

**NEUTRALIZATION OF ARUBA RATTLESNAKE (*CROTALUS UNICOLOR*)
VENOM ACTIVITY USING A POLYVALENT (ANTI-VIPERIDAE)
VENEZUELAN ANTIVENIN**

**EL VENENO DE LA CASCABEL DE ARUBA (*CROTALUS UNICOLOR*) ES
NEUTRALIZADO USANDO UN ANTISUERO POLIVALENTE (ANTI-
VIPERIDAE) VENEZOLANO**

Alexis Rodriguez-Acosta y Luis F. Navarrete

Universidad Central de Venezuela, Instituto de Medicina Tropical, Sección de Inmunología, Caracas, Venezuela. Apartado 47423, Caracas 1041, Venezuela Email: rodriguf@camelot.rect.ucv.ve

SUMMARY

Crotalus unicolor is an endemic species of the Neotropical rattlesnakes, which it is only found on the Island of Aruba, located off the coast of Venezuela. In a comparative study using SDS-PAGE *C. unicolor* venom has similar bands as several Venezuelan rattlesnake venoms. At least 6 different bands of approximately 210, 116, 114, 110, 96 and 52 kDa were shared among *C. unicolor* and other Venezuelan rattlesnakes tested. Precipitation lines in an immunodiffusion assay exhibited many antigen-antibodies determinants in venom proteins and antivenin.

RESUMEN

Crotalus unicolor es una especie de crotálo Neotropical endémica de la Isla de Aruba, localizada al frente de la costa de Venezuela. En un estudio comparativo usando la técnica de SDS-PAGE se observó que el veneno del *C. unicolor* comparte bandas similares con varios venenos de crotalos venezolanos. Por lo menos 6 bandas de aproximadamente 210, 116, 114, 110, 96 y 52 kDa eran compartidos entre *C. unicolor* y los otros crotalos. Las líneas de precipitación en un ensayo de inmunodifusión exhibió muchos determinantes antígeno-anticuerpo en proteínas de veneno contra el suero antiofídico.

Keywords: *Crotalus unicolor*, Aruba rattlesnake, antivenin, venom, rattlesnake ecology.

Palabras clave: *Crotalus unicolor*, cascabel de Aruba, antiveneno, veneno, ecología de cascabel.

INTRODUCTION

The Aruba Island rattlesnake (*Crotalus unicolor*) belongs to the Viperidae family (Vanzolini, 1986). This snake has a heat-sensing cavity behind and below their nares (nostrils) (Grenard, 1994). Besides rattlesnakes, some other kinds of pit vipers are: *Bothrops*, *Bothriechis*, *Bothriopsis*, *Porthidium* and *Lachesis* (Fuentes and Rodriguez-Acosta, 1997).

Crotalus unicolor is an endemic species of the Neotropical rattlesnakes, which it is only found on the Island of Aruba, located off the coast of Venezuela. It is the only rattlesnake taxon included in the "International Endangered Species Captive Breeding Program". The distribution area for the

snakes is only 32 km² of the island's centre dry interior at 0-50 meters of altitude; vegetation in this region is very scarce and the pluvial annual precipitation is 444 mm (Campbell and Lamar, 1989). The mean temperature is 28 °C and atmospheric humidity is less than 60%. Its climate corresponds to the North xerophytic Venezuelan pattern. The pit viper when intimidated, its rattle can be perceived. Its activity is mainly in the evening and early in the morning. They are very timid and the risk for a human to come across this snake is remote. However, physicians in Aruba when need to treat this envenomation they have used imported serum from the United States of America.

In this work we would like to emphasize that the Venezuelan antiserum is highly superior to the

North American rattlesnakes antiserum to treat a *C. unicolor* envenomation. These North American rattlesnakes, in general are haemorrhagic, proteolytic and poorly neurotoxic venomous snakes.

MATERIALS AND METHODS

Polyvalent antiserum (anti-*Crotalus/Bothrops*)

Polyvalent antiserum was purchased from the Pharmacy Faculty of the Universidad Central de Venezuela. It is prepared in horses immunised with *C. durissus cumanensis*, *C. vegrandis*, *Bothrops colombiensis* and *Bothrops venezuelensis* venoms.

Venom

C. unicolor venom was donated by Mr. Roeland de Kort, Director of the Parke Nacional Arikok. Animals were milked and then the venom transported to Venezuela at 0 °C until liophylisation was carried out.

Snake description and habitat

The maximum size for *C. unicolor* is 95 cm. A typical adult specimen weighs 1 to 1.5 kg and they can live from 10 to 22 years. In nature Aruba rattlesnake probably feed on lizards, rodents and birds. Like other rattlesnakes, *C. unicolor* injects their prey with a complex biochemical mixture that kills the victim and start to digest it.

The soil area of *C. unicolor* is very poor and the vegetation is mainly Cactaceae, *Opuntia* and scattered trees of many varieties (*Mimosa* spp, *Prosopis juliflora*, etc.) (Campbell and Lamar, 1989). The rattlesnake is usually found in small clusters of grass or among the boulder of rocks.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SDS-PAGE was carried out conforming to the Laemmli method (1970), using 12.5 % gels under reducing conditions. Molecular weight markers (Bio-Rad) were run in parallel and gels were stained with Coomassie Blue R-250 (Hudson and Hay, 1976). The molecular weight was determined by Multi-Analyst TM/PC version 1.1 program (Bio-Rad).

Double diffusion in two dimensions (Ouchterlony, 1970)

In this procedure venom (antigen) and antiserum (antibody) were allowed to migrate towards each other in a gel and a line of precipitation was formed where two reactants met. As routine technique is described elsewhere. Briefly, of *C. unicolor* venom (1mg/mL) was poured in the centre well of seven wells of previously prepared agar slide. Each of the six wells left was filled with a sample (20 µL) of non-diluted polyvalent antivenin (Pharmacy Faculty of Universidad Central de Venezuela).

RESULTS

Adult specimen of *C. unicolor* in its natural environment is showed in Fig.1.

The comparative SDS-PAGE where *C. unicolor* venom has similar bands as other Venezuelan rattlesnake venoms is showed in Fig. 2. At least 6 different bands of approximately 210, 116, 114, 110, 96 and 52 kDa were shared among *C. unicolor* and other Venezuelan rattlesnakes tested.

Precipitation lines that have fused completely exhibiting the presence of many determinants in venom proteins is showed in Fig. 3

DISCUSSION

This rattlesnake lives only on the dry, stony hillsides of Aruba Island. It was once heeded to be just a subspecies of another rattlesnake species common to South America. However, further studies have verified it is a distinct species in its own right.

Campbell and Lamar (1989) refer it as a subspecies (*Crotalus durissus unicolor*). Taking into account its morphological, ecological, and venom constitution characteristics, for them it seemed to be a subspecies that was developed in an isolated islander environment. The transfer of rattlesnakes from Venezuela to Aruba, throughout a hundred of year's mercantile traffic between both

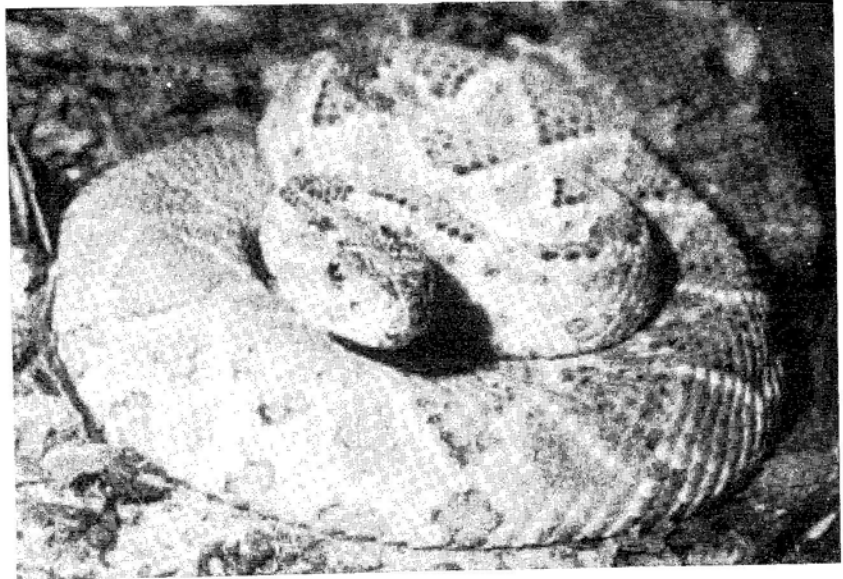


Figure 1. Adult specimen of *Crotalus unicolor* in its natural environment.

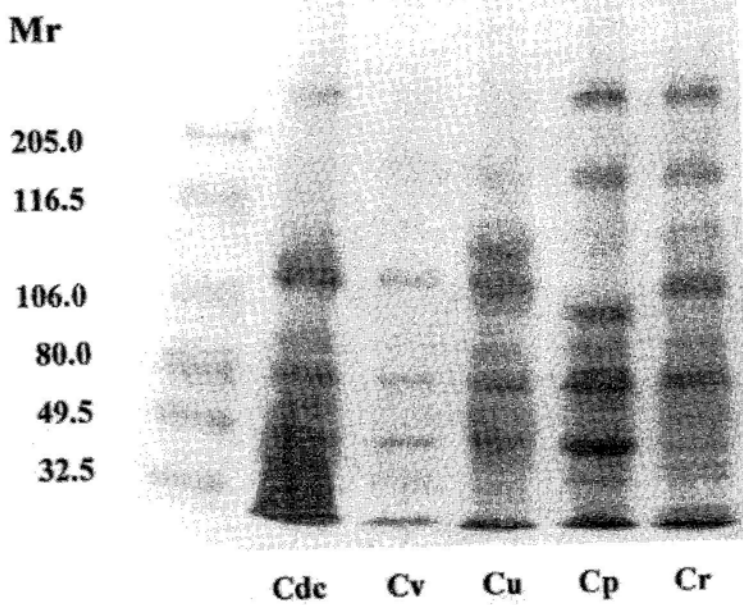


Figure 2. Polyacrylamide gel electrophoresis comparative bands of *Crotalus durissus cumanensis* (Cdc), *Crotalus vegrandis* (Cv), *Crotalus unicolor* (Cu), *Crotalus ruruima* (Cr) and *Crotalus pifanorum* (Cp).

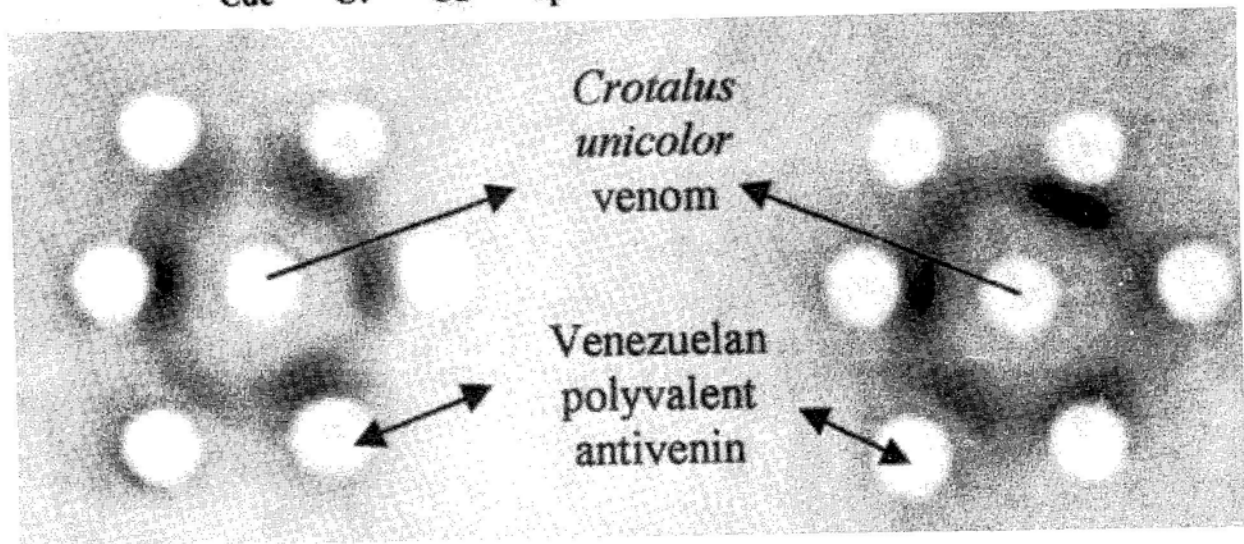


Figure 3. Immunodiffusion (Ouchterlony) assay. In the centre well 10 (L (1mg/ml5) of *C. unicolor* was tested against 10 (L of polyvalent antivenin. At least 6 bands were shared among *Crotalus* venom tested.

countries, appears to be certain. However, up to now nobody has described a rattlesnake in the island with similar morphological characteristics to the Venezuela common rattlesnake (*Crotalus durissus cumanensis*).

When *C. unicolor* venom fractions SDS-PAGE analysis was carried out, it was evidenced a high number of precipitation bands that were shared among the different venoms of Venezuelan rattlesnakes. *C. durissus cumanensis*, *C. vegrandis*, *Crotalus ruruima* and *C. pifanorum* shared at least 10 bands with *C. unicolor*. This allowed us to affirm that Venezuelan antiophidic serum prepared against continental rattlesnakes necessarily had to neutralise *C. unicolor* venom. Immuno-diffusion assays ratified the high precipitation of both antiophidic serum and *C. unicolor* venom that we already suspected with the previous technique.

In any reasonable snake venom emergency a physician must manage snakebite appropriately. For every snake maintained in captivity, one must keep or have access to antivenom in sufficient quantity to treat two «moderate» bites. People working or studying rattlesnakes in their natural environment should not rely on accessing antivenom from a zoo or regional hospital, they must possess or have direct access to the appropriate antivenom for the snake under study. In Venezuela antivenom for the rattlesnake is available in most hospitals in regions where rattlesnakes are indigenous.

Human intrusion on Aruba, mainly tourism, almost led this species to extinction. Now the island's people are prepared about this snake's role in the environment. A nature reserve (Parque Nacional Arikok) has been established, and the snake is protected.

LITERATURE CITED

CAMPBELL, J. A. AND W. W. LAMAR

1989. *Venomous reptiles of Latin America*. Cornell Univ. Pub. New York.

GRENARD, S.

1994. Snakes. In: *Medical herpetology*. NG Publishing Inc. USA. 53-86.

LAEMMLI, U. K.

1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

HUDSON, L AND F. HAY

1976. *Practical immunology*. Blackwell Sc. Pub. London. 298 pp.

OUCHTERLONY, O.

1970. *Handbook of immunodiffusion and immunoelectrophoresis*. Ann Arbor. Sc. Pub. Inc. USA. 133 pp.

FUENTES, O. AND A. RODRIGUEZ-ACOSTA

1997. Sobre los géneros *Bothriechis*, *Bothriopsis*, *Bothrops* y *Porthidium* (SERPENTES: CROTALIDAE) existentes en Venezuela. Claves para su identificación de interés biomédico. *Acta. Biol. Ven.*, 17 (3): 31-38.

VANZOLINI, P. E.

1986. *Catalogue of the Neotropical Squamata*. Smithsonian Institution Press. Washington D.C & London. 465 pp.