Reviews* 2014, Issue 12. Art. No.: CD011428. DOI: 10.1002/14651858.CD011428.

8. 2015. **Maduwage K**, Buckley NA, de Silva HJ, Lalloo DG, Isbister GK. Snake antivenom for snake venom induced consumption coagulopathy (Review). *Cochrane Database of Systematic Reviews*, Issue 6. Art. No.: CD011428. DOI: 10.1002/14651858.CD011428.pub2.

9. 2014**. Maduwage K**, Isbister GK. Current Treatment for Venom-Induced Consumption Coagulopathy Resulting from Snakebite. *PLoS Neglected Tropical Diseases*. 8 (10): e3220. doi:10.1371/journal.pntd.0003220.

LIST OF OTHER PUBLICATIONS DURING CANDIDATURE

(Not included as a part of the thesis)

1. 2015. Isbister GK, **Maduwage K**, Saiao A, Buckley NA, Jayamanne SF, Seyed S, et al. Population Pharmacokinetics of an Indian F(ab')2 Snake Antivenom in Patients with Russell's Viper (*Daboia russelii*) Bites. *PLoS Neglected Tropical Diseases* 9(7): e0003873. doi:10.1371/journal.pntd. 0003873.

2. 2015. O'Leary MA, **Maduwage K**, Isbister GK. Detection of venom after antivenom administration is largely due to bound venom. *Toxicon*. 93: 112-115.

3. 2014. Isbister GK, **Maduwage K**, Page CB. Antivenom cross neutralisation in a suspected Asian pit viper envenoming causing severe coagulopathy. *Toxicon* 90: 286-90.

4. 2013. Isbister GK, **K. Maduwage**, S. Shahmy, F. Mohamed, C. Abeysinghe, H. Karunathilake, CA Ariaratnam, NA Buckley. Diagnostic 20-min whole blood clotting test in Russell's viper envenoming delays antivenom administration. *Quarterly Journal of Medicine* 106(10):925-32.

5. 2013. O'Leary M, **Maduwage K**, Isbister, GK. Use of immunoturbidimetry to detect venom-antivenom binding using snake venoms. *Journal of Pharmacology and Toxicological Methods* 67: 171-181.

6. 2013. **Maduwage K**, Isbister GK, Silva A, Bowatta S, Mendis S, Gawarammana I. Epidemiology and clinical effects of Hump-nosed pit viper (Genus: *Hypnale*) envenoming in Sri Lanka *Toxicon* 61: 11-15.

7. 2016. **Maduwage K**, O'Leary M, Silva A, Isbister GK. Detection of Snake Venom in Post-Antivenom Samples by Dissociation Treatment Followed by Enzyme Immunoassay *Toxins 8*, 130; doi:10.3390.

CONFERENCE PRESENTATIONS DURING CANDIDATURE

1. 2015. Snake antivenom for snake venom induced consumption coagulopathy. *Cochrane Database of Systematic Reviews*. Asia Pacific Association of Medical Toxicology (APAMT), Perth, Australia. (Poster presentation). Conference proceedings, page 56.

2. 2015. Effect of pro-coagulant snake venoms on different animal plasma. Asia Pacific Association of Medical Toxicology (APAMT), Perth, Australia. (Platform presentation). Conference proceedings, page 25.

3. 2015. Snake antivenom for snake venom induced consumption coagulopathy. *Cochrane Database of Systematic Reviews* Australian Society for Medical Research (ASMR) conference, Sydney, Australia (Poster presentation). Conference proceedings, page 40.

4. 2015. Effect of pro-coagulant snake venoms on different animal plasma. Australian Society for Medical Research (ASMR) conference, Hunter Medical Research Institute, Newcastle (March) Australia (Poster presentation). Conference proceedings, page 25.

5. 2015. Efficacy of two Indian polyvalent snake antivenoms against coagulopathy and neurotoxicity of Sri Lankan snake venoms. Australian Society for Medical Research (ASMR) conference, HMRI, Newcastle (March) Australia (Platform presentation). Conference proceedings, page 24.

6. 2014 Diagnosis of snake envenoming using simple phospholipase A_2 assay. North American Centre for Clinical Toxicology (NACCT) conference, New Orleans, USA (Platform presentation, (**Informa Health Care Award for 2015 for best Platform presentation**). *Clinical Toxicology*, 52, page 686.

7. 2014 Diagnosis of snake envenoming using simple phospholipase A_2 assay. The Australian Society for Medical Research New South Wales conference, Sydney Australia. (Poster presentation, (**ASMR Award for Best overall scientific presentation**). Conference proceedings, page 49.

8. 2014. Measurement of venom and clotting functions of Russell's viper envenoming and response to antivenom treatment. European Association of Poisons Centres and Clinical Toxicology (EAPCCT), Brussels, Belgium (Platform presentation). *Clinical Toxicology* (Supplement) 52; 347.

9. 2014 Diagnosis of snake envenoming using simple phospholipase A_2 assay. Conference of Toxicology and Poisoning network of Australasia (TAPNA), Newcastle, Australia (Platform presentation). Conference proceedings, page 15.

10. 2014 Diagnosis of snake envenoming using simple phospholipase A_2 assay. The Australian Society for Medical Research Satellite Scientific Meeting, Hunter Medical Research Institute, Newcastle, Australia. (Poster presentation, **John Morris Scientific Award for the Best overall poster presentation**). Conference proceedings, page 34.

11. 2013. Venom recurrence in Russell's viper (*Daboia russelii*) envenoming in Sri Lanka. 12th Asia Pacific Association of Medical Toxicology Conference (APAMT), Dubai (Platform presentation). Conference proceedings, page 94.

12. 2013. Assessment of efficacy and effectiveness of snake antivenoms in Asia. 12th Asia Pacific Association of Medical Toxicology Conference (APAMT), Dubai, (**Keynote presentation**). Conference proceedings, page 43.

13. 2013. Assessing the efficacy of antivenoms for Sri Lankan venomous snakes. Conference of Toxicology and Poisoning network of Australasia (TAPNA), Newcastle, Australia. (**Keynote presentation**). Conference proceedings, page 14.

14. 2012. Clotting studies and factor deficiencies of Hump-nosed pit viper (*Hypnale hypnale*) envenoming in Sri Lanka, 11th Asia Pacific Association of Medical Toxicology Conference, Hong-Kong (Platform presentation). Hong Kong Journal of emergency Medicine. 19 (6); 427.

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SYNOPSIS

Coagulopathy is the commonest systemic effect of snake envenoming. Despite this there is limited information on the severity and time course of venom-induced consumption coagulopathy (VICC) and the effect of antivenom. Evidence of the efficacy and effectiveness of antivenom is vital to continue antivenom treatment for envenoming. There is increasing evidence that early administration of antivenom is essential, but there is a lack of diagnostic tests of envenoming that can be used to decide on antivenom administration.

The broad aim of this project was to investigate the procoagulant effects of Sri Lankan snake venoms, and the efficacy and effectiveness of antivenoms against these effects. In addition, the study aimed to explore novel methods of testing for envenoming and for the presence of venom in blood.

Snake envenoming cases in Sri Lanka were used with the collection of serial clinical and laboratory data, and blood samples from patients admitted to hospitals in Sri Lanka. Coagulopathy from hump-nosed pit viper and Russell's viper envenoming was investigated by analyzing citrated samples from envenomed patients. Identification of the snake species was by venom specific sandwich enzyme immunoassay (EIA). Antivenom efficacy was assessed in a series of *in-vitro* and *in-vivo* animal studies. Antivenom effectiveness was assessed by undertaking two systematic reviews: Cochrane review of placebo randomized controlled trials and a systematic review of prospective and other controlled trials of antivenom for VICC.

The results provide a much better description of VICC using clotting times and factor levels in both hump-nosed pit viper and Russell's viper envenoming, showing prolonged clotting times and different factor deficiencies. Phospholipase A_2 enzyme levels were investigated as a diagnostic test for snake envenoming and will be key to improving outcomes in snake bite cases as it will allow early identification of envenomed patients so antivenom can be given when it is most effective. The efficacy of two Indian antivenoms was assessed, which showed one to be more efficacious but more importantly explored the difference between lethality studies and clinically focused *in vitro* studies. Two systematic reviews and antivenom for VICC revealed a lack of placebo controlled randomized trials, but that some comparative clinical trials and observational studies provide information on the effectiveness of antivenom.

OVERVIEW

Aims and Objectives

The broad aims of this project were to investigate the coagulant effects of venoms from Sri Lankan snakes, and to investigate diagnostic tests for snake envenoming, as well as the efficacy and effectiveness of antivenoms. To fulfil these aims, the proposed PhD study program consisted of three main study themes.

1. A description of the coagulopathy in hump-nosed pit viper and Russell's viper envenoming including clotting times and clotting factor concentrations.
2. Development of diagnostic tests for envenoming.
3. Investigation of the efficacy and effectiveness of snake antivenoms

Methodology

Ethical approval for demographic data, clinical data and the human blood sample collection from Sri Lankan snake envenomed patients was sought and approval granted by the Ethical Review Committee, Faculty of Medicine, University of Peradeniya, Sri Lanka (approval number: 2011/EC/46 and 2008/EC/26) and and Animal Ethical Review Committee (2012/008), Monash University, Australia. Ethical approval was granted by the University of Newcastle, Australia, (approval number: H2010-1060) for the work done at the University of Newcastle. Informed written consent was obtained from all patients who provided blood samples and clinical information. All subjects had the right to withdraw their participation at any time during the study if they wished.

The majority of the data used for the thesis was from snake envenoming cases in Sri Lanka. This involved the collection of serial clinical and laboratory data, and blood samples from patients admitted to hospitals in Sri Lanka with snake bites. The investigation of coagulopathy in Sri Lankan snake envenoming (Hump-nosed pit viper and Russell's viper) was undertaken by analyzing citrated samples from envenomed patients, including measuring clotting times and clotting factor levels. Development of a diagnostic test for snake envenoming used the same serial blood collection, but used serum samples in which venom concentrations and phospholipase A_2 enzyme levels were measured. Identification of the snake species involved in cases was by a venom specific sandwich enzyme immunoassay (EIA), which has been used widely in the

past to confirm snake identity. Antibodies to Sri Lankan snake venom were prepared especially for this project.

A major issue with evaluating the benefits of antivenom is separating the assessment of the 'efficacy' and 'effectiveness' of antivenoms. We defined the 'efficacy' of antivenom as the ability to bind and neutralise venom-mediated effects under ideal conditions (*in vitro* studies and animal studies of binding and neutralisation) and the 'effectiveness' of antivenom as its ability to reverse or prevent envenoming in human patients and ultimately improve patient outcomes (Isbister, 2010a). Antivenom efficacy was assessed in a series of *in vitro* and *in vivo* animal studies: measurement of the *in vitro* ability of antivenom to bind venom; measurement of antivenom neutralization of *in vitro* coagulopathy and neurotoxicity; and the measurement of the lethal dose 50 (LD_{50}) and effective dose 50 (ED_{50}) in mice. Antivenom effectiveness for the treatment of coagulopathy was assessed by undertaking two systematic reviews: 1) a Cochrane review of placebo randomized controlled trials of antivenom for venom-induced consumption coagulopathy (VICC); and 2) a systematic review of prospective and other controlled trials of antivenom for VICC.

To fulfil these aims, the PhD program consisted of nine chapters covering three sections of study as follows:

1. Coagulopathy in snake envenoming:
    a. Investigate the coagulopathy of hump-nosed pit viper envenoming in Sri Lanka
    b. Investigate the coagulopathy of Sri Lankan Russell's viper envenoming
    c. Explore the correlation between recurrence of venom and the coagulopathy in Russell's viper envenoming

2. Diagnostic tests of snake envenoming:
    a. Investigate the use of snake venom phospholipase A_2 enzyme levels as a diagnostic test of snake envenoming.

3. Efficacy and effectiveness of snake antivenom
    a. Assess the efficacy of Indian snake antivenoms in binding Sri Lankan snake venoms in neutralising coagulopathy and neurotoxicity they cause and preventing lethality.
    b. Explore the susceptibility of various animal plasmas to different procoagulant snake venoms.

c. Cochrane systematic review of snake antivenom for venom-induced consumption coagulopathy.
d. Systematic review of other study designs for antivenom in snake venom induced consumption coagulopathy.

Outcomes

The results of this thesis will provide a much better description of venom-induced consumption coagulopathy using clotting times and factor levels in both hump-nosed pit viper and Russell's viper envenoming. This will provide the basis for evaluating the effectiveness of treatments in VICC, including the use of antivenom and factor replacement. Development of phospholipase A_2 enzyme levels as a diagnostic test for snake envenoming is a key to improving outcomes in snake bite because it will allow the early identification of envenomed patients so that antivenom can be given when it is most effective. Assessment of the efficacy and the effectiveness of antivenoms will provide evidence for the use of antivenom in snake bite and therefore improve clinical outcomes. Understanding the difference between efficacy and effectiveness will result in antivenom being used where it is going to produce the most benefit. In this thesis the efficacy of two Indian antivenoms, VINS and BHARAT, was assessed against four Sri Lankan snake venoms. An important issue that arose during these studies was the role of animals in assessing efficacy for human envenoming, and in particular against coagulopathy. It was found that different animal plasmas had different susceptibility to procoagulant snake venoms, and that only rabbit plasma had a similar susceptibility to human plasma. A systematic review of both placebo randomized controlled trials, comparative clinical trials and observational studies was used to assess the effectiveness of antivenom for VICC.

Link to publications

1. 2013. **Maduwage K**, Scorgie FE, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, Lincz LF, Gnanathasan CA, Isbister GK. **Factor deficiencies in Hump-nosed pit viper (*Hypnale hypnale*) envenoming**. *Clinical Toxicology* 51: 527-31.

Although hump-nosed pit viper bites are the commonest cause of snake bite in Sri Lanka, there is limited information on the coagulopathy that occurs. In this paper we measured serial clotting time tests and clotting factor levels of proven hump-nosed pit

viper envenomings in Sri Lanka to understand the effect of venom on the clotting cascade. The study describes the pattern and severity of factor deficiencies and clotting times to better characterise the severity of hump-nosed viper coagulopathy and the potential pathophysiology.

2. 2015. Isbister GK, **Maduwage K**, Scorgie FE, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, O'Leary MA, Gnanathasan CA, Lincz LF. **Venom Concentrations and Clotting Factor Levels in a Prospective Cohort of Russell's Viper Bites with Coagulopathy**. *PLoS Neglected Tropical Diseases* 9(8): e0003968. doi:10.1371/journal.pntd.0003968.

Few studies have been published on the severity and time course of coagulopathy in Russell's viper envenoming, despite procoagulant toxins from Russell's viper venom being well characterised as factor V and factor X activators. The recovery of clotting factor levels and clotting times following antivenom administration was poorly characterised with most studies using whole blood clotting times. This paper reports the initial severity and range of abnormal clotting times and factor levels (fibrinogen, factor V, factor VIII and factor X) and then the recovery of after antivenom treatment. In addition, the study found initially high levels of factor VII, VIII and IX which was unusual and thought to be a result of the toxin interfering with the assays *in vitro*.

3. 2014. **Maduwage K**, O'Leary MA, Scorgie FE, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, Lincz LF, Gnanathasan CA, Isbister GK. **Detection of Venom after Antivenom Is Not Associated with Persistent Coagulopathy in a Prospective Cohort of Russell's Viper (*Daboia russelii*) Envenomings**. *PLoS Neglected Tropical Diseases* 8(12): e3304. doi:10.1371/journal.pntd.0003304.

Venom recurrence (i.e. detection of venom following undetectable venom immediately post-antivenom) has been reported after envenoming by a number of different vipers and is believed to be associated with recurrence of envenoming due to insufficient antivenom. It has been reported a number of times with Russell's viper envenoming and its significance remains unclear. This study demonstrates that the detection of venom recurrence is not associated with the recurrence of coagulopathy but does occur in patients with initially higher venom concentrations. The explanation for "venom recurrence" appears to be a result of the venom assay measuring bound venom which is shown in an associated publication (2015. O'Leary MA, **Maduwage K**, Isbister GK. Detection of venom after antivenom administration is largely due to bound venom. *Toxicon*. 93: 112-115.)

4. 2014. **Maduwage K**, O'Leary M, Isbister GK. **Diagnosis of snake envenomation using a simple Phospholipase A2 assay.** *Nature Scientific Reports* 4 (4827): DOI:10.1038/srep04827 (2014).

Currently there is no single test that can be used to diagnose envenoming in snake bite patients. The decision to give antivenom has to be based on a constellation of clinical features and laboratory tests, which in some cases may be falsely negative or falsely positive (e.g. whole blood clotting time for coagulopathy - 2013 Isbister GK, **K. Maduwage**, S. Shahmy, F. Mohamed, C. Abeysinghe, H. Karunathilake, CA Ariaratnam, NA Buckley. Diagnostic 20-min whole blood clotting test in Russell's viper envenoming delays antivenom administration. *Quarterly Journal of Medicine*), or develop too late (signs of paralysis). The ability to give antivenom treatment before the development of clinical effects is vital to prevent irreversible effects. This paper is a proof of concept study that shows the potential use of phospholipase A_2 (PLA_2) enzyme levels to diagnose snake envenoming in a range of Sri Lankan snakes and one Australian snake. There was a highly significant correlation between PLA_2 levels and venom concentrations.

5. 2015. **Maduwage KP**, Scorgie FE, Lincz LF, O'Leary MA, Isbister GK. **Procoagulant snake venoms have differential effects in animal plasmas: Implications for antivenom testing in animal models**, *Thrombosis Research*, 137; 174–177.

Although animal models are routinely used to assess the efficacy of snake antivenom, there is little data to support the relevance of animal studies to human envenoming. There is no published information on the susceptibility of different animal plasmas to the procoagulant toxins in snake venoms. This paper describes the wide variation in the susceptibility of seven different animal plasmas to four different procoagulant snake venoms. It demonstrates the significant potential limitation of using animal models to assess the efficacy of antivenom for venom-induced consumption coagulopathy.

6. 2016. **Maduwage K,** Silva A, O'Leary M, Hodgson WC, Isbister GK. **Efficacy of Indian polyvalent snake antivenoms against Sri Lankan snake venoms: lethality studies or clinically focussed *in vitro* studies**. *Nature Scientific. Reports*. **6**, 26778; doi: 10.1038/srep26778 (2016).

Despite Indian antivenom being used for decades to treat snake bite in Sri Lanka, there are no published studies on the assessment of these antivenoms for Sri Lankan snake venoms. In addition, the World Health Organisation currently recommends ED_{50} studies as the 'gold standard' for testing antivenom efficacy. However, such testing ignores the fact that venoms may affect animals and humans in different ways. This paper assesses the efficacy of two Indian antivenoms against Sri Lankan snake venoms and demonstrates that one is superior to the other based on *in vitro* binding, coagulation and neurotoxicity studies. It also demonstrates that ED_{50} studies were inferior in comparing the efficacy of the two antivenoms and future studies should use binding and clinically relevant *in vitro* studies.

7. 2014. Maduwage K, Buckley NA, de Silva HJ, Lalloo DG, Isbister G. **Snake antivenom for snake venom induced consumption coagulopathy (Protocol).** *Cochrane Database of Systematic Reviews*, Issue 12. Art. No.: CD011428. DOI: 10.1002/14651858.CD011428.

8. 2015. **Maduwage K**, Buckley NA, de Silva HJ, Lalloo DG, Isbister GK. **Snake antivenom for snake venom induced consumption coagulopathy (Review).** *Cochrane Database of Systematic Reviews* 2015, Issue 6. Art. No.: CD011428. DOI: 10.1002/14651858.CD011428.pub2.

A Cochrane systematic review was undertaken to investigate the evidence for antivenom effectiveness in VICC. The study found an absence of published placebo randomized controlled trials of antivenom for VICC.

9. 2014**. Maduwage K**, Isbister GK. **Current Treatment for Venom-Induced Consumption Coagulopathy Resulting from Snakebite**. *PLoS Neglected Tropical Diseases*. 8 (10): e3220. doi:10.1371/journal.pntd.0003220.

This systematic review further investigated the effectiveness of antivenom for VICC but included a broader range of studies and study designs including randomised comparative studies, non-randomised studies comparing antivenom to no treatment

and prospective observational studies. The review found few studies that compare antivenom to no antivenom treatment, and that antivenom appears to be effective for VICC in some snakes (Australian elapids), but not for others (*Echis* spp. – carpet vipers).

LIST OF ABBREVIATIONS

aPTT	Activated partial Thromboplastin Time
ED50	Effective dose 50
EIA	Enzyme immunoassay
FFP	Fresh Frozen Plasma
INR	International Normalized Ratio
LD50	Lethal dose 50
PLA_2	Phospholipase A_2
PT	Prothrombin time
MCD	Minimum Clotting Dose
MDD	Minimum Defibrinogenating Dose
MHD	Minimum Hemorrhagic Dose
MMD	Minimum Myotoxic Dose
MND	Minimum Necrotic Dose
SHR	Systemic Hypersensitivity Reactions
TCT	Thrombin Clotting Time
TLEs	Thrombin like enzymes
VICC	Venom induced consumption coagulopathy
WBCT20	20 Minutes Whole Blood Clotting Test
WHO	World Health Organisation

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Table 12. Clinical effects of hump-nosed pit vipers from two different studies. (Ariaratnam et al., 2008), Maduwage et al., 2013).

Table 13. Summary of clinical effects of Sri Lankan snake envenoming based (Kularatne 2003, 2009; Ariartnam et al, 2008; Kularatne et al, 2008; Kularatne et al., 2011; Maduwage et al., 2013 and Gnanathasan et al., 2012).

Table 14. Different preclinical antivenom assessment tests (Anonymous, 2010, Gutierrez et al., 1985, Theakston and Reid, 1983, Laing et al., 1992, Jene et al., 1989, Gutierrez et al., 1992, Chettya et al., 2004).