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A genetically detoxified version of Sphingomyelinase D engineered in insect biofactories for the production of antivenoms against loxoscelism

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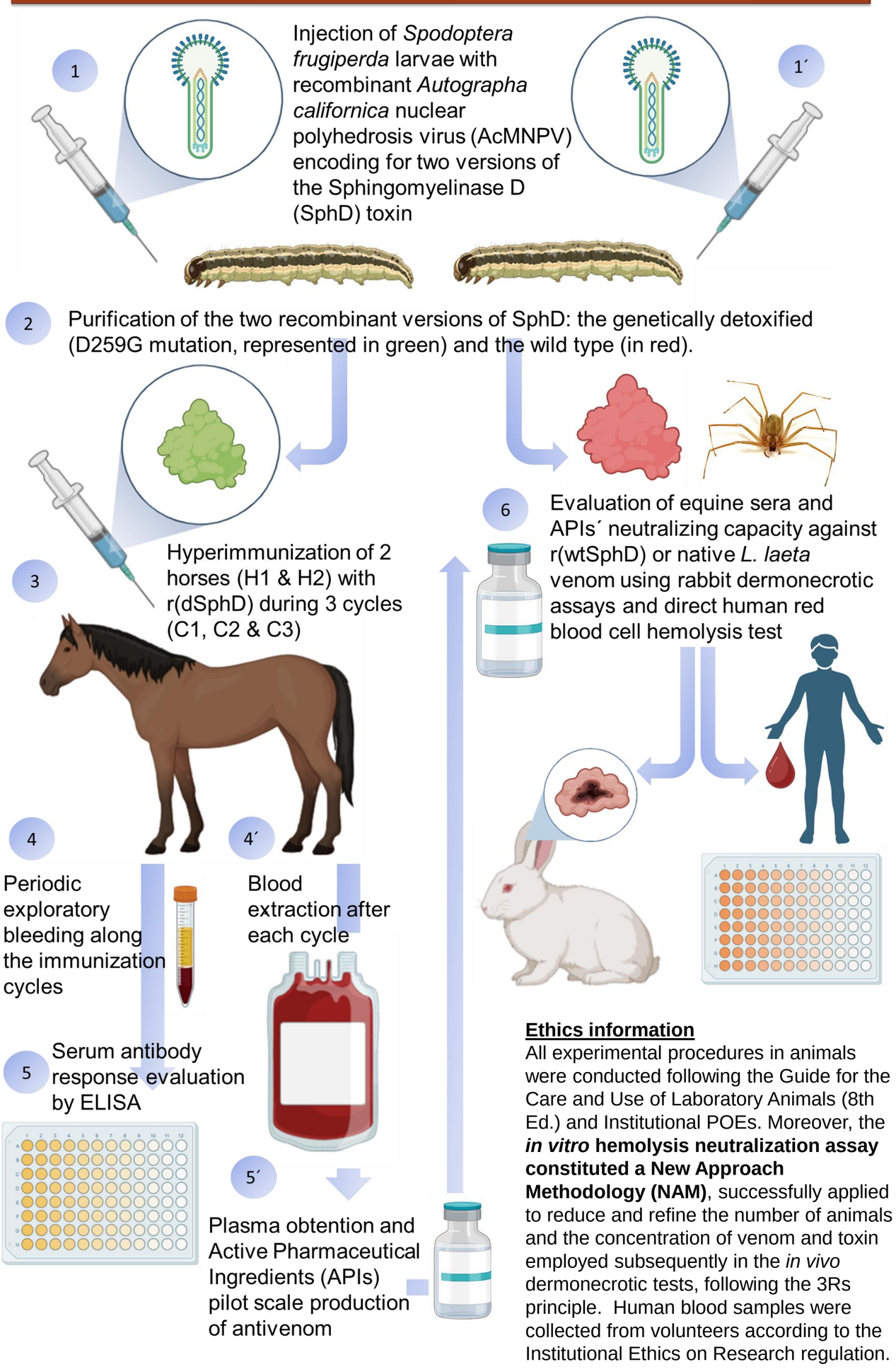
INTRODUCTION & AIM

- Systemic loxoscelism is caused by the venom of the *Loxosceles laeta* spider and can be life-threatening.
- The main toxin responsible for the clinical manifestations of loxoscelism is Sphingomyelinase D (SphD).
- Current specific treatment relies on the administration of antivenom produced from the plasma of horses hyperimmunized with spider venom.
- Limited venom availability is the major production bottleneck.

This study aims to evaluate whether a genetically detoxified version of SphD, produced in the baculovirus-insect larvae (BIL) platform, could improve antivenom production.

METHOD



Ethics information

All experimental procedures in animals were conducted following the Guide for the Care and Use of Laboratory Animals (8th Ed.) and Institutional POEs. Moreover, the ***in vitro* hemolysis neutralization assay constituted a New Approach Methodology (NAM)**, successfully applied to reduce and refine the number of animals and the concentration of venom and toxin employed subsequently in the *in vivo* dermonecrotic tests, following the 3Rs principle. Human blood samples were collected from volunteers according to the Institutional Ethics on Research regulation.

RESULTS & DISCUSSION

A **specific immune humoral response** was developed after each immunization cycle. Higher levels of antibodies were detected in Horse 1 (Fig. 1).

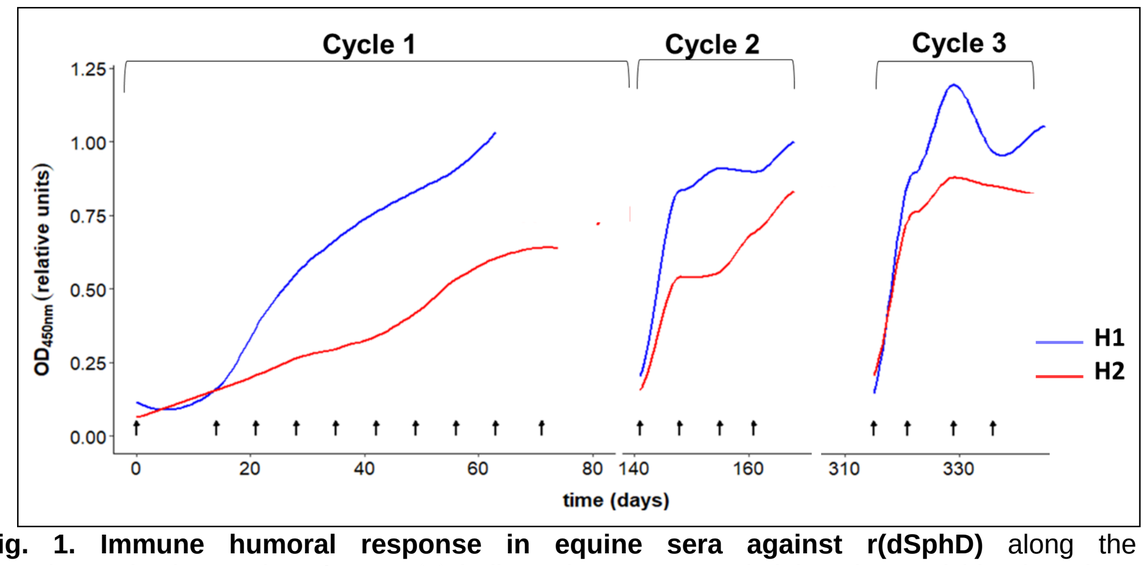


Fig. 1. Immune humoral response in equine sera against r(dSphD) along the 3 hyperimmunization cycles. Arrows (↑) indicate immunogen administration and blood exploratory sampling.

Equine **sera and APIs** from Horse 1 at cycles 2 and 3 **effectively neutralized the r(wtSphD)´s hemolytic activity**, while Horse 2 only showed similar neutralization capacity at cycle 3 (Fig. 2).

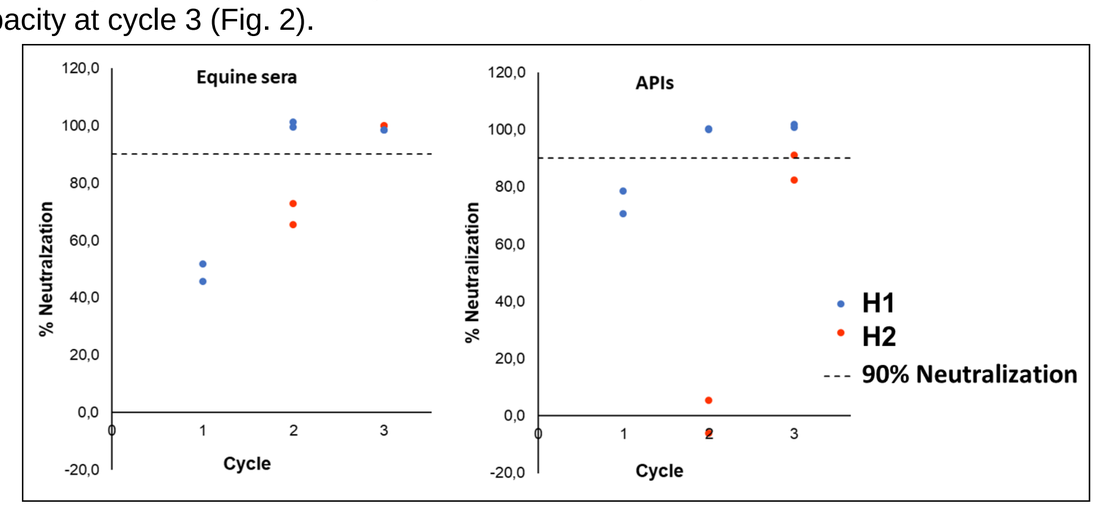


Fig. 2. Neutralizing activity of equine sera and APIs against r(wtSphD) tested *in vitro*.

Dermonecrotic injuries of native *L. laeta* venom were partially neutralized by the sera from both animals. Moreover, **APIs** from Horse 1 at cycles 2 and 3, and API from Horse 2 at cycle 3 **effectively neutralized the r(wtSphD)´s dermonecrotic activities** (Table 1).

Table 1: Neutralizing activity of APIs against r(wtSphD) tested *in vivo*.

APIs		Protein concentration (g/100 ml)	Neutralizing potency (200 DMN/ml)
Animal	Cycle		
Horse 1	1	43	No
	2	64	**Yes**
	3	42	**Yes**
Horse 2	2	33	No
	3	56	**Yes**

CONCLUSION

These promising results demonstrate that BIL platform can be used to produce a genetically detoxified version of SphD, capable of inducing an effective immune response capable of neutralizing its toxic homolog.

FUTURE WORK

Further evaluation of other detoxified isoforms of Sphingomyelinase D will be carried out to broaden protection against *L. laeta* venom. Recombinant antigen production in the BIL platform will be optimized, and its efficacy validated in preclinical models.

This strategy could enable scalable and accessible antivenom manufacturing by reducing the use of animals and venom extraction in the process, significantly enhancing the availability and safety of treatments for loxoscelism.