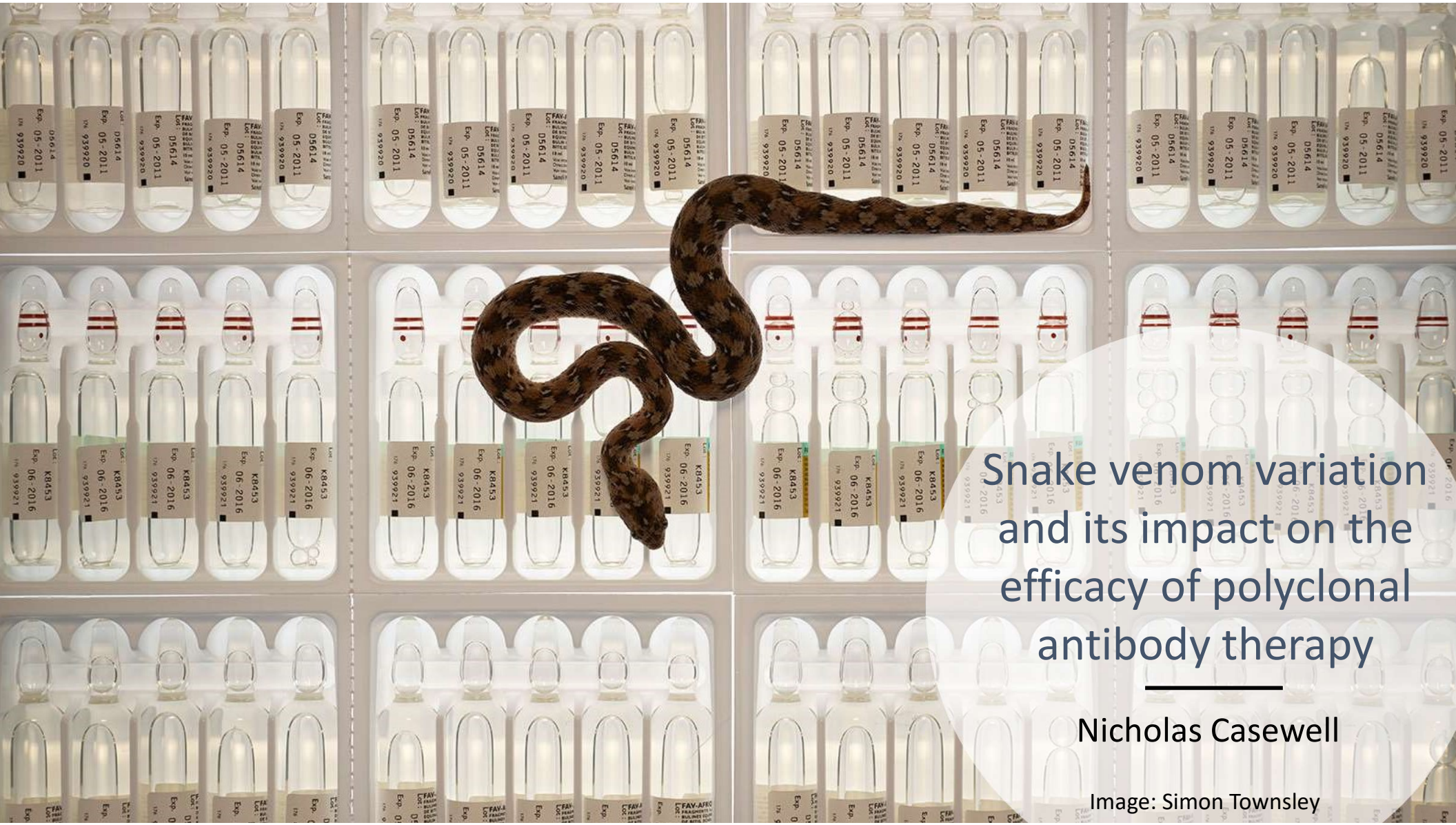
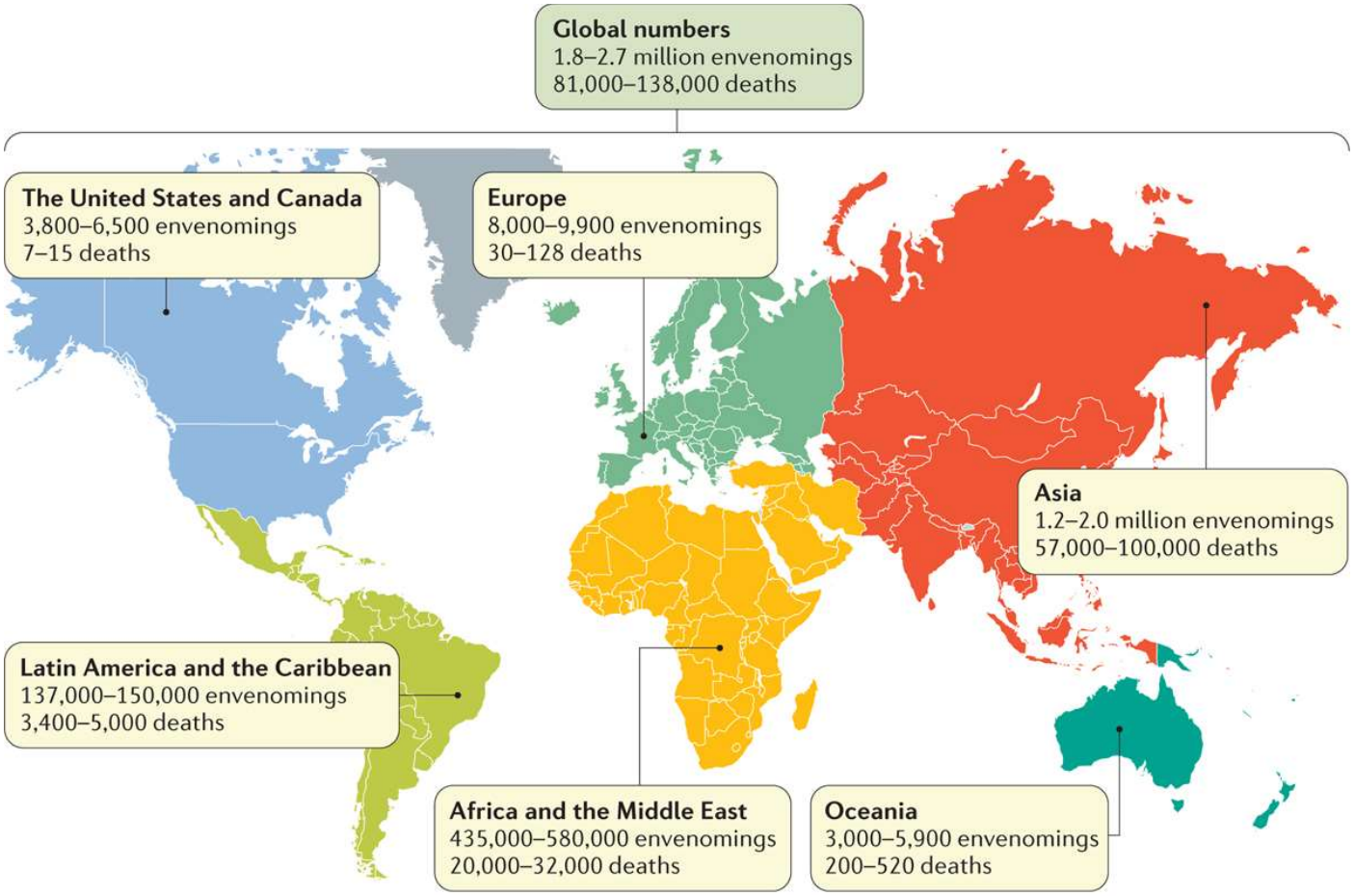


Snake venom variation and its impact on the efficacy of polyclonal antibody therapy

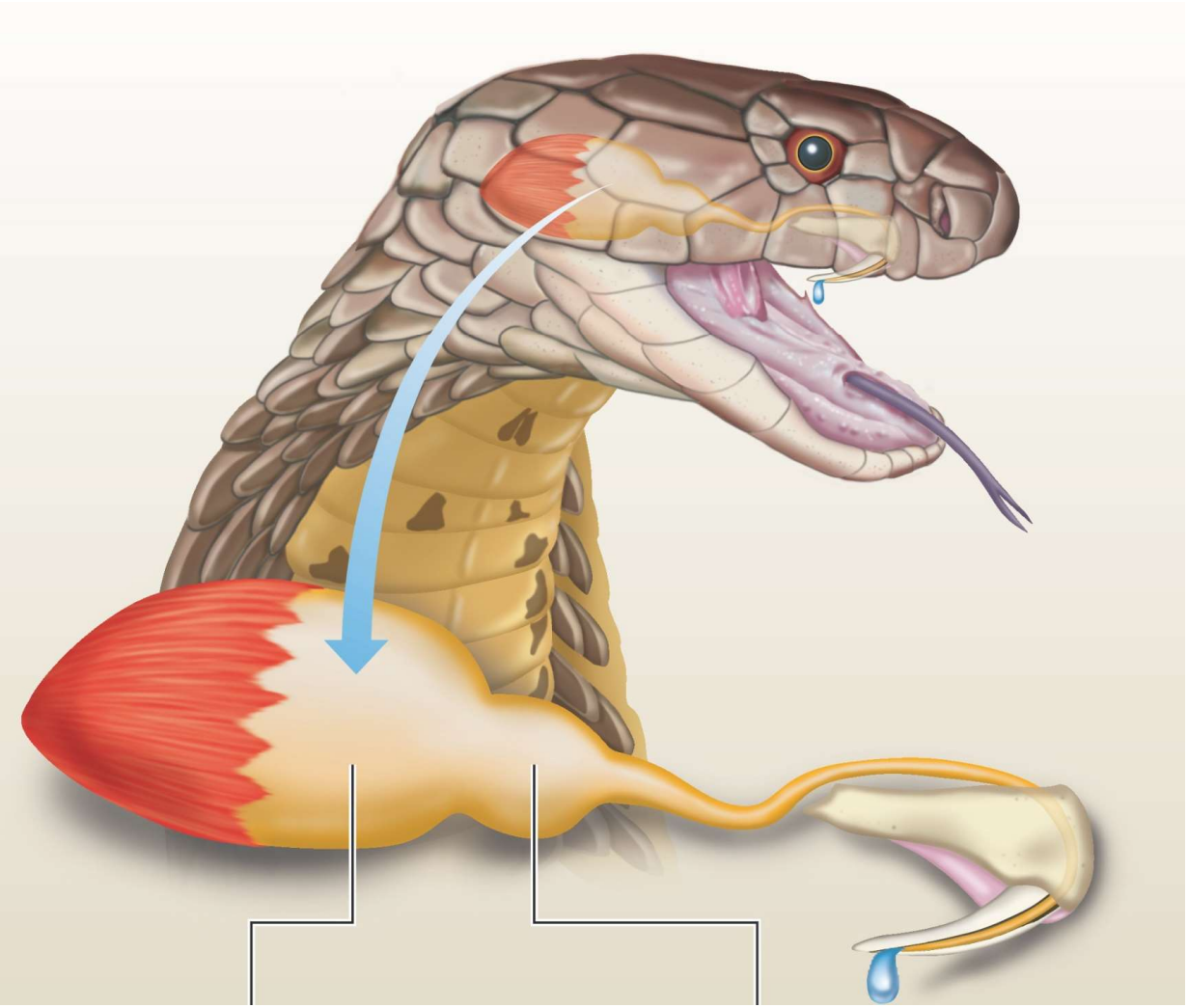
Nicholas Casewell

Image: Simon Townsley

Global burden of snakebite

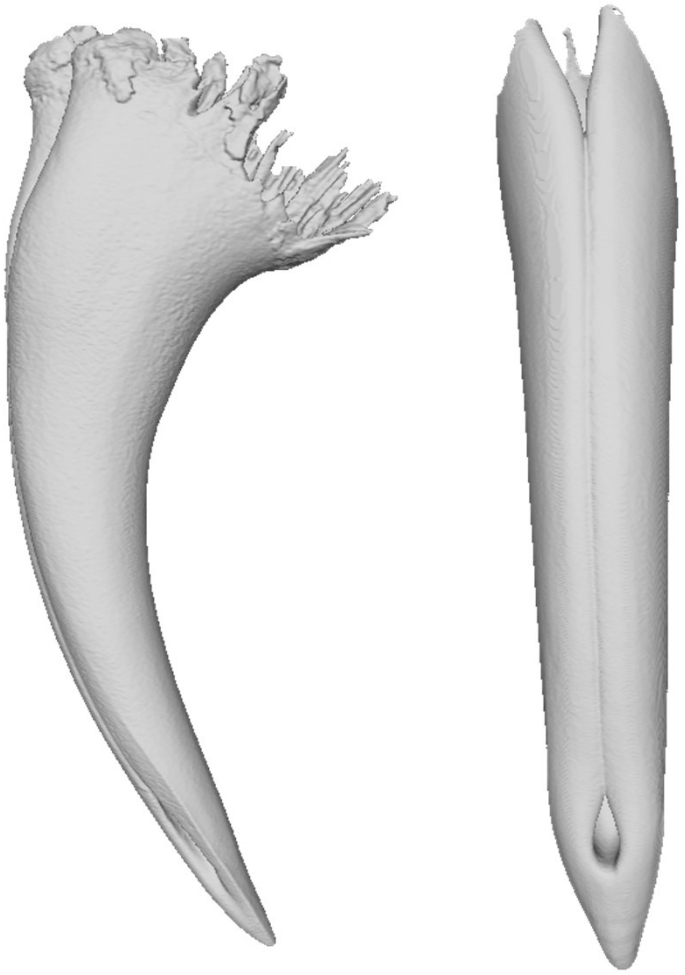


Gutiérrez *et al.* 2017. *Nat. Rev. Dis. Primers*



venom gland

accessory gland



fangs

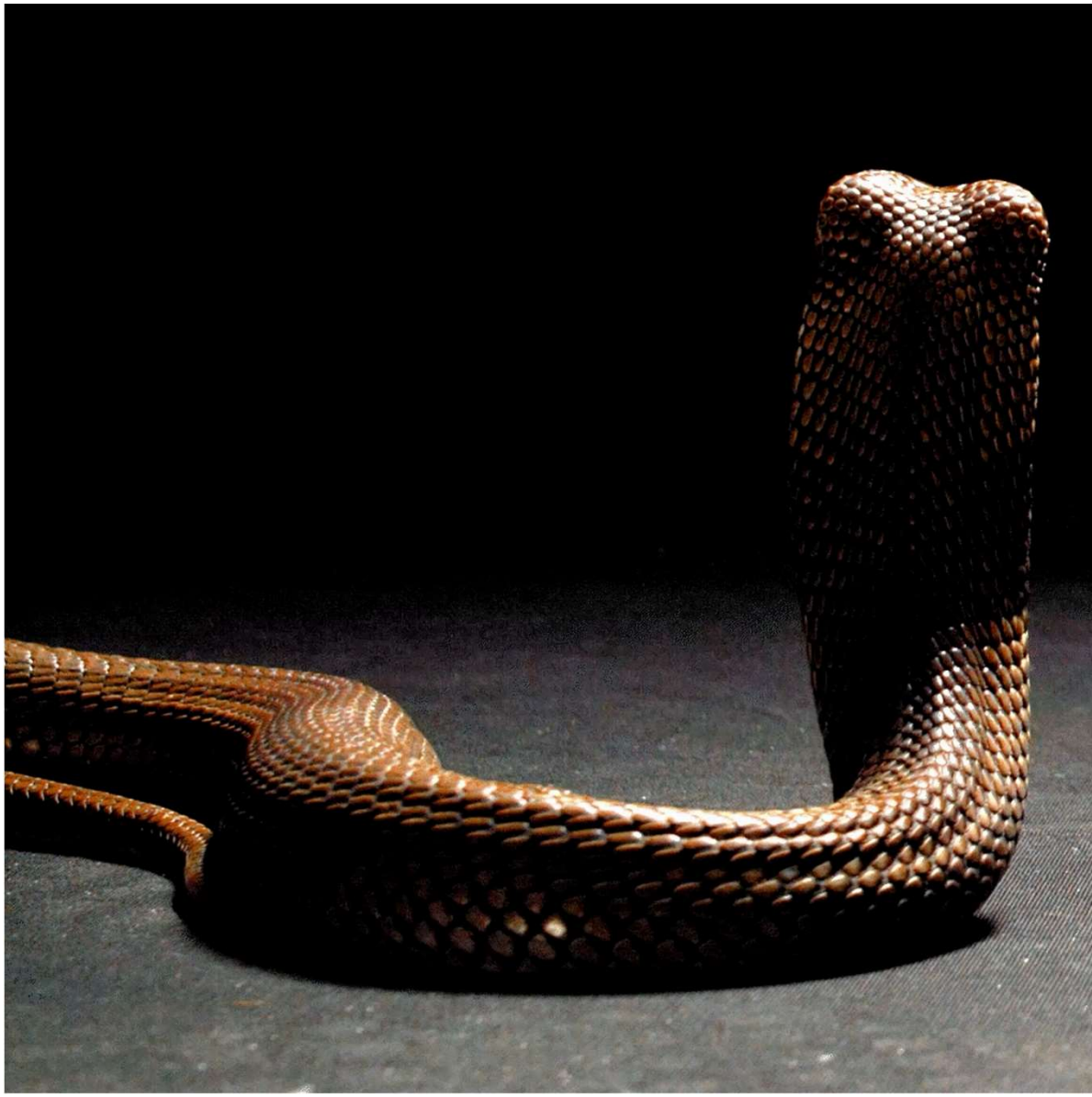
Medically important (front fanged) snakes

Elapids

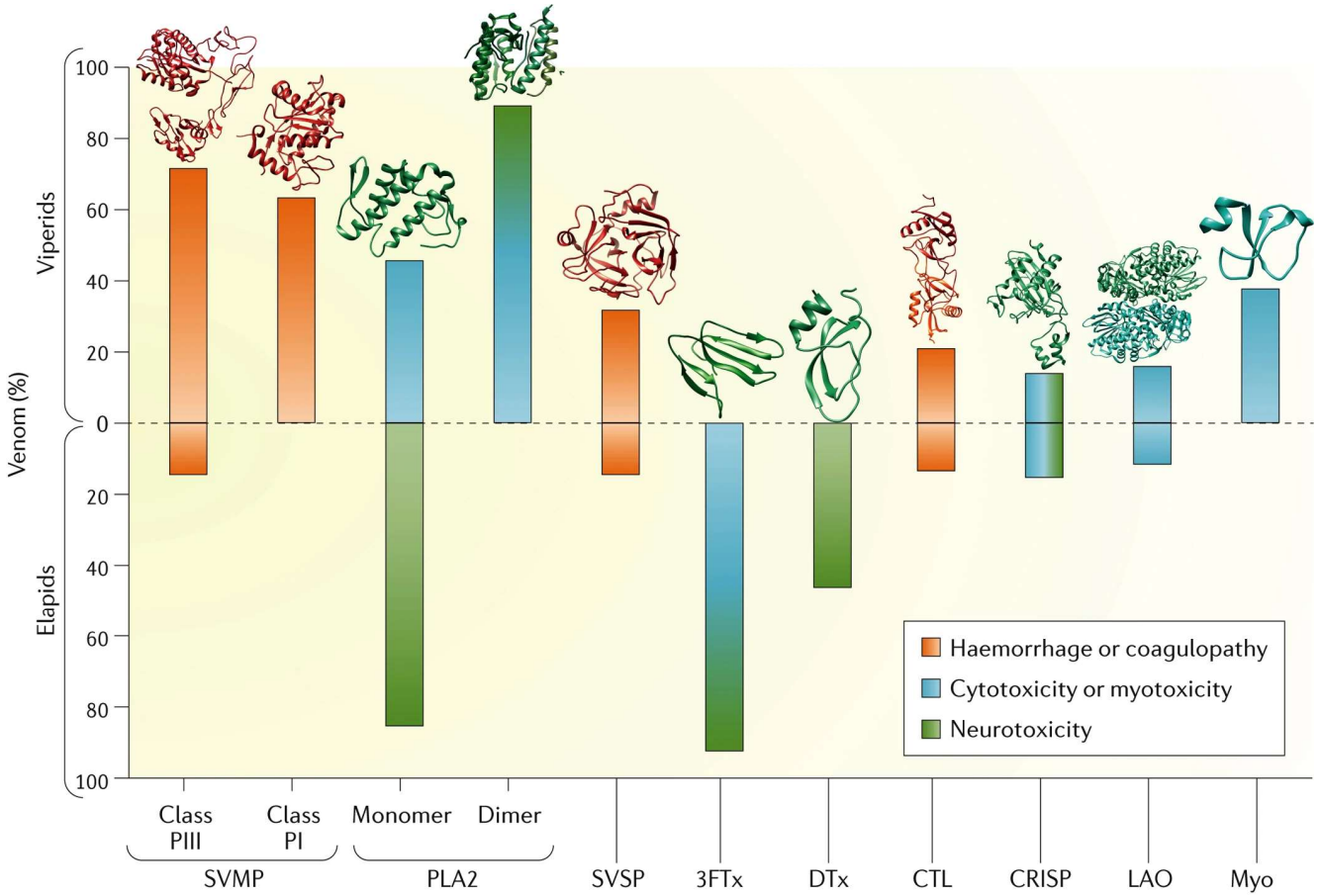
Vipers

Snake venoms

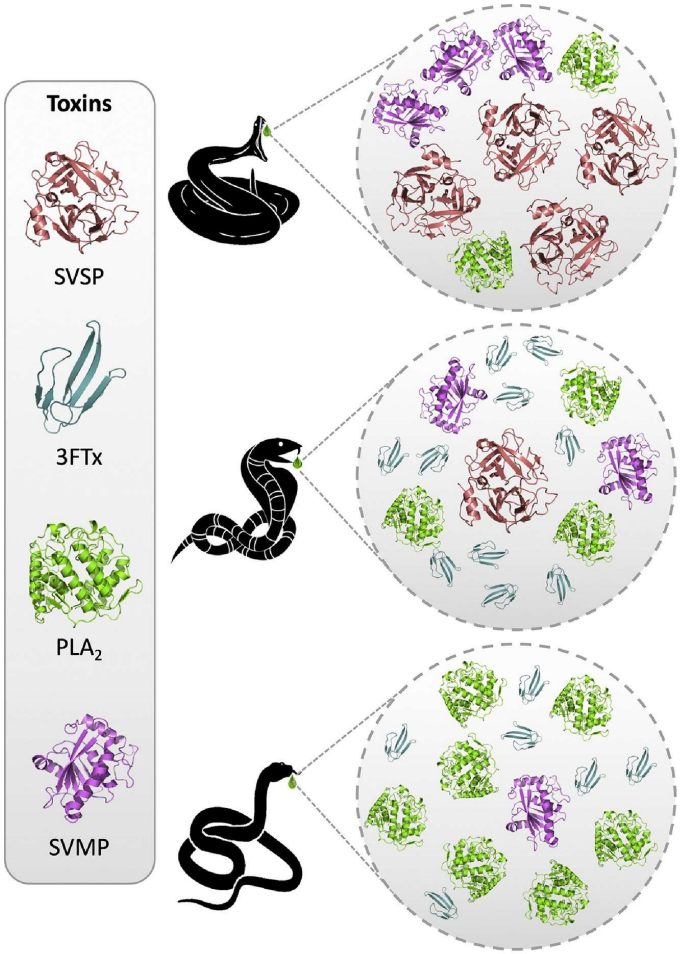
- Mixtures of proteins and peptides
- Venoms vary in composition
    - Inter-specifically
    - Intra-specifically
- Primarily used for prey capture
    - Composition linked to diet
- BUT, also used for defence
    - >100,000 human deaths/year
    - (and some can spit venom defensively too!)



Snake venom variation

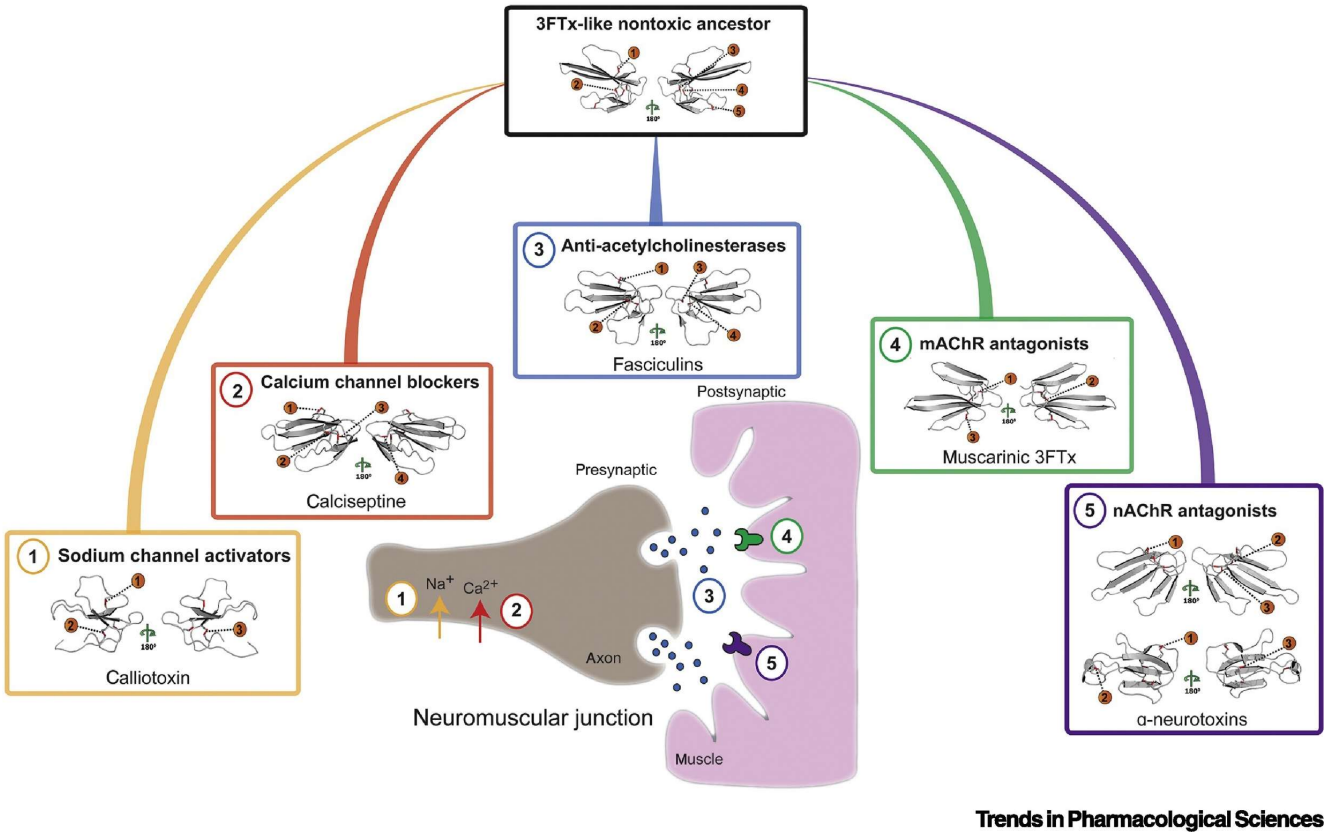


Gutiérrez *et al.* 2017. *Nat. Rev. Dis. Primers*



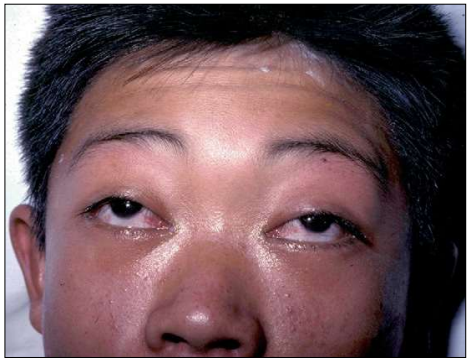
Casewell *et al.* 2020. *Trends Pharmacol. Sci.*

Toxin composition dictates pathology

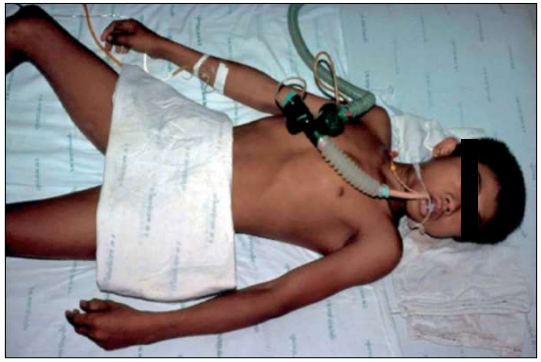


Casewell et al. 2020. *Trends Pharmacol. Sci.*

Elapid snakes



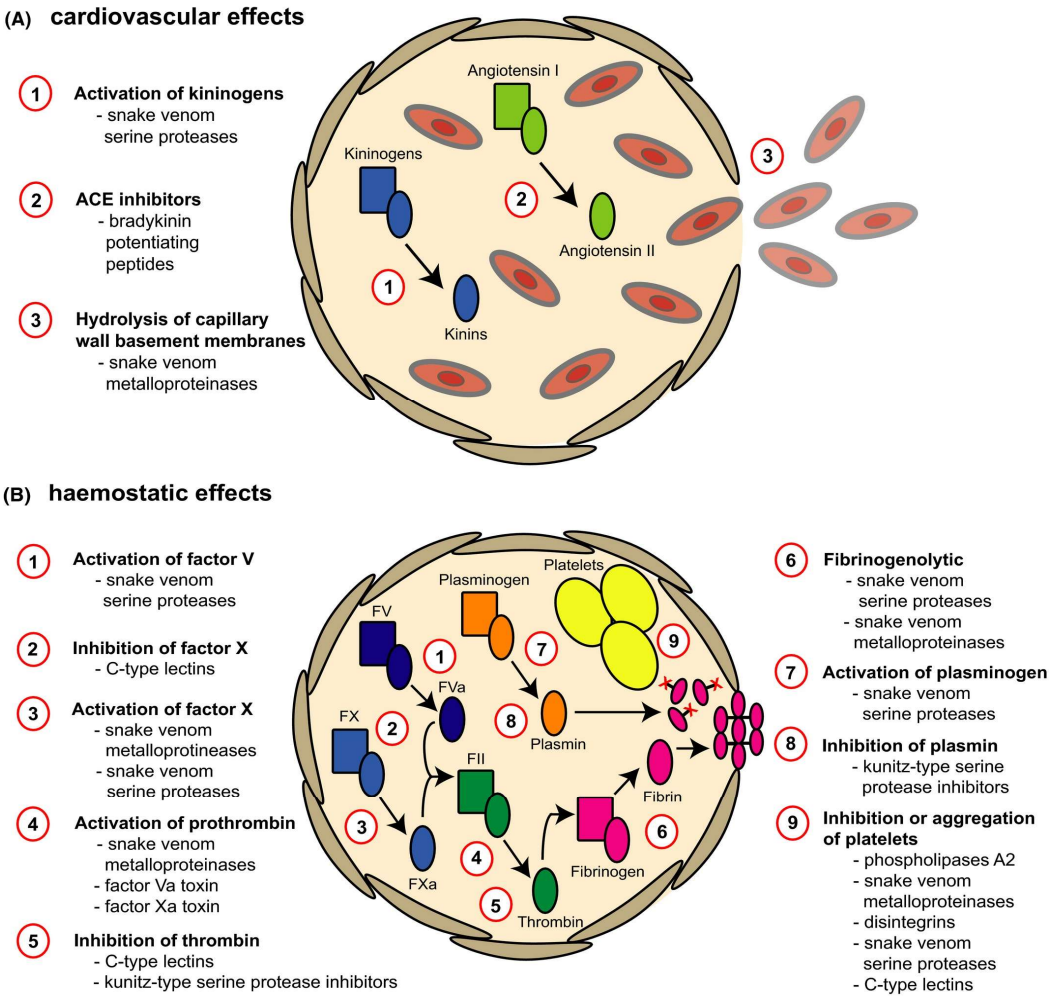
Ptosis



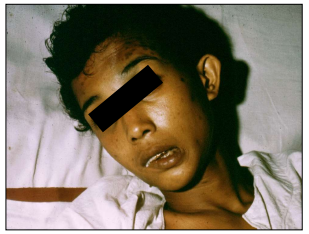
Respiratory failure

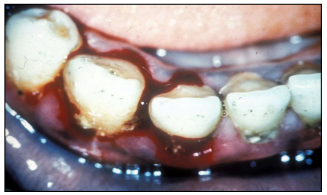
neuromuscular paralysis

Toxin composition dictates pathology

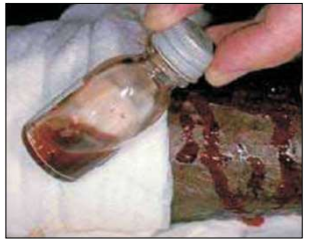


Viperid snakes

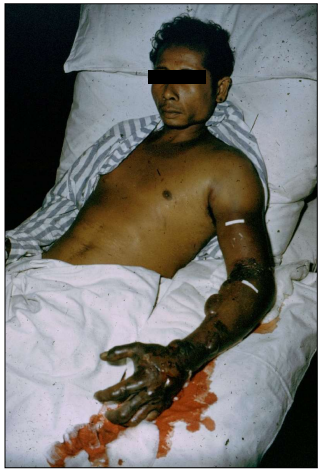




Intra-cranial haemorrhage



Non-clotting blood (VICC)



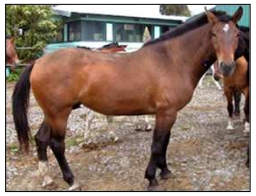
systemic haemorrhage

Slagboom et al. 2017. *British Journal Haematology*

Venom variation impacts on antivenom efficacy

1) Extract venom

2) Immunise



3) Extract blood

4) Purify antibodies

5) Formulate

Venom variation → low paraspecific efficacy against different biting species

The more venoms used in manufacture:

- the more distinct IgGs
- the less IgG to each venom
- the more vials required for cure
- the greater the potential for adverse effects
- the greater the cost

Many antivenom manufacturers globally

Europe
Manufacturers in:
France, Poland, UK, Russia, Croatia

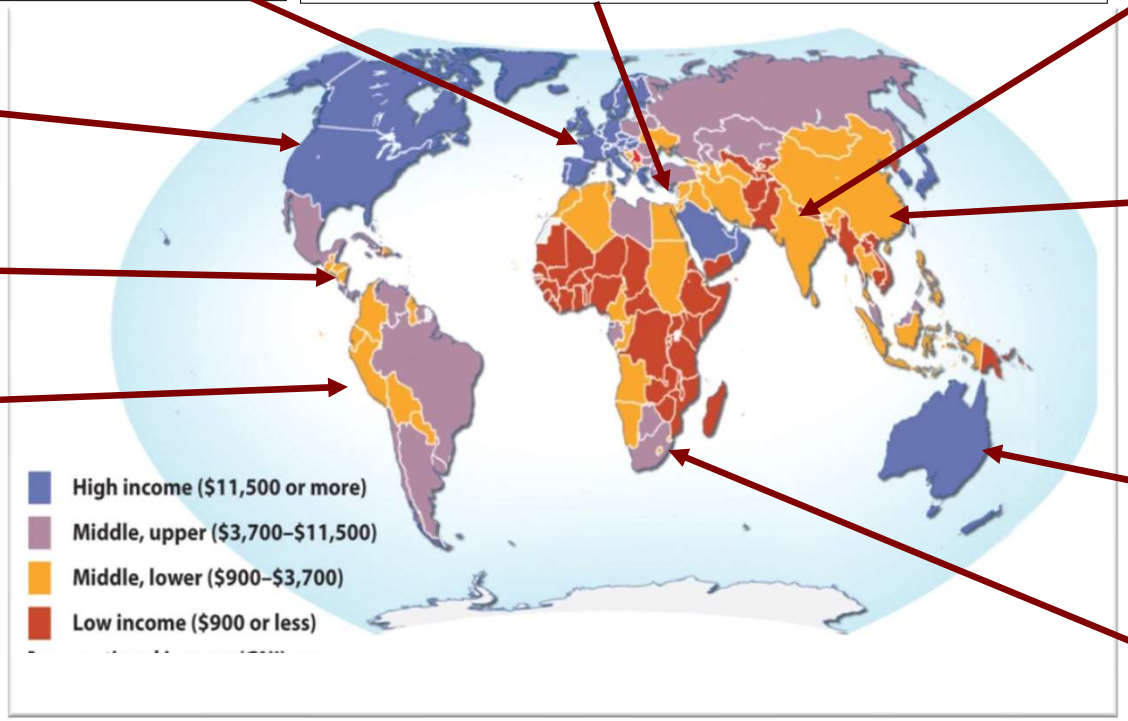
Middle East/N Africa
Manufacturers in:
Iran, Saudi Arabia, Israel, Egypt, Tunisia, Algeria

India
Five manufacturers

N America
Manufacturers in:
USA
Mexico

C America
Manufacturers in:
Costa Rica

S America
Manufacturers in:
Brazil
Colombia
Argentina
Peru
Venezuela
Uruguay
Ecuador



Asia
Manufacturers in:
Thailand
Japan
China
Indonesia
Phillipines
Korea
Myanmar
Pakistan

Australasia
Manufacturer in:
Australia

Sub-Saharan Africa
Manufacturer in:
South Africa

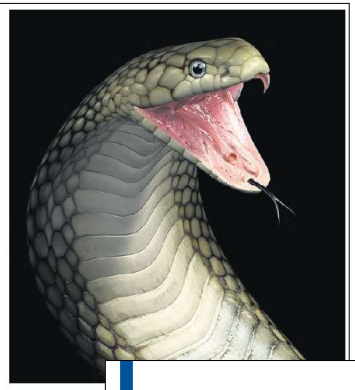
http://apps.who.int/bloodproducts/snakeantivenoms/database/

The result is a fragmented and vulnerable market

The Financial Times (10/10/2014)

Snake bite anti-venom remains crude and expensive

Research Fear and stigma is hampering progress on treatment for a killer condition. By *Andrew Jack*



On a farm at an undisclosed location in Utah, a British company milks venom from 500 carefully nurtured snakes every month. It ships the venom to Australia to inject into thousands of cossetted sheep, then processes in Wales the immunoglobulin that the sheep produce, before sending it on to the US for distribution to doctors.

"Those sheep are the biggest and happiest you'll ever meet," jokes Andy Burrows, vice-president of corporate and investor relations at BTG, the company which produces CroFab anti-venom to treat more than 5,000 potentially lethal pit-viper bites every year in North America. "Lambs are usually slaughtered before they are 18 months old. Ours are euthanised after nine or 10 years, when they have tooth decay so they can no longer eat."

On paper, the business sounds lucrative. CroFab sells for $2,000 a vial - and a course of treatment can require more than 24 vials. Yet BTG, which generates $100m a year from the product, is investing little in diversifying into treatments for other poisonous snake bites or expanding its existing treatment to other countries.

This complicated chain reflects the difficulties of producing affordable, effective and innovative treatments around the world for snake bites, one of the more neglected medical conditions of the poor, and estimated to cause 100,000 deaths globally each year. Amputation and psychological trauma is a consequence for many more.

"For every death, there are possibly four times more left handicapped," says David Warrell, emeritus professor of tropical medicine at Oxford, who is hosting an international conference next year to draw wider attention to the problem. "There is not just mortality and morbidity but psycho-morbidity. The experience of being bitten as a child often leaves a lasting impression."

He describes being "laughed out of court" by experts for years when he raised concerns over snake bites - by donors, academics and clinicians in the west and even in countries such as India, where snake bites are common. "People find it very difficult to take seriously."

He suggests the reasons include "a primal, biblical, morbid fear of snakes" and stigma, as most snake bites occur in poor, rural areas. Only a handful of authorities, including Myanmar, have given the problem significant political attention.

Dr Robert Harrison, head of the Alistair Reid venom research unit at the Liverpool School of Tropical Medicine, says that most funders have failed to show interest - from the Gates Foundation to the European Commission, which recently inadvertently excluded snake bites by unveiling programmes earmarked specifically for "infectious" diseases.

"Snake bites have no epidemic potential," he says. "They have been treated in the [World Health Organisation]

Snake bite incidence and mortality rate

	Incidence ('000)
Tropical disease	
Chagas disease	21
Cholera	17
Dengue Haemorrhagic fever	7
Leishmaniasis	1,69
Japanese encephalitis	4
Schistosomiasis	5,73
Snake bite envenoming	420-2,68
Yellow fever	0.1-2.1

Source: www.thelancet.com

statistics alongside road tr
dents as injuries - something
very little about. It's very d
change that perception."

Both researchers are cautio
mistic, as they point to recen
ments in prevention, diagn
treatment that could help r
burden. The launch of the Glo

Editorial

The Lancet (18/09/2015)

Snake bite—the neglected tropical disease

Last week, Médecins Sans Frontières drew attention to the fact that by mid-2016 sub-Saharan Africa will run out of one of the most effective treatments for snake bite, Fav-Afrique. Sanofi Pasteur stopped manufacturing the antivenom last year, and stockpiles will expire in June, 2016. Fav-Afrique is the only antivenom proven to be safe and effective to treat envenoming by all the different types of snake in sub-Saharan Africa, where an estimated 30 000 people die from snake bite and 8000 end up with amputations every year. No replacement product is likely to be available for at least 2 years.

Globally, about 5 million people are estimated to be bitten by snakes every year, causing about 125 000 to die and 400 000 to be permanently disabled or disfigured. Snake bite has a mortality rate equivalent to one fifth of deaths due to malaria worldwide, and half of that due to HIV/AIDS in India. Yet snake bite is largely invisible to WHO, other international and national health agencies, many African governments, and to the big donors, and has been marginalised even within the neglected tropical diseases community.

With its triad of high mortality, high
substantial psychological morbidity, snak
high priority research. Robust data are nee
donors and governments to subsid
development and distribution. It costs
treat one patient with Fav-Afrique (each
two to four are needed per bite). Cheap, safe, effective, and non-cold chain-dependent antivenoms are desperately needed. Other products by South African, Costa Rican, Mexican, and Indian producers need to be used in combination to neutralise different species' venoms, and need further research.

Snake bite largely affects those aged 10–30 years old living in the poorest, most rural areas of the world, where health-care facilities are few and far between. Identifying key actions to reduce the public health neglect of snake bite victims is the aim of the 2-day meeting next week funded by the Wellcome Trust. Urgent implementation of the actions will be needed, which can only happen with the aid of major donors. ■ *The Lancet*

For **MSF on snake bite** see http://www.msf.org/article/global-health-community-slithers-away-snakebite-crisis-antivenom-runs-out

For **snake bite data** see http://www.lstmed.ac.uk/research/centres-and-units/the-alistair-reid-venom-research-unit

The snakebite fight

Snakes kill tens of thousands of people each year. But experts can't agree on how best to overcome a desperate shortage of antivenom.

BY CARRIE ARNOLD

Nature News (01/09/2016)

Nature News (18/09/2015)

IN FOCUS NEWS

GLOBAL HEALTH

Africa braced for snakebite crisis

Health specialists warn that stocks of antivenom will run out in 2016.

BY QUIRIN SCHIERMEIER

PAUL STAROSTA/CORBIS

Rural Africa is facing a resurgence of a persistent plague that rarely makes headlines: snakebite.

By June next year, stockpiles of the antivenom that is most effective against Africa's vipers, mambas and cobras are expected to run out because the only company that makes the medicine has stopped production. With no adequate replacement in sight, the death toll from bites is set to rise, specialists warned at a tropical-medicine congress last week in Basel, Switzerland.

"We're dealing with a neglected health crisis that is turning into a tragedy for Africa," says Gabriel Alcoba, a medical adviser with the international humanitarian group Médecins spokesman. Sanofi Pasteur is working to enable the transfer of know-how to companies willing to take over production of Fav-Afrique, he says.

Pharmaceutical companies in South Africa, India, Mexico and Costa Rica are among those marketing cheaper products — some of which work well against snakes in their host nations. But their safety and effectiveness against the large variety of species in Africa have not yet been established in clinical trials. To speed up the process, MSF is offering two of its hospitals in the Central African Republic (CAR) and South Sudan as study sites. But it will take at least two years to validate the products in development, and none is as broadly efficient as Fav-Afrique, Alcoba says.

NEGLECTED THREAT

The real-world consequences can be disastrous

- Fake products
- Dilute products
- Geographically inappropriate products

= disastrous outcomes for snakebite patients

CAF: ↑ from 0.4% to 10.0%

Ghana: ↑ from 1.8% to 12.1%

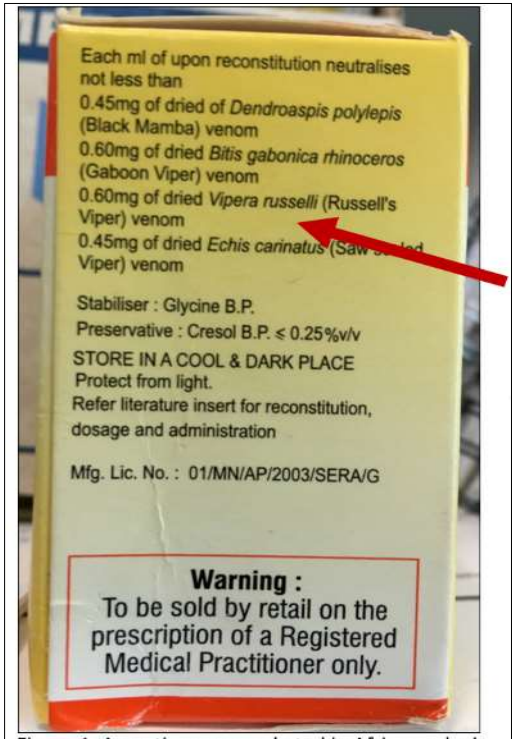


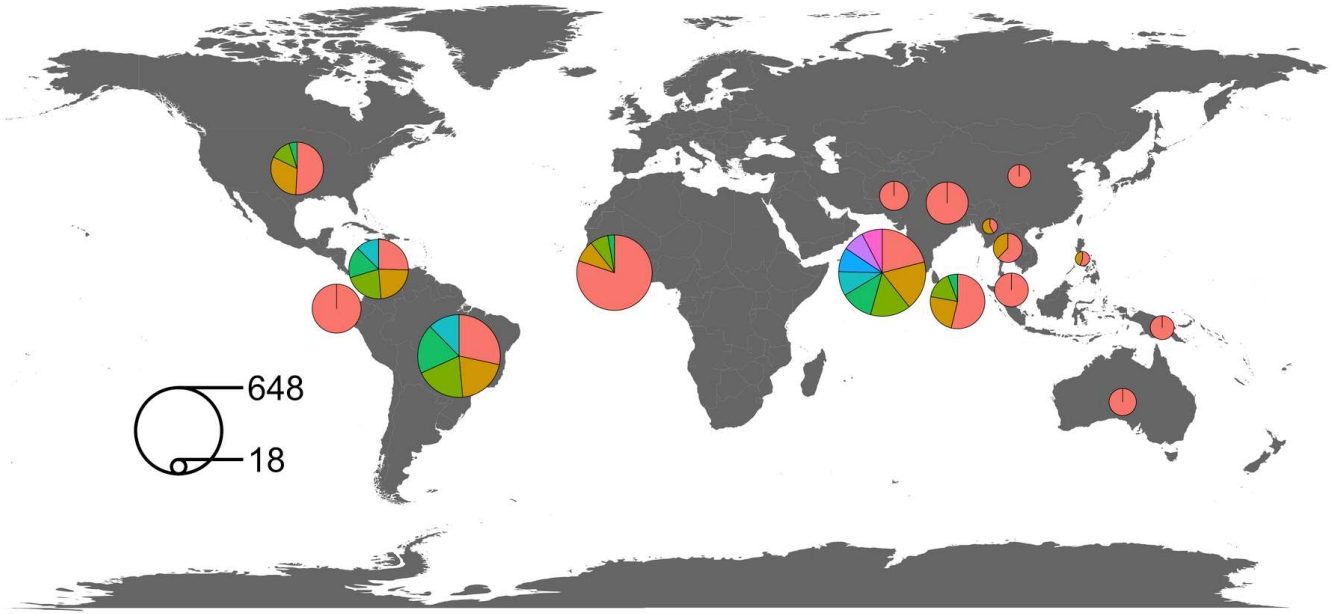
Figure 1. An antivenom marketed in Africa – why is an Indian snake included in its efficacy statement?

Alirol et al. 2015. *PLOS NTD*

Visser et al. 2008. *Trans. R. Soc. Trop. Med. Hyg.*

How do we know which products are effective?

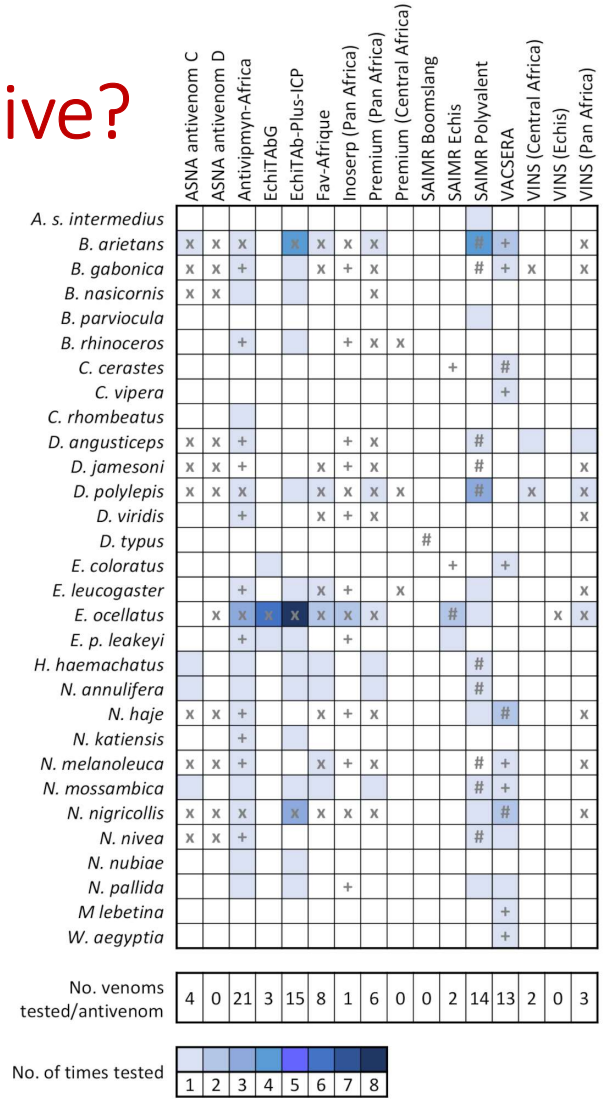
- Weak regulatory frameworks
- Limited robust clinical trials
  - Difficult to perform for multiple biting species
  - Outcome measures highly variable



Abouyannis et al. 2021.
PLOS NTD

How do we know which products are effective?

- Weak regulatory frameworks
- Limited robust clinical trials
- Antivenom efficacy reliant on preclinical testing
  - Models are limited
  - Testing restrictive



Ainsworth et al. 2020. *PLOS NTD*

Antivenom efficacy is unpredictable

- SAIMR polyspecific **'gold standard'**
  - (South African Vaccine Producers)
- FavAfrique
  - (Sanofi Pasteur)
- Snake Venom Antiserum (African)
  - (VINS Bioproducts Ltd)
- Polyvalent Snake Venom Antiserum (PAN AFRICA)
  - (Premium Serums and Vaccines Ltd)
- Inoserp PANAFRICAIN
  - (Inosan Biopharma)
- SAIMR *Echis* monospecific
  - (South African Vaccine Producers)

Dose efficacy is unpredictable

saw-scaled viper

SAIMR *Echis* or Premium

puff adder

SAIMR poly or Premium

black mamba

SAIMR poly

cobras

FavAfrique or Premium

FavAfrique or Premium

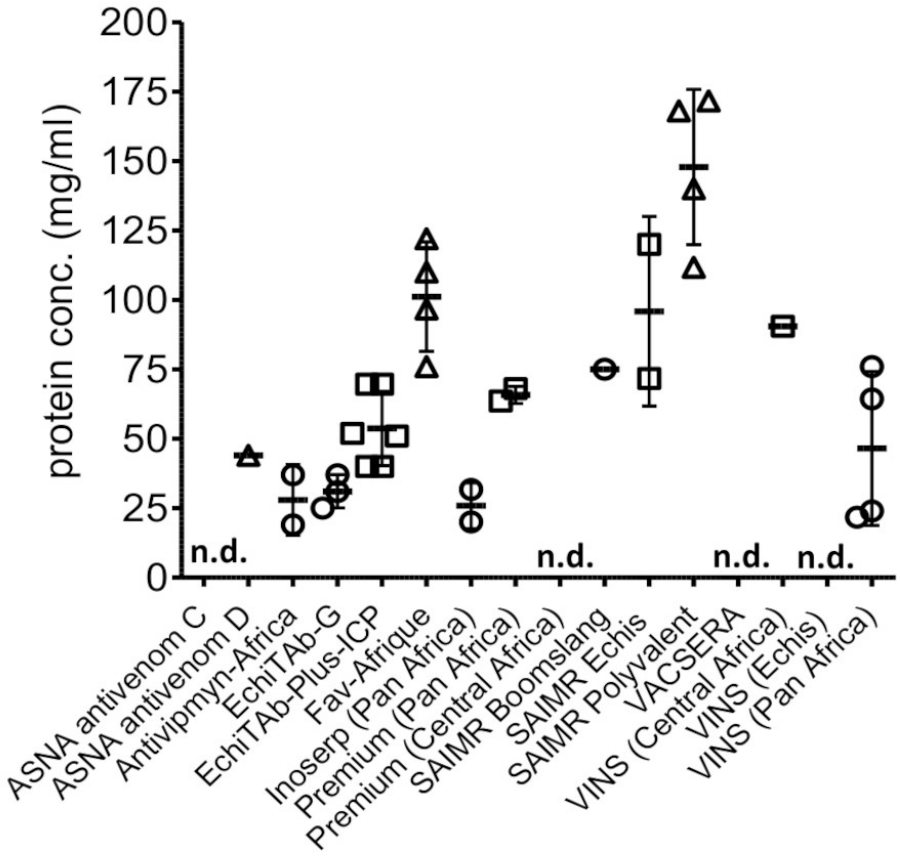
SAIMR poly

Harrison et al. 2017. *PLOS NTD*

Antibody content likely plays a major role

Antivenom	US $/vial	Antibody (mg/ml)
Premium - PAN AFRICA poly	$ 84	63.3
VINS – African	$ 48	21.7
Inosan – Inoserp PANAFRICAIN	$ 105	31.7
Sanofi – FavAfrique	$ 79-99	96.7
SAVP - SAIMR polyvalent	$ 315	111.7
SAVP - SAIMR *Echis*	$ 315	71.7

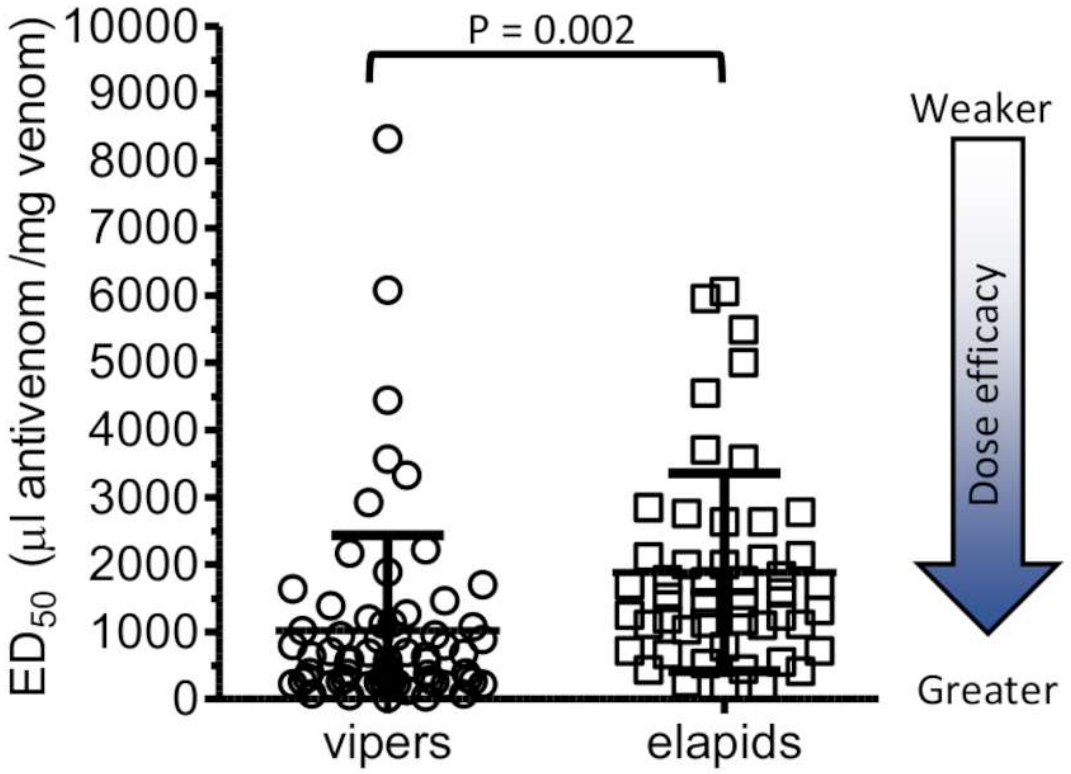
Harrison et al. 2017. *PLOS NTD*



Ainsworth et al. 2020. *PLOS NTD*

But immunogen composition influences dose efficacy

- Elapid venoms have a higher proportion of low molecular weight toxins
- These are poorly immunogenic compared with larger viper enzymes
- Results in polyvalent antivenoms having weaker dose efficacy against elapid venoms



Ainsworth et al. 2020. *PLOS NTD*

How many venom immunogens do we need?

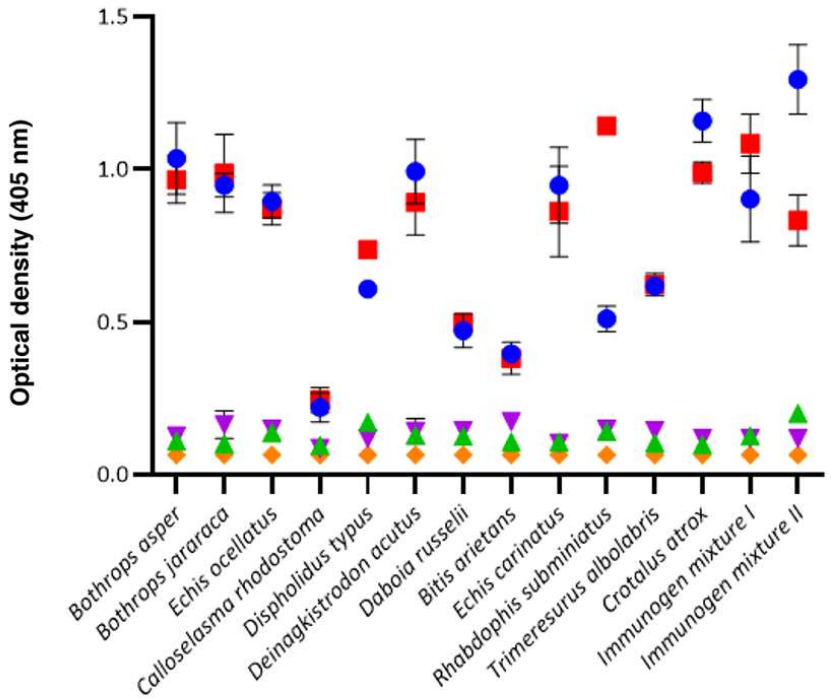
- Generic anti-haemotoxic polyvalent antivenom
- Used two different immunising mixtures – 7 and 12 venoms
- Immunised sheep, compared responses *in vitro* and venom neutralisation *in vivo*

	Species	Sub-family	Geographical region	Venom origin
Immunogen mixture I (resulting in EAV 1)	*Bothrops asper*	Crotalinae	Central America	Costa Rica
	Bothrops jararaca	Crotalinae	South America	Brazil
	Echis ocellatus	Viperinae	West Africa	Nigeria
	Calloselasma rhodostoma	Crotalinae	Southeast Asia	Captive bred
	Dispholidus typus	Colubrinae	sub-Saharan Africa	South Africa*
	Deinagkistrodon acutus	Crotalinae	East Asia	Captive bred
	Daboia russelii	Viperinae	South Asia	Sri Lanka
Immunogen mixture II (resulting in EAV 2)	The same 7 venoms in immunogen mixture I plus:			
	Bitis arietans	Viperinae	sub-Saharan Africa	Nigeria
	Echis carinatus	Viperinae	Middle East & South Asia	India**
	Rhabdophis subminiatus	Natricinae	Southeast Asia	Hong Kong
	Trimeresurus albolabris	Crotalinae	Southeast Asia	Captive bred
	Crotalus atrox	Crotalinae	North America	USA

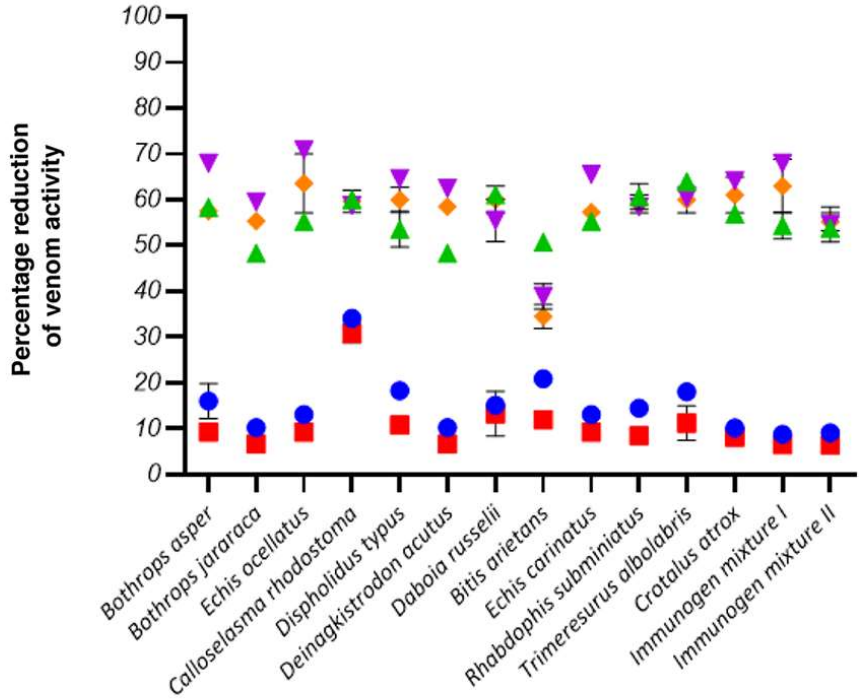
Alomran et al. 2021. *PLOS NTD*

Different immunogen diversity = comparable *in vitro* binding

End-point titration ELISA



Avidity ELISA

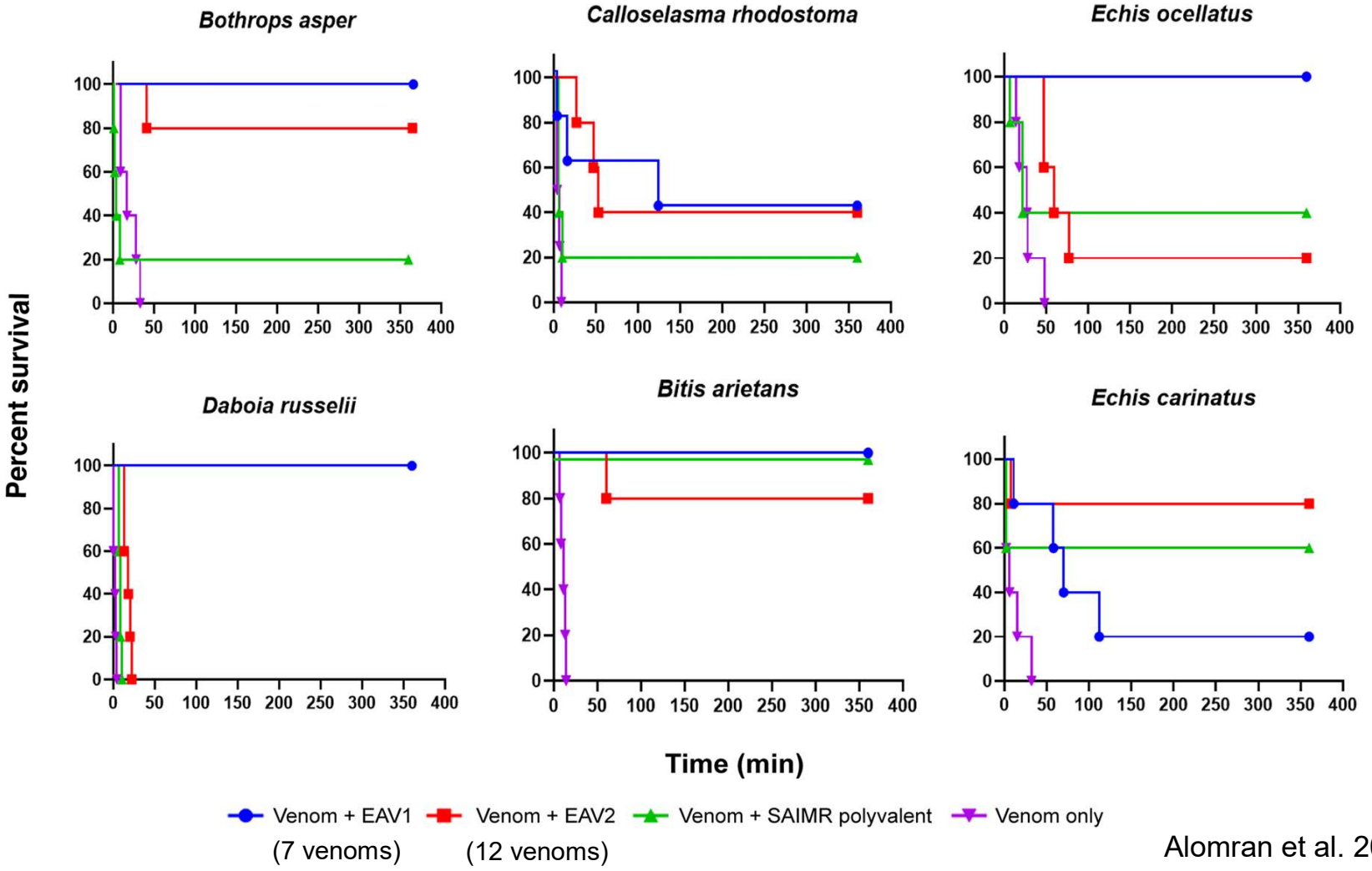


EAV1 EAV2 SAIMR polyvalent Normal sheep IgG Normal horse IgG

(7 venoms) (12 venoms)

Alomran et al. 2021. *PLOS NTD*

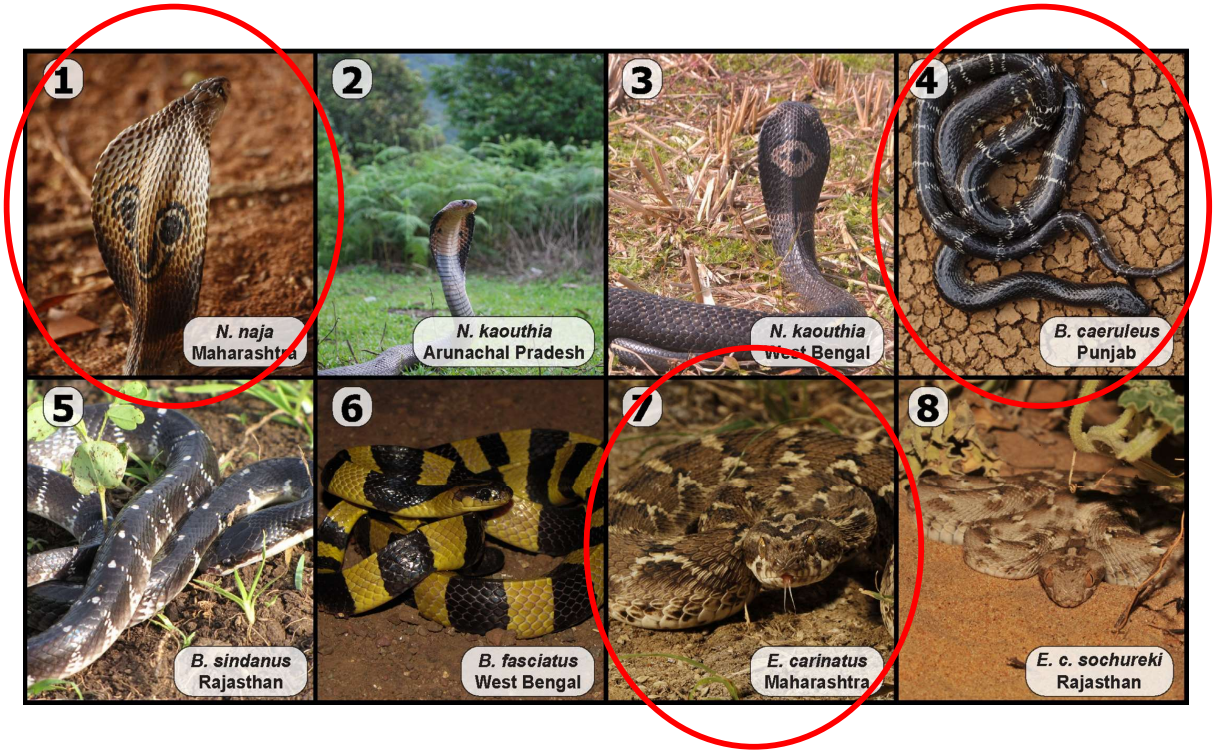
Fewer immunogens = superior dose efficacy and breadth



Alomran et al. 2021. *PLOS NTD*

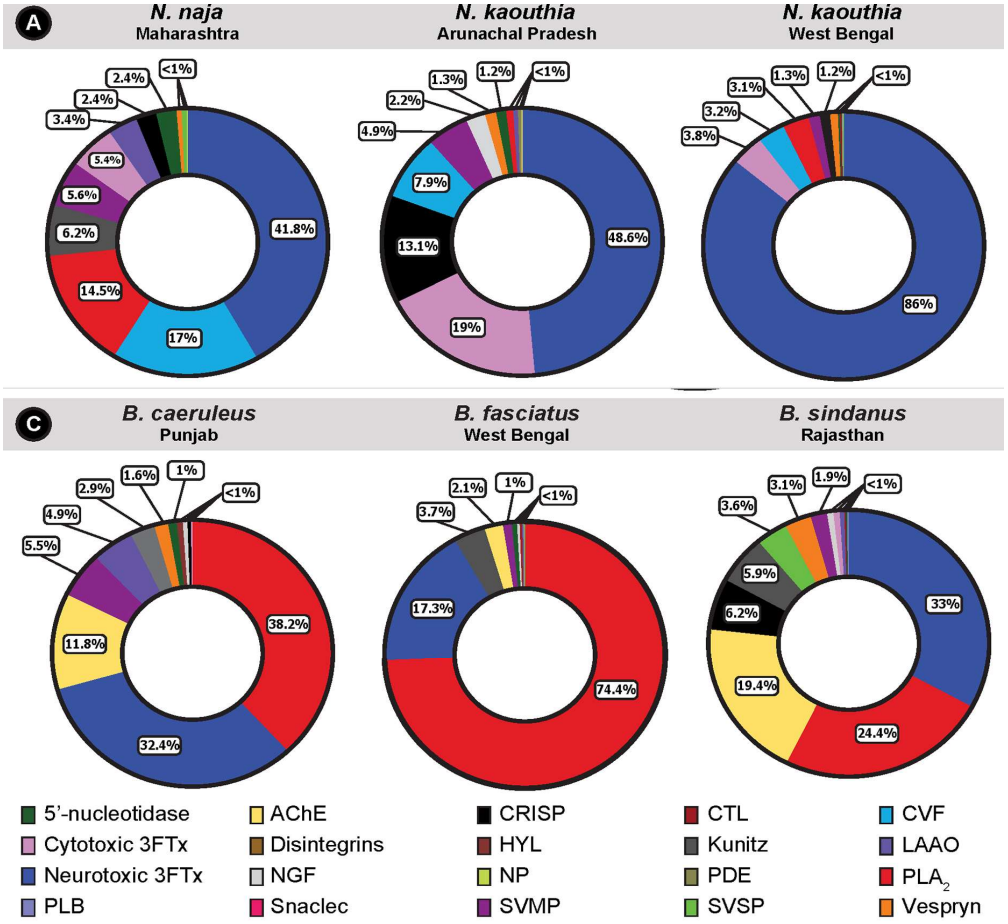
But insufficient immunogen breadth can lead to inefficacy

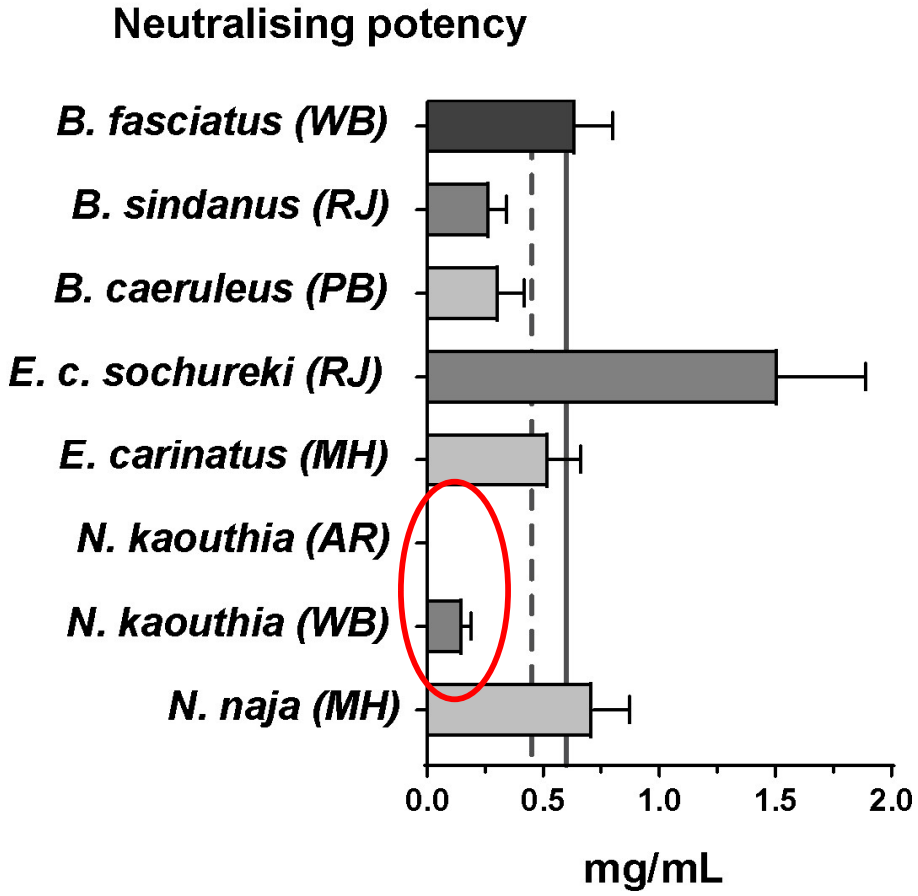
- In India there are ~58,000 snakebite deaths annually
- 'big four biting species'
  - *Naja naja* (cobra)
  - *Bungarus caeruleus* (krait)
  - *Echis carinatus* (carpet viper)
  - *Daboia russelii* (Russell's viper)
- All antivenoms made using these four venoms, sourced from SE India, as immunogens



Laxme et al. 2019. *PLOS NTD*

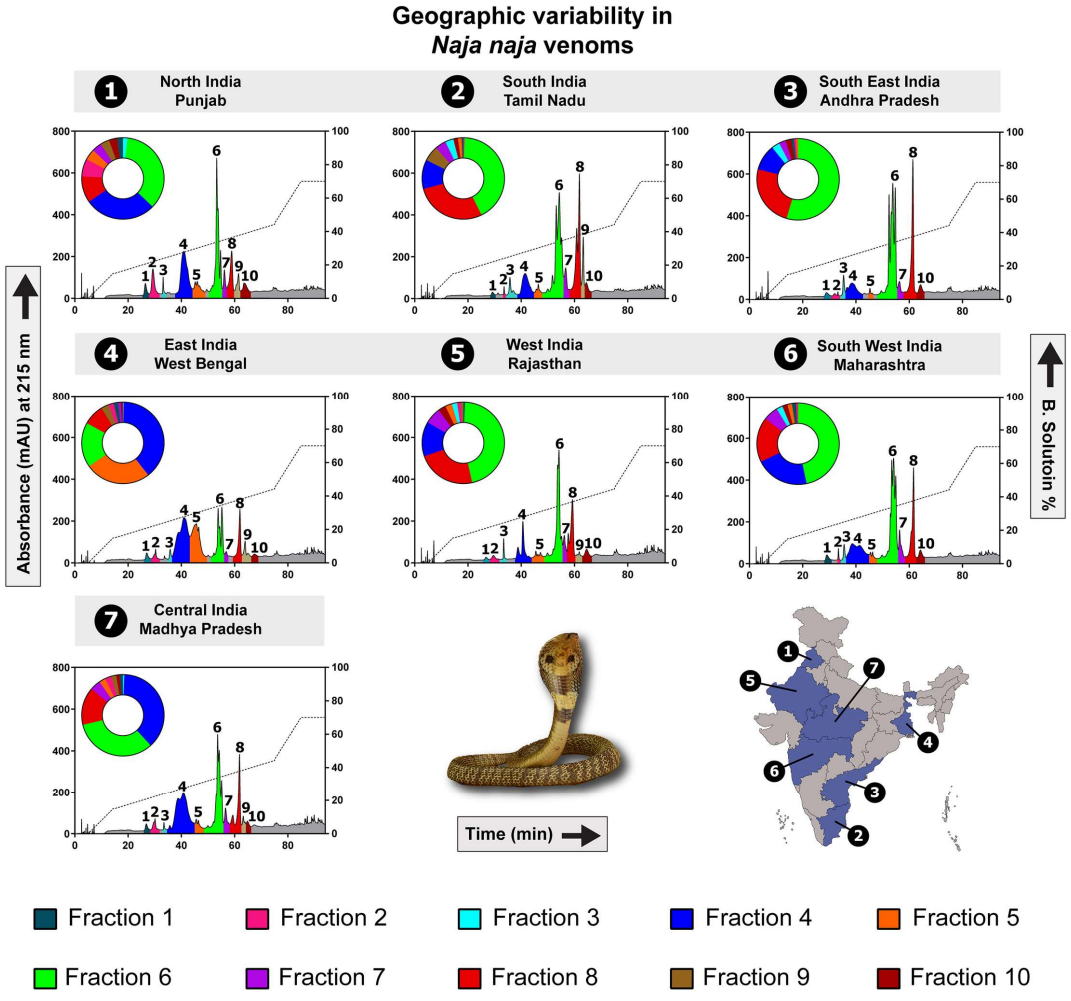
Inter-specific venom variation undermines efficacy

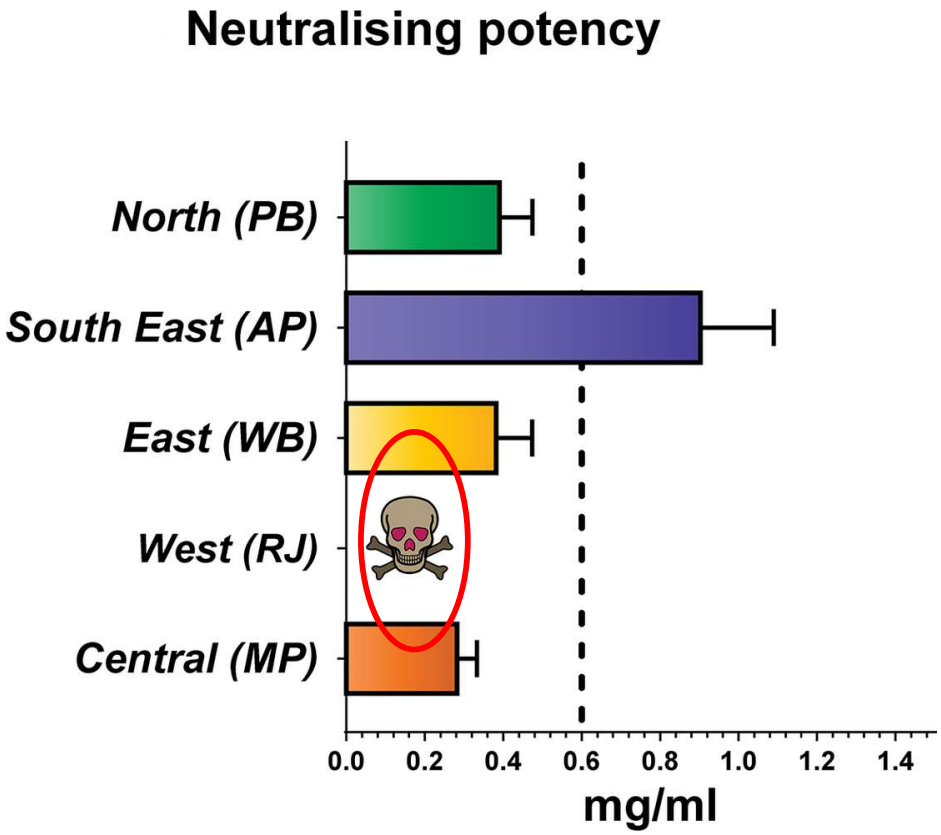




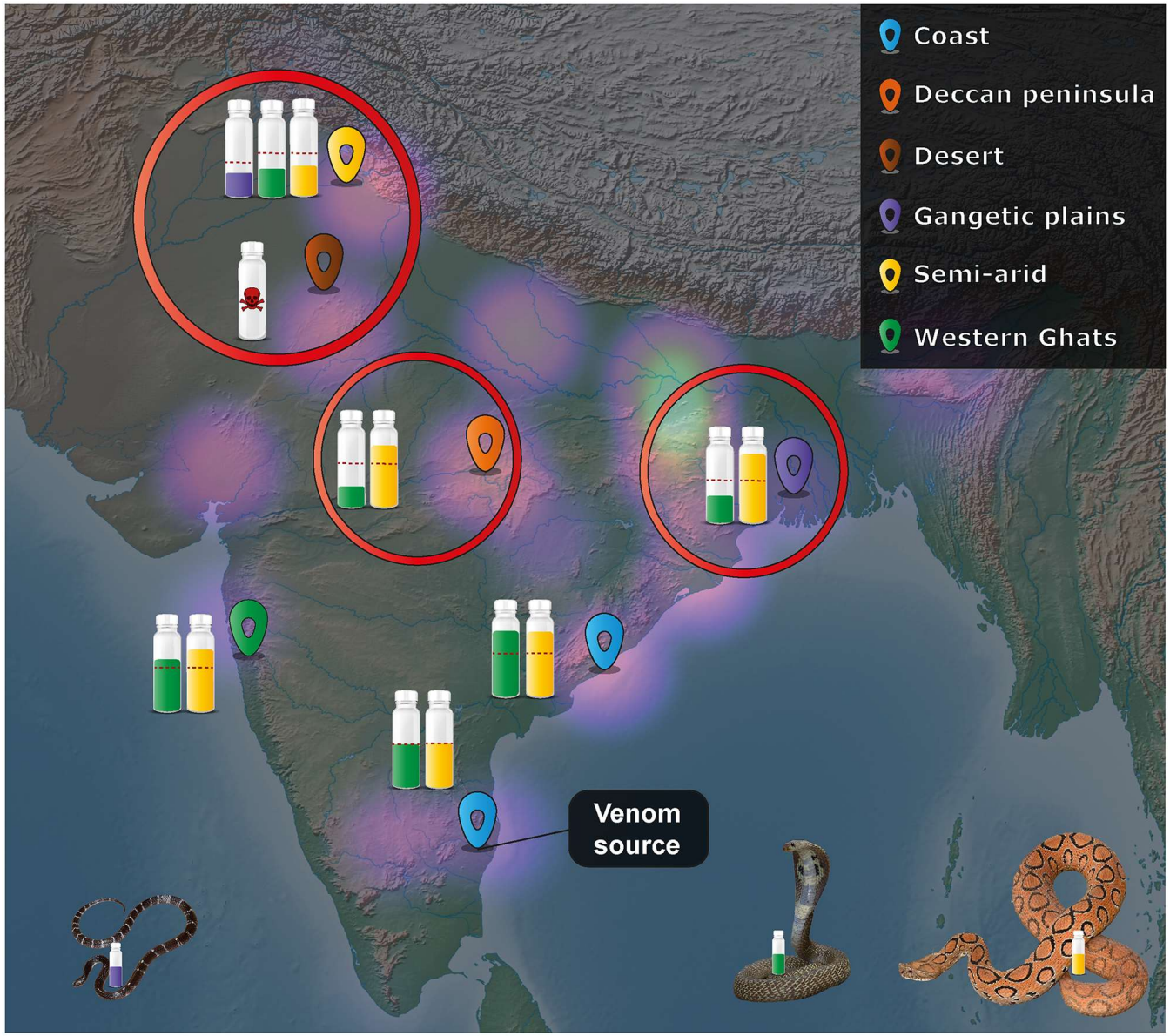
Laxme et al. 2019. *PLOS NTD*

Intra-specific venom variation undermines efficacy





Laxme et al. 2021. *PLOS NTD*



Laxme et al. 2021.
PLOS NTD

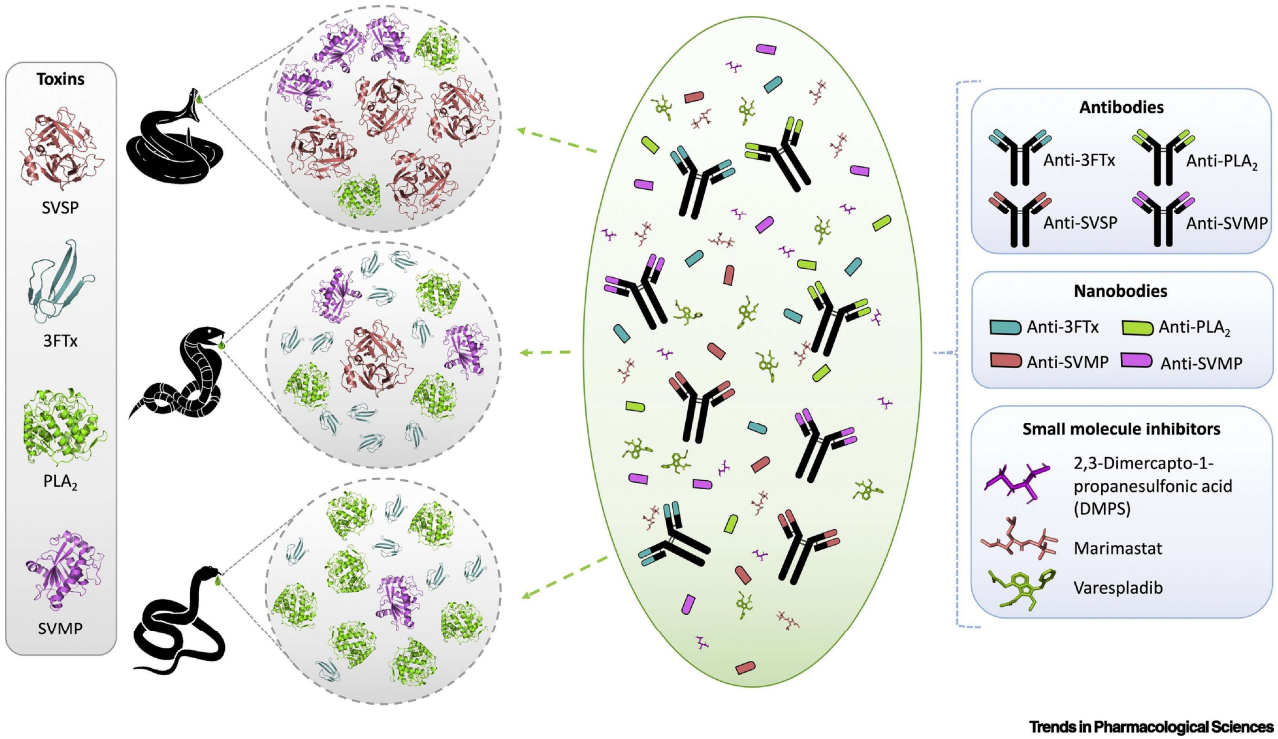
Challenges posed by venom variation

- Inter- and intra-species venom variation can dramatically reduce the efficacy of antivenom
- Convergent evolution of similar venom profiles can result in unexpected efficacy against unrelated snake species
- But predicting the efficacy of existing treatments is challenging, and even more so without knowledge of venom composition
- Robust testing is therefore required to ensure appropriate antivenom efficacy across desired geographical indication

New approaches are needed to improve/enhance antivenoms

- Toxin specific antibodies (mAbs, nanobodies, etc)
- DNA aptamers
- ADDomer virus like particle toxin binding molecules
- Receptor mimicking peptides/proteins
- Small molecule toxin inhibitors (“drugs”)

Multiple formats likely required to tackle the diversity of toxins found across geographically distinct venoms



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