Production of an alternative antivenom based on egg yolk antibodies against *Crotalus durissus terrificus*

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INTRODUCTION

Antivenoms are considered essential medicines for treating snakebite envenomings. Conventional production is based on the immunization of large animals, mainly horses, with mixtures of representative venoms from a determined geographical area. Due to its advantages regarding animal welfare and lower costs of production, an alternative production of antivenoms may be based on the use of egg yolk antibodies –IgY technology – by immunizing laying hens.

OBJETIVE

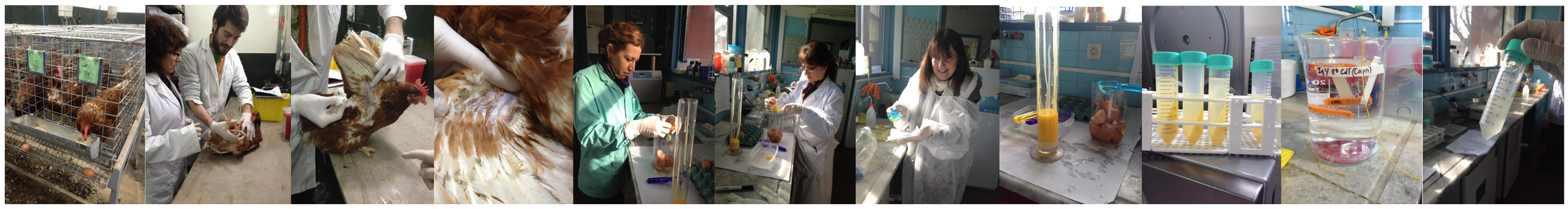
The aim of this work was to produce an IgY-based antiserum against *Crotalus durissus terrificus (C.d.terrificus)* venom from Argentina, by using different purification methods and to evaluate its efficacy in an murine model of envenoming.

Habitat of *Crotalus dusissus terrificus* in Argentina (North and center of the country)

MATERIAL AND METHODS

Animals: laying hens (n=2)
Immunization: via *i.m.* with *C. d. terrificus* whole venom (pool of venoms from Argentina) at days 0, 14, 28, 71, 237, 289, 304, 473 and 487).
Collection of eggs : during 10 days after the 7th ,8th and 9th immunization.
Methods of purification evaluated: 1) Ammonium sulphate double-precipitation (AMS) (24 and 26% w/v).
2) *PEG-8000* (12% w/v)
3) Caprylic Acid (7% v/v), 0.01% (w/v) thimerosal was added for preservation.
Efficacy of the antivenom (*in vivo* neutralization assay): Median effective dose (ED_{50}) according to WHO guidelines (2017).

IgY production process This study meets the ARRIVE and WHO guidelines . Experiments were approved by the IACUC from CICVyA-INTA



RESULTS

Median Effective Dose

Nº Immunization	Methods of purification					
	PEG-8000		Caprylic acid		AMS	
	Hen 1	Hen 2	Hen 1	Hen 2	Hen 1	Hen 2
in vivo Neutralization (µg/mL)						
7°	*	0,031	< 40	< 40	395	395
8°	79,53	79,53	< 40	< 40	395	395
9°	158,11	158,11	< 40	< 40	395	395

Table1- *In vivo* neutralization of *C.d.terrificus* venom by IgY obtained after the 7th, 8th, 9th immunizations with three methods.
* Not data, sample contaminated

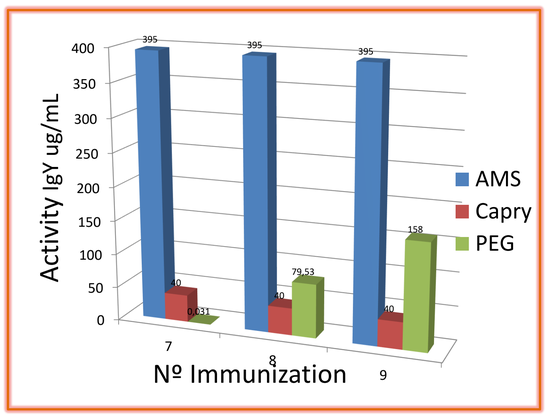


Figure 1- Activity IgY vs. Nº Immunization

Performance IgY

Hen 1	Hen2
Amount of eggs :10	Amount of eggs : 12
V_0 of yolk : 130 mL	V_0 of yolk : 150 mL

Methods	PEG	Capry	AMS	PEG	Capry	AMS
V_0 (mL) after purification	50	40	40	50	50	50
Total proteins (mg/ml)	*	9,15	51	13,9	16,17	26,1

Table 2- IgY yield- proteins- according to purification methods

The optimal ED_{50} was obtained by sulphate ammonium precipitation rather than using PEG-8000 and caprylic acid.

CONCLUSIONS

We produced **IgY-based antivenoms** with DE_{50} similar to the ones obtained by immunizing horses (400 ug/mL), capable of neutralizing the lethal activity of ***Crotalus durissus terrificus*** at a preclinical level, showing a higher yield when using ammonium sulfate in the purification process. IgY- technology may allow to produce effective and affordable antivenoms.