Snakebites in the Brazilian Amazon: Neglecting effective antivenoms to a neglected population

Dr. Manuela Pucca
**SNKEBITES**

- 2,7 million bites
- 130,000 deaths
- 400,000 disabilities

*Snakebites predominantly affect the poor*

2017 - WHO categorized snakebite envenoming into the Category A - Neglected Tropical Diseases

*Incidence et mortalité par animaux venimeux dans les pays tropicaux* by Jean-Philippe Chippaux, Médecine Tropicale, 2008, 68(4), 334–39
Brazil registers about 30,000 snakebites each year, with high impact in the Brazilian Amazon.

**INCIENCE per 100,000 people (2020)**

- **Brazil**: 17
- **BR Amazon**: 48
- **Roraima**: 65 (State - highest snakebite incidence in Brazil)
- **Yanomami**: 200 (Cancer (190 / 100,000))
LEGAL AMAZON

33% Savannas

67% Tropical Forest

Rio Branco
(White River)
40% Yanomami

- 11 indigenous ethnicities
- 32 indigenous communities
- 8 different spoken languages

INCIIDENCE per 100,000 people (2020)

<table>
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<tr>
<th>Region</th>
<th>Incidence</th>
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<td>Brazil</td>
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Snakebites in Roraima
The administration of antivenom based on polyclonal antibodies produced in horses is the only specific treatment available against snakebites.

Questions and obscure information regarding antivenoms
ISSUE 1: Are the heterologous polyvalent antivenoms effective?

The literature says that polyvalent antivenoms are only 25-30% effective (while that is actually not proven).

- Crotalid antivenom (recommended for all Brazilian rattlesnakes)
  
  Produced by horses immunized with *C. d. terrificus* and *C. d. collilineatus*.

- Bothropic antivenom (recommended for all pitvipers from *Bothrops* genus)
  
  Produced by horses immunized with 5 species of *Bothrops*: *B. neuweid*, *B. moojeni*, *B. jararacussu*, *B. alternatus*, *B. jararaca*. 
Brazil: 6 subspecies (*Crotalus* genus)

We are conducting clinical studies in Roraima to show evidences that the antivenom is much less effective for our unique rattlesnake.
Brazilian Pitvipers
Brazil: 29 species (Bothrops genus)

Campos et al., Toxicon 76 (2013) 1–10
How an unique polyclonal antivenom formulation can neutralize all these toxins from all these species?

If death occurs after antivenom administration, how to confirm the cause of death – caused from venom or antivenom?
ISSUE 2: Since we do not have any clinical studies, what confirms the effectiveness & safety of antivenoms?

In Brazil, none randomized, placebo-controlled, double-blind trial have ever been conducted.

Clearly, there are also limitations in the design of this study because the product varies in each delivery.
How could a regulatory agency evaluate efficacy and approve different products?

Horses are a biological system with a diverse genetic and immune system which cannot provide an uniform product quality at the end of the manufacturing process.
We have many health issues because of foreign bodies injected into human bodies.

Anaphylaxis (10%, 40%)

Pirogenic reaction

Serum Sickness (7-15%, 56%)

70% are non-neutralizing Abs

ISSUE 3: Are heterologous antivenoms safe?
ISSUE 4: Antivenom shortage specially for population in remote regions.

- For indigenous communities, it may take days to obtain health care.
- Subsequently, with this delay, the antivenom is no longer useful in reversing the effects of envenoming.

Itineraries of the study participants expressed graphically from the time from the bite to hospital admission.

ISSUE 5: The use of equines as living producers of antivenoms confront us with ethical concerns.

1894 - A revolutionary treatment to cure diphtheria wrote heterologous serotherapy into the history of modern medicine.

In early 1895, the Pasteur Institute had about 136 horses, and a production of over 7,000 litters of blood/month.

“...equine are the victims to our progress in immunology”
Over a three-month period, twenty-four injections totalling an amount of venom equivalent to 1,512 scorpion stings was inoculated into one horse.

The horse's reactions throughout the immunisation process were described in detail:

“With each injection, [the horse] demonstrated intense reaction to pain, showing widespread trembling, breathlessness, nasal and tear hypersecretion, a rise in body temperature, intense sweating. Such symptoms lasted no more than 12 hours”.

This study also presents one of the rare records on deaths of horses used as serum producers by the Butantan Institute, during the years 1947 and 1948.
Weight loss, unresponsiveness to keep title of antibodies, and fractures due to the accidental falls taken by weakened animals, indicated that the horse would be submitted to ‘TOTAL BLEEDING’.

**TOTAL BLEEDING**: consisted of inoculating in the animal saline solution that kept circulatory mechanism, delaying haemorrhagic shock, to allow the animal to survive until all the blood could be withdrawn, in a procedure that could take as long as two days.

The intense worldwide demand for hyperimmunized plasma has written a crude trajectory of horse exploitation which are still not entirely dimensioned.
How come antivenoms are being administered extensively & promoted as if it is well proven and following the horse welfare?
Do we have an alternative treatment???

Questions and obscure information regarding antivenoms

Is it real effective?

Is it safe?

Is it an ethical therapy?

Is it available for all?
WHO – MAY 2019

Snakebite Envenoming
A strategy for prevention and control

Fig. 2: Strategic objectives, target and implementation phases
What are we doing to change the ancient use of horses as an antibody machine?

There were many progresses in antibody discovery technologies, antibody engineering approaches, and antibody manufacturing.

We need novel antivenoms composed of mAbs to eliminate animal-derived antibodies and to make available more effective and safe antivenoms.
Phage Display (since 2006)

Discovered of fully human mAbs targeting animal-derived toxins

Although many efforts have been done, it is far to have effective human antivenoms available
1998 – MRC – Medical Research Council

PhD, MD, José Elpidio Barbosa

Expression of Human Recombinant Antibody Fragments Capable of Partially Inhibiting the Phospholipase Activity of Bothrops jararacussu venoms

Human antibody fragments specific for Bothrops jararacussu venoms reduce the toxicity of other Bothrops sp. venoms

Therapeutic monoclonal antibodies: scFv patents as a marker of a new class of potential biopharmaceuticals

Human scFv antibodies (Afribumabs) against Africanized bee venom: Advances in melittin recognition

Expression of recombinant human antibody fragments capable of inhibiting the phospholipase and myotoxic activities of Bothrops jararacussu venom

Serrumbas: A human monoclonal antibody that counters the biochemical and immunological effects of Tityus serrulatus venom

Serrumbas: A novel human single chain-fragment antibody with multiple scorpion toxin-neutralizing capacities

Production of Human Antibody Fragments Binding to Melittin and Phospholipase A2 in Africanised Bee Venom: Minimising Venom Toxicity

History of Envenoming Therapy and Current Perspectives

In vivo Update: Current Knowledge on Bee Venom and Bee Envenoming Therapy

Identification of cross-reactive human single chain variable fragments against phospholipase A2 from Bothrops jararacussu and Bothrops sp. venom

Biochimica et Biophysica Acta (BBA) - General Subjects

Discovery of human scFvs that cross-neutralize the toxic effects of B. jararacussu and B. d. terrificus venoms

Acta Tropic
BASIC
- Bioprospecting venom compounds
- Performing Genomics and Proteomics
- Discovering fully human antivenoms

CLINICAL
- Following snakebite victims in hospital and health centers
- Inside the Yanomami Community

EDUCATIONAL PROGRAM
- Educating the local population (including indigenous)
- Training the health professionals
- Mitigate the local Snakebite Problem
Antibody Engineering & Therapeutics

Europe

Delivered as a Hybrid Event, 7 - 9 June, 2023
Live & In-Person Event: 7 - 9 June, Pestana Hotel Amsterdam. Digital Experience: All Sessions Livestreamed 7 - 9 June

PEGiação de toxinas animais:
Um passo à frente para aplicações terapêuticas?

MODERADORA
Dr. Manuela B. Fucca
(coordenadora do projeto Snakebite Roraima)

PALESTRANTE
Dr. Ernesto L. Pinheiro-Júnior
(KU Leuven, Bélgica)
• Snakebite Webinars

Patofisiologia dos envenenamentos por serpentes na América Latina
DIA 7 DE NOVEMBRO DE 2020
9:30 hs (GMT-4)

Panorama do ofídismo na Amazônia Brasileira
DIA 5 DE DEZEMBRO DE 2020
9:30 hs (GMT-4)

Produitos derivados de toxinas de serpentes: da bancada ao paciente
DIA 8 DE FEVEREIRO DE 2021
9:30 hs (GMT-4)

Serpentes peçonhentas, acidentes ofídicos e etnobiologia no oeste da Amazônia
DIA 27 DE MARÇO DE 2021
9:30 hs (GMT-4)

Toxinas botrônicas: identificação, ação e neutralização
DIA 24 DE ABRIL DE 2021
9:30 hs (GMT-4)

Epidemiologia dos acidentes ofídicos no Estado do Espírito Santo
DIA 28 DE MAIO DE 2021
9:30 hs (GMT-4)

Cobras corais: explorando a peçonha e o tratamento
DIA 28 DE JUNHO DE 2021
9:30 hs (GMT-4)

Coagulopatias nos envenenamentos botrônicos
DIA 8 DE JULHO DE 2021
9:30 hs (GMT-4)

Avaliação local e cuidados com a lesão após acidente ofídico
DIA 18 DE AGOSTO DE 2021
9:30 hs (GMT-4)
Snakebite Training Program

- High School
- Agrotechnical School
- Medical School
- Nursing School
- Physicians, nurses and health staff
- Health team – Indigenous Community
• **Snakebite Prevention and Control Program**

Provide basic information on preventive measurements in different Roraima communities

- Rural Population
- Indigenous
- Army
- Venezuelan migrants (>50.000)
- Others
ILLUSTRATED SNAKEBITE INFORMATIVE MATERIAL

- Portuguese ✓
- English ✓
- Spanish ✓
- Yanomami ✓
- Wapichana ✓
- Macuxi ✓
- Yek’wana ✓

Different Languages

Free

Distribution & Download
MASS MIDIA
Prevention, first aid, effective and safe treatment, and well-trained medical staff to allow many victims to return more quickly to good health and lives.
Dr. Isadora S. de Oliveira

BACKGROUND
Since 2017, snakebites are classified as category A of the Neglected Tropical Diseases (NTDs), affecting more than 10 million people worldwide, with around 100,000 deaths and around 300,000 amputations or other permanent disabilities. In Brazil, snakebite envenomings are mostly caused by pit vipers from Bothrops genus (~60%), distributed throughout the national territory. Pit-viper-derived venoms are mainly composed of metalloproteases (~50-70%), phospholipases (~7-40%) and serine proteases (~2-24%), varying quantitatively between them, making the venoms hemorrhagic, proteolytic, and coagulant, characteristics that are responsible for many of their local and systemic manifestations. Moreover, it is known that metalloproteases are one of the components responsible for pain induced during envenomation, and this pain does not stop even with the administration of antivenom. Unfortunately, the unique treatment for these envenomings (e.g. heterologous antivenom) is of scarce availability, since they only can be produced in a few laboratories, and this does not happen in all countries, where there is no access to antivenom. For this reason, new technologies for antivenom development are necessary, such as the phage display technique, which allows the selection of fully human antibodies against the most usual antigens.

GOALS
In this study, we aimed the selection and production of fragments of human monoclonal antibodies (scFvs) against jararhagin, a hemorrhagic metalloproteinase from Bothrops jararaca, through phage display.

METHODS
Jararhagin
Rabbit

RESULTS

CONCLUSION AND FUTURE STEPS
This study reports the selection of the first human antibody against metalloproteases from Bothrops snakes, which could be included in the formulation of a new generation of antivenom serum. These monoclonal phage-antibodies will be used for in vitro and in vivo experiments, with the aim of evaluating their potential to produce suitable anti-toxin antibodies, and their ability to bind toxins will be evaluated by monoclonal scFv ELISA. Furthermore, their ability to inhibit metalloproteases will be tested in vitro and in vivo.

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Contact Information: isadora.souza@usp.br
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Snakebite Roraima Team

UFRR Professors
Felipe A. Cerni, Ph.D
Eliseu A. Sandri, Ph.D
Bruna K. Bassoli, Ph.D.
Jacqueline Maciel, Ph.D.
Allex Jardins, Ph.D.
Fabrício Barreto, Ph. D.

Collaborators
Ana Moura da Silva, Ph.D (Instituto Butantan)
Fan Hui Wen, Ph.D (Instituto Butantan)
Wuelton Monteiroi, PhD (FMT-HVD)
Denise Tambourgi, Ph.D (Instituto Butantan)
Eliane C. Arantes, Ph.D (FCFRP - USP)
Isadora Oliveira Ph.D (FCFRP - USP)
Marco Sartim, Ph.D (FMT-HVD)
Paulo Bernarde, Ph.D (UFAC)
Rui Seabrarra, Ph.D (UNESP)
José María Gutiérrez, Ph.D (ICP, Costa Rica)
Jan Tytgat, Ph.D (KULeuven, Belgium)
Andreas Laustsen-Kiel, Ph. D (DTU, Denmark)
Loic Quinton, Ph.D (ULiège, Belgium)
Darín Rokyta, Ph.D (FSU, USA)

Students
Allan Quadros
Henrique Machado
Samuel Vieira
Vitória Santos
Poliana Lucena
Jamil Calderado
Mário Jorge Filho
Karlos Daniel
Hidyan Lima
Isabella
Maria Eugênia
Paulo Frassinete
Rommel Monte
Michelle Franco
Gabriel Melo
Katinyane Zolin
Vitória Silva
Carla balenzuela
Thays Prado
Amanda Cunha
Kiara Cardenas
Hellen
Ana Paula Lobato
Altair Neto
João Nicolau
Organizing Committee

Paul Parren
Professor of Molecular Immunology
Leiden University Medical Center

Chelcie Bird
Senior Operations Coordinator
Informa Connect

Michael Keenan
Project Manager and Production
Informa Connect