Comunicación breve

CHARACTERIZATION OF BOTHROPS VENOMS OF ARGENTINA BY SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

N. O. Stanchi[1], C. Dilorenzo[1], C.M. Gómez[1,2], C. Grisolia[3], J. Copes[1]

[1]Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.
[2.]Sector Sueros. Laboratorio Central de Salud Pública.
Ministerio de Salud. Provincia de Buenos Aires.
[3]Laboratorio y Museo de Animales Venenosos (LYMAV), Facultad de Ciencias Médicas.
Universidad Nacional de La Plata.

ABSTRACT: *Electrophoretic pattern of* Bothrops alternatus, B.neuwiedi, B. ammodytoides, B. jararaca *and* B. jararacussu *of Argentina by SDS-Polyacrylamide gel electrophoresis was carried out. Venoms used in this study came from animals (including juvenile) which live in different geographical zones. Special care was taken to not have cross-contamination of samples with blood and tissue fluids. All the snakes were milked with a minimum starvation period of 30 days. SDS-PAGE may be useful as a complementary method for characterization of snake venoms.*

Key Words: *Bothrops*, venom, electrophoresis

CARACTERIZACIÓN DE VENENOS DE BOTHROPS POR ELECTROFORESIS EN GELES DE SDS-POLIACRILAMIDA

RESUMEN: *Un estudio mediante electroforesis en geles de poliacrilamida fue realizado en venenos de* Bothrops alternatus, B. neuwiedi, B. ammodytoides, B. jararaca *y* B. jararacussu *procedentes de Argentina. Los venenos usados en este estudio fueron obtenidos a partir de animales procedentes de diferentes zonas geográficas, habiendo incluido animales jóvenes en este estudio. Se tomó especial precaución en el momento de extracción de los mismos evitando todo tipo de contaminación cruzada con sangre y otros fluidos animales. Todas las serpientes fueron «ordeñadas» con un período mínimo de ayuno de 30 días. Se desprende del análisis de este estudio que la técnica de SDS-PAGE puede ser de utilidad como método complementario para la caracterización de venenos de ofidios.*

Palabras Clave: *Bothrops*, venenos, electroforesis

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Dirección para correspondencia:
Carlos M. Gómez, CC 296 (B1900AVW) La Plata, ARGENTINA. Tel/Fax: 54-21-257980
E-mail: cgomez@fcv.medvet.unlp.edu.ar

INTRODUCTION

Bothrops genus comprises different species of snakes, but from medical and toxicological points of view there are five which have great importance in Argentina. *Bothrops alternatus*, *B. neuwiedi*, and *B. ammodytoides* are wide distributed in the country. On the other hand, *B. jararaca*, and *B. jararacussu* are found only in Misiones Province; this province resembles to south Brazil biogeographically. In ispite that *B. mooenii* is present in Argentina, it was not included in this study because it has no importance in the production of antisera.

Electrophoretic studies of snake venoms, particularly those regarding *Bothrops* genera, have been conducted by different investigators. With the approach of more advanced electrophoretic techniques during the past decade, several studies have been carried out. These investigations used paper electrophoretic (1), cellulose acetate (2,3), and polyacrylamide gel electrophoresis (PAGE) (4, 5, 6).

As verification of venom identity is a necessity, mainly when the production of antisnake venom serum with material from different sources is achieved, the present paper describes the electrophoretic PAGE pattern of five snake venoms of Argentina, with the purpose of its use as a guide to certify the identity and purity.

MATERIAL AND METHODS

Venoms

Snake venom samples of *Bothrops alternatus*, *B. neuwiedi*, *B. ammodytoides*, *B. jararaca*, and *B.jararacussu* were obtained from LYMAV (Laboratorio y Museo de Animales Venenosos) with the methodology described previously (7, 8) milking one animal species per day. Each lot has in composition venoms of animals of different age (including juvenile specimens) and have originated from different geographical zones of the country. Each specie comprise 50 animals for *B.alternatus*, *newviedii* and *ammodytoides*, and 20 for *jararaca* and 12 for *jararacussu*. Immediately after milking, venoms were desiccated under vacuum, phosphorus pentoxide and ambient temperature conditions; then it was pooled and stored at 4°C until processed (no more than 30 days). Resuspension was made on distilled water at 1% (w/v) concentration.

Electrophoresis

Sodium dodecil sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% w/v) in Tris-glycine buffer, pH 8.6 was performed in slab according to the method of Laemmli (9), with 2.5% (w/v) stacking gel. Briefly, 20 µl of each venom were mixed with sample buffer (12.5 mM Tris-HCl, pH 6.8; 4.6 % sodium dodecil sulfate; 2%-Mercaptoethanol and 0.1 Bromophenolblue) and denatured by treatment at 95ºC for 5 min. Runs were in parallel with 20 mA per slab and bromophenol blue was applied as tracking dye. Gels were stained for 30 min at room temperature with 0.05% (w/v) Coomasie brilliant blue R in 12.5% (v/v) acetic acid and 40% (v/v) methanol, and destained for 4 days with several changes of 7.5% acetic acid and 5% (v/v) methanol. Molecular weigh marker were also used (Sigma Co.). A densitometry of gel was carried out.

RESULTS

The results of SDS-PAGE are shown in Fig. I. The venoms are clearly differentiated from each other. *Bothrops alternatus* show 11 important bands, *B. neuwiedi* 9, *B. ammodytoides* 9, *B. jararaca* 8 and *B. jararacussu* 6 bands. Common bands are seen in different venoms (see arrow). All these different patterns are clearly defined by gel densitometry (Fig. II).

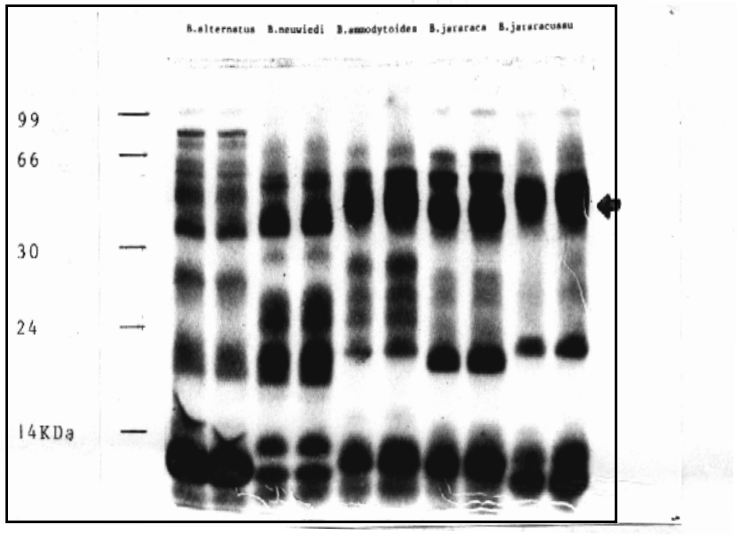

Figure I. SDS-Polyacrylamide gel electrophoresis (12% w/v) of *Bothrops* venoms (1% w/v, sample: 10 µl). Runs were carried out in parallel.
Figura I. Electroforesis en Geles de Poliacrilamida con SDS (12 % p/v) de venenos de *Bothrops* (1% p/v, muestra 10 µl). Las corridas se realizaron por duplicado.

DISCUSSION

As proteins and their derivatives constitute important aspects in the toxic effectiveness of most venoms, electrophoretic techniques offer a valuable contribution to show differences in venom composition.

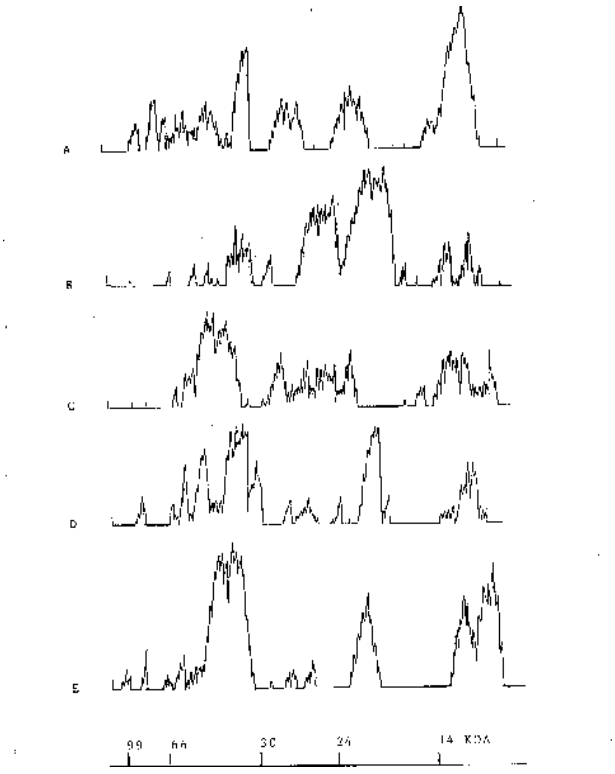

Figure II.Densitometry of SDS-Polyacrilamide gel electrophoresis: A. *B.alternatus*, B. *B.neuwiedi*, C. *B. ammodytoides*, D. *B. jararaca*, E. *B. jararacussu*.

Figura II.Densitometría de electroforesis en geles de SDS-

When animals are milked, a careful proceeding should be observed, in order to avoid the presence of contaminating proteins. Most of the reaction observed between anti-venom and snake sera can be explained by assuming that the venom used to immunize horses for antivenin production has been contaminated with snake blood or tissue fluid (10). According to this, when contaminating proteins are present, the electrophoretic pattern may change.

Juvenile and adult specimens were also included because differences in venom composition have been found in different investigations (11, 12, 13, 14). It is relevant to mention that the animals used in this study were milked once every 6 months, and with a minimum starvation period of 30 days to preserve all venom components, as described previously (7,8). There is unparalleled regeneration of snake venom components in successive milking (15) causing a decrease in the protein production of the gland and in the enzyme activity (16).

Stored venoms used for hiperimmunization of animals for anti-snake venom must be identical on antigenic properties such as fresh venom, because electrophoretic changes have been found under different storage conditions (17). They must also comprise venoms from animals of different geographical zones. There are important differences found in the composition of venoms in animals of the same geographical zones (18, 19, 20, 21).

Electrophoresis of venoms in SDS-PAGE has a similar number of bands to those obtained by Siles Villaroel (2, 3) with cellulose acetate («Cellogel»), except for *B. jararaca*. In this venom we found 10 bands, while in Brazilian snakes 15 bands have been observed; however, the former technique allows greater resolution with a simple differentiation of bands. On the other hand, Perrone et al (6) in Brazil, found different SDS-PAGE pattern, for both *B. neuwiedi* and *B. jararacussu* in comparison with our work. Apart from the difference of the technique employed by Siles Villaroel, in both cases this disagreement may be explained through the induction of variation of venom composition mentioned above. *B. ammodytoides* is present only in Argentina and we have no other reports to evaluate differences or similarities with other papers.

SDS-PAGE may be a suitable technique to verify venom identity and/or quality, when venom arrives to the laboratory from different serpentaria. Besides from that, this method of characterization of snake venom may be a valuable help as a complement to the method described by Theakston and Reid (22) for the same purpose.

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REFERENCES

1.Suzuki T. Separation methods of animal venoms constituents. Mem Inst Butantan 1966, 33,2:389-410.

2.Siles Villaroel M, Rolin Rosa R., Schwindt-Furlaneto R. Zelante F., Estudo electroforetico em «Cellogel» de venenos do gênero *Bothrops*. Mem Inst Butantan 1973, 37:83-90.

3.Siles Villaroel M. Contribuçao ao estudo de venenos de serpentes do gênero *Bothrops* (*Bothrops jararaca, B.alternatus, B.insularis, B.jararavussu, B.atrox* e *B.cotiara*). Tese Doutor. Instituto de Ciências Biomédicas. Universidade de São Paulo. Brasil. 1972.

4.Basu AS, Parker R, O'connor RO. Disc electrophoresis of glycoproteins in snake venoms. Toxicon 1970, 8: 279-284.

5.Mandelbaum FR, Assajura MT, Reichl AP. Characterization of two hemorrhagic factors isolated from venom of *Bothrops neuwiedi* (Jararaca pintada). Toxicon 1984, 22,3: 193-206.

6.Perrone MAL, Siles Villaroel M, Furtado M de FD. Estudo comparativo entre os venenos de serpentes do gênero *Bothrops* procedentes do estado de São Paulo e do Estado do Parana com algumas espécies morfologicamente duvidosas. Mem Inst Butantan 1989, 51,1: 25-32.

7.Grisolia C, Stanchi NO, Peluso F. Relación entre el período de ayuno y la cantidad de veneno extraída de ofidios en cautiverio. Rev Arg Prod Animal 1986, 6, 7/8:513-516.

8.Grisolia C, Peluso F, Stanchi NO. Informe sobre la actividad del Laboratorio y Museo de Animales Venenosos de la Facultad de Ciencias Médicas, UNLP. Rev Arg Prod Animal 1986, 6, 7/8:517-519.

9.Laemmly UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 1970, 227: 680.

10.Minton SA. Common antigens in snake sera and venoms. Toxicon 1970, 8,2: 143.

11.Bonilla CA, Faith MR, Minton SA Jr. L-aminoacid oxidase, phosphodiesterase, total protein and other properties of juvenile timber rattlesnake (*C.h.horridus*) venom at different stage of growth. Toxicon 1973, 11: 301.

12.Dominguez Furtado M de F, Contribuçao ao estudo do veneno de *Bothrops moojeni* (Hoge, 1965) (Serpentes, Viperidae, Crotalinae) em Tese Doutor. Universidade de São Paulo, 1987.

13.Gutierrez JM, Chavez F, Bolaños R. Estudio comparativo de veneno de ejemplares recién nacidos y adultos de *Bothrops asper*. Rev Biol trop 1980, 28:341.

14.Lomonte B, Gene JA, Gutierrez JM, Cerdas L. Estudio comparativo de los ejemplares de serpiente cascabel (*crotalus durissus durissus*) de ejemplares adultos y recién nacidos. Toxicon 1983, 21,3: 379-384.

15.Schenberg S, Pereira Lima FA, Nogueira Schiripa LN, Nagamori A. Unparalleled regeneration of snake components in successive milking. Toxicon 1970, 8,2:152.

16.Sobol Brown R, Boolah A, Kochva E, Brown MB. Secretion of enzymes in the venom glands of *Vipera palaestinae* (Viperidae, Ophidia). Toxicon 1966, 8,2:156.

17.Gene JA, Lomonte B, Gutierrez JM, Cerdas L. Cambios en el patrón electroforético del veneno de la serpiente cascabel muda (*Lachesis muta stenophys*) almacenado bajo diferentes condiciones. Rev Biol trop 1985 33,1:63-65.

18.Aragon F, Gubensek P. *Bothrops asper* venom from the Atlantic and Pacific zones of Costa Rica. Toxicon 1981, 19: 797-805.

19.Glenn J, Straight RC, Wolfe MC, Haryd L. Geographical variation in *Crotalus scutulatus scutulatus* (Mojave rattlesnake) venom properties. Toxicon 1983, 21, 1:119-130.

20.Jimenez Porras JM. Intraspecific variations in composition of venoms of the jumping viper, *Bothrops nummifera*. Toxicon 1964, 2: 187-195.

21.Jimenez Porras JM. Differentiation between *Bothrops nummifer* and *Bothrops picadori* by means of the biochemical properties of their venoms. Toxicon 1967, 4:300-322.

22.Theakston RDG, Reid MA, Development of simple standard assay procedure for the characterization of snake venoms. Bull. WHO 1983, 61,6:949-956.