A preclinical model to substantially refine and reduce the number of animals subjected to severe procedures in snakebite envenoming research

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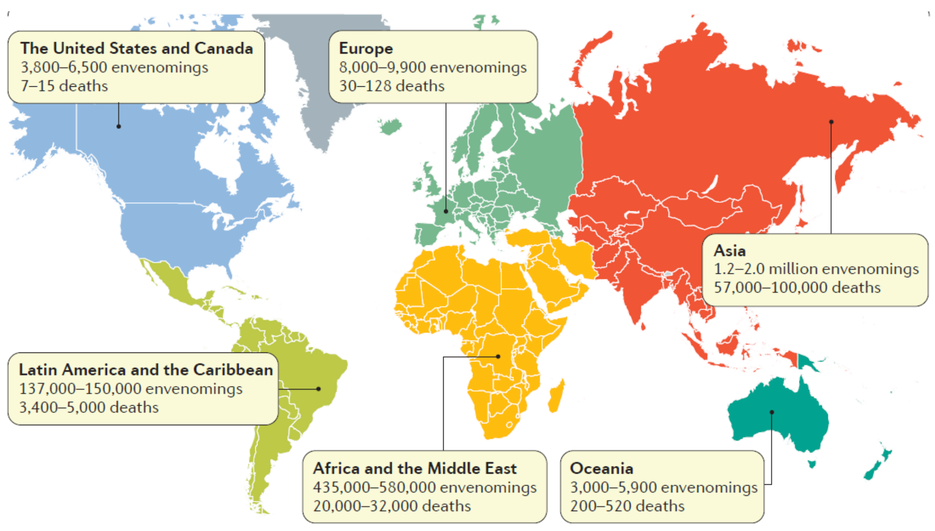
Snakebite

- Snakebite envenoming is a WHO recognised Neglected Tropical Disease
- Responsible for **85,000-130,000 deaths** and **maiming >400,000** people annually
- Those living in the world's rural and disadvantaged communities are the most at risk
- Antivenom is currently the only specific therapy for envenoming

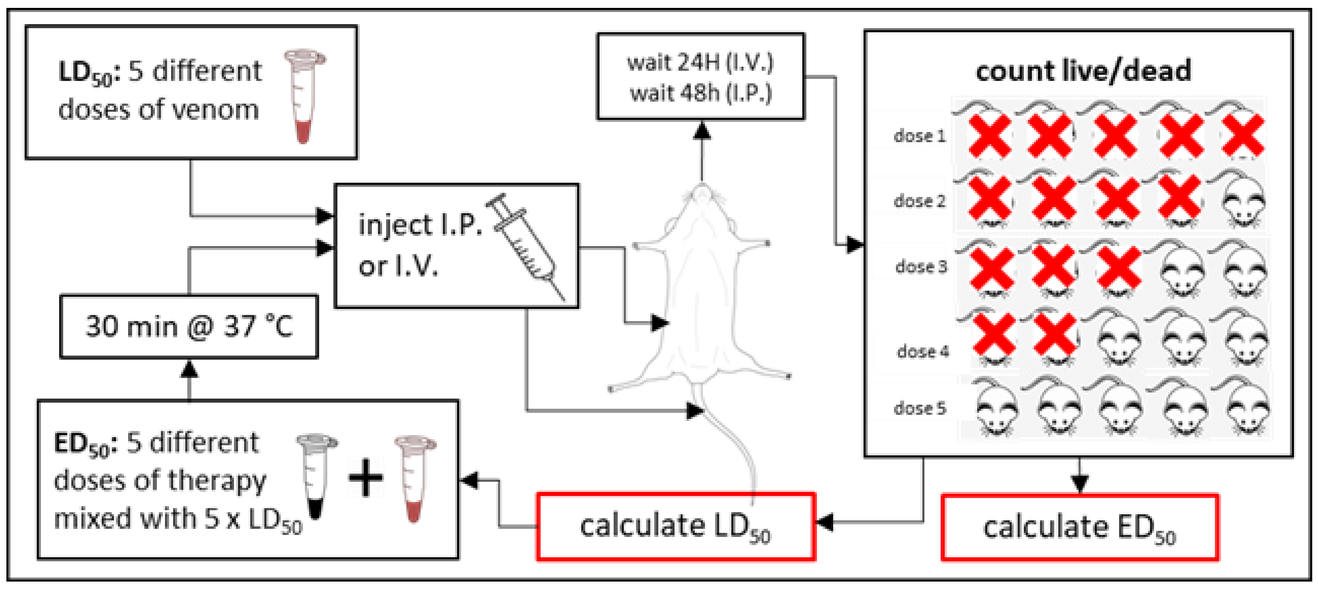


Gutiérrez, J.et al. *Nat Rev Dis Primers* 3, 17063 (2017).

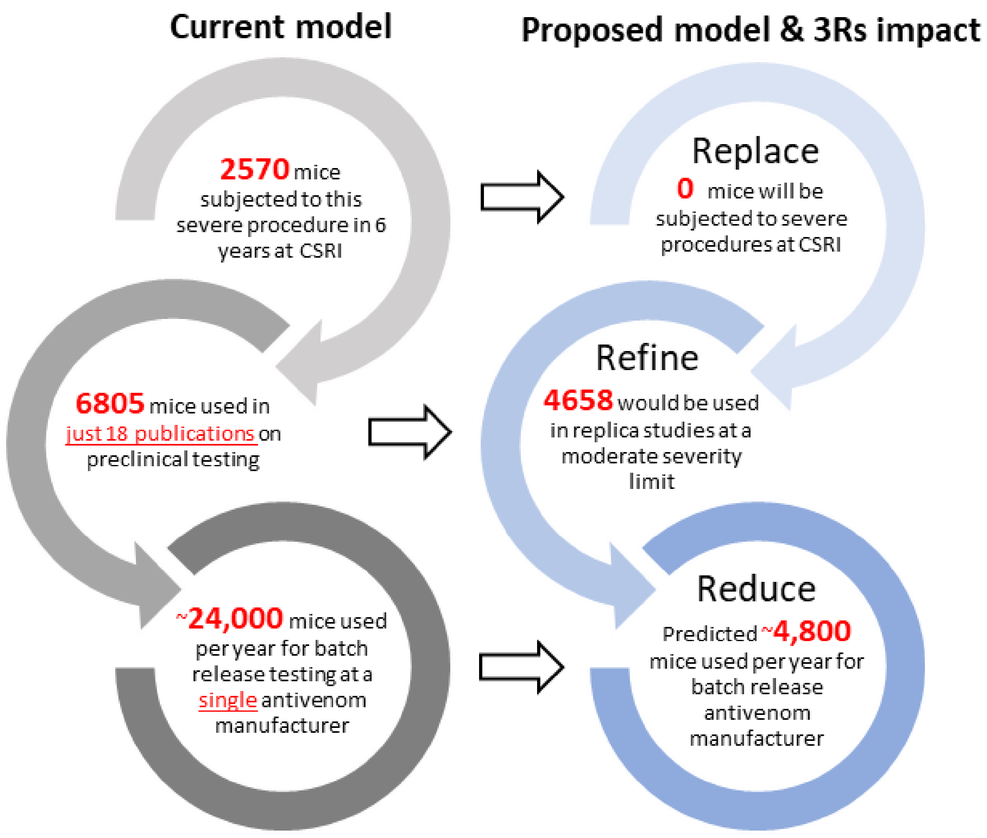
Photo: Wolfgang Wuster

The current model

- Antivenoms are unusual human medicines in that they are normally released for clinical use without needing to pass clinical trials
- All antivenom licensing is based on their performance in the murine preclinical "neutralisation of venom lethality assay"
- This assay, endorsed by the WHO and listed in many pharmacopeia, is 40 years old and is used globally
- Whilst simple, the assay is
  - not reflective of human envenoming
  - requires large numbers of mice (n=25/experiment/venom)
  - Relies on death as an outcome
  - Is highly distressing for mice (rated severe in the UK) .



A 21st century model

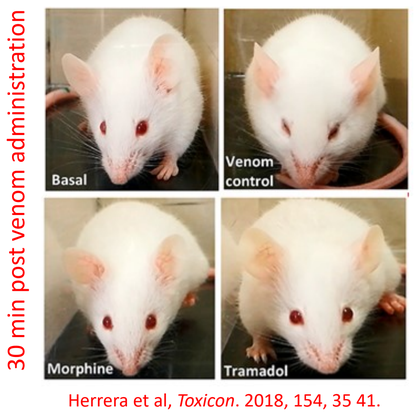


- There is increasing consensus that the current standard assay for assessing envenoming therapy efficacy does not meet modern requirements.
- Several regulatory authorities are now putting restrictions on its practice. This is a major concern given pre-clinical models are the primary assessment of envenoming therapy prior to their clinical use.
- There is an urgent need to develop assays to enable envenoming therapies to be rigorously assessed, with maximal information retrieval, whilst subjecting mice to the lowest possible level of suffering.
- We have recently secured NC3Rs funding to develop a new assay of murine envenoming to address this:
- Our aims are to develop a refined replacement procedure that better reflects real-world envenoming and will allow:
  1. flexibility in testing different therapeutic formats at different timepoints post-envenoming,
  2. a 40% reduction of the total number of animals required for robust efficacy testing,
  3. a maximum 'moderate' severity rating to be applied, once established, and validated.

Is this achievable?

From our experience with & refining the current pre-clinical model, there are four main reasons why we are optimistic this is achievable:

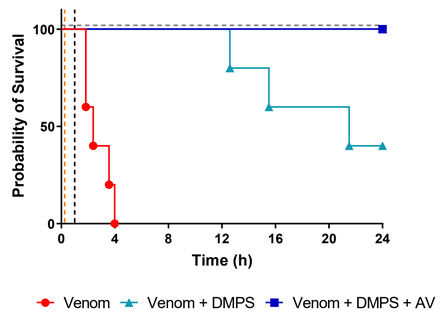
1. Analgesia



Herrera et al, *Toxicon*. 2018, 154, 35 41.

- We have demonstrated that pre-emptive IP dosing of mice with morphine, followed by regular oral dosing with Oramoph, can reduce murine pain during envenomation as indicated in reduction in mouse grimace scale scores.

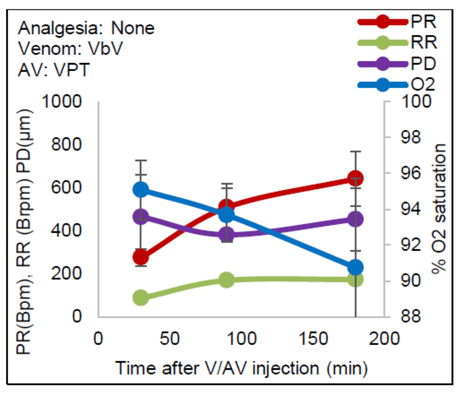
2. Severity progression



Albulescu et al, *Sci. Transl. Med.* 2020. 12, eaay8314.

- Mice display normal behavioural activity/mild symptoms of envenoming before progressing to more moderate, then severe symptoms.
- This presents a window in which to assess envenoming parameters before mice display overt symptoms.

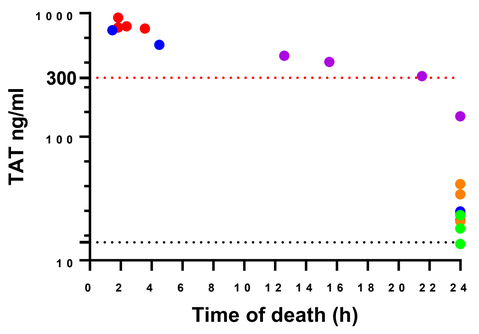
3. Vital Sign Monitoring



Bolton, PhD Thesis, 2017. Uni. of Liverpool.

- Previous work at CSRI has suggested that monitoring of vital signs (0_2 saturation, BP, Heart Rate) could be beneficial for identifying early humane endpoints.
- We wish to build on this work using non-invasive vital sign monitoring techniques

4. Biological Markers



Albulescu et al, *Sci. Transl. Med.* 2020. 12, eaay8314.

- CSRI has performed research to attempt to identify clinical markers in mice for coagulopathic envenoming
- Some markers (e.g. Thrombin-AntiThrombin (TAT) complex) strongly correlate with envenoming
- We wish to develop the use of blood chemistry testing (e.g. ROTEM) to identify early measures of efficacy and endpoints

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