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

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Image: Simon Townsley
Global burden of snakebite

**Global numbers**
1.8–2.7 million envenomings
81,000–138,000 deaths

**The United States and Canada**
3,800–6,500 envenomings
7–15 deaths

**Europe**
8,000–9,900 envenomings
30–128 deaths

**Asia**
1.2–2.0 million envenomings
57,000–100,000 deaths

**Latin America and the Caribbean**
137,000–150,000 envenomings
3,400–5,000 deaths

**Africa and the Middle East**
435,000–580,000 envenomings
20,000–32,000 deaths

**Oceania**
3,000–5,900 envenomings
200–520 deaths

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


Toxin composition dictates pathology

Elapid snakes

Ptosis

Respiratory failure

Toxin composition dictates pathology

(A) cardiovascular effects
1. Activation of kininogens - snake venom serine proteases
2. ACE inhibitors - bradykinin potentiating peptides
3. Hydrolysis of capillary wall basement membranes - snake venom metalloproteinases

(B) haemostatic effects
1. Activation of factor V - snake venom serine proteases
2. Inhibition of factor X - C-type lectins
3. Activation of factor X - snake venom metalloproteinases - snake venom serine proteases
4. Activation of prothrombin - snake venom metalloproteinases - factor Va toxin - factor Xa toxin
5. Inhibition of thrombin - C-type lectins - kunitz-type serine protease inhibitors
7. Activation of plasminogen - snake venom serine proteases
8. Inhibition of plasmin - kunitz-type serine protease inhibitors
9. Inhibition or aggregation of platelets - phospholipases A2 - snake venom metalloproteinases - disintegrins - snake venom serine proteases - C-type lectins

Viperid snakes

Intra-cranial haemorrhage

Non-clotting blood (VICC)

Slagboom et al. 2017. *British Journal Haematology*
Venom variation impacts on antivenom efficacy

1) Extract venom

Venom variation → low paraspecific efficacy against different biting species

2) Immunise

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

3) Extract blood

4) Purify antibodies

5) Formulate
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

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

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

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 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%

Alirol et al. 2015. *PLOS NTD*
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
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

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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

FavAfrique
or Premium

cobras

puff adder

SAIMR
_poly or
Premium

FavAfrique
or Premium

black mamba

SAIMR poly

SAIMR poly

Harrison et al. 2017. _PLOS NTD_
Antibody content likely plays a major role

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

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*

<table>
<thead>
<tr>
<th>Immunogen mixture I (resulting in EAV 1)</th>
<th>Species</th>
<th>Sub-family</th>
<th>Geographical region</th>
<th>Venom origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bothrops asper</td>
<td>Crotalinae</td>
<td>Central America</td>
<td>Costa Rica</td>
<td></td>
</tr>
<tr>
<td>Bothrops jararaca</td>
<td>Crotalinae</td>
<td>South America</td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Echis ocellatus</td>
<td>Viperinae</td>
<td>West Africa</td>
<td>Nigeria</td>
<td></td>
</tr>
<tr>
<td>Calloselasma rhodostoma</td>
<td>Crotalinae</td>
<td>Southeast Asia</td>
<td>Captive bred</td>
<td></td>
</tr>
<tr>
<td>Dispholidus typus</td>
<td>Colubrinae</td>
<td>sub-Saharan Africa</td>
<td>South Africa*</td>
<td></td>
</tr>
<tr>
<td>Deinogkistrodon acutus</td>
<td>Crotalinae</td>
<td>East Asia</td>
<td>Captive bred</td>
<td></td>
</tr>
<tr>
<td>Daboia russellii</td>
<td>Viperinae</td>
<td>South Asia</td>
<td>Sri Lanka</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunogen mixture II (resulting in EAV 2)</th>
<th>The same 7 venoms in immunogen mixture I plus:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitis arietans</td>
<td>Viperinae</td>
</tr>
<tr>
<td>Echis carinatus</td>
<td>Viperinae</td>
</tr>
<tr>
<td>Rhabdophis subminiatus</td>
<td>Natricinae</td>
</tr>
<tr>
<td>Trimeresurus albolabris</td>
<td>Crotalinae</td>
</tr>
<tr>
<td>Crotalus atrox</td>
<td>Crotalinae</td>
</tr>
</tbody>
</table>

Alomran et al. 2021. *PLOS NTD*
Different immunogen diversity = comparable *in vitro* binding

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

Neutralising potency

- **B. fasciatus (WB)**
- **B. sindanus (RJ)**
- **B. caeruleus (PB)**
- **E. c. sochureki (RJ)**
- **E. carinatus (MH)**
- **N. kaouthia (AR)**
- **N. kaouthia (WB)**
- **N. naja (MH)**

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

Casewell et al. 2020 Trends Pharmacological Sciences
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