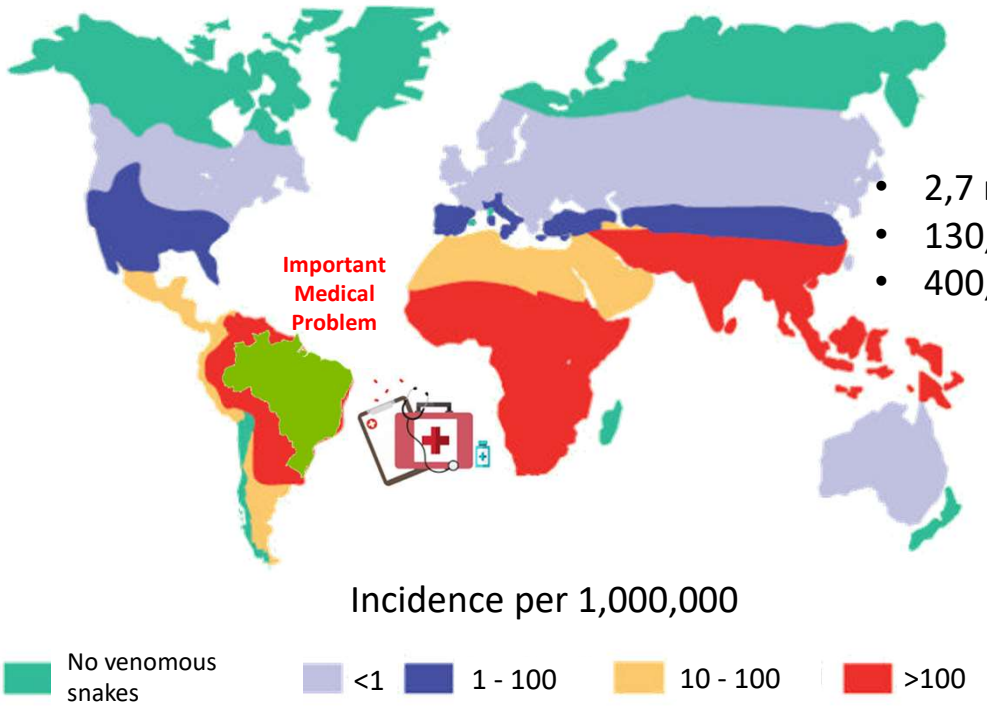
Snakebites in the Brazilian Amazon:

Neglecting effective antivenoms to a neglected population

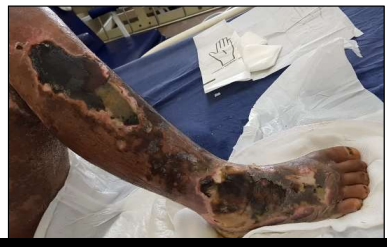
Dr. Manuela Pucca

SNAKEBITES



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

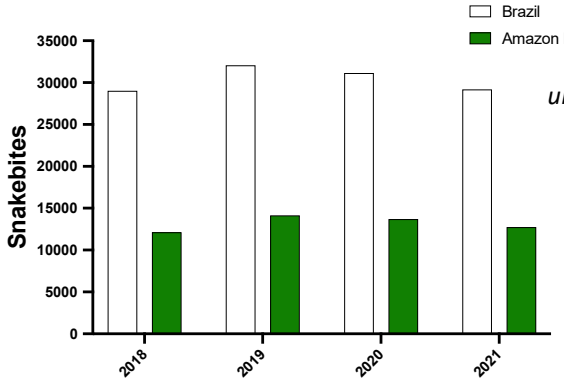
Snakebites predominantly affect the poor



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

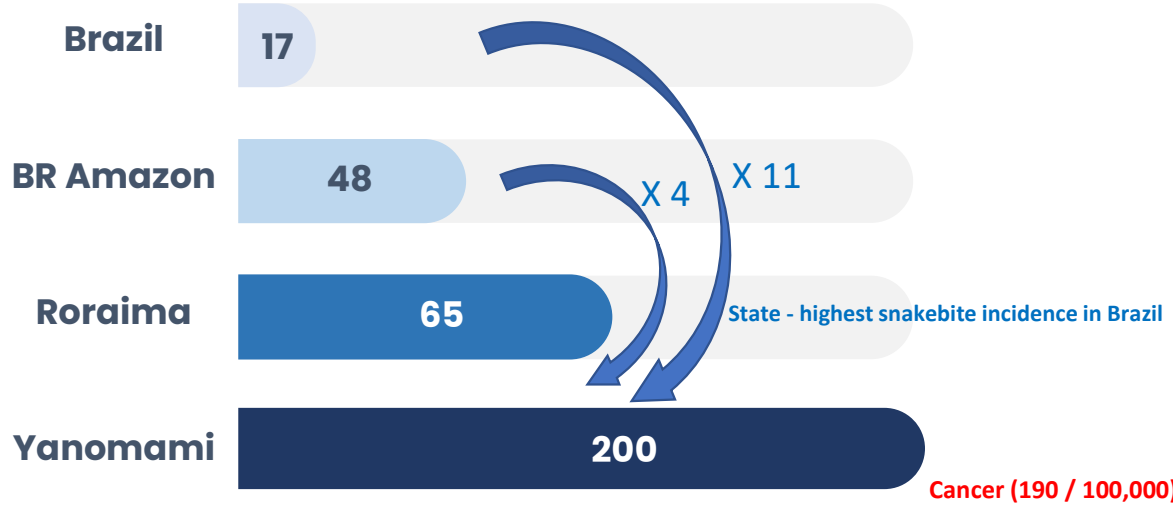
Incidence et mortalité par animaux venimeux dans les pays tropicaux" by Jean-Philippe Chippaux, Médecine Tropicale, 2008, 68(4), 334–39

Brazil registers about 30,000 snakebites each year, with high impact in the Brazilian Amazon



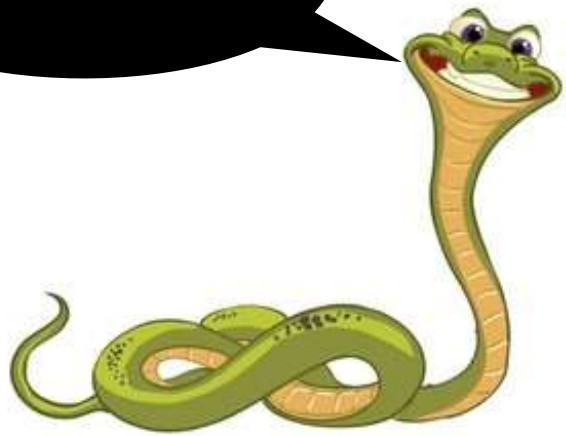
underreported accidents

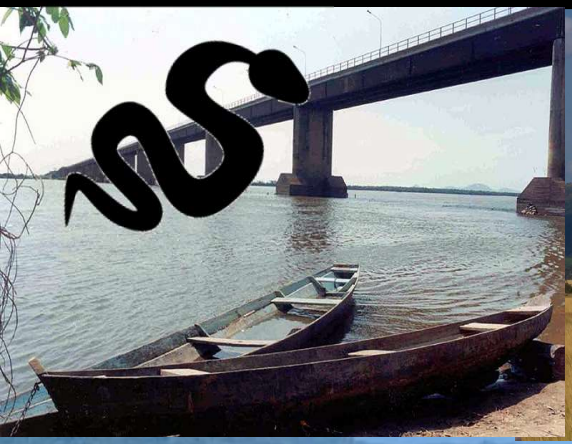
INCIDENCE per 100,000 people (2020)

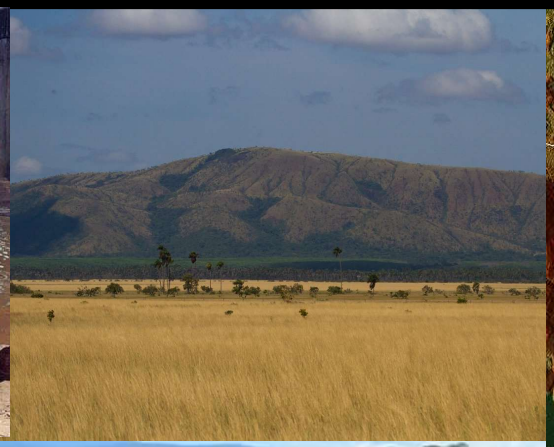


SNAKEBITE
RORAIMA

RORAIMA



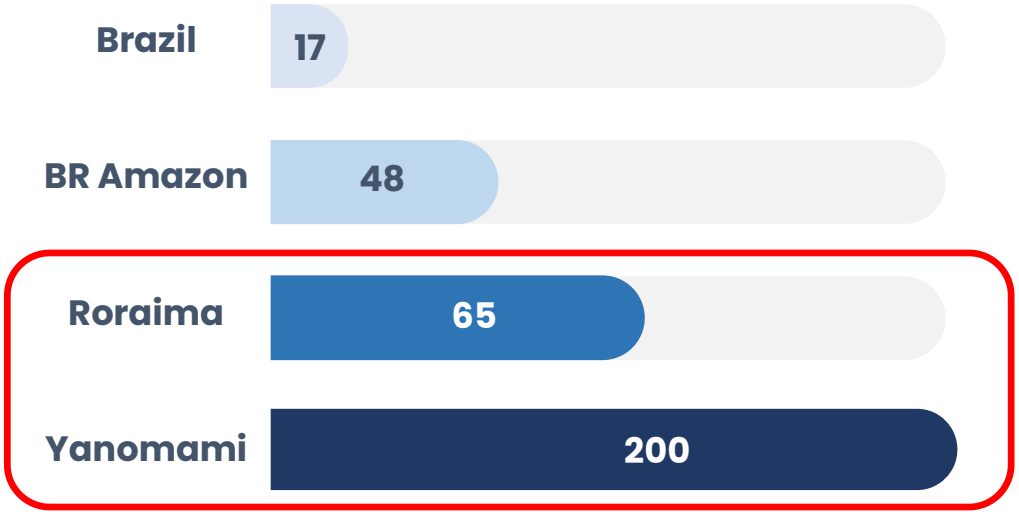




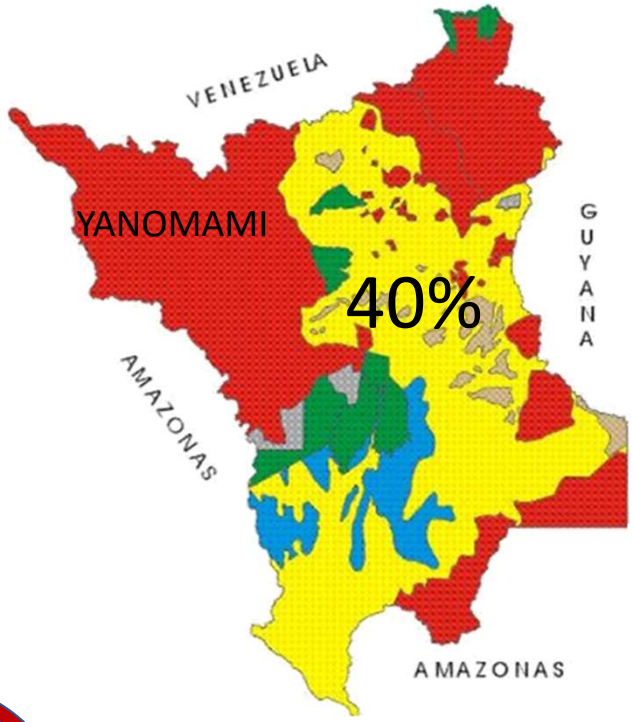

Rio Branco
(White River)


LEGAL AMAZON
33% Savannas
67% Tropical Forest

INCIDENCE per 100,000 people (2020)

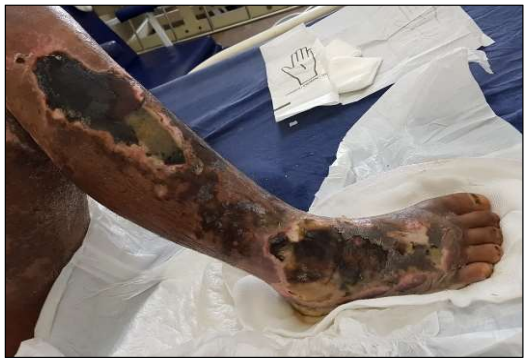


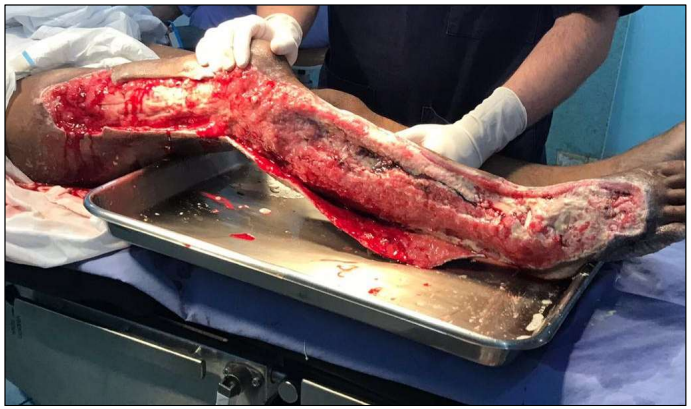
RORAIMA

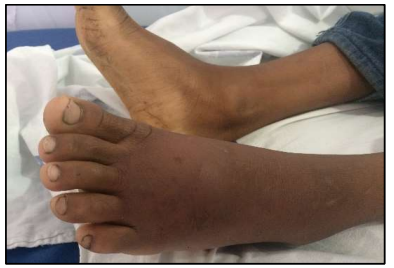


- *11 indigenous ethnicities*
- *32 indigenous communities*
- *8 different spoken languages*

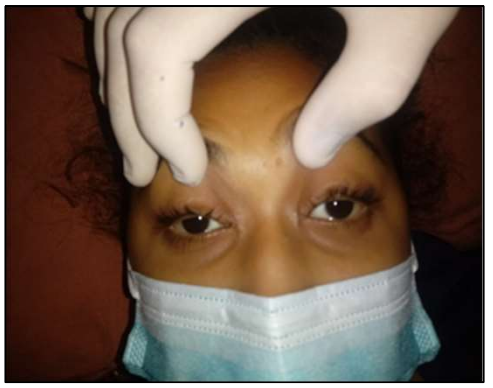
Snakebites in Roraima

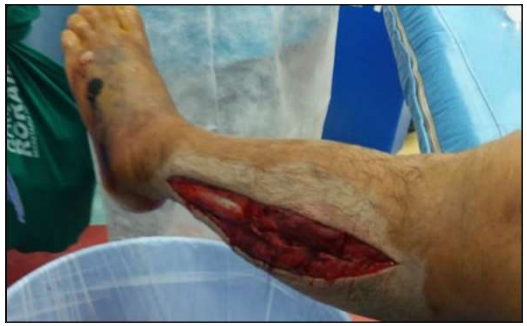


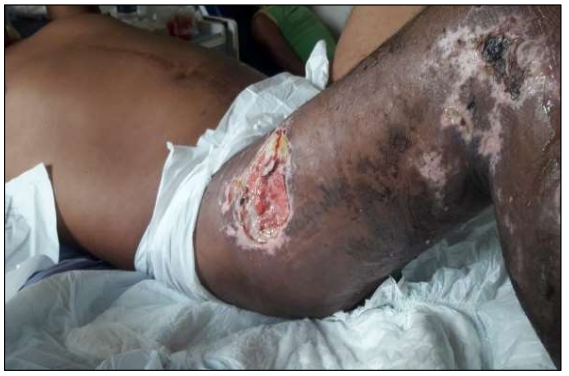


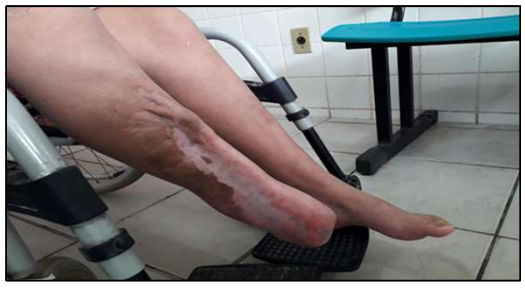


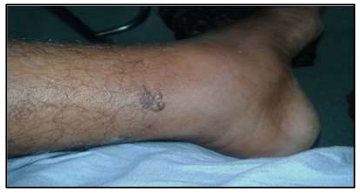
SNAKEBITE
RORAIMA



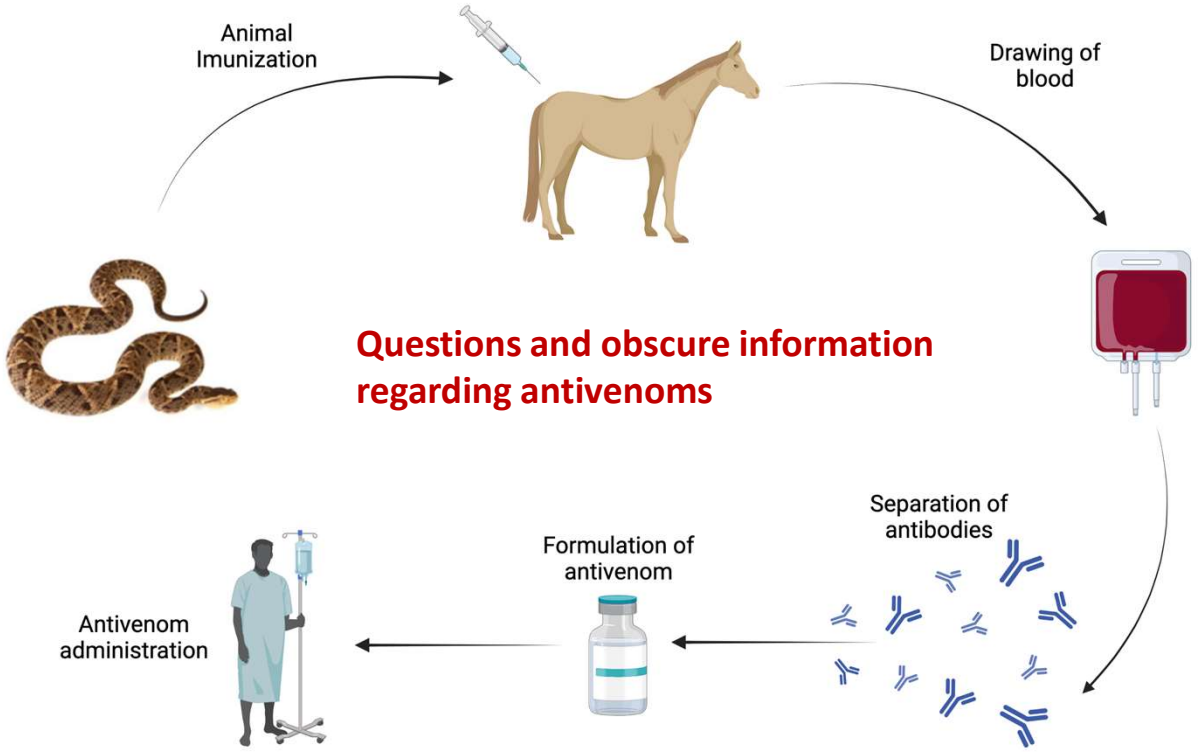






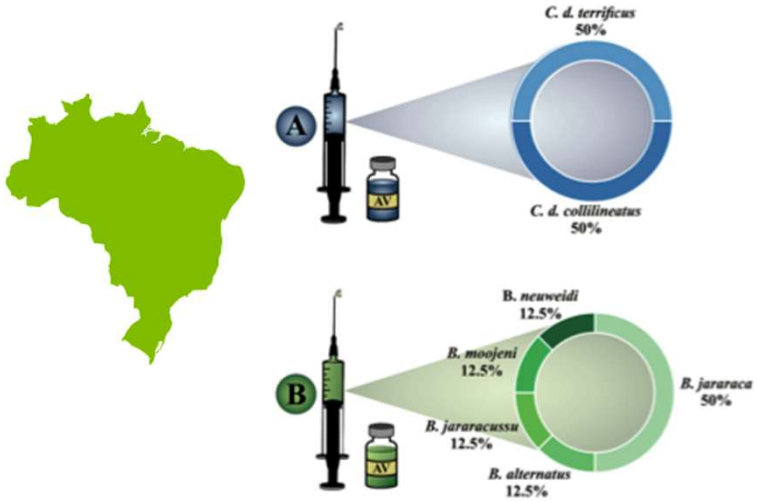


The administration of antivenom based on polyclonal antibodies produced in horses is the only specific treatment available against snakebites



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.*

2021

Brazilian Rattlesnakes

Brazil: 6 subspecies (*Crotalus* genus)

Crotalus d. durissus		RR	**AP**	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	RJ	SP	PR	SC	RS
Crotalus d. cascavella	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	**MA**	**PI**	**CE**	**RN**	**PB**	**PE**	**AL**	**SE**	**BA**	ES	**MG**	RJ	SP	PR	SC	RS
Crotalus d. collilineatus		RR	AP	AC	RO	**AM**	PA	**TO**	**MT**	**MS**	**GO**	**DF**	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	RJ	**SP**	PR	SC	RS
Crotalus d. marajoensis	x	RR	AP	AC	RO	AM	**PA**	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Crotalus d. ruruima		**RR**	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Crotalus d. terrificus		RR	AP	AC	**RO**	AM	PA	**TO**	**MT**	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**					

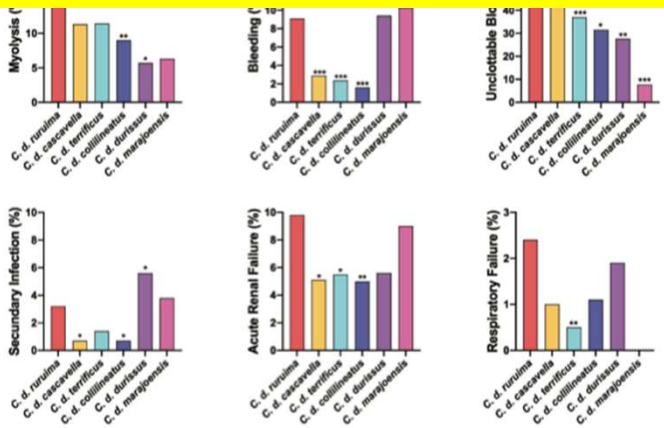
TABLE 1 | Venomic comparison of Brazilian *Crotalus durissus* subspecies.

C. durissus	Crotoxin	SVSP	CTL	SVMP			
				I	II	III	IV
ruruima	82.7	8.1	4.3	–	–	2.9	–
cascavella	72.5	1.2	<0.1	–	–	<0.1	–
collilineatus	67.4	1.9	<0.1	–	–	0.4	–
durissus							NR

We are conducting clinical studies in Roraima to show evidences that the antivenom is much less effective for our unique rattlesnake

Crotalus Durissus Ruruima: Current Knowledge on Natural History, Medical Importance, and Clinical Toxinology

Manuela B. Pucca[1], Paulo Sérgio Bernarde[2], Anderson Maciel Rocha[3], Patrik F. Viana[4], Raimundo Erasmo Souza Farias[4], Felipe A. Cerni[1,5], Isadora S. Oliveira[5], Isabela G. Ferreira[5], Eliseu A. Sandri[6], Jacqueline Sachett[7,8], Fan Hui Wen[9], Vanderson Sampaio[10], Andreas H. Laustsen[11], Marco A. Sartim[10,12] and Wuelton M. Monteiro[7,10*]*

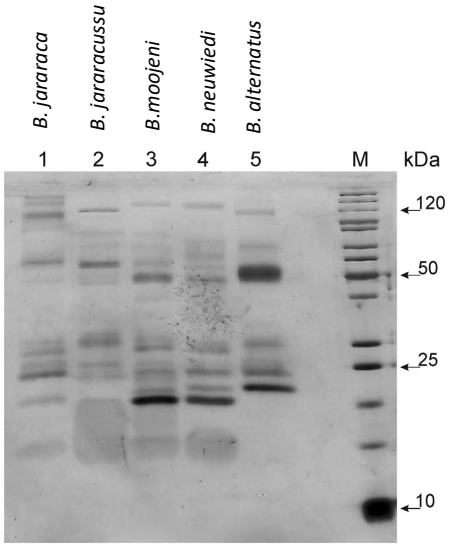


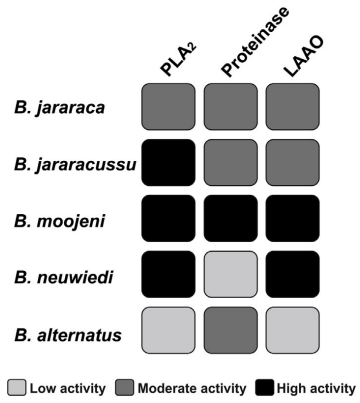
2021

Brazilian Pitvipers

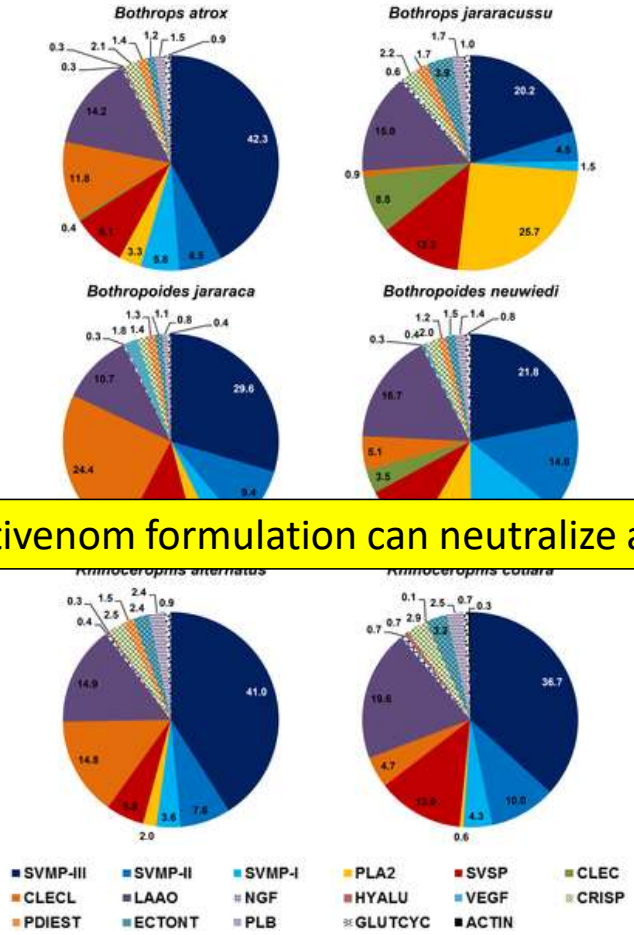
Brazil: 29 species (*Bothrops* genus)

Bothrops alcatraz	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	PR	SC	RS
Bothrops alternatus		RR	AP	AC	RO	AM	PA	TO	MT	**MS**	**GO**	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	**RJ**	**SP**	**PR**	**SC**	**RS**
Bothrops atrox		**RR**	**AP**	**AC**	**RO**	**AM**	**PA**	**TO**	**MT**	MS	GO	DF	**MA**	PI	CE	RN	PB	PE	AL	!	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops b. bilineatus		**RR**	**AP**	AC	**RO**	**AM**	**PA**	TO	**MT**	MS	GO	DF	MA	PI	CE	RN	PB	?	**AL**	SE	**BA**	**ES**	**MG**	**RJ**	SP	PR	SC	RS
Bothrops b. smaragdinus		RR	AP	**AC**	**RO**	**AM**	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops brazili		RR	!	**AC**	**RO**	**AM**	**PA**	TO	**MT**	MS	GO	DF	?	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops cotiara		RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	**PR**	**SC**	**RS**
Bothrops diporus		RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	**PR**	**SC**	**RS**
Bothrops erythromelas	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	**PI**	**CE**	**RN**	**PB**	**PE**	**AL**	**SE**	**BA**	ES	MG	RJ	SP	PR	SC	RS
Bothrops fonsecai	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	**RJ**	**SP**	PR	SC	RS
Bothrops insularis	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	PR	SC	RS
Bothrops itapetiningae	x	RR	AP	AC	RO	AM	PA	TO	MT	**MS**	**GO**	**DF**	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	RJ	**SP**	**PR**	SC	RS
Bothrops jararaca		RR	AP	AC	RO	AM	PA	TO	MT	MS	**GO**	DF	MA	PI	CE	RN	PB	PE	AL	SE	**BA**	**ES**	**MG**	**RJ**	**SP**	**PR**	**SC**	**RS**
Bothrops jararacussu		RR	AP	AC	RO	AM	PA	TO	MT	**MS**	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	**BA**	**ES**	**MG**	**RJ**	**SP**	**PR**	**SC**	**RS**
Bothrops leucurus	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	**CE**	**RN**	**PB**	**PE**	**AL**	**SE**	**BA**	**ES**	**MG**	RJ	SP	PR	SC	RS
Bothrops lutzi	x	RR	AP	AC	RO	AM	PA	**TO**	MT	MS	**GO**	**DF**	MA	**PI**	**CE**	RN	!	**PE**	AL	SE	**BA**	ES	**MG**	RJ	!	PR	SC	RS
Bothrops marajoensis	x	RR	**AP**	AC	RO	AM	**PA**	TO	MT	MS	GO	DF	**MA**	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops marmoratus	x	RR	AP	AC	RO	AM	PA	**TO**	MT	MS	**GO**	**DF**	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	RJ	SP	PR	SC	RS
Bothrops mattogrossensis		RR	AP	AC	**RO**	**AM**	PA	**TO**	**MT**	**MS**	**GO**	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	PR	SC	RS
Bothrops moojeni		RR	AP	AC	RO	AM	PA	**TO**	**MT**	**MS**	**GO**	**DF**	MA	**PI**	CE	RN	PB	PE	AL	SE	**BA**	ES	**MG**	RJ	**SP**	**PR**	SC	RS
Bothrops muriciensis	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	**AL**	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops neuwiedi	x	RR	AP	AC	RO	AM	PA	!	!	MS	**GO**	**DF**	MA	PI	CE	RN	PB	PE	AL	SE	**BA**	ES	**MG**	**RJ**	**SP**	**PR**	**SC**	**RS**
Bothrops oligobalius		RR	**AP**	AC	RO	**AM**	**PA**	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS
Bothrops otavioi	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	**SP**	PR	SC	RS
Bothrops pauloensis		RR	AP	AC	RO	AM	PA	!	**MT**	**MS**	**GO**	**DF**	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	**MG**	RJ	**SP**	**PR**	SC	RS
Bothrops pirajai	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	**BA**	ES	MG	RJ	SP	PR	SC	RS
Bothrops pubescens		RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	**SC**	**RS**
Bothrops sazimai	x	RR	AP	AC	RO	AM	PA	TO	MT	MS	GO	DF	MA	PI	CE	RN	PB	PE	AL	SE	BA	**ES**	MG	RJ	SP	PR	SC	RS
Bothrops taeniatus		**RR**	**AP**	**AC**	**RO**	**AM**	**PA**	TO	**MT**	MS	GO	DF	**MA**	PI	CE	RN	PB	PE	AL	SE	BA	ES	MG	RJ	SP	PR	SC	RS





Campos et al., Toxicon 76 (2013) 1–10



How an unique polyclonal antivenom formulation can neutralize all these toxins from all these species?

Sousa et al. (2013) Comparison of Phylogeny, Venom Composition and Neutralization by Antivenom in Diverse Species of Bothrops Complex. PLoS Negl Trop Dis 7(9): e2442.

If death occurs after antivenom administration, how to confirm the cause of death – caused from venom or antivenom ?

reports

MDPI

Case Report

Fatal Rattlesnake Envenomation in Northernmost Brazilian Amazon: A Case Report and Literature Overview

Jilvando M. Medeiros [1], Isadora S. Oliveira [2], Isabela G. Ferreira [2], Gabriel Melo Alexandre-Silva [1], Felipe A. Cerni [2], Umberto Zottich [1] and Manuela B. Pucca [1,*]

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[2] Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto 14049-900, SP, Brazil; isadora_so@yahoo.com (I.S.O.); igobboferreira@usp.br (I.G.F.); felipe_cerni@hotmail.com (F.A.C.)

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Received: 30 January 2020; Accepted: 5 April 2020; Published: 8 April 2020

check for updates

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.

Why?

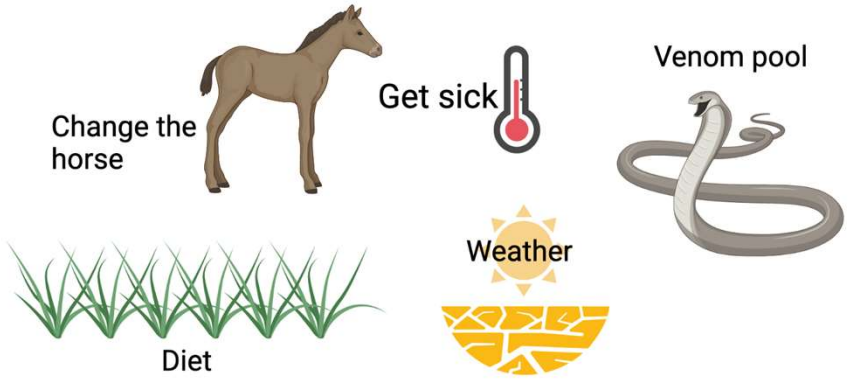
Antivenoms in the Market since 1901

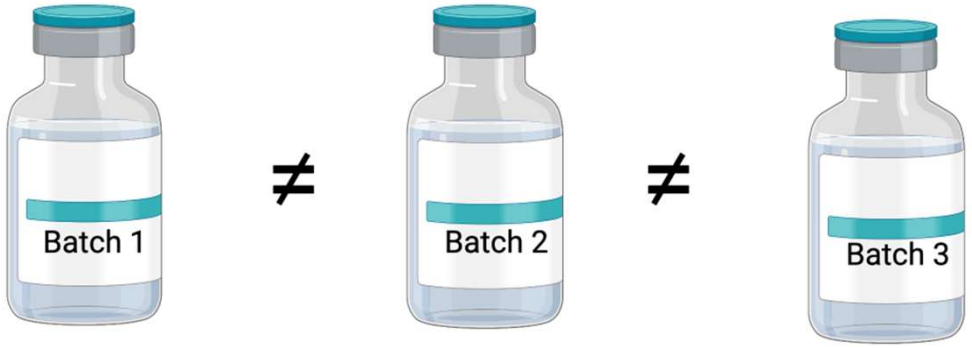
ANVISA

The Brazilian Health Regulatory Agency

Criated 1999

Clearly, there are also **limitations** in the design of this study because the **product varies** in each delivery.



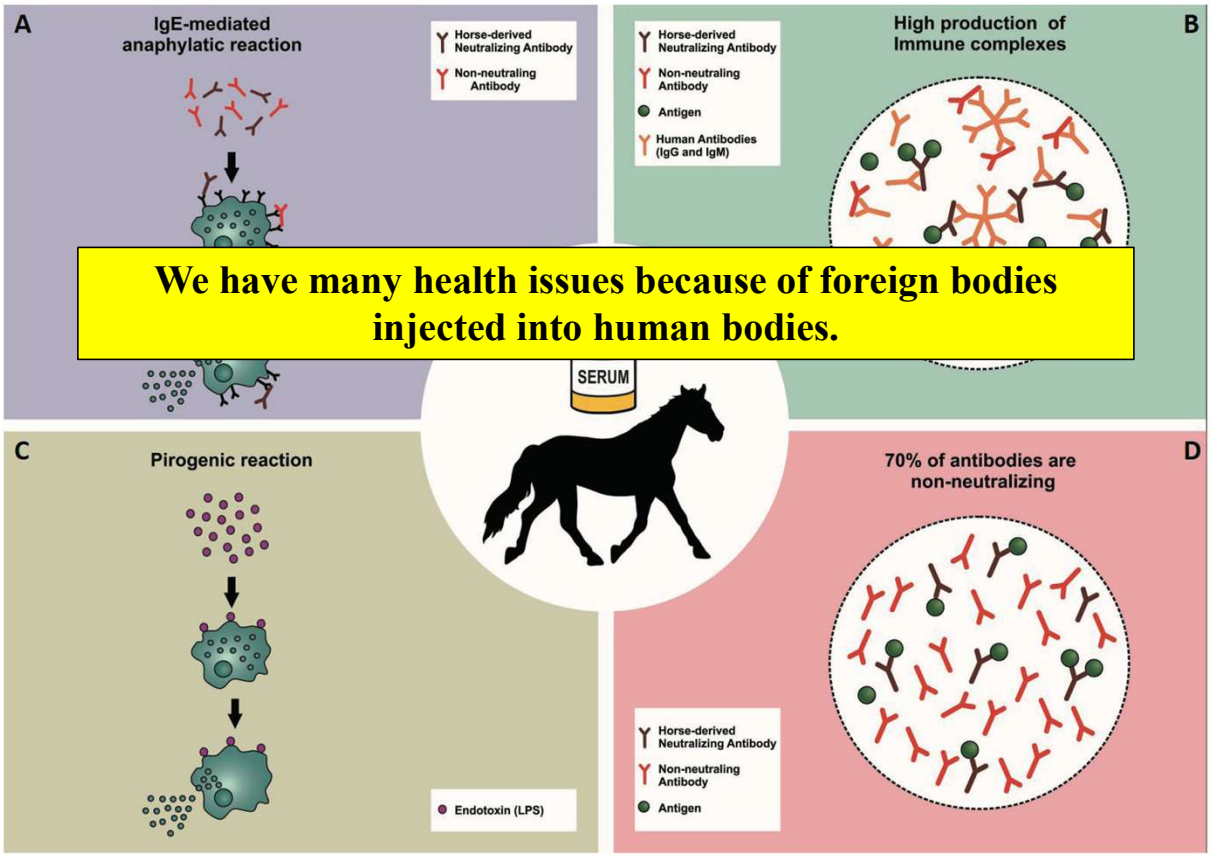


Horses are a biological system with a diverse genetic and immune system which cannot provide an uniform product quality at the end of the manufacturing process.

ISSUE 3: Are heterologous antivenoms safe?

Anaphylaxis (10%, 40%)

Pirogenic reaction

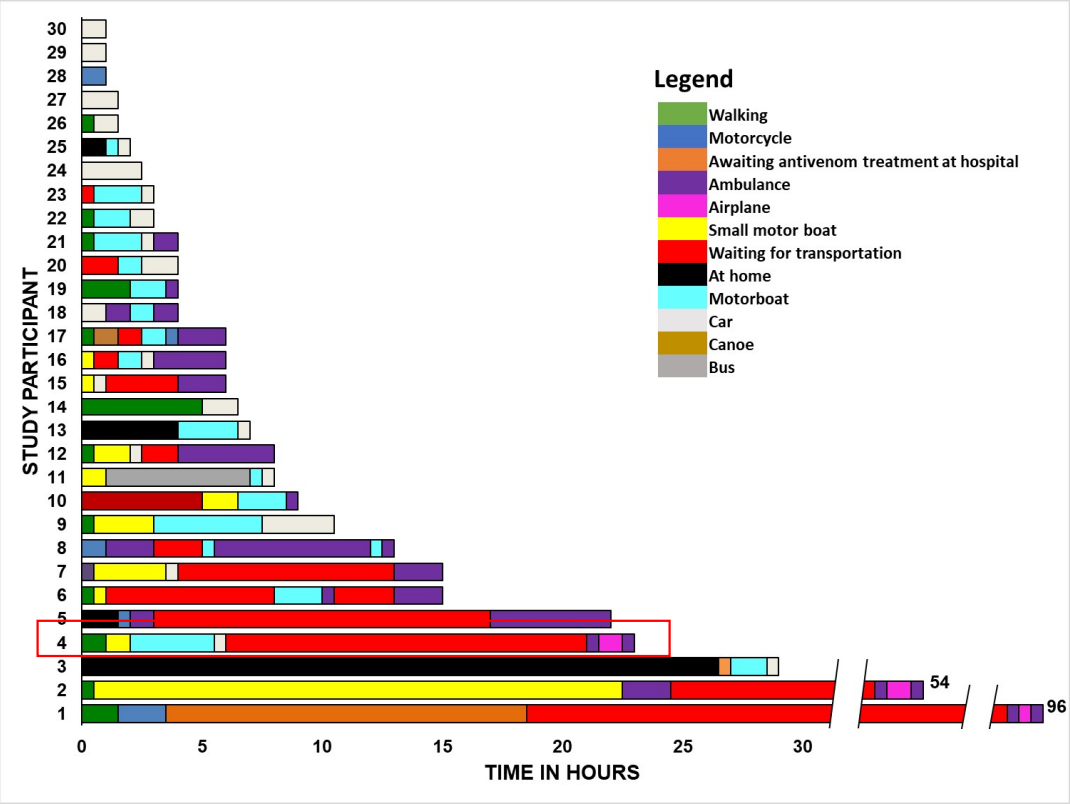


Serum Sickness (7-15% , 56%)

70% are non-neutralizing Abs

Camphora & Pucca, International Animal Health Journal 8 (4) (2022).

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

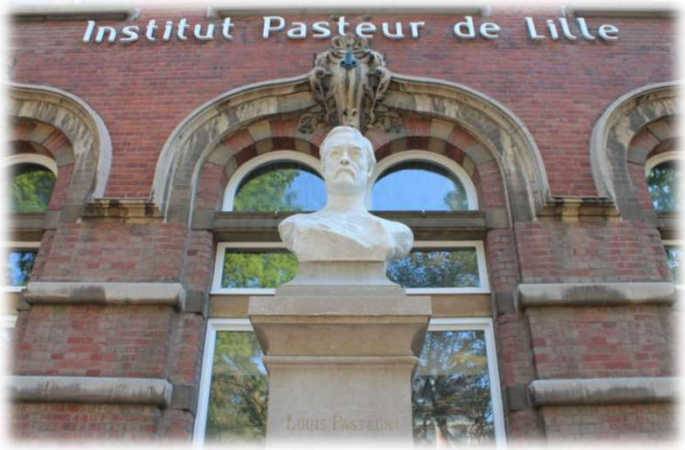
Itineraries of the study participants expressed graphically from the time from the bite to hospital admission.

Cristino et al. (2021) A painful journey to antivenom: The therapeutic itinerary of snakebite patients in the Brazilian Amazon (The QUALISnake Study). PLoS Negl Trop Dis 15(3): e0009245.

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.

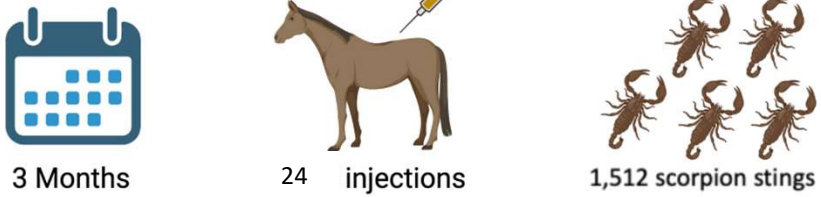


"….equine are the victims to our progress in immunology"

OBSCURE INFORMATION REGARDING THE USE OF HORSES

Rare descriptions of experimental procedures in horses

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*".

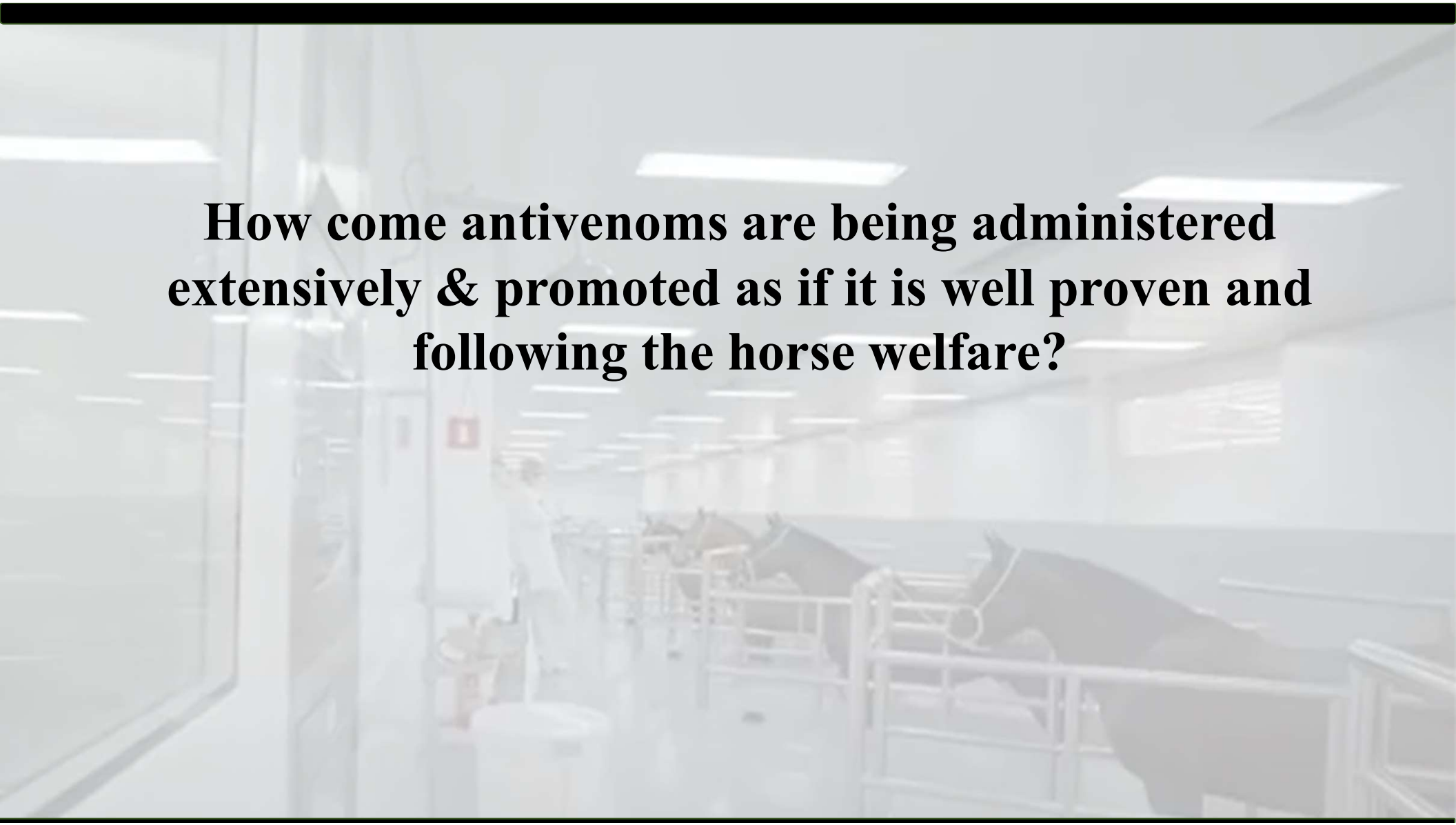
This study also presents one of the rare records on deaths of horses used as serum producers by the Butantan Institute, during the years 1947 and 1948.

Recovering the diphtheria serum from horse blood in Marburg, Germany, drawn from nature by Fritz Gehrke, 1890s

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'.**

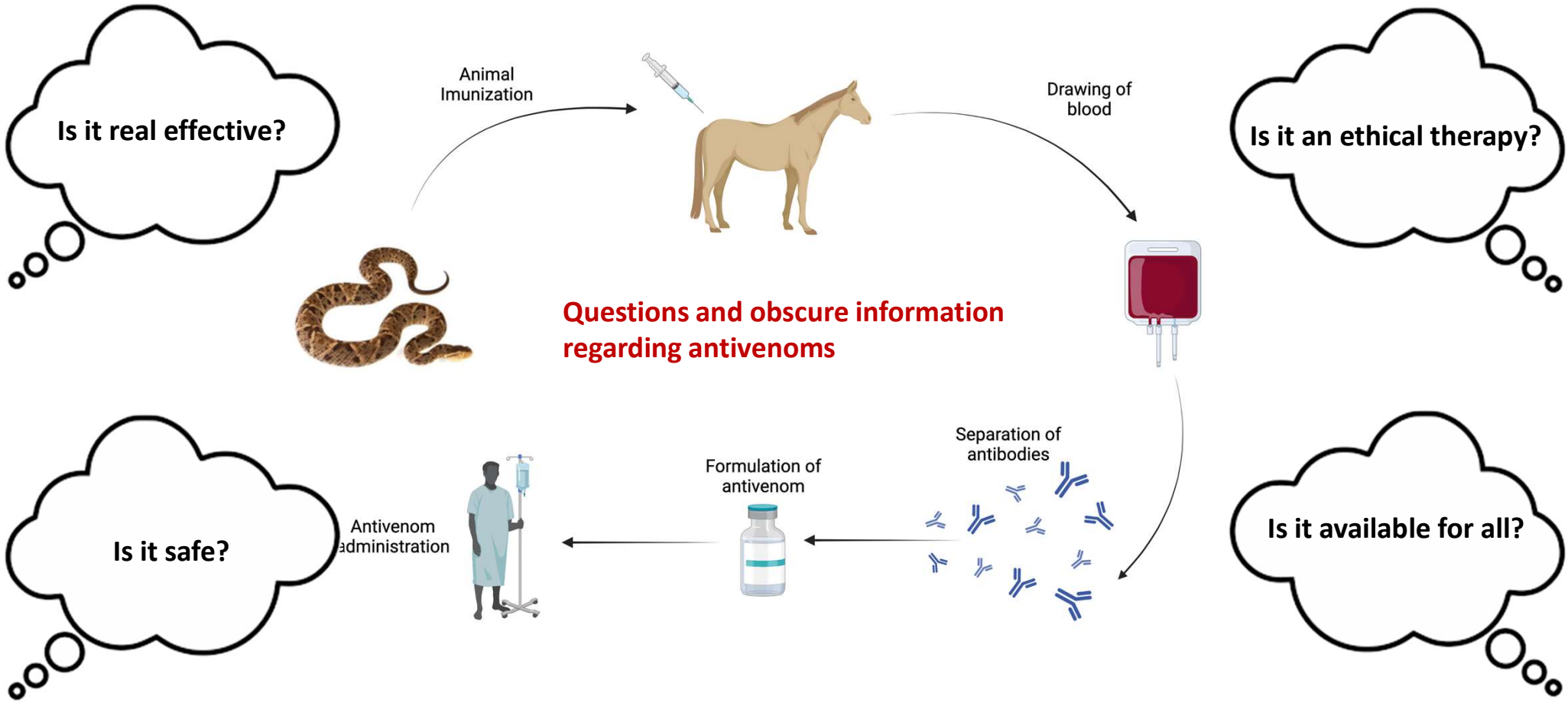
TOTAL BLEEDING: *consisted of inoculating in the animal saline solution that kept circulatory mechanism, delaying haemorrhagic shock, to allow the animal to survive until all the blood could be withdrawn, in a procedure that could take as long as two days.*

The intense worldwide demand for hyperimmunized plasma has written a crude trajectory of horse exploitation which are still not entirely dimensioned.



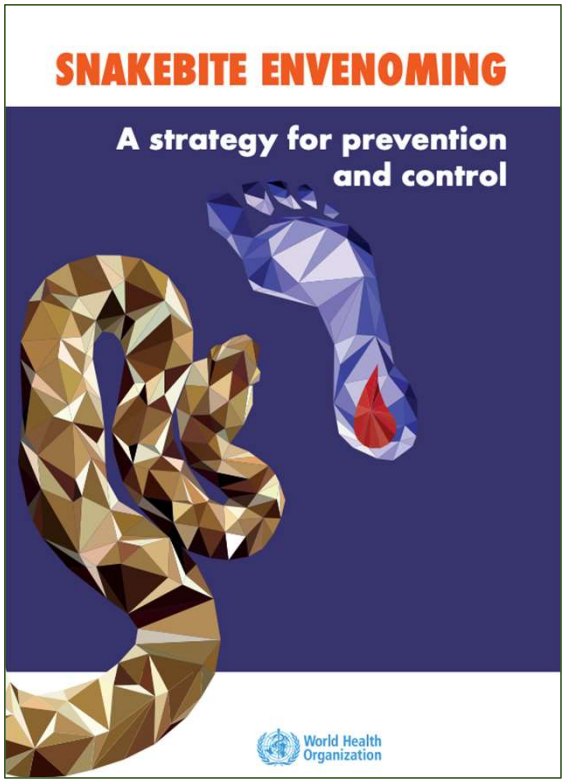
How come antivenoms are being administered extensively & promoted as if it is well proven and following the horse welfare?

Do we have an alternative treatment???



WHO – MAY 2019

World Health Organization



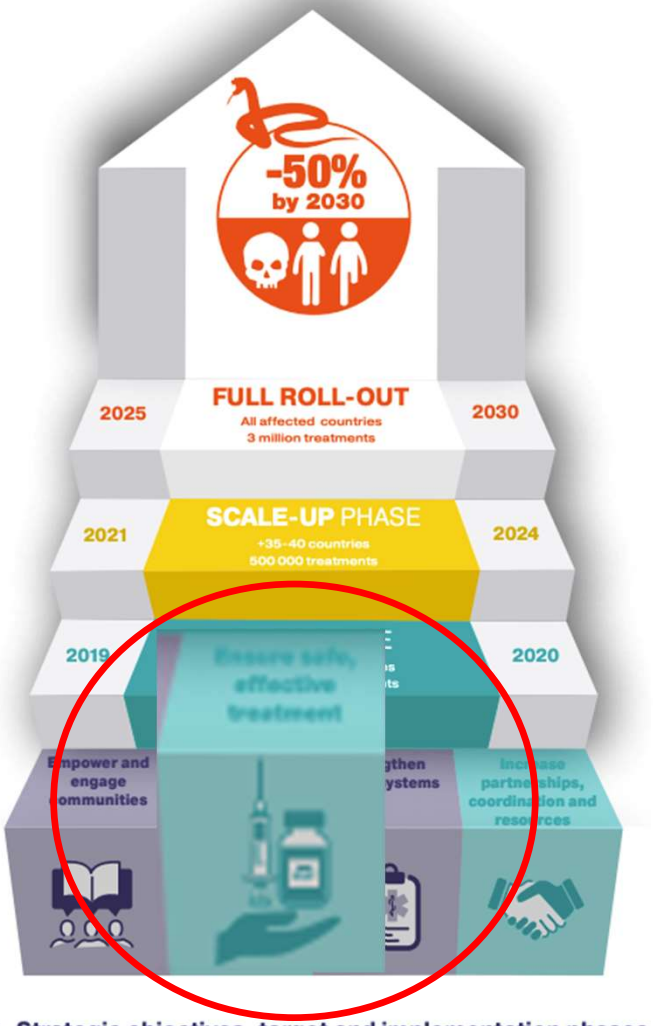
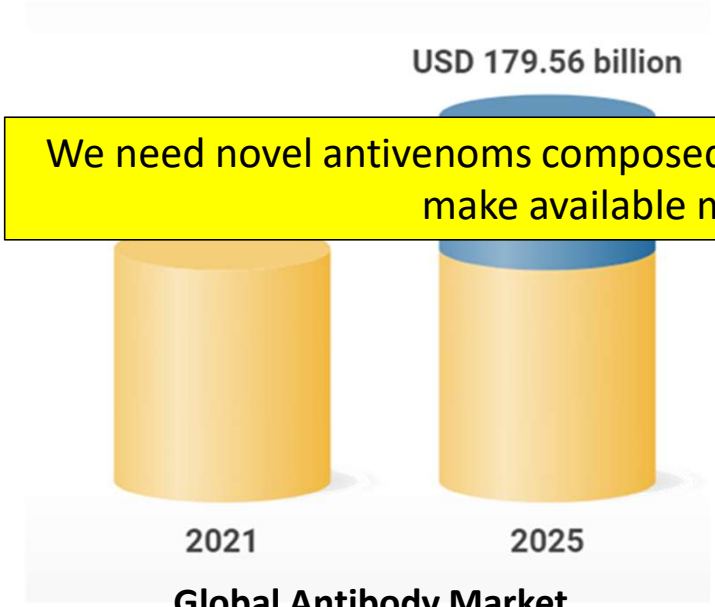


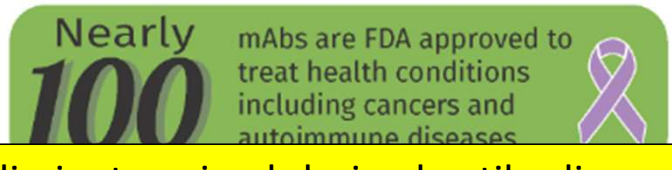
Fig. 2. **Strategic objectives, target and implementation phases**

What are we doing to change the ancient use of horses as an antibody machine?

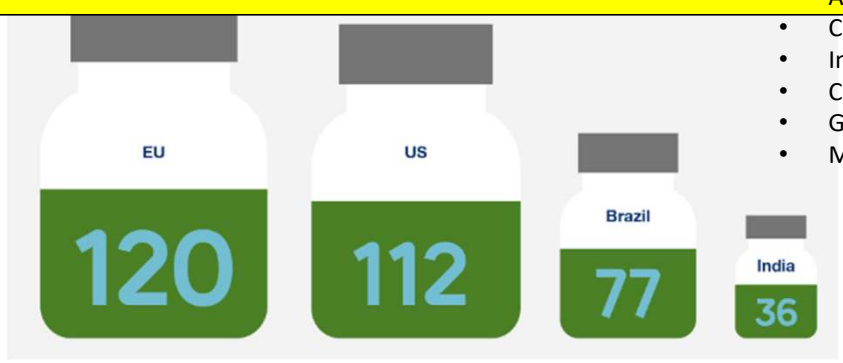
There were many progresses in antibody discovery technologies, antibody engineering approaches, and antibody manufacturing.



Global Antibody Market



We need novel antivenoms composed of mAbs to eliminate animal-derived antibodies and to make available more effective and safe antivenoms

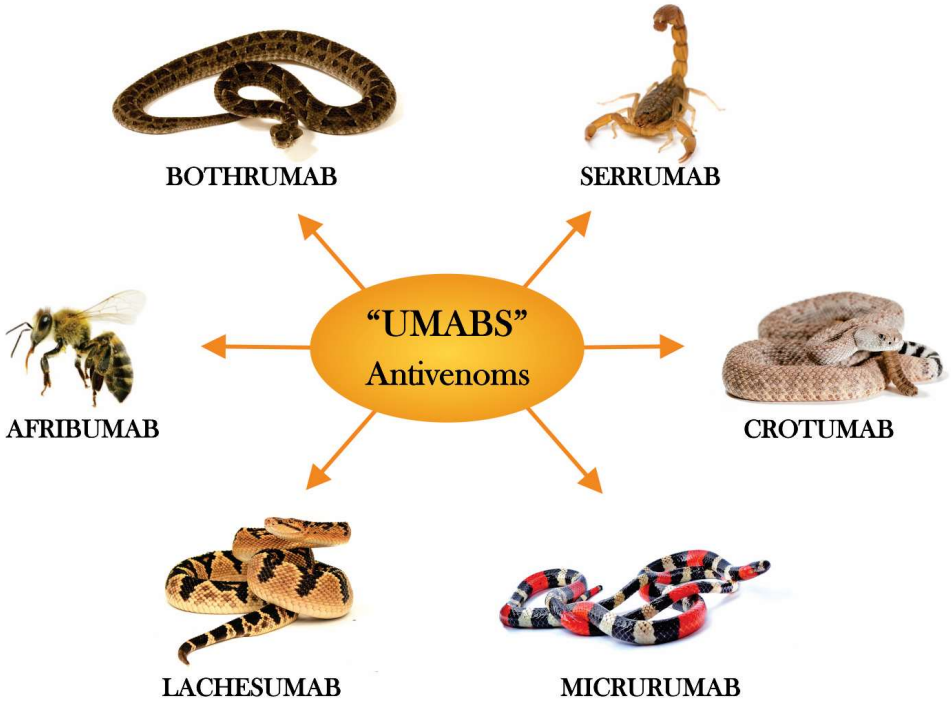


- Autoimmune diseases
- Cancer
- Infections disease
- Chronic diseases
- Genetic diseases
- Metabolic diseases

Neglected Tropical Diseases???

Phage Display (since 2006)

Discovery 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

2019

ELSEVIER

Toxicon

Volume 112, 15 March 2016, Pages 59–67

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

Gabriela Pessenda[a], Luciano C. Silva[a], Lucas B. Campos[a], Elenice M. Pacello[a], Manuela B. Pucca[b], Edson Z. Martinez[c], José E. Barbosa[a]

ELSEVIER

Toxicon

Volume 93, January 2015, Pages 79–84

Mini-review

Phage display as a novel promising antivenom therapy: A review

Eduardo Crosara Roncolato, Lucas Benício Campos, Gabriela Pessenda, Luciano Costa e Silva, Gilvan Pessoa Furtado, José Elpidio Barbosa

BJPS

Brazilian Journal of Pharmaceutical Sciences
vol. 47, n. 1, jan./mar., 2011

Article

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

Manuela Berto Pucca[1,*], Thaís Barboza Bertolini[1], José Elpidio Barbosa[1], Simone Vasconcelos Ribeiro Galina[2], Geciane Silveira Porto[3]

frontiers in Immunology

REVIEW

published: 06 September 2019
doi: 10.3389/fimmu.2019.02090

Bee Updated: Current Knowledge on Bee Venom and Bee Envenoming Therapy

Manuela B. Pucca[1,2*], Felipe A. Cerni[2,3], Isadora S. Oliveira[1], Timothy P. Jenkins[2], Lidia Argemi[2], Christoffer V. Sørensen[2], Shirin Ahmadi[2,4], José E. Barbosa[1] and Andreas H. Laustsen[2*]

frontiers in Immunology

REVIEW

published: 10 July 2019
doi: 10.3389/fimmu.2019.01598

BCPT

Basic & Clinical Pharmacology & Toxicology

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Expression of Human Recombinant Antibody Fragments Capable of Partially Inhibiting the Phospholypase Activity of *Crotalus durissus terrificus* Venom

Juliana G. Oliveira, Sandro G. Soares, Andreimar M. Soares, José R. Giglio, José E. Teixeira, José E. Barbosa

Research article

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

Eduardo Crosara Roncolato, Manuela Berto Pucca, Jaqueline Carlos Funayama, Thaís Barboza Bertolini, Lucas Benício Campos & José Elpidio Barbosa

Contents lists available at ScienceDirect

Toxicon

journal homepage: http://www.elsevier.com/locate/toxicon

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Short communication

Identification of cross-reactive human single-chain variable fragments against phospholipases A_2 from *Lachesis muta* and *Bothrops* spp venoms

Lucas B. Campos[1], Manuela B. Pucca[1,2,*], Luciano C. Silva[1,3], Gabriela Pessenda[1], Bruno A. Filardi[1], Felipe A. Cerni[2,4], Isadora S. Oliveira[1], Andreas H. Laustsen[2], Eliane C. Arantes[1], José E. Barbosa[1,*]

Journal of Immunotoxicology

http://informahealthcare.com/imt
ISSN: 1547-691X (print), 1547-6901 (electronic)
J Immunotoxicol, 2014; 11(2): 133–140
© 2014 Informa Healthcare USA, Inc.
DOI: 10.3109/1547691X.2013.809175

informa healthcare

RESEARCH ARTICLE

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

Manuela Berto Pucca[1], Felipe Augusto Cerni[2], Steve Peigneur[3], Eliane Candiani Arantes[2], Jan Tytgat[3], and José Elpidio Barbosa[1]

Journal of Immunotoxicology, 2012; 9(2): 173–183
© 2012 Informa Healthcare USA, Inc.
ISSN 1547-691X print/ISSN 1547-6901 online
DOI: 10.3109/1547691X.2011.649220

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RESEARCH ARTICLE

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

Manuela Berto Pucca[1], Karina Furlan Zoccal[2], Eduardo Crosara Roncolato[1], Thaís Barboza Bertolini[1], Lucas Benício Campos[1], Camila Takeno Cologna[1], Lúcia Helena Faccioli[2], Eliane Candiani Arantes[3], and José Elpidio Barbosa[1]

BCPT

Basic & Clinical Pharmacology & Toxicology

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

Jaqueline C. Funayama, Manuela B. Pucca, Eduardo C. Roncolato, Thaís B. Bertolini, Lucas B. Campos, José E. Barbosa

ELSEVIER

Biochimica et Biophysica Acta (BBA) - General Subjects

Volume 1760, Issue 9, September 2006, Pages 1450–1457

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

M.B. Tamarozzi[a], S.G. Soares[a], S. Marcussi[b, c], J.R. Giglio[c], J.E. Barbosa[c]

History of Envenoming Therapy and Current Perspectives

Manuela B. Pucca[1,2], Felipe A. Cerni[2,3], Rahel Janke[2], Erick Bermúdez-Méndez[4], Line Ledsgaard[2], José E. Barbosa[3] and Andreas H. Laustsen[2*]

[1] Medical School, Federal University of Roraima, Boa Vista, Brazil, [2] Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark, [3] Department of Biochemistry and Immunology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, [4] Facultad de Farmacia, Universidad de Costa Rica, San José, Costa Rica

ELSEVIER

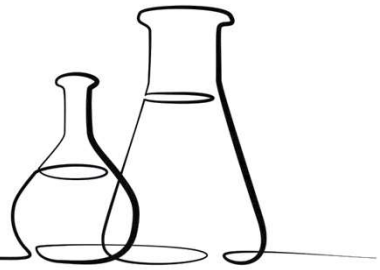
Acta Tropica

Volume 177, January 2018, Pages 66-73

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

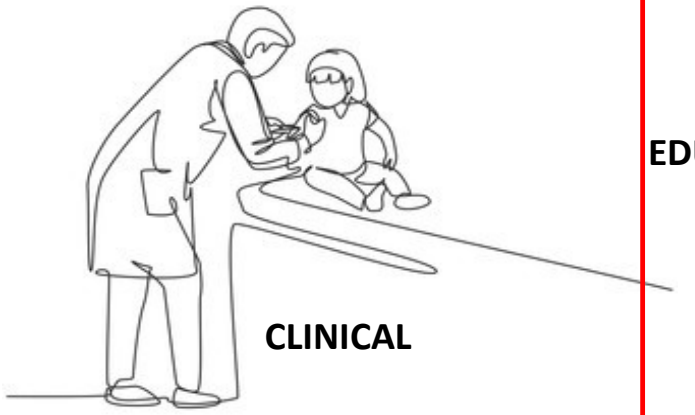
Luciano C. Silva[a, 1], Manuela B. Pucca[b, 1], Gabriela Pessenda[a], Lucas B. Campos[a], Edson Z. Martinez[c], Felipe A. Cerni[d], José E. Barbosa[a]

SNAKEBITE
RORAIMA



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

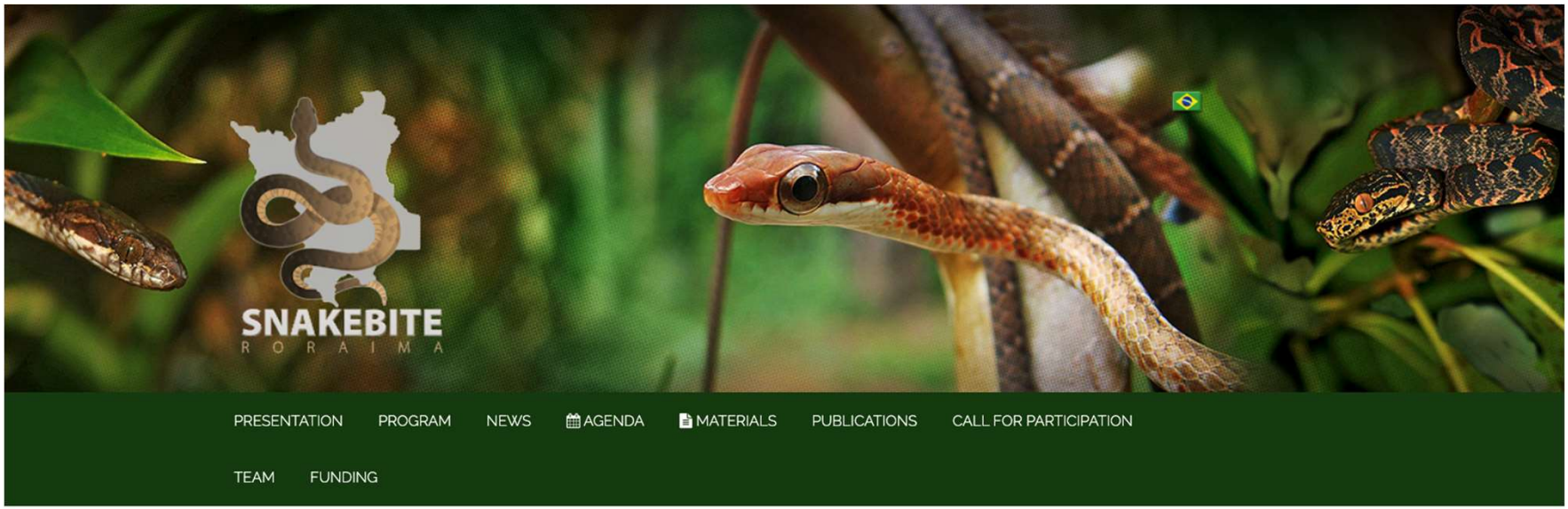
EDUCATIONAL PROGRAM

- Educating the local population (including indigenous)
- Training the health professionals

Mitigate the local Snakebite Problem

SNAKEBITE

EDUCATIONAL PROGRAM



Antibody Engineering Euro...

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

MODERADORA

Dra. Manuela B. Pucca

(coordenadora do projeto Snakebite Roraima)

PALESTRANTE

Dr. Ernesto L. Pinheiro-Junior

(KU Leuven, Bélgica)

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Portuguese: www.snakebiteroraima.com.br

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- Agrotechinical School
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- Nursing School
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- Health team – Indigenous Community

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- Indigenous
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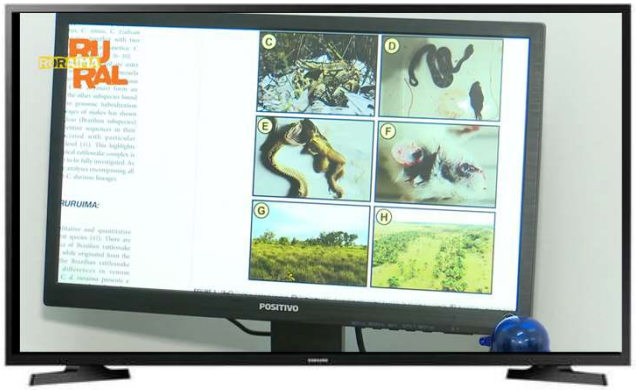
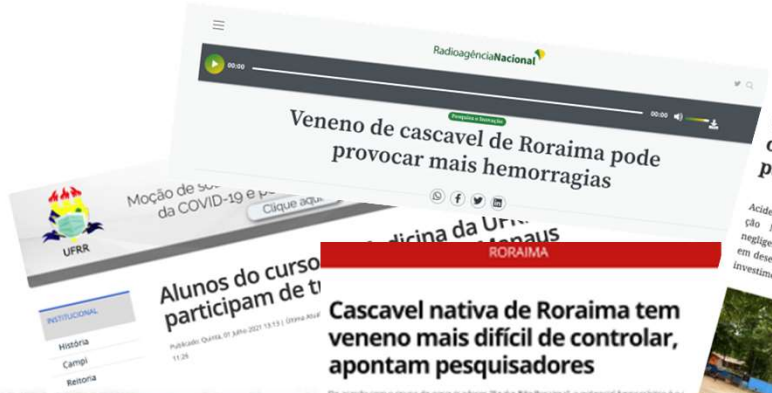




Grupo Snakebite Roraima: educação, pesquisa e combate ao ofidismo no extremo norte do país

Acidentes ofídicos (picada de cobra) foram considerados, pela Organização Mundial da Saúde, parte da lista de doenças tropicais negligenciadas. Essas doenças ocorrem predominantemente em países em desenvolvimento, não são alvo de muitas pesquisas devido ao baixo investimento, mas acometem mais de 5 milhões de indivíduos por ano.

NEWS

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

Developing of monoclonal antibody fragments targeting metalloproteases from pit viper

Isadora Sousa de Oliveira[1], Manuela Berto Pucca[2], Ana Maria Moura-da-Silva[3], Eliane Candiani Arantes[1]

[1]*Laboratory of Animal Toxins, Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil;*
[2]*Medical School, Federal University of Roraima, Boa Vista, RR, Brazil;*
[3]*Laboratory of Immunopathology, Butantan Institute, São Paulo, SP, Brazil*

BACKGROUND

Since 2017 snakebites are classified as category A of the Neglected Tropical Diseases (NTDs), affecting more than 5 million people worldwide per year, which results in more than 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 (80-90%), distributed throughout the national territory.

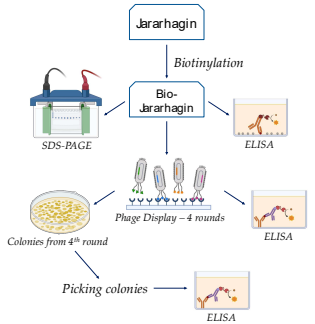
Pit viper-derived venoms are mainly composed of metalloproteases (~30-70%), phospholipases (~7-40%) and serine proteases (~2-24%), varying quantitatively between them, making the venoms haemorrhagic, proteolytic, and coagulant, characteristics that are responsible for many of their local and systemic 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 administration) has several disadvantages regarding its use, since they can trigger anaphylaxis and serum sickness.

Thus, new technologies for antivenom development are necessary, such as the phage display technique, which allows the selection of fully human antibodies against the most varied 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



RESULTS

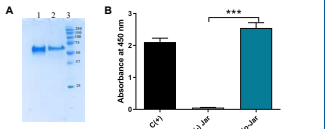


Figure 1. Jararhagin biotinylation. Jararhagin was biotinylated for better antigen presentation during panning via streptavidin, in a ratio of 1:5 (toxin:biotin). **(A)** SDS-PAGE (13,5%) in reduced conditions, and stained with Coomassie Brilliant Blue G-250. 1: Jararhagin; 2: Jararhagin biotinylated, or bio-jararhagin; 3: molecular weight markers. **(B)** Bio-jararhagin analyzed by ELISA. 96-well plates were sensitized with bio-jararhagin (2 µg, bio-Jar). The commercial antibiotin peroxidase-labeled (1:10.000) was used to evaluate the biotinylation of jararhagin. Absorbance reading was performed at 450 nm. C(+): wells sensitized with PEG4-biotin.; C(-) Jar: wells sensitized with jararhagin. Data are presented as mean ± SD, which were analyzed by ANOVA and Tukey's multiple comparison test (quadruplicate assay). ***$p < 0.0001$ when compared to C(-).

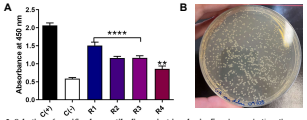


Figure 2. Selection of specific phage-antibodies against jararhagin. For phage selection, the cross-panning method was performed, with a human phage-antibody library, Griffin, with diversity of 10^8 different scFvs, and the selection was made in four panning rounds. **(A)** Phage-antibodies were analyzed by ELISA. 96-well plates were sensitized with phage-antibodies from panning (10 µL), and bio-jararhagin (1 µg) was added for immunorecognition. The commercial antibiotin peroxidase-labeled (1:5.000) was used to evaluate this assay. Absorbance reading was performed at 450 nm. C(+): wells sensitized with bio-jararhagin (1 µg); C(-): wells sensitized with VCSM13. Data are presented as mean ± SD, which were analyzed by ANOVA and Tukey's multiple comparison test (triplicate assay). **$p < 0.01$ when compared to C(-); ****$p < 0.0001$ when compared to C(-). **(B)** Culture plates demonstrate *E. coli* TG1 bacteria colonies growth, which were infected with phage-antibodies from the 4th round of panning.

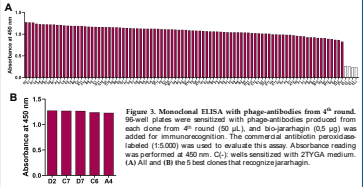


Figure 3. Monoclonal ELISA with phage-antibodies from 4th round. 96-well plates were sensitized with phage-antibodies produced from each clone from 4th round (50 µL), and bio-jararhagin (0.5 µg) was added for immunorecognition. The commercial antibiotin peroxidase-labeled (1:5.000) was used to evaluate this assay. Absorbance reading was performed at 450 nm. C(-): wells sensitized with 2TYGA medium. **(A)** All and **(B)** the 5 best clones that recognize jararhagin.

CONCLUSION AND FUTURE STEPS

This study reports the selection of the first human antibody against metalloprotease from *Bothrops* snakes, which could be included in the formulation of a new generation of antibotropic serum. These monoclonal phage-antibodies will be used for infection of *E. coli* bacteria, which will be properly induced to produce soluble scFv antibody fragments, 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*.

Financial Support: FAPESP Process n. 2020/13176-3

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SNAKEBITE

Snakebite Clinics and Pathogenesis: From Preclinical to Resource Mapping Studies

Guest Editors
Dr. Manuela Berto Pucca
Dr. Wuelton M. Monteiro
Dr. Hui Wei Fan
Dr. Ana Maria Moura-Da-Silva

Deadline
30 June 2022

Special Issue
Invitation to submit

VENORAIMA

VENOMOUS ANIMALS IN RORAIMA

November 24th and 25th

INTERNATIONAL CONGRESS OF
Venomous Animals in Roraima

ACKNOWLEDGE

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Loic Quinton, Ph.D (ULiège, Belgium)
Darin Rokyta, Ph.D (FSU, USA)

ACKNOWLEDGE

Organizing Committe

Paul Parren

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THANKS









SNAKEBITE
RORAIMA

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