Development of new-generation anti-snake venom horse antiserum

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Outline

- Snakebite envenoming : Neglected Tropical Disease
- Antivenom : anti-snake venom horse antiserum
- Development of new antivenom

    Targeting principle toxins of venom

    Recombinant technology

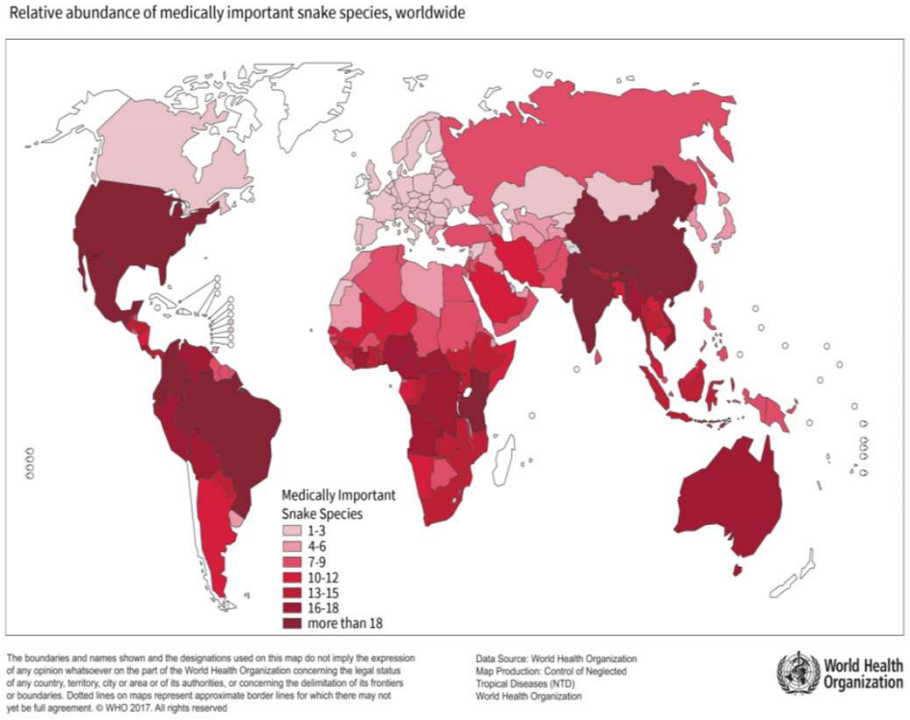
    Cross neutralization evaluation

Snakebite envenoming

- Definition:
  Injection of a mixture of venom following the bite of a venomous snake.

- There are currently more than 3000 species of snakes in the world, approximately 250 of these are listed by WHO as being of medical importance because of the harm their venoms can do.

- The highest burden of snakebites is in South Asia, Southeast Asia, and sub-Saharan Africa

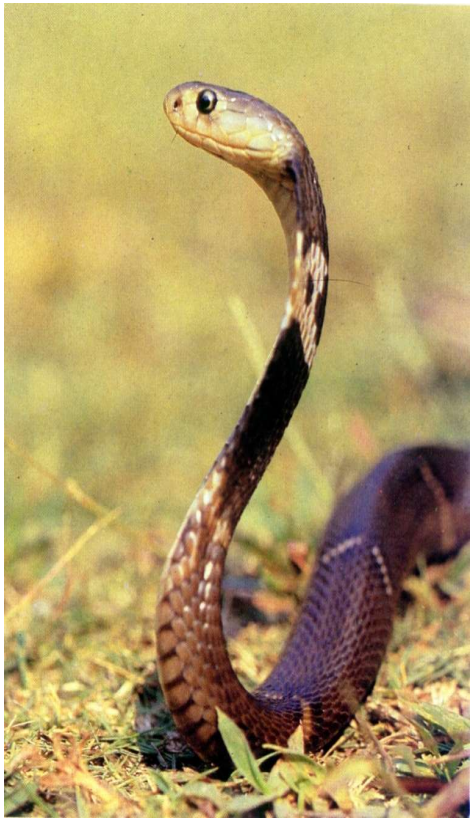


Threaten of snakebite envenoming

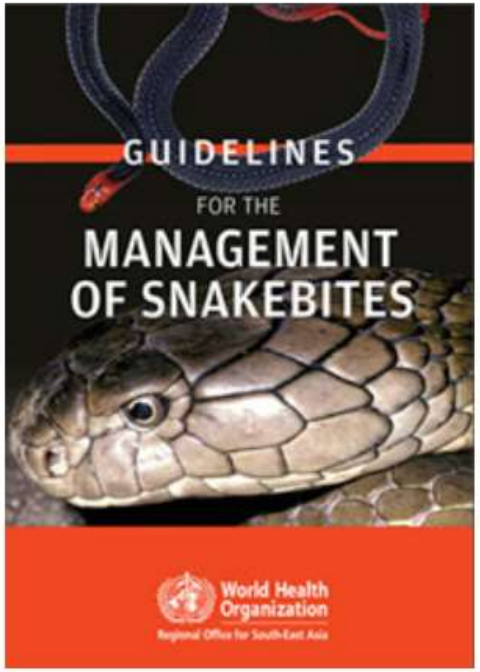
- About 50–55% of all snakebites result in envenoming.
- Near 5 million snakebite envenomings and 20,000-94,000 deaths occur worldwide each year
- 30-45% of victims are women and children.
- WHO added snakebite envenoming to the list of Neglected Tropical Diseases(NTDs) in 2017.

Venomous snakes in Taiwan

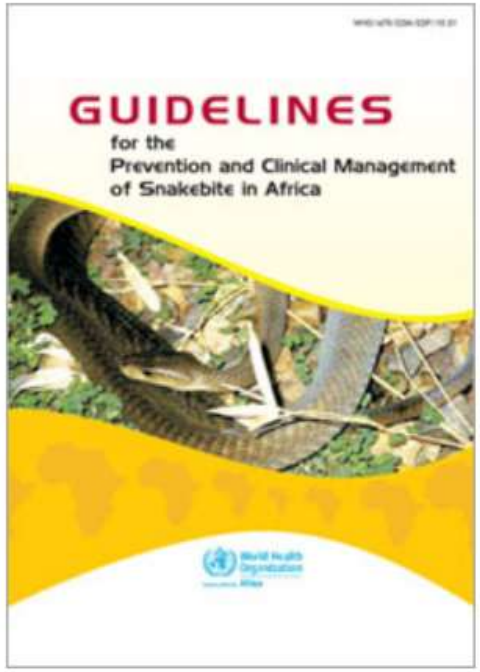
- Neurotoxicity
  - *N. naja atra* (Taiwan cobra)
  - *B. multicicinctus (Manybanded Krait)*
- Hemotoxicity
  - *D.actus (*Hundred-pace pit viper*)*
  - *T. stejnegeri (Taiwan bamboo viper)*
  - *T. mucrosquamatus (Taiwan Habu)*
- Multitoxcicity
  - *D. siamensis (Russelii viper)*



If you suspect a snake bite,



WHO Guidelines for the management of snake bites in South-East Asia



WHO Guidelines for the prevention and clinical management of snakebite in Africa

Principles:

1. Transported to a health facility without delay.
2. Administration of correct antivenom.

What is antivenom?

Albert Calmette
(1863-1933)

In 1894,

Crude venom

Horse immunization

Anti-snake venom horse antiserum

The only effective antidote for snake venom

Manufacturing process of current antivenom

Venom immunized horse

↓

Collect horse plasma

↓

WFI Dilution

↓

Pepsin digestion

↓

Ammonium sulfate precipitation

↓

Tangential flow filtration and dialysis

↓

Formulation

↓

Filling

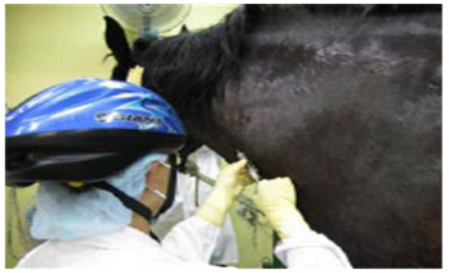
↓

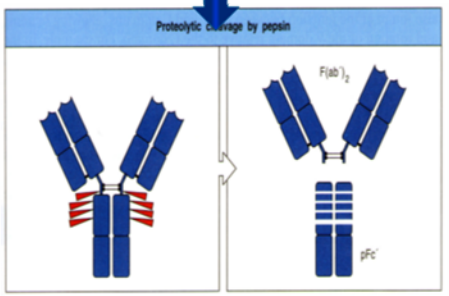
Antivenom product

Upstream

Downstream

Bulk filling





FDA 食品藥物管理署 Food and Drug Administration

PIC/S GMP

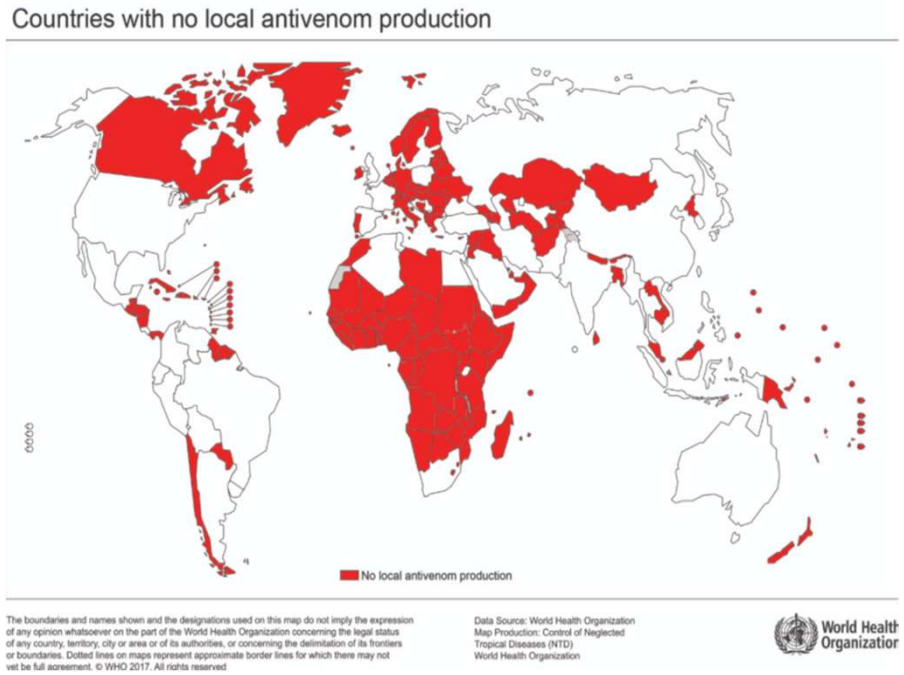
Challenges on current antivenom

- The WHO has estimated that 10 million vials of antivenoms are needed each year.

  Geneva: WHO; 2007, 1-38

- Only 46 antivenoms manufacturers globally. The number is declining.

- The use of a particular antivenom product is very much limited to only one or several species of a certain region.



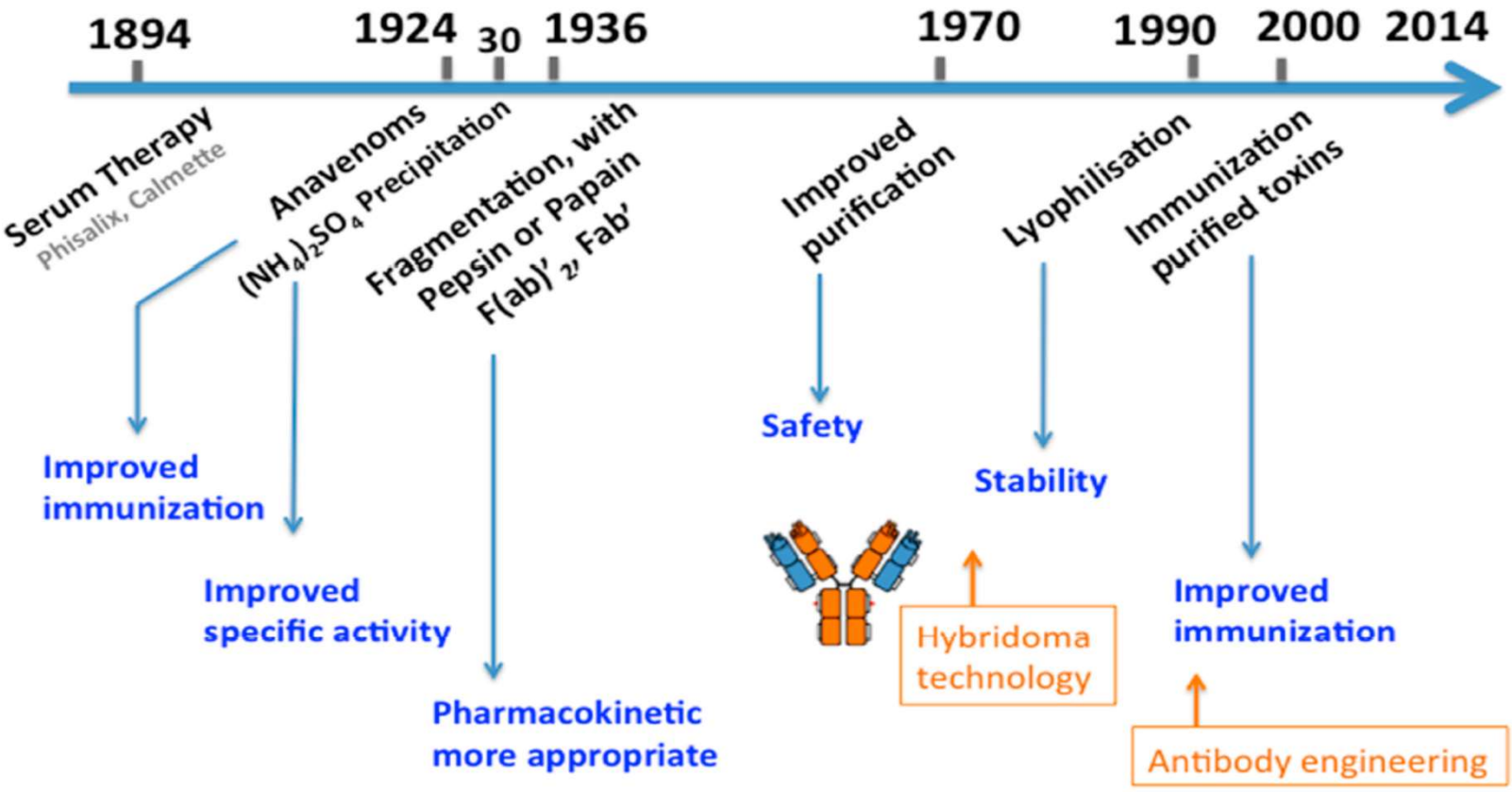
Challenges on current antivenom

Naja atra	Equine antibody 1600~2400 mg
Viper	Equine antibody 1000mg
Colorectal cancer	Human antibody 300 mg

Antivenom contains 5–36% effective antibodies directed against venom components

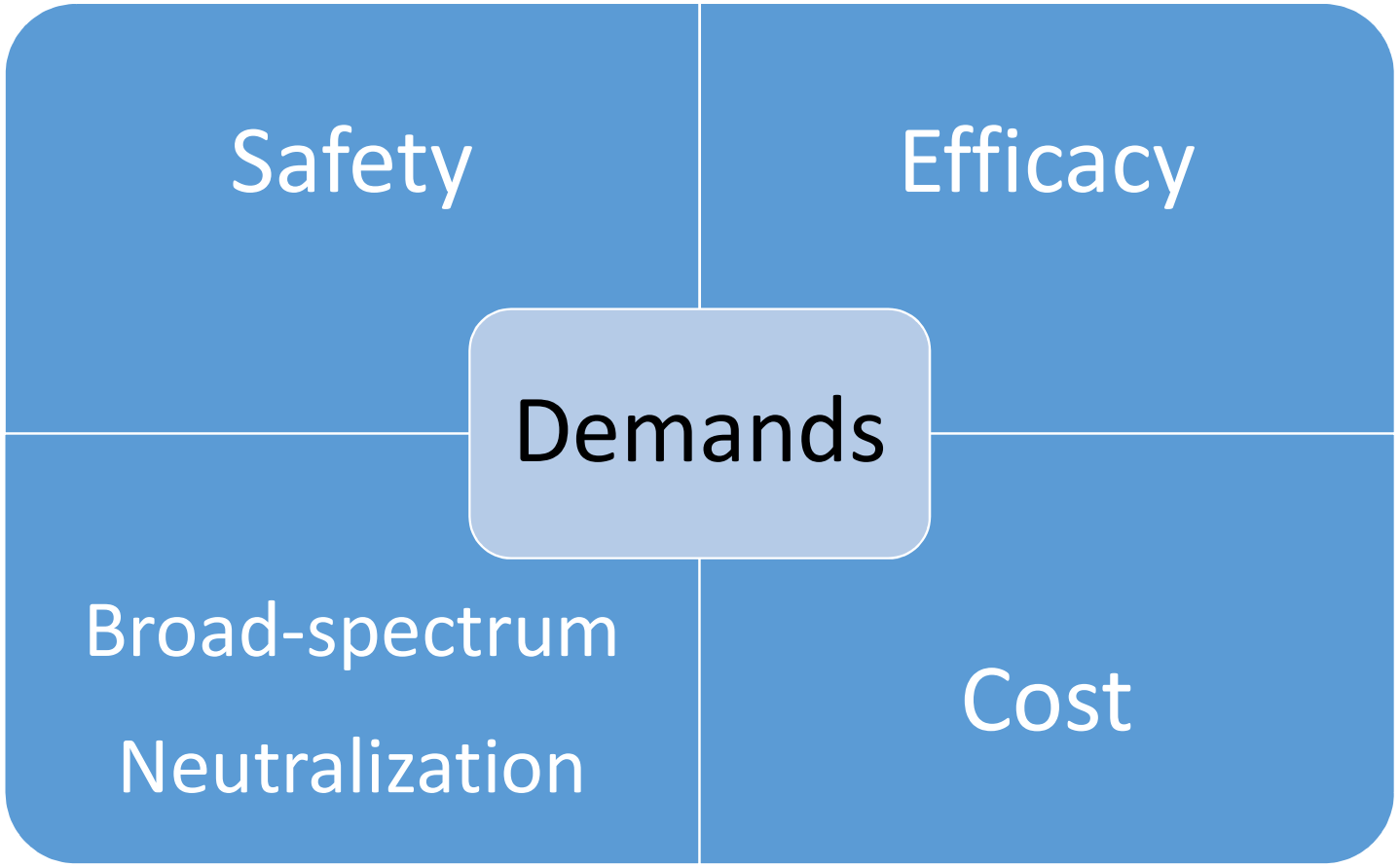
PLoS Negl Trop Dis. 2017, e0005361

Timeline of antivenom development



Toxins 2014, *6*, 2541-2567

Vision of new generation antivenom



Cobra bite envenoming

- *Naja* is a genus of venomous elapid snakes known as cobras that inflict approximately 10% of snakebite evenoming in Pan-Asia annually.

- Cobra venom contains high abundant neurotoxic and cytotoxic toxins, which could induce neurotoxic manifestations and severe local tissue damage on snakebite victims

- The neutralization potency of antivenoms toward cobra venoms are consistently low, being in the range of less than 1 to 2mg/mL.

J. Proteom. **2011**, 74, 1735–1767.

Maja cobra species in East and Southeast Asia

Naja kaouthia (孟加拉眼鏡蛇)

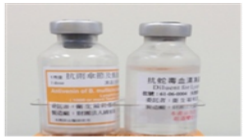
Naja atra (中華眼鏡蛇)

Graphs adapted from the Reptile Database. http://reptile-database.reptarium.cz/

Venomic and antivenomic analysis

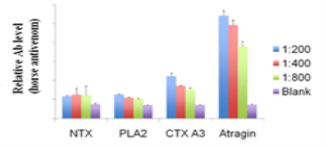
Identification of principle toxins of cobra venoms

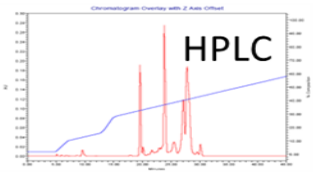
Cobra venom

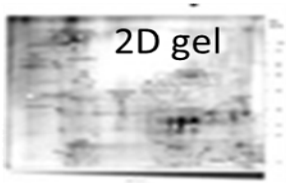


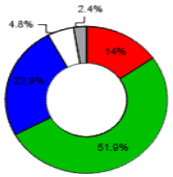
ELISA assay

Specific Ab titer



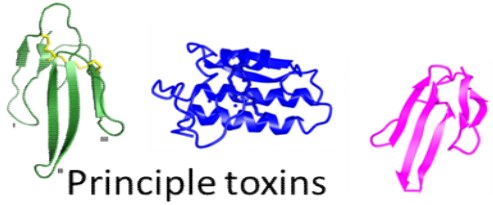






LCMS/MS

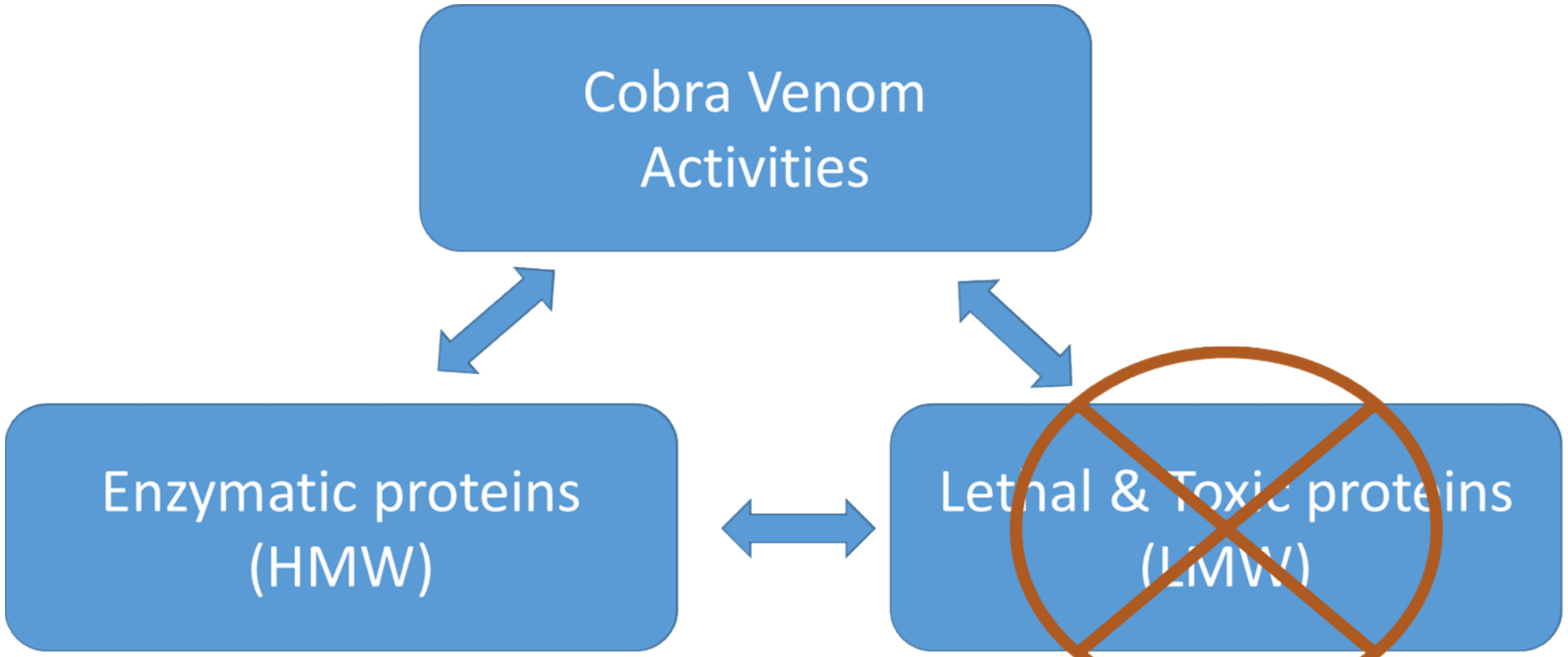
Protein identification



Principle toxins

Three finger toxins (3FTXs)

Toxin	Lethality	LD50
3FTX	High	Low
PLA2	weak	High
SVMP	weak	ND

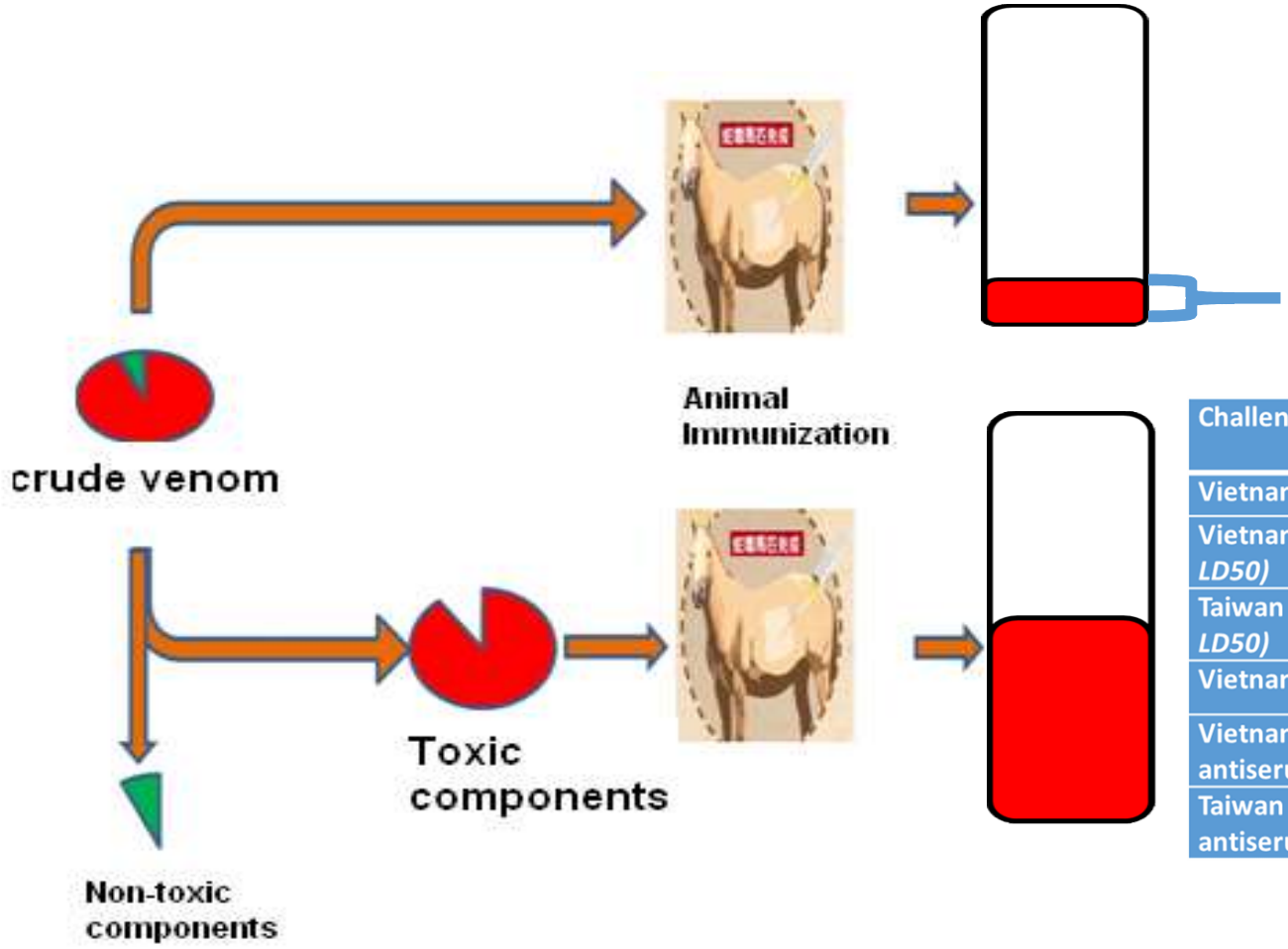


Toxins (Basel). 2016 Apr; 8(4): 86.
Published online 2016 Mar 26. doi: 10.3390/toxins8040086
PMCID: PMC4848613

Neutralization of the Principal Toxins from the Venoms of Thai *Naja kaouthia* and Malaysian *Hydrophis schistosus*: Insights into Toxin-Specific Neutralization by Two Different Antivenoms

Kae Yi Tan,[1] Choo Hock Tan,[2,*] Shin Yee Fung,[1] and Nget Hong Tan[1]

Generation of new antivenom using principle toxins



Challenge Venom	Survival rate 3 hr	Survival rate 48 hr	Survival rate %
Vietnam *N. naja (5 x LD50)*	0/6	0/6	0%
Vietnam *N. kaouthia (5 x LD50)*	0/6	0/6	0%
Taiwan *N. naja atra (3x LD50)*	2/6	0/6	0%
Vietnam *N. naja* + antisera	6/6	6/6	100%
Vietnam *N. kaouthia* + antiserum	6/6	6/6	100%
Taiwan *N. naja atra* + antiserum	6/6	6/6	100%

Potential of

Recombinant venom toxin

Alternative source of venom toxin

Reduce the cost and risk

Higher flexibility

Broaden neutralization targets

Fulfill the need for QC antigen

NEWS IN FOCUS

Milking snakes is a key part of producing conventional antivenom.

MEDICINE

Synthetic biology tackles antivenom

Artificial antibodies could ease global snakebite burden.

BY CARRIE ARNOLD

When the medical charity Médecins Sans Frontières called the worldwide shortage of snake antivenom a public-health crisis last September, Brazilian biochemist Paulo Lee Ho wasn't surprised. He has spent his career at São Paulo's Butantan Institute searching for better ways to create antivenom to treat bites from coral snakes.

Conventional methods rely on natural coral-snake venom, which is hard to come by: the snakes produce only small amounts with each bite and are hard to raise in captivity. So Ho and others have turned to proteomics and synthetic biology in the hope of improving the quality and availability of antivenom. "We need a new way to meet the demand for antivenom from the Ministry of Health," he says.

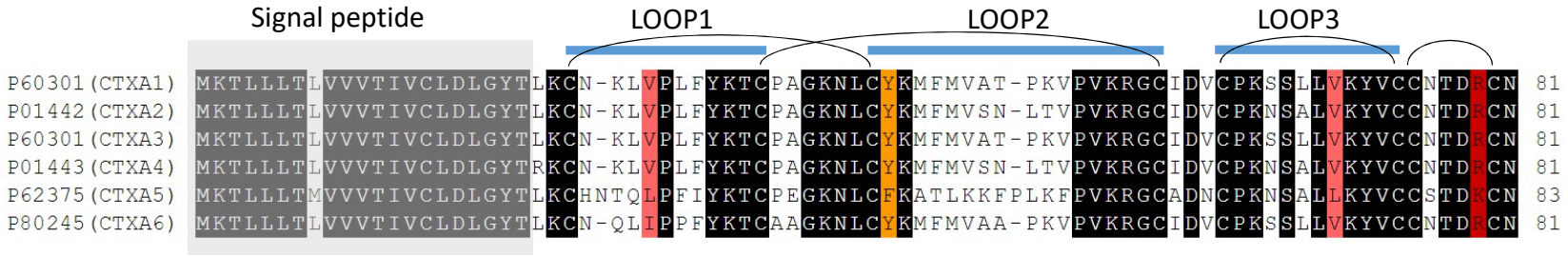
These efforts are bearing fruit. Last month, Ho and his colleagues reported[1] that they had engineered short pieces of DNA that, when injected into mice, triggered antibodies against coral-snake venom. The scientists then boosted the animals' immune response by injecting them with small pieces of synthetic venom antibodies synthesized in *Escherichia coli* bacteria. In a separate study[2], another group of researchers in Brazil used synthetic antibody fragments to neutralize the effects of bites by the pit viper *Bothrops jararacussu*.

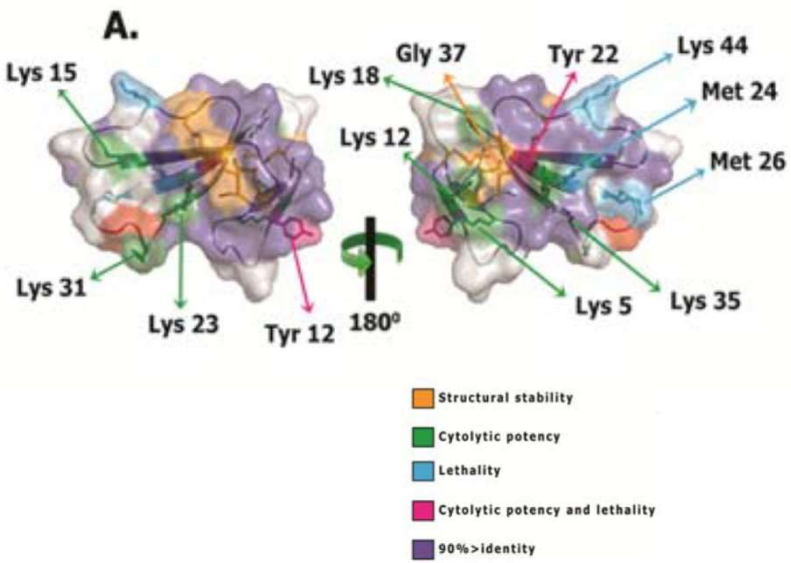
Such progress is encouraging, given the severe medical burden caused by snakebites in the developing world, says Robert Harrison, head of the Alistair Reid Venom Research Unit at the Liverpool School of Tropical Medicine, UK. Each year, around 90,000 people die after being bitten by venomous snakes[3].

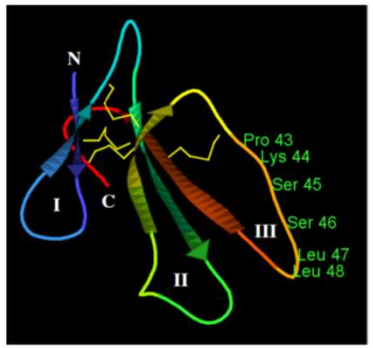
Yet antivenoms are still made using a method that has not changed for more than a century. Large animals, typically horses, are injected with small amounts of purified proteins extracted from snake venom, which prompts the production of antibodies. Plasma containing these antibodies is then given to snakebite victims.

But this life-saving treatment is limited in important ways. Each antivenom is effective against only a single species or, at most, a small

Sequence alignment of 3FTXs isomers

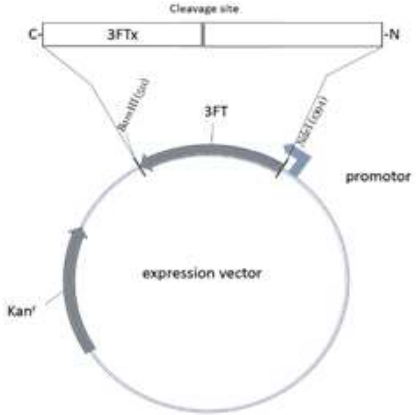






cid sequences of eight cardiotoxin (CTX) isoforms from Taiwan cobra (*Naja naja*

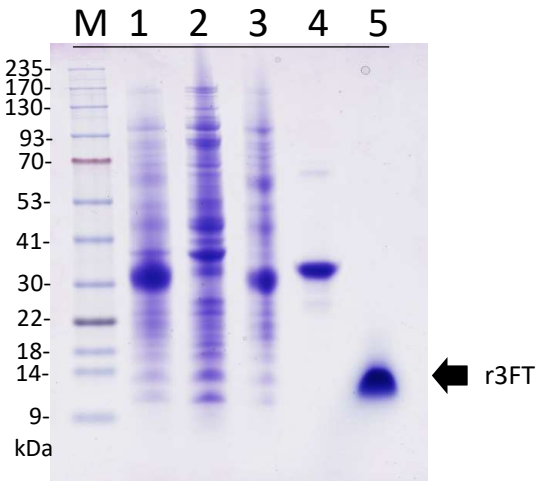
Generation of native-like recombinant toxin



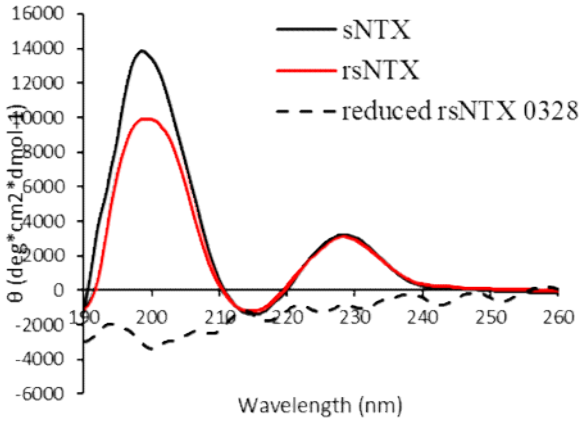
Ni affinity column Purification
Enzyme protease cleavage

Purification

E.coli expressed 3FTx

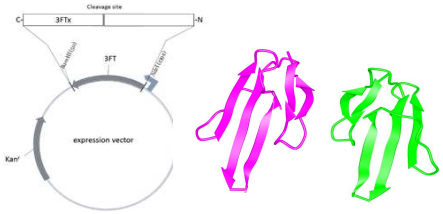


1. Induction
2. Total lysate w/o IPTG induction
3. Soluble fraction
4. uncleavged-SNTX
5. r3FTx

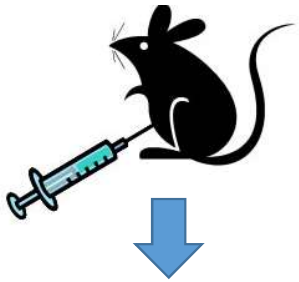


Toxin	Recombinant	Native
LD50 (μg/mouse)	4.91	4.62

Cross neutralization potency of mice antisera



Recombinant toxins of cobra venoms



Crude venom challenge
3 xLD50

Challenge with 3xLD50 *N. atra* venom:

Group	Immunogen	Adjuvant	Survival % (3h)	Survival (48h)
1	N. atra venom	CFA/IFA	83.3 (5/6)	83.3 (5/6)
2	N. kaouthia venom	CFA/IFA	83.3 (5/6)	83.3 (5/6)
3	N. atra + N. kaouthia venom	CFA/IFA	57.1 (4/7)	57.1 (4/7)
4	Native toxins	CFA/IFA	100 (7/7)	100 (7/7)
5	Recombinant toxins	CFA/IFA	83.3 (5/6)	83.3 (5/6)

Challenge with 3xLD50 *N. kaouthia* venom:

Group	Immunogen	Adjuvant	Survival % (3h)	Survival (48h)
1	N. atra venom	CFA/IFA	0 (0/6)	0 (0/6)
2	N. kaouthia venom	CFA/IFA	100 (6/6)	100 (6/6)
3	N. atra + N. kaouthia venom	CFA/IFA	100 (7/7)	100 (7/7)
4	Native toxins	CFA/IFA	100 (7/7)	100 (7/7)
5	recombinant toxins+ rLNTX	CFA/IFA	100 (6/6)	100 (6/6)

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  Huang, Hsuan-Wei

  Chiang, Liao-Chun

- Assistants

  Jiang, Bo-Rong

  Li, Chi-Han

- Grants

1. NHRI
2. MOST

Thank you for your attention