Snakebite envenoming

Tackling a biting neglected tropical disease

- Snakebite envenoming is a pressing global public health concern – an unfortunately common medical emergency caused by the bite of a venomous snake.
- Despite its prevalence and seriousness, snakebite envenoming is a neglected tropical disease (NTD).
- Current treatment for snake envenoming is antivenom, but it has limitations.
- Dr Amy Marriott and Dr Stuart Ainsworth from the Centre for Snakebite Research and Interventions at the Liverpool School of Tropical Medicine, UK, are addressing vital issues in regulations and testing.
- The researchers are exploring novel ways to lessen the impact on mice used in antivenom experiments.

Humans and snakes have a long symbolic and cultural history. However, snakebite envenoming, which has plagued humankind since antiquity, still kills between 85,000 to 130,000 people and maims a further 400,000 each year and is classed as a neglected tropical disease (NTD). NTDs affect a significant proportion of the world's population – but are underserved in terms of resources, research, and treatment needed to halt their devastating impact on extremely disadvantaged and socioeconomically vulnerable communities.

Snake envenoming disproportionately affects the most disadvantaged communities and is especially prevalent in India, sub-Saharan Africa, Latin America, and southeast Asia. This common – but devastating – disease causes death, disability, deprivation, and destitution for hundreds of thousands of people each year.

New research by Dr Amy Marriot and Dr Stuart Ainsworth from the Centre for Snakebite Research and Interventions at the Liverpool School of Tropical Medicine, UK, is aiming to assist with an antidote to this long-overlooked NTD. Ainsworth and Marriott are developing research techniques to improve the efficacy of antivenoms, and while doing so are developing alternative models to substantially increase mouse welfare in antivenom pre-clinical experiments.

Snakebite envenoming

Snakebite envenoming occurs when someone is bitten by a venomous snake. Snakebites are always accidental in nature and are most common amongst agricultural workers, who may disturb snakes while working in farmland, or in individuals walking at night (when snakes are harder to see and to avoid). Once injected, snake venom can cause a myriad of potentially lethal effects on the human body. In the aftermath of a bite, symptoms range from uncontrollable bleeding, tissue damage, paralysis, and ultimately death. The effects of envenoming can be localised (primarily affecting the bite site) or systemic (rapidly spreading throughout the body) or, if you're unlucky, both. Snakebite envenoming is therefore a medical emergency, and the time taken to receive medical treatment is critical in terms of positive outcomes. Once a victim is bitten, toxic effects can occur in as little as 30 minutes.

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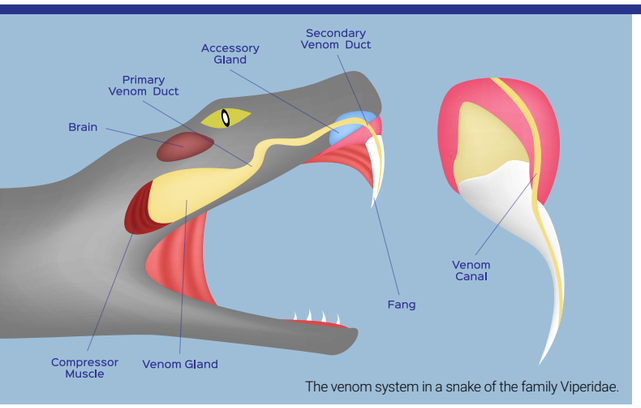
Antivenom: more research needed

Research on treating snakebite envenoming has been miniscule compared to other NTDs. The only treatment currently available for snakebite envenoming is antivenom, which consists of antibodies purified from horses immunised with non-toxic quantities of venom. When given to a snakebite victim, the antibodies in the antivenom will bind and neutralise the potentially fatal effects of the venom's toxins. As the World Health Organization (WHO) states, 'Good-quality antivenoms can literally make a difference between life and death' for snakebite victims.

Snake venoms and their toxins vary from species to species, meaning that different antivenoms are required for each variety of snake. This has made developing a safe and universally effective antivenom extremely difficult. The resources needed to develop, produce, store, and administer species-specific antivenoms mean that the costs of treatments are high for the populations most affected. Many snakebite victims are simply unable to afford the resulting huge medical bills that plunge them further into poverty. Unfortunately, even if victims can afford antivenom, the antivenom they receive may not be as effective as they hoped or even claimed by the manufacturer.



The venom system in a snake of the family Viperidae.

A 'crude' model

Like most other drugs, methods for testing an antivenom's effectiveness and safety start with animal experiments using mice. However, unlike most other drugs, which will then go through phases of human clinical trials, antivenoms are unusual in that they completely rely on these animal experiments to predict their potential efficacy in humans.

Unfortunately, the mouse model, whilst simple, is not 100% reliable in its ability to predict an antivenoms performance in humans. Marriott and Ainsworth explain that 'The current model is very crude, relying on mixing venom and antivenom together and injecting it into a mouse. In very basic terms, if the animal survives, the therapy is considered effective.' This approach, they say, 'Does not reflect real-world envenoming, as venom and therapy would never be premixed or injected directly' and runs the risk of overestimating the effectiveness of an antivenom. The premixing of venom and antivenom also makes detailed assessments of the antivenom's pharmacokinetic properties, something that is routinely assessed for other drugs, nearly impossible.

Naturally, questions remain as to the and reliability of antivenoms assessed in this way. Ainsworth and Marriott argue that while many antivenoms are effective, the limitations with the current model can result in 'Ineffective antivenoms being licensed and reaching patients – ultimately resulting in unnecessary deaths.'

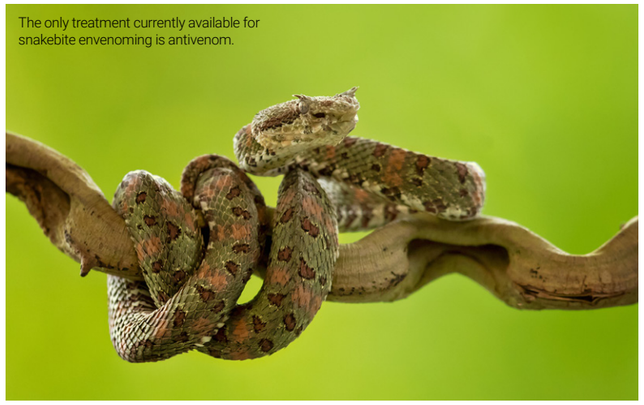
Importantly, Marriott and Ainsworth highlight the high cost of these experiments on mouse welfare: 'The current model is outdated in terms of the large number of mice used to determine if an antivenom may be effective or not, and the substantial pain and distress the mice experience during the experiment.' To combat this, the researchers want to bring the science of antivenom preclinical testing – both in terms of being able to accurately predict an antivenom's effectiveness in humans and in terms of animal welfare – into the 21st century.

> **Marriott and Ainsworth are spearheading work to improve testing procedures for antivenoms – that will benefit both the patient and the animals involved in saving their lives.**

There is a general agreement on the need for a coordinated effort between government and research institutions and agencies, antivenom manufacturers, and international regulating bodies to tackle the issues. The current model has been used for many decades with little to no modification. 'There has been attempts to improve the assay in terms of mouse welfare, for instance introducing painkillers which clearly make a difference, but still the model relies on a crude live/dead readout.' These types of severe models are becoming substantially less common globally as researchers search for better or animal-free models. Fortunately, Marriott and Ainsworth are spearheading work to improve testing procedures for antivenoms – that will benefit both the patient and the animals involved in saving their lives.

New research on snake envenoming

Marriott and Ainsworth say that animal-free testing of antivenom is still some way off. 'The current reality of antivenoms not having to go through human clinical trials means that regulators are likely to insist on animal models for assessing envenoming therapies in the foreseeable future.' However, they say 'This does not mean we should not strive to make the required animal testing as stress-free and painless for the mice as possible.' The NC3Rs is a UK government funding agency whose remit is to fund research which will work towards the replacement, refinement, and reduction of animal use in research, and Marriot and Ainsworth recently obtained NC3Rs funding to develop a new model mouse of envenoming. Ainsworth explains, 'We hope to use our knowledge of venom toxins and their modes of action to rigorously assess antivenoms, preventing ineffective therapies reaching snakebite victims, while using substantially fewer mice and subjecting them to the lowest possible level of suffering.'



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The key to their new models is to use the smallest possible dose of venom which can induce a measurable change in biomarkers, such as blood fibrinogen levels or prothrombin time, which may also be used to clinically assess human snakebite victims and their response to antivenom. The use of assays and non-invasive monitoring equipment means a much more detailed and rigorous readout can be obtained versus the current binary and not very informative 'alive or dead' readout. Importantly, while still in the early days of development, the welfare of the animals in the new models seem much improved compared to the current model. Marriott explains 'The mice receiving such small quantities of venom do not show any classical signs of pain or distress and outwardly behave no differently from control non-envenomed mice.'

Future snakebite therapies

All of this paves the way towards exciting alternative antivenom therapies, such as small molecule inhibitors and monoclonal antibodies, which are showing great promise in terms of efficacy, safety and cost. However, Ainsworth notes 'Unlike traditional snakebite therapies, these newer therapies will be required to undergo normal regulatory assessment, meaning rigorous and expensive human clinical trials'. The current mouse model does not lend itself to supporting these clinical trials, as its readout does not have the resolution to distinguish between promising therapies and ones which might fail in the clinic – again, a process which is routine for most drugs under pharmaceutical development. Marriott and Ainsworth's new models will hopefully allow better early identification of promising and not-so-promising candidates and thereby speed up the translation of exciting new therapeutics to clinical study in humans.

Personal response

What sparked your interest in snakebite envenoming?

Ainsworth: It's always been the human aspect for me. It is difficult to comprehend the level of suffering snakebite inflicts, which has, until very recently, been ignored by the global community or just assumed to be a fact of life.

Marriott: The human impact for me too – the devastating impact the snake venom toxins can cause systemically following a bite, and how difficult it can be for patients to receive effective treatments quickly. I've always been interested in improving the welfare of animals and the development of improved animal models to be used in scientific research. With the current mouse model being outdated, I really wanted to help develop a new model better reflective of a human snake bite, to allow us to find better treatments for snakebite patients.

Are there antivenoms available to cover most snake species, and what do we still need to develop in this field?

The majority of highly medically important snake species will have antivenoms available. However, the main issue is access – in many places, despite antivenoms existing, those who need it most simply cannot access them. Much research and coordinated implementation, for instance by the World Health Organization, is being pursued in this area to improve accessibility to effective antivenoms.

Could you tell us a bit more about the alternative antivenom therapies, such as small molecule inhibitors and monoclonal antibodies?

These therapies are really to address the shortcomings of current antivenoms, which are highly specific for the biting snake, need to be administered intravenously in a hospital, are poorly dose effective, and have poor safety profiles.

Small molecule inhibitors are classes of drugs which act in a generic manner to inhibit whole classes of toxins independent of the species they come from. For instance, Varespladib is a generic inhibitor of phospholipase A2 toxins, while Unithiol is a generic inhibitor of venom metalloproteinase toxins via chelation of metal ions. In the case of Varespladib and Unithiol, these are repurposed drugs, so we already have a wealth of human safety data available. Both drugs are safe, cheap to manufacture and excitingly, both are orally available – which means one day you may be able to carry them with you as tablets and start treatment immediately following a bite.

Monoclonal antibodies, or recombinant antivenoms, are similar to existing antivenoms in that the active ingredient is the same, anti-venom antibodies. However, monoclonal antibodies can be selected for their potency and specificity and engineered for desirable pharmaceutical properties like increased half-lives or superior tissue penetration or increased safety profiles. This means in theory you can generate recombinant antivenoms which are substantially superior in terms of species coverage, dose potency and safety. Several groups are working towards the production of such therapies with very exciting advances made in recent years, especially by researchers at the Technical University of Denmark.

How long do you think it might be before alternative antivenom therapies are tested on humans in clinical trials?

Very excitingly, alternatives to antivenom therapies are already going through clinical trials. The Broad-spectrum Rapid Antidote: Varespladib Oral for Snakebite (BRAVO) is currently in phase II clinical trial, while colleagues at CSRI are close to completion of the *Trial of Repurposed Unithiol for snakebite Envenoming phase* 1 (TRUE-1) clinical trial. Recombinant antivenoms are not as advanced in their clinical progression, but I wouldn't be surprised if a recombinant antivenom will be in human clinical trials by the end of the decade.

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Details

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Bio

Dr Stuart Ainsworth is a career track lecturer and UKRI Future Leader Fellow based in the Centre for Snakebite Research & Interventions at the Liverpool School of Tropical Medicine, UK. His research is focused on the improvement of antivenoms and their preclinical regulation.

Dr Amy Marriott is a postdoctoral research associate at the Centre for Snakebite Research and Interventions at the Liverpool School of Tropical Medicine, UK. Dr Marriott's expertise is in developing and establishing new in vivo models of Tropical Diseases and techniques to assist in the reduction and refinement of animal testing.

Funding

- NC3Rs
- UKRI FLF

Further reading

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