Ciências Biológicas

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Individual variation in venom from the left and right glands of snakes of the species *Bothrops atrox*[1]

Matheus Felipe Ketes Bergamin[2*], Ilia Gilmara Carvalho dos Santos[3], Maria Carolina Scheffer de Souza[4], Hipócrates de Menezes Chalkidis[5], Rafael Luckwu de Sousa[6], Keyla Emanuelle Ramos de Holanda [7], Valéria Mourão de Moura[8], Maria Cristina Dos-Santos[9]

Abstract

Snakebite envenomation is considered a neglected public health problem in Brazil. Most of such accidents are attributed to different species of the genus *Bothrops*, of which *Bothrops atrox* is responsible for most cases. Snake venoms consist primarily of proteins, each of which performs a biological activity or acts synergetically to help snakes to survive by capturing and digesting prey and to defend themselves against attacks by predators. Studies have reported intraspecific and intrapopulational variability that depends on the age (ontogenetic variation), sex and size of the snake as well as geographic region. In the present study, variations in venom produced by the left and right glands of the same individual were assessed. Phospholipase A_2 (PLA_2) and coagulant activities and protein profiles were analyzed, and the immunogenic proteins in venom from each gland were titrated. *In vitro* results for venom from seven snakes of the species *B. atrox* (14 samples; one from each gland of seven snakes) revealed a statistically significant difference in PLA_2 activity and coagulant activity between venom from the left and right glands in one snake. In addition, minor differences in protein profile were observed in SDS-PAGE and in the minimum concentration of proteins reactive to a polyclonal antiserum against *B. atrox* venom in a Dot-ELISA. The results therefore suggest that the same snake may be able to produce biochemically distinct venoms. However, the frequency with which these variations occur, their extent and whether they can cause clinical differences *in vivo* are not known.

Keywords: snake venom; individual variability; *Bothrops atrox*; snake antivenom.

[1] Part of the work and experiments carried out by the scholarship student during his project of the Scientific Initiation Program (PIBIC).

[2] Bolsista PIBIC/FM/UFAM, correspondence matheusbergamin@hotmail.com
[3] Lab. Imunoquímica, Depto Parasitologia, ICB/UFAM, iliagilmara@hotmail.com
[4] Lab. Imunoquímica, Depto Parasitologia, ICB/UFAM, mcarol.biotec@gmail.com
[5] Lab Pesquisas Zoológicas, CUA – UNAMA, chalkidis@hotmail.com
[6] Lab Imunoquímica, Depto Parasitologia, ICB/UFAM, rafalucksousa@gmail.com
[7] Lab Imunoquímica, Depto Parasitologia, ICB/UFAM, ramos.keyla@gmail.com
[8] PPGRNA, LabBBEx, UFOPA, mouraovm@yahoo.com.br
[9] Profa Titular, Lab. Imunoquímica, Departamento de Parasitologia, ICB/UFAM, mcsantos@ufam.edu.br

Ciências Biológicas

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Variabilidade individual entre serpentes *Bothrops atrox* em relação aos venenos de suas glândulas direita e esquerda. O envenenamento por picada de serpente é considerado um problema de saúde pública negligenciado no Brasil. Dentre as serpentes causadoras, ao gênero *Bothrops* sp. é atribuída a maioria dos acidentes. Na região Amazônica, a espécie *Bothrops atrox* lidera a lista de casos por envenenamentos. A composição química da peçonha é, predominantemente, proteica e cada componente vai desempenhar uma atividade biológica ou agir sinergicamente auxiliando a serpente a sobreviver pela apreensão, captura, digestão de presas e na defesa contra-ataques predadores. Dentre as pesquisas já realizadas, foram constatadas as variabilidades intraespecíficas nas peçonhas de acordo com sexo, tamanho, localização geográfica e variações intrapopulacionais. No presente estudo, avaliou-se as variações existentes, em um mesmo indivíduo, nos venenos produzidos por suas glândulas direita e esquerda, através das atividades fosfolipásica A_2, coagulante, pelo perfil proteico e pela titulação das proteínas imunogênicas. A partir dos resultados obtidos *in vitro*, com os venenos de sete serpentes *Bothrops atrox* (14 amostras, sendo uma de cada glândula das 07 serpentes), foi encontrada uma diferença estatística entre a peçonha das glândulas direita e esquerda de apenas uma serpente em relação às atividades fosfolipásica A_2 e coagulante. Além disso, também foram encontradas discretas diferenças no perfil proteico analisado em SDS-PAGE e na quantificação das proteínas imunogênicas pelo Dot-ELISA. Portanto, segundo estes resultados, uma mesma serpente pode ser capaz de produzir venenos bioquimicamente distintos. Contudo, não se sabe com qual frequência e grau estas variações ocorrem e se são possíveis de causar diferentes manifestações clínicas *in vivo*.

Palavras-chaves: variação individual; variabilidade intraespecífica; soro antiofídico; *Bothrops atrox*.

1. Introduction

According to the Brazilian Unified Health System Information Technology Department (DATASUS), around 265,000 accidents involving venomous animals were recorded in 2019 in Brazil. Of these, more than 25,000 involved snakes (Ministry of Health – MH, 2020). Because of its high incidence, snakebite envenomations are considered a public health problem in various countries with tropical and underdeveloped regions, such as Brazil, and are classified as a neglected problem by the WHO (Hui Wen et al., 2015; Fry B.G., 2018; WHO, 2019; Da-Silva et al., 2020).

Despite the great size of the Brazilian Amazon and the variety of genera and species of venomous snakes found there (*Bothrops* – ten species; *Bothrocophias* – two species; *Crotalus durissus* – five subspecies; *Lachesis muta*; *Leptomicrurus* – three species; and *Micrurus* – 26 species), the majority of accidents are attributed to *Bothrops atrox* and *Lachesi muta*, the former being responsible for over 80% of cases (Feitosa et al., 2015; Sociedade Brasileira de Herpetologia, 2018; Monteiro et al., 2020).

Snake venoms contain a complex mixture of many different compounds, primarily proteins (phospholipases A_2, serine proteases

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

and metalloproteinases) and biologically active peptides that have a range of biological activities and trigger different clinical manifestations depending on the amount of venom and of each component injected into the victim when he/she is bitten (Sousa et al., 2013; Wang et al., 2020).

Phospholipases A_2 (PLA_2s), enzymes that catalyze the hydrolysis of some phospholipids depending on their structure, can induce effects such as neurotoxicity, myotoxicity, cardiotoxicity, activation or inhibition of platelet aggregation, hemorrhage, edema, convulsion and hypotension, interfering with homeostasis in humans (Gutiérrez and Lomonte, 1995).

The coagulant activity of snake venoms is due essentially to the presence of two types of enzymes: metalloproteinases and serine proteases. The former can hydrolyze factor X, which is common to the intrinsic and extrinsic pathways of the coagulation cascade, and prothrombin, while the latter can convert fibrinogen to fibrin (thrombin-like action). Marked consumption of these clotting factors is responsible for the incoagulability of blood observed in patients bitten by snakes of the genus *Bothrops*. (Santoro et al., 2008).

The signs and symptoms of Bothropic envenomation, the most common in Brazil, are varied and are the result of local tissue damage caused by the toxins in the venom. Victims usually present with pain, edema, bleeding, blisters and necrosis in the region of the bite (Oliveira et al., 2010). Systemic manifestations may also be present and may include hemorrhage, coagulation disorders and kidney and heart damage depending on the severity of the accident and the time that elapses between the snakebite and administration of the antivenom (Salazar et al., 2007; Moretto Del-Rei et al., 2019).

According to various studies in which functional proteomic analysis of venoms was performed, intraspecific variability of venom can be observed in snakes such as *B. atrox* (Sousa et al., 2017), *B. asper* (Vélez et al., 2017), *B. alternatus* (Rocco et al., 2013), *Crotalus durissus* (Oliveira et al., 2019) and *Micrurus frontalis* (Sanz et al., 2019).

It would appear therefore that the composition of the venom is determined mainly genetically, and that very minor structural modifications occur in the proteins as a result of the substitution of certain amino acids. However, there are environmental factors that lead to qualitative and quantitative variations in the venom produced (Salazar et al., 2007; Oliveira et al., 2018). The factors which influence the composition of the venom of snakes of the same species (intraspecific variations) can include the age (ontogenetic variations), size, sex and diet of the snake, as well as the geographic region where the snake is found and seasonal variations (Chippaux et al., 1991; Amazonas et al., 2018).

As scientific research has progressed, it has become increasingly necessary to gain a better understanding of intraspecific variations to develop more complete antivenoms that can neutralize all the protein variants of a particular venom. It is therefore important to have a pool of standardized venoms to produce effective antivenoms that can minimize complications due to

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

envenomation (Salazar et al., 2007; Farias et al., 2018).

It has been shown that the same individual can produce different types of venom simultaneously (Deoras, 1963), and a study in 1987 by Johnson et al. with rattlesnakes from the South Pacific coast showed a difference in venom produced by a single snake, i.e., an individual variation. The authors found that the left and right glands did not produce the same venom: the right gland produced white venom while the left gland produced yellow venom. Furthermore, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed significant differences in the protein composition of the two venoms (Johnson et al., 1987).

In another more recent study (Smith and Mackessy, 2020) with *Crotalus viridis viridis*, one of the snakes studied was shown to lose much of the functionality of the toxins in venom from one gland when it reached adulthood. In 2014, venom from the left and right glands was similar, while in 2017 venom from the left gland had lost many functions previously observed and looked very like white venom. This was considered asymmetrical expression of venom from a single snake (Smith and Mackessy, 2020).

Another study reported that these differences could be related to the ability of one gland to eject more venom than another and to the possibility of "toxin replenishment" occurring at different rates in each gland. A certain toxin could therefore be present in a lower concentration than another at a particular time, leading to this functional difference (Harris et al., 2020).

In light of the need to expand our understanding of interspecific variations and the dearth of studies on individual variability, the present study sought to analyze *in vitro* possible intraspecific and individual variations (between left and right glands) in PLA_2 and coagulant activities of Bothropic venom from snakes of the species *B. atrox*. We also analyzed protein profiles using SDS-PAGE and determined the minimum concentrations of the proteins in the venoms that are reactive to PABa, a polyclonal antiserum developed in our laboratory against *B. atrox* venom.

2. Materials and Methods

2.1. Venoms

The poisons were kindly provided by the Doctor Heitor Vieira Dourado Tropical Medicine Foundation. Bothropic venoms were collected from the left and right glands of seven adult *B. atrox* specimens, giving a total of 14 samples, and then freeze dried. The venoms were diluted to a final concentration of 1 mg/mL in saline. The animals were bred in captivity under the same conditions in the Museum of the Amazon (MUSA) (Manaus, Brazil) with approval from the Authorization and Biodiversity Information System (SISBIO) (ref. no. 2065059) and the Brazilian Institute of the Environment and Natural Resources (IBAMA) (ref. no. 02005.001444/2006-06). The animals were all captured in municipalities in the interior of the state of Amazonas between 2010 and 2013. The venoms were collected in 2017 with approval from the Committee for Ethics in the Use of Animals at the Dr Heitor Vieira Dourado Tropical Medicine Foundation (ref. no. 001552/2017.011/FMT-HVD/CEUA).

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

2.2. Phospholipase A_2 Activity

PLA_2 activity was assessed by the indirect hemolysis method using a Petri dish with agarose gel and the following reagents: 2 mL 0.01M $CaCl_2$; 2.4 mL egg yolk diluted 1:3 in PBS; 2 g 1% agarose; 200 mL 0.9% NaCl pH 7.2; 40 µL 0.005% sodium azide; and 2.4 mL human erythrocytes. Based on a previous study, 2.5 µg, the equivalent of 2 x MiHD (minimum indirect hemolytic dose) (Moura et al., 2014), of each of the 14 venom samples were placed in duplicate in wells measuring approximately 3 mm. The reaction was monitored after incubation of the plates for 16 hours, and the diameters of the halos formed were measured. PLA_2 cleaves the lecithin in the egg yolk, forming lysolecithin, which causes the red blood cells to lyse and a lighter halo to form around the well. The PLA_2 activity of each sample can thus be assessed visually based on the size in cm^2 of the respective halo (Gutiérrez et al., 1988).

2.3. Coagulant Activity

Coagulant activity was evaluated according to the method described by Theakston and Reid (1983) with modifications. A 200 µL volume of human plasma (2.8 g/L in PBS pH 7.2) was previously incubated at 37°C. Next, 10 µL of venom samples at a concentration of 1 mg/mL were added to the mixture, and the time required for a fibrin mesh (clot) to form was observed visually and measured in seconds. The absence of a fibrin mesh after 10 minutes was considered to indicate an absence of coagulant activity in the samples.

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The protein profiles of the individual venoms of *Bothrops atrox* were analyzed by SDS-PAGE following the protocol described by Laemmli (1970) with modifications. The electrophoresis was run at 100 V, and the running gel and stacking gel concentrations were 15% and 5%, respectively. The following proteins (7 µL each) were used as molecular weight markers: myosin (192 kDa), β-galactosidase (102.1 kDa), bovine serum albumin (58.1 kDa), ovalbumin (41 kDa), carbonic anhydrase (27.9 kDa), soybean trypsin inhibitor (20.4 kDa), lysozyme (15.1 kDa) and aprotinin (6.4 kDa) (Bio Rad® Prestained SDS-PAGE Standards, broad range, USA, catalog no. 161-0318). After the gels were run in the Mini-PROTEAN system (Bio Rad®) (Bio-Rad, USA), they were stained for two hours with Coomassie Brilliant Blue R-250 (Bio Rad ®) and left in a destaining solution for 24 hours to enable the protein bands to be visualized and the protein migration to be measured with a millimeter-scaled ruler. The samples were applied to two gels and run separately, each with seven samples, to visualize the protein bands for all 14 samples. Gel 1 contained samples from snakes 1 to 4 while gel 2 contained samples from snakes 5 to 7. The molecular weights of the five proteins in the visible bands were determined from the displacement in mm of the bands and the graphs constructed with the molecular-weight markers. ImageJ® free image processing and analysis software was used to convert pixel concentration into protein density.

Ciências Biológicas

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

2.5. Dot-ELISA

Dot-ELISA was used to determine the minimum concentrations of the proteins reactive to PABa (Towbin and Gordon 1984). A 2 µL volume of different concentrations ($2x10^{-5}$ µg; $1x10^{-5}$ µg; $0.5x10^{-5}$ µg; $0.25x10^{-5}$ µg; $0.125x10^{-5}$ µg; $0.0625x10^{-5}$ µg; $0.03125x10^{-5}$ µg; $0.015625x10^{-5}$ µg) of the individual venoms was applied to a nitrocellulose membrane. After the membrane had dried, it was blocked with 5% Molico® Nestlé skim milk in PBS pH 7.2 at 4 °C for 24 hours. The membrane was then incubated at room temperature for one hour with PABa diluted 1:400 in PBS pH 7.2, washed with PBS pH 7.2 and incubated for an hour with peroxidase-conjugated anti-mouse IgG (Thermo Fisher Scientific®) diluted 1:1000 in PBS pH 7.2. Next, the membrane was washed again with PBS pH 7.2, and a developing solution consisting of an H_2O_2 substrate in the presence of the chromogen 3'3 diaminobenzidine (DAB – Bio-Rad) diluted in PBS pH 7.2 was added to detect the antigen-antibody reaction. This allowed the minimum concentration of proteins sharing the same epitopes recognized by the immunoglobulins in the PABa in the different venom samples to be determined.

2.6. Statistical analysis

The PLA_2 and coagulant activity experiments were performed in duplicate so that the mean and standard deviation of the results could be determined. The results were then analyzed with two-way ANOVA and Sidak's multiple comparison test.

3. Results

Figures 1 and 2 show the PLA_2 and coagulant activities of the 14 venom samples from the seven *B. atrox* snakes (left and right glands of everyone) (Figures 1 and 2).

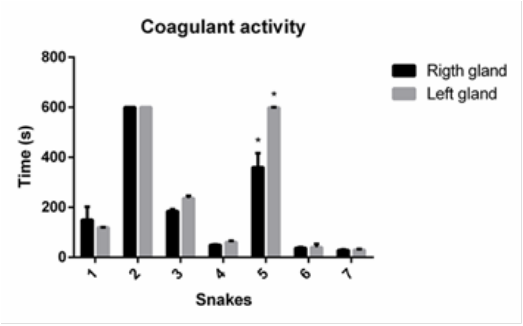

Figure 1. Results of two-way ANOVA and Sidak's multiple comparison test for coagulant activity of the left and right glands of the seven snakes. (*) Statistically significant difference found in coagulant activity between left and right glands of snake five (5). The vertical axis shows the time in seconds before coagulant activity was observed. The horizontal axis shows snakes number 1 to 7.

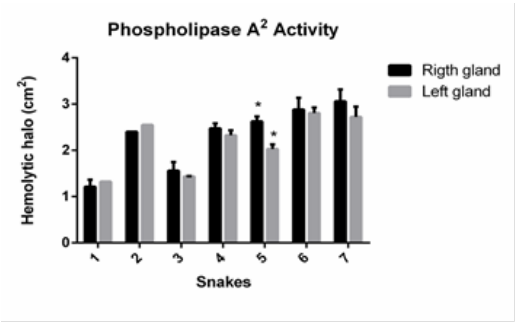

Figure 2. Results of two-way ANOVA and Sidak's multiple comparison test tor PLA_2 activity of the left and right glands of the seven snakes. (*) Statistically significant difference found in PLA_2 activity between left and right glands of snake 5. The vertical axis shows the hemolytic halo in cm^2. The horizontal axis shows snakes 1 to 7.

The figures show that there is a statistically significant difference in PLA_2 and coagulant activities between the left and right glands of

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

snake 5. No statistically significant differences in these activities were observed for the other six snakes (Figures 1 and 2).

Figure 3 shows the protein bands detected by SDS-PAGE in the 14 samples in two gels. There is a subtle difference between the stained protein bands for the left and right glands. In sample 3R, for example, band 4 was much less dense than in sample 3L. However, a greater difference is observed between snakes, suggesting that the composition of the venom varies between snakes of the same species (Figure 3).

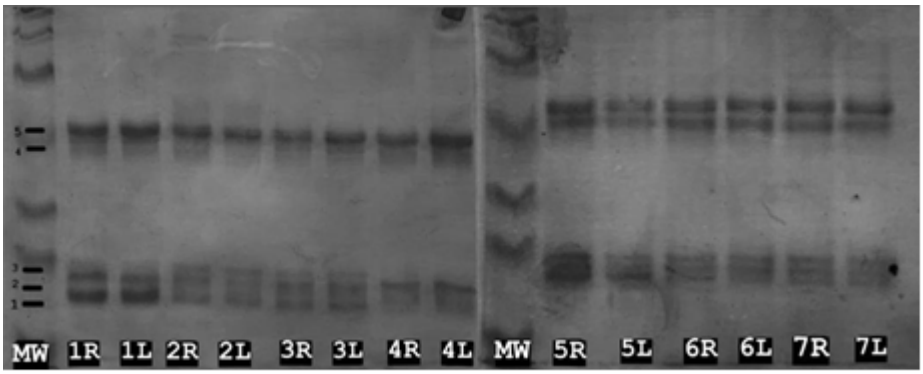

Figure 3. Results for the two SDS-PAGE gels. Gel 1, on the left, with samples from snakes 1 to 4. Gel 2, on the right, with samples from snakes 5 to 7. A total of 14 samples with the five most visible protein bands numbered 1 to 5 on the left of the figure. MW: molecular weight markers; 1R: venom sample from the right gland of snake 1; 1L: venom sample from the left gland of snake 1. The other samples follow the same pattern (2R, 2L etc.).

In the analysis of the minimum concentration of proteins that share the same epitopes detected by PABa, variations were also found between snakes and between their left and right glands. For example: in snake 1, the minimum concentration was 1R = $0.125x10^{-5}$ µg and 1L = $0.062x10^{-5}$ µg; in snake 3, 3R = $0.25x10^{-5}$ and 3L = $0.125x10^{-5}$ µg; in snakes 5 and 6, 5R and 6R = $0.125x10^{-5}$ µg and 5L and 6L = $0.25x10^{-5}$; and in snake 7, 7R = $0.062x10^{-5}$ µg and 7L = $0.125x10^{-5}$ µg. No statistically significant differences in the minimum concentrations between left and right glands were observed for snakes 2 and 4.

4. Discussion

An understanding of intraspecific variability is important not only to elucidate the process by which snakes adapt as they grow and their food preferences and habitat change, but also to improve management of the different clinical manifestations patients may present with depending on the snake involved in the snakebite (Amazonas et al., 2019).

The recommended treatment for envenomation is administration of specific snake antivenom as soon as the snake has been identified (Gutiérrez et al., 2017). Populations that have access to fewer facilities and are farther from the nearest health center are therefore more affected by disabling and even fatal sequelae. This risk group includes children, males, fishermen, riverine communities, indigenous peoples, farmers, shepherds, and individuals with few years of schooling (da-Silva et al., 2020).

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

In Brazil, *Bothrops* antivenom is produced in reference institutions using plasma from horses immunized with specific antigens from five species of the genus *Bothrops* from different geographic regions: *B. jararaca* (50%), *B. alternatus* (12.5%), *B. moojeni* (12.5%), *B. neuwedi* (12.5%) and *B. jararacuçu* (12.5%). The pool of reference antigens therefore does not contain venom from *B. atrox*, the species responsible for most snakebites in the Amazon region (Farias et al., 2018).

The three main biological effects of Bothropic venom are hemorrhagic, coagulant, and proteolytic. The hemorrhagic effect of Bothropic venom is induced by toxins known as hemorrhagins; the coagulant activity is a result of substances with thrombin-like activity; and the proteolytic effect is a result of the activity of proteases, hyaluronidases and PLA_2s. Because of intraspecific variations, each animal's venom may contain components in higher or lower concentrations, resulting in biological manifestations that differ between snakebites. The antivenom must therefore effectively neutralize all the toxins released in the region of the bite and in the patient's bloodstream to ensure that the victim suffers less severe complications. The development of a more inclusive pool of reference venoms is therefore of utmost importance (Galizio et al., 2018).

Corroborating studies by Smith and Mackessy (2020), Johnson et al. (1987) and Deoras (1963), we also found differences between samples from the same individual (left and right glands): as mentioned earlier, differences in PLA_2 and coagulant activities were observed in snake 5 although the adult snakes used in this study, which were captured in the state of Amazonas, were kept under the same conditions. It appears, however, that the differences observed here are not as marked as those reported in the firsts of the two studies mentioned above (Smith and Mackessy, 2020 and Johnson et al., 1987), in which a single snake was able to produce white and yellow venoms, the latter indicating the presence of L-amino acid oxidase. Furthermore, it is not known whether these differences in venom from a single snake result in quite different clinical manifestations *in vivo*, and the intensity and frequency with which these variations occur is also unknown.

5. Conclusion

Our *in vitro* findings show that intraspecific variations in the composition of *B. atrox* venom can occur, as suggested in other studies. This corroborates the idea that the venom is determined mainly genetically but that under certain conditions expression of a particular protein may be favored over that of another.

As far as individual variability is concerned, it is not possible to infer how often this occurs, what repercussions it has for envenomations and what the cause could be as different amounts of protein may have been injected in prey by each gland before the venom was extracted since venom glands in snakes are positioned on opposite sides and can eject venom independently.

There is therefore a need for further studies of individual variations (between glands) and the importance of understanding these for both public health and immuno-

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

chemistry, particularly so that ever more effective snake antivenoms can be developed.

Acknowledgments

The authors would like to thank CNPq Brazil (the National Council for Scientific and Technological Development) for providing undergraduate students at the Federal University of Amazonas with an incentive to contribute to the scientific literature by making student fellowships for undergraduate research programs (PIBIC) available. The authors are also indebted to the Museum of the Amazon (MUSA, Manaus, Brazil) and Doctor Heitor Vieira Dourado Tropical Medicine Foundation for kindly providing the venoms studied here. Finally, the author would like to thank all the staff at the Immunochemistry Laboratory at the Institute of Biological Sciences, Federal University of Amazonas, for making this research possible. MCS is a recipient of a CNPq productivity fellowship (ref. 303032 / 2016-2 and 480892/ 2019-2).

Divulgation

This article is unpublished and is not being considered for any other publication. The authors and reviewers did not report any conflict of interest during this evaluation. Therefore, the Scientia Amazonia magazine owns the copyrights, has the approval and the permission of the authors for disclosure, of this article, by electronic means.

References

Amazonas, Diana R., Luciana A. Freitas-De-Sousa, Daniele P. Orefice, Leijiane F. de Sousa, Melissa G. Martinez, Rosa H.V. Mourão, Hipócrates M. Chalkidis, Plínio B. Camargo, and Ana M. Moura-Da-Silva. 2019. "Evidence for Snake Venom Plasticity in a Long-Term Study with Individual Captive Bothrops Atrox." *Toxins* 11 (5). https://doi.org/10.3390/toxins11050294

Amazonas, Diana R., José A. Portes-Junior, Milton Y. Nishiyama-Jr, Carolina A. Nicolau, Hipócrates M. Chalkidis, Rosa H.V. Mourão, Felipe G. Grazziotin, et al. 2018. "Molecular Mechanisms Underlying Intraspecific Variation in Snake Venom." *Journal of Proteomics*, 2018. https://doi.org/10.1016/j.jprot.2018.03.032

Chippaux, J. P., Williams, V. & White, J. 1991. Article, Review. "Review Article Snake Venom Variability: Methods of Study, Results and Interpretation" 29 (I): 1279–1303.

Brasil. Ministério da Saúde (MS). Tecnologia da Informação a Serviço do SUS (DATASUS). 2019. "Acidentes Por Animais Peçonhentos – Notificações Segundo Tipo de Acidente No Brasil Em 2019 [Página Na Internet]." 2019.

Costa, H. C., & Bérnils, R. S. 2018. "Répteis Do Brasil e Suas Unidades Federativas: Lista de Espécies." *Revista Herpetologia Brasleira* 7(1): 11–57.

Deoras, P. J. 1963. *Studies on Bombay Snakes; Snake Farm Yield Records and Their Probable Significance. Venomous and Poisonous Animals and Noxious Plants of the Pacific Region*. (KEEGAN, H.

Farias, Iasmim Baptista de, Karen de Morais-Zani, Caroline Serino-Silva, Sávio S. Sant'Anna, Marisa M.T.da Rocha, Kathleen F. Grego, Débora Andrade-Silva, Solange M.T. Serrano, and Anita M. Tanaka-Azevedo. 2018. "Functional and Proteomic Comparison of Bothrops Jararaca Venom from Captive Specimens and the Brazilian Bothropic Reference Venom." *Journal of Proteomics* 174 (October 2017): 36–46. https://doi.org/10.1016/j.jprot.2017.12.008

Feitosa, Esaú Samuel, Vanderson Sampaio, Jaqueline Sachett, Daniel Barros De Castro, Maria das Dores Nogueira Noronha, Jorge Luis López Lozano, Emiro Muniz, Luiz Carlos Lima De Ferreira, Marcus Vinícius Guimarães De Lacerda, and Wuelton Marcelo Monteiro. 2015. "Snakebites as a Largely Neglected Problem in the Brazilian

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Amazon: Highlights of the Epidemiological Trends in the State of Amazonas." *Revista Da Sociedade Brasileira de Medicina Tropical* 48 (Suppl I): 34–41. https://doi.org/10.1590/0037-8682-0105-2013

Fry, Bryan G. 2018. "Snakebite: When the Human Touch Becomes a Bad Touch." *Toxins* 10 (4): 1–24. https://doi.org/10.3390/toxins10040170

Galizio, Nathália da Costa, Caroline Serino-Silva, Daniel Rodrigues Stuginski, Patrícia Antônia Estima Abreu, Sávio Stefanini Sant'Anna, Kathleen Fernandes Grego, Alexandre Keiji Tashima, Anita Mitico Tanaka-Azevedo, and Karen de Morais-Zani. 2018. "Compositional and Functional Investigation of Individual and Pooled Venoms from Long-Term Captive and Recently Wild-Caught Bothrops Jararaca Snakes." *Journal of Proteomics* 186 (July): 56–70. https://doi.org/10.1016/j.jprot.2018.07.007

Gutiérrez, José María, Alexandra Rucavado, Teresa Escalante, Cristina Herrera, Julián Fernández, Bruno Lomonte, and Jay W. Fox. 2018. "Unresolved Issues in the Understanding of the Pathogenesis of Local Tissue Damage Induced by Snake Venoms." *Toxicon* 148: 123–31. https://doi.org/10.1016/j.toxicon.2018.04.016

Gutiérrez, José María, Gabriela Solano, Davinia Pla, María Herrera, Álvaro Segura, Mariángela Vargas, Mauren Villalta, et al. 2017. "Preclinical Evaluation of the Efficacy of Antivenoms for Snakebite Envenoming: State-of-the-Art and Challenges Ahead." *Toxins* 9 (5): 1–22. https://doi.org/10.3390/toxins9050163

Gutiérrez, JoséMaría, Claudio Avila, Ermila Rojas, and Luis Cerdas. 1988. "An Alternative in Vitro Method for Testing the Potency of the Polyvalent Antivenom Produced in Costa Rica." *Toxicon* 26 (4): 411–13. https://doi.org/10.1016/0041-0101(88)90010-4

Gutiérrez, JoséMaría, and Bruno Lomonte. 1995. "Phospholipase A2 Myotoxins from Bothrops Snake Venoms." *Toxicon* 33 (11): 1405–24. https://doi.org/10.1016/0041-0101(95)00085-Z

Harris, Richard J., Christina N. Zdenek, Amanda Nouwens, Charlotte Sweeney, Nathan Dunstan, and Bryan G. Fry. 2020. "A Symmetry or Asymmetry: Functional and Compositional Comparison of Venom from the Left and Right Glands of the Indochinese Spitting Cobra (Naja Siamensis)." *Toxicon: X* 7: 100050. https://doi.org/10.1016/j.toxcx.2020.100050

Hui Wen, Fan, Wuelton Marcelo Monteiro, Ana Maria Moura da Silva, Denise V. Tambourgi, Iran Mendonça da Silva, Vanderson S. Sampaio, Maria Cristina dos Santos, et al. 2015. "Snakebites and Scorpion Stings in the Brazilian Amazon: Identifying Research Priorities for a Largely Neglected Problem." *PLoS Neglected Tropical Diseases* 9 (5): 1–11. https://doi.org/10.1371/journal.pntd.0003701

Johnson, Edward K., Kenneth V. Kardong, and Charlotte L. Ownby. 1987. "Observations on White and Yellow Venoms from an Individual Southern Pacific Rattlesnake (Crotalus Viridis Helleri)." *Toxicon* 25 (11): 1169–80. https://doi.org/10.1016/0041-0101(87)90135-8

Laemmli, U. K. 1970. "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4." *Nature* 227 ((5259)): 680–85. https://doi.org/10.1038/227680a0

Monteiro, Wuelton Marcelo, Jorge Carlos Contreras-Bernal, Pedro Ferreira Bisneto, Jacqueline Sachett, Iran Mendonça da Silva, Marcus Lacerda, Allyson Guimarães da Costa, et al. 2020. "Bothrops Atrox, the Most Important Snake Involved in Human Envenomings in the Amazon: How Venomics Contributes to the Knowledge of Snake Biology and Clinical Toxinology." *Toxicon: X* 6. https://doi.org/10.1016/j.toxcx.2020.100037

Moretto Del-Rei, Tiago H., Leijiane F. Sousa, Marisa M.T. Rocha, Luciana A. Freitas-de-Sousa, Silvia R. Travaglia-Cardoso, Kathleen Grego, Sávio S.

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Sant'Anna, Hipócrates M. Chalkidis, and Ana M. Moura-da-Silva. 2019. "Functional Variability of Bothrops Atrox Venoms from Three Distinct Areas across the Brazilian Amazon and Consequences for Human Envenomings." *Toxicon* 164 (March): 61–70. https://doi.org/10.1016/j.toxicon.2019.04.001

Mourão De Moura, Valéria, Adrielle N. Serra Bezerra, Rosa Helena Veras Mourão, Juliana L. Varjão Lameiras, Juliana D. Almeida Raposo, Rafael Luckwu De Sousa, Antônio Luiz Boechat, Ricardo Bezerra De Oliveira, Hipocrátes De Menezes Chalkidis, and Maria Cristina Dos-Santos. 2014. "A Comparison of the Ability of Bellucia Dichotoma Cogn. (Melastomataceae) Extract to Inhibit the Local Effects of Bothrops Atrox Venom When Pre-Incubated and When Used According to Traditional Methods." *Toxicon* 85: 59–68. https://doi.org/10.1016/j.toxicon.2014.04.009

Oliveira, Fagner Neves, Monalisa Taveira Brito, Isabel Cristina Oliveira de Morais, Sayonara Maria Lia Fook, and Helder Neves de Albuquerque. 2010. "Accidents Caused by Bothrops and Bothropoides in the State of Paraiba: Epidemiological and Clinical Aspects." *Revista Da Sociedade Brasileira de Medicina Tropical* 43 (6): 662–67. https://doi.org/10.1590/s0037-86822010000600012

Oliveira, Isadora Sousa, Iara Aimê Cardoso, Karla de Castro Figueiredo Bordon, Sante Emmanuel Imai Carone, Johara Boldrini-França, Manuela Berto Pucca, Karina Furlani Zoccal, et al. 2019. "Global Proteomic and Functional Analysis of Crotalus Durissus Collilineatus Individual Venom Variation and Its Impact on Envenoming." *Journal of Proteomics* 191 (January): 153–65. https://doi.org/10.1016/j.jprot.2018.02.020

Oliveira, Paula Regina Simões de, Felipe Silva de França, Isadora Maria Villas Boas, Marisa Maria Teixeira da Rocha, Sávio Stefanini Sant'Anna, Maria de Lourdes Bastos, and Denise V. Tambourgi. 2018. "Snake Venoms from Angola: Intra-Specific Variations and Immunogenicity." *Toxicon* 148: 85–94. https://doi.org/10.1016/j.toxicon.2018.04.013

Organization, World Health. 2019. "Snakebite Envenoming: A Strategy for Prevention and Control: Executive Summary." 2019. https://doi.org/10.1016/S0140-6736(18)32745-4

Rocco, Daniela M., Gustavo Reati, Vanessa Costa de Oliveira, Laura C. Lanari, Rodrigo D. Laskowicz, and Adolfo R. de Roodt. 2013. "Caracterización Tóxica Del Veneno de Bothrops (Rhinocerophis) Alternatus de Diferentes Regiones de La Provincia de Córdoba (Argentina)." *Revista de La Facultad de Ciencias Médicas (Córdoba, Argentina)* 70 (1): 7–13.

Salazar, Ana Maria, Alexis Rodriguez-Acosta, Maria E. Girón, Irma Aguilar, and Belsy Guerrero. 2007. "A Comparative Analysis of the Clotting and Fibrinolytic Activities of the Snake Venom (Bothrops Atrox) from Different Geographical Areas in Venezuela." *Thrombosis Research* 120 (1): 95–104. https://doi.org/10.1016/j.thromres.2006.07.004

Santoro, Marcelo L., Ida S. Sano-Martins, Hui W. Fan, João L.C. Cardoso, R. David G. Theakston, and David A. Warrell. 2008. "Haematological Evaluation of Patients Bitten by the Jararaca, Bothrops Jararaca, in Brazil." *Toxicon* 51 (8): 1440–48. https://doi.org/10.1016/j.toxicon.2008.03.018

Sanz, Libia, Lucas N. de Freitas-Lima, Sarai Quesada-Bernat, Viviane K. Graça-de-Souza, Andreimar M. Soares, Leonardo de A. Calderón, Juan J. Calvete, and Cleópatra A.S. Caldeira. 2019. "Comparative Venomics of Brazilian Coral Snakes: Micrurus Frontalis, Micrurus Spixii Spixii, and Micrurus Surinamensis." *Toxicon* 166 (March): 39–45. https://doi.org/10.1016/j.toxicon.2019.05.001

Silva, Ageane Mota da, Mônica Colombini, Ana Maria Moura-Da-Silva, Rodrigo Medeiros de SOUZA, Wuelton Marcelo Monteiro, and Paulo Sérgio Bernarde. 2020. "Epidemiological and Clinical Aspects of

Scientia Amazonia, v. 10, n.1, CB1-CB12, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Snakebites in the Upper Juruá River Region, Western Brazilian Amazonia." *Acta Amazonica* 50 (1): 90–99. https://doi.org/10.1590/1809-4392201901561

Silva Souza, Anderson da, Jacqueline de Almeida Gonçalves Sachett, João Arthur Alcântara, Monique Freire, Maria das Graças Costa Alecrim, Marcus Lacerda, Luiz Carlos de Lima Ferreira, Hui Wen Fan, Vanderson de Souza Sampaio, and Wuelton Marcelo Monteiro. 2018. "Snakebites as Cause of Deaths in the Western Brazilian Amazon: Why and Who Dies? Deaths from Snakebites in the Amazon." *Toxicon* 145: 15–24. https://doi.org/10.1016/j.toxicon.2018.02.041

Smith, Cara Francesca, and Stephen P. Mackessy. 2020. "Asymmetrical Expression of Toxins between the Left and Right Venom Glands of an Individual Prairie Rattlesnake (Crotalus Viridis Viridis)." *Toxicon* 186 (August): 105–8. https://doi.org/10.1016/j.toxicon.2020.08.005

Sousa, Leijiane F., Carolina A. Nicolau, Pedro S. Peixoto, Juliana L. Bernardoni, Sâmella S. Oliveira, José Antonio Portes-Junior, Rosa Helena V. Mourão, et al. 2013. "Comparison of Phylogeny, Venom Composition and Neutralization by Antivenom in Diverse Species of Bothrops Complex." *PLoS Neglected Tropical Diseases* 7 (9). https://doi.org/10.1371/journal.pntd.0002442

Sousa, Leijiane F., José A. Portes-Junior, Carolina A. Nicolau, Juliana L. Bernardoni, Milton Y. Nishiyama-Jr, Diana R. Amazonas, Luciana A. Freitas-de-Sousa, et al. 2017. "Functional Proteomic Analyses of Bothrops Atrox Venom Reveals Phenotypes Associated with Habitat Variation in the Amazon." *Journal of Proteomics* 159: 32–46. https://doi.org/10.1016/j.jprot.2017.03.003

Sousa, Leijiane F., Christina N. Zdenek, James S. Dobson, Bianca op den Brouw, Francisco C.P. Coimbra, Amber Gillett, Tiago H.M. Del-Rei, et al. 2018. "Coagulotoxicity of Bothrops (Lancehead Pit-Vipers) Venoms from Brazil: Differential Biochemistry and Antivenom Efficacy Resulting from Prey-Driven Venom Variation." *Toxins* 10 (10). https://doi.org/10.3390/toxins10100411.

Theakston, R. D.G., and H. A. Reid. 1983. "Development of Simple Standard Assay Procedures for the Characterization of Snake Venoms." *Bulletin of the World Health Organization* 61 (6): 949–56.

Towbin, H., and J. Gordon. 1984. "Immunoblotting and Dot Immunobinding - Current Status and Outlook." *Journal of Immunological Methods* 72 (2): 313–40. https://doi.org/10.1016/0022-1759(84)90001-2

Vélez, Sara María, Marcos Salazar, Hildaura Acosta de Patiño, Leandra Gómez, Abdiel Rodriguez, David Correa, Julio Saldaña, et al. 2017. "Geographical Variability of the Venoms of Four Populations of Bothrops Asper from Panama: Toxicological Analysis and Neutralization by a Polyvalent Antivenom." *Toxicon* 132: 55–61. https://doi.org/10.1016/j.toxicon.2017.04.002

Wang, C. Ruth, Emily R. Bubner, Blagojce Jovcevski, Parul Mittal, and Tara L. Pukala. 2020. "Interrogating the Higher Order Structures of Snake Venom Proteins Using an Integrated Mass Spectrometric Approach." *Journal of Proteomics* 216 (November 2019): 103680. https://doi.org/10.1016/j.jprot.2020.103680 .