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Original Article

Ontogenetic and sexual differences in the venom of *Bothrops moojeni*: insights from a litter and its mother

Diferenças ontogenéticas e sexuais no veneno de *Bothrops moojeni*: percepções de uma ninhada e de sua mãe

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Abstract

Variability in snake venom composition is well-documented and crucial for understanding snake ecology and predicting snakebites. In this study, we characterize the venom composition and biological activities of newborn female and male *Bothrops moojeni* and their mother. Our results reveal significant differences between the venom of newborn females and males, demonstrating a broad and diverse range of proteins. The venoms of newborn females showed higher serine protease effects, increased hemorrhagic activity, and greater lethality compared to the venom of newborn males. However, no differences were observed in phospholipase A_2 and coagulant activity. The differences in protein composition and toxic activities between maternal and neonatal venom, as well as between the venoms of newborn females and males, contribute to understanding the diverse outcomes of snakebites. These results underscore the importance of considering sex and ontogeny in understanding venom composition in snakes.

Keywords: snakes, viperidae, *Bothrops moojeni*, newborn, venom.

Resumo

A variabilidade na composição dos venenos de serpentes é bem documentada e crucial para a compreensão da ecologia das serpentes ou do prognóstico dos envenenamentos ofídicos. Nesse estudo, caracterizamos a composição e a atividade biológica do veneno de fêmeas e machos recém-nascidos e sua mãe. Nossos resultados revelaram diferenças significativas entre o veneno de fêmeas e machos recém-nascidos, demonstrando uma ampla e diversificada gama de proteínas. Os venenos de fêmeas recém-nascidas apresentaram mais efeitos de serina protease, aumento da atividade hemorrágica e maior letalidade em comparação ao veneno dos machos recém-nascidos. No entanto, nenhuma diferença foi observada na fosfolipase A_2 e na atividade coagulante. As diferenças na composição proteica e nas atividades tóxicas entre o veneno materno e neonatal, bem como entre os venenos de fêmeas e machos recém-nascidos contribuem para a compreensão dos diversos resultados dos acidentes ofídicos. Esses resultados ressaltam a importância de considerar o sexo e a ontogenia na compreensão da composição do veneno desses animais.

Palavras-chave: serpentes, viperidae, *Bothrops moojeni*, récem-nascidos, veneno.

1. Introduction

Over the past century, snakebites in Brazil have shown a consistent epidemiological pattern, with snakes of the genus *Bothrops* accounting for over 90% of reported cases and predominantly affecting the lower limbs of rural males (Bochner and Struchiner, 2003; Brasil, 2023). The highest incidence of snakebites is concentrated in central-western and northern Brazil, with the state of Tocantins ranking third in reported cases (Queirós et al., 2020; Brasil, 2023).

Bothrops species, commonly known as jararacas, are morphologically and ecologically diverse and are widely distributed in Brazil (Bernarde, 2014; Guedes et al., 2023; Duque et al., 2023). *Bothrops moojeni* is a medically important species found in the Caatinga, Cerrado and Pantanal with records in the states of Tocantins, Mato Grosso, Mato Grosso do Sul, Goiás, Maranhão, Distrito Federal, Piauí, Bahia, Minas Gerais, São Paulo, and Paraná

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(Guedes et al., 2023). Although four species of *Bothrops* occur in Tocantins (Silva et al., 2019), *B. moojeni* is the main responsible for snakebites in the state.

Bothrops envenomation triggers local and systemic effects that can lead to serious sequelae, including death. Many studies have demonstrated the severity of the effects induced by *B. moojeni* venom, which include hemostatic disorders, local bleeding, and severe local manifestations, such as edema, ecchymosis, and blisters, sometimes evolving to local necrosis and sequelae in the affected area (Oliveira et al., 2013; Mamede et al., 2016).

The symptoms and severity of snakebites are modulated by the type and quantity of toxins in the venom. Due to the consequences of envenomations and the epidemiological importance of snakebites, numerous studies have sought to characterize the proteomic composition of the venom of various snake species (Sunagar et al., 2014; Sunagar et al., 2016; Amazonas et al., 2018). The proteome of *B. moojeni* venom is mainly composed of metalloproteinases, serine proteases, phospholipases A_2, L-amino acid oxidases, and cysteine-rich proteins (Amorim et al., 2017; Amorim et al., 2018). Both ontogeny and sex influence the composition of *Bothrops* venom. Comparisons between young and adult *B. jararaca*, *B. jararacussu*, *B. moojeni*, and other congeners have revealed variations in biological activities and venom composition (Antunes et al., 2010; Freitas-de-Sousa et al., 2020; Hatakeyama et al., 2021). Moreover, juvenile snake venom has been shown to induce more severe coagulopathy in cases of human envenomation (Freitas-de-Sousa et al., 2020).

Given the proteomic characteristics and differences between neonatal and maternal venoms (Amorim et al., 2018), studies contributing to understanding the severity of snakebites are of great importance, especially for the state of Tocantins. In this context, we addressed the following questions: Is there sexual variation in the venom of siblings? Do the venom components of the mother and its offspring vary significantly? Thus, this study characterizes the composition and activity of the venom of a female *B. moojeni* and its litter.

2. Material and Methods

2.1. Venom source

A pregnant female *B. moojeni* (135 cm total length) was collected in the Araguaína municipality, Tocantins state (northern Brazil), and kept in captivity. After parturition, venom was obtained from both the mother and her litter. Capture authorization was granted by the Biodiversity Authorization and Information System (SISBIO, No. 52416-1). Procedures adhered to Normative Instruction No. 03/2014, and the biological material was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen No. A2C170C). Venom extraction, lyophilization, and storage (−20 ºC) were conducted at the Herpetology Laboratory of the Instituto Butantan (São Paulo state, southeastern Brazil) by one of us (Sávio Stefanini). The venom extracted from seven newborn female *B. moojeni* (28.8–31.2 cm total length) was pooled to create the newborn female venom pool, and the venom extracted from 10 newborn males (28.0–31.4 cm total length) was pooled to create the newborn male venom pool. The newborn venom was compared to the maternal venom.

2.2. Chemical characterization of venoms

2.2.1. Determination of protein content

Protein concentration was determined using the method described by Markwell et al. (1978). Bovine serum albumin was used as the standard, following the manufacturer's protocol. Absorbance readings at 660 nm were used for quantification.

2.2.2. SDS-PAGE electrophoresis

Venom protein profiles were analyzed using polyacrylamide gel electrophoresis (SDS-PAGE) following Laemmli (1970). Venom samples (25 µg) were applied in the presence of β-mercaptoethanol and a molecular mass standard (14.4–94.0 kDa - GE Healthcare) was run in parallel. The electrophoresis gel was then stained with Coomassie Brilliant Blue R-250. Densitometric analysis was performed using the Gel Analyzer software.

2.2.3. High-performance liquid chromatography (HPLC)

Venoms were fractionated following Sousa et al. (2017) using a semi-preparative reverse-phase column (C-18 column, Vydac, 10 µm, 4.6 mm i.d. × 250 mm). Five-milligram venom samples were dissolved in 500 µL of 0.1% trifluoroacetic acid in water. After centrifugation at 18,400×g for 10 minutes, the supernatant was applied to the column previously equilibrated with the same solution. Elution occurred at a flow rate of 2 mL/min using a gradient system of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B) for 100 minutes. The gradient consisted of the following steps: 5% B for 5 min, followed by 5–15% B for 10 min, 15–45% B for 60 min, 45–70% B for 10 min, 70–100% B for 5 min, and 100% B for 10 min. Fractions were monitored with UV 214 nm detection.

2.2.4. Determination of coagulant activity on plasma

The minimum coagulant dose (MCD) was determined using a pool of citrated equine plasma. Venom samples were diluted in a 0.85% saline solution at a concentration of 1.0 mg/ml and then distributed in serial portions, ranging from 1000 to 0.2 µg of venom. Using a BBL Fibrosystem fibrometer®, 200 µL aliquots of the plasma solution (at 37 ºC) were combined with 100 µL of each venom solution. The test was conducted in triplicate, and the MCD was calculated using linear regression. MCD is defined as the lowest venom concentration capable of clotting plasma within 60 seconds at 37 ºC (Theakston and Reid, 1983).

2.2.5. Serine protease activity

Serine protease activity was assessed following the method described by Knittel et al. (2016). This process involved adding 40 µL of 4 mM BAPNA dissolved in dimethyl sulfoxide (DMSO), 40 µL of 50 mM Tris-HCl (pH 8.0), and 20 µL of venom at a concentration of 5 µg

to 96-well plates. The mixture was incubated at 37 °C for 40 minutes, and the release of the product (p-nitroaniline) was monitored using a microplate spectrophotometer (Kasuaki DR-200BS), at 405 nm. Results were expressed in Abs/min/mg of venom. The experiment was conducted in triplicate, with *B. jararaca* venom as the control.

2.2.6. Phospholipase A_2 activity

Phospholipase A_2 activity was assessed following Knittel et al. (2016). Twenty micrograms of each venom were incubated with 320 µM of chromogenic synthetic substrate (NOBA). After a 40-minute incubation at 37 °C, absorbance of the samples was measured using a microplate spectrophotometer (Kasuaki DR-200BS) at 425 nm. Phospholipase activity was expressed in nmoles/min of the released chromophore. The positive control for this assay was the venom of *B. jararacussu*, and the experiment was conducted in triplicate.

2.3. Biological activities

2.3.1. Animals

Experimental protocols for assessing biological activities in male Swiss mice (18–22 g) were approved by the Ethics Committee of the Butantan Institute (CEUAIB Protocol No. 3873030817). The procedures followed ethical standards and requirements for research involving laboratory animals.

2.3.2. Toxicity test

The toxicity of the venom samples was determined using the single-dose procedure as described by Aird and Kaiser (1985). The individual dose was determined by averaging the last three LD_{50} records from the Quality Control of the Herpetology Laboratory of the Butantan Institute, namely 135 µg/animal. Venom toxicity was assessed in mice groups (n = 4) through intraperitoneal injection of venom in 0.5 mL of saline solution. Animal mortality was monitored for 48 hours, and venom toxicity was assessed by recording the time and number of deaths at 24- and 48-hours post-venom injection (Finney, 1971).

2.3.3. Hemorrhagic activity

To assess hemorrhagic activity, a dose of 2 µg/animal was used (Lomonte and Gutiérrez, 1989). The hemorrhagic effect of the venoms was assessed following Kondo et al. (1960), with modifications by Gutiérrez et al. (1985). Groups of five mice were intradermally inoculated in the abdominal region with venom diluted in saline (100 µL). After 3 hours, the mice were euthanized, and their ventral skins were excised. Digital photographs were taken to examine the formation of the hemorrhagic halo and measure its size in pixels. The margin was traced, and the intensity of the hemorrhage was quantified. Hemorrhagic activity was assessed by calculating the mean and standard deviation (SD) of the diameters of the bleeding areas (mm^2) and expressed in hemorrhagic units (HeU) (Jenkins et al., 2017). The bleeding halos (mm^2) and their color intensity (pixels - RGB) were measured using Inkscape software versions 0.92 and 0.93.

2.4. Statistical analysis

Data are expressed as mean ± SD. Statistical analyses were performed using one-way ANOVA, followed by Tukey's test, with GraphPad PRISM software, version 8.0.

3. Results

The electrophoretic profile of newborn *B. moojeni* venoms revealed 12 protein bands ranging from 15 to 94 kDa (Figure 1). Notably, newborn venoms exhibited

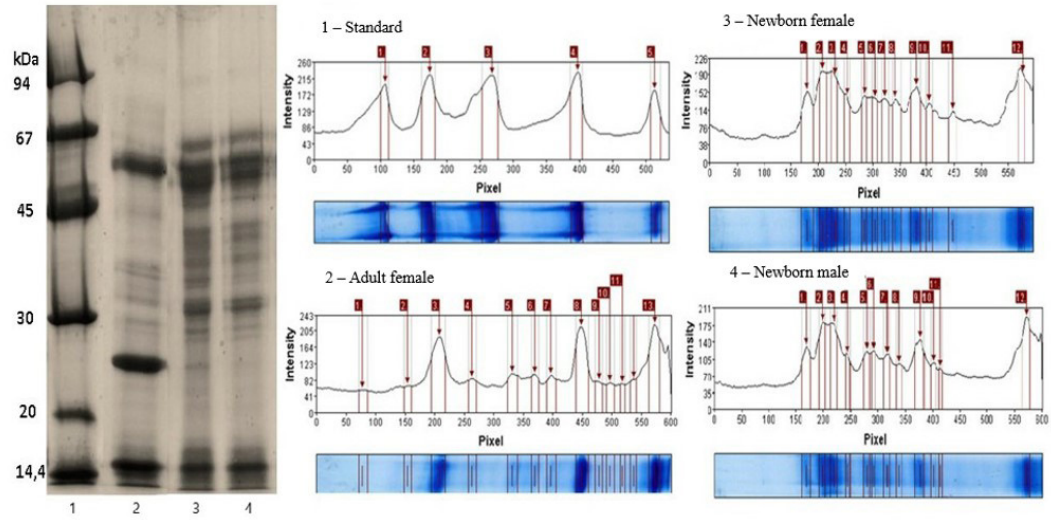


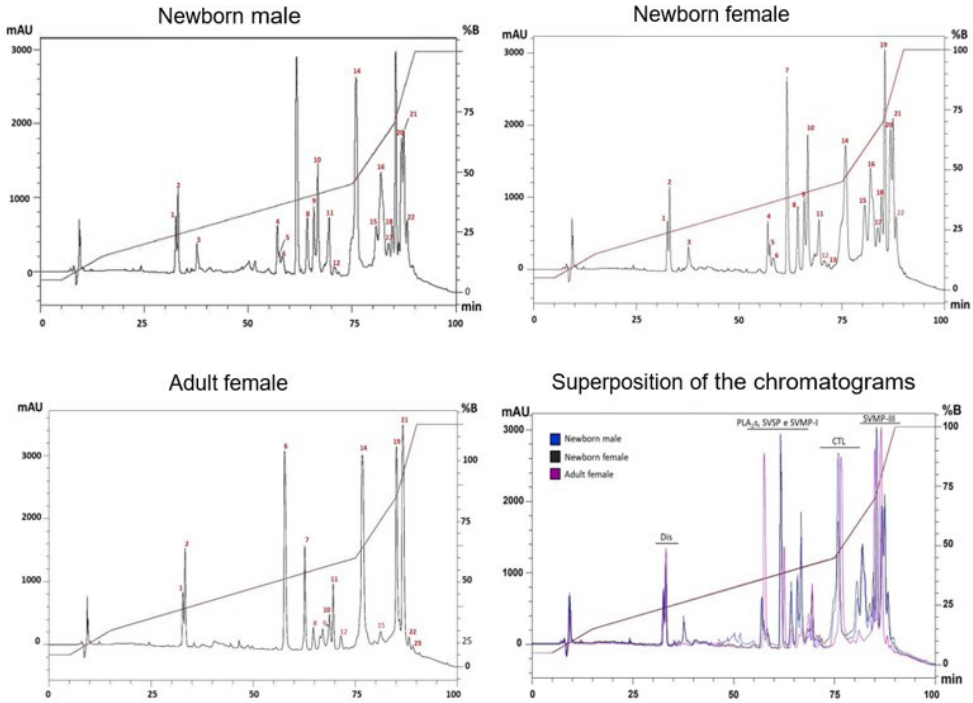
Figure 1. SDS-PAGE gel electrophoresis (with β-mercaptoethanol) and densitometric analysis of the venom bands of newborn *Bothrops moojeni*. 1 - standard; 2 – adult female; 3 – newborn female; 4 – newborn male. Standard: 14.4 kDa (α-Albumin), 20.1 kDa (trypsin inhibitor), 30 kDa (carbonic anhydrase), 43 kDa (ovalbumin), 67 kDa (albumin), 94 kDa (phosphorylase B) (Pharmacia).

a higher concentration of proteins with electrophoretic mobility between 30 and 67 kDa, whereas the maternal venom contained three bands at approximately 50, 28, and 15 kDa. Interestingly, the 28 kDa band was nearly absent in newborn venoms.

Chromatographic analysis revealed 22 proteomic peaks in the venom of newborn females and 21 peaks in the venom of newborn males, with the most prominent peaks in both groups being 7, 14, 16, and 19. Fifteen peaks were identified in the maternal venom, with the most prominent ones being 6, 7, 14, 19, and 21 (Figure 2). The main proteomic families were identified based on their elution time. Proteins classified as disintegrins (Dis) were eluted between 25 and 35 minutes. Proteins from the alkaline phospholipase (K49-PLA_2) group were eluted between 55 and 60 minutes. Proteins from the alkaline and acidic phospholipase (D49-PLA_2) type, serine proteases (SVSPs), and metalloproteinases (SVMP-I) were eluted between 60 and 70 minutes. C-type lectins (CTLs) were eluted between 70 and 85 minutes, and mainly SVMP-III metalloproteases were observed after 80 minutes of elution.

When comparing the spectra of the neonatal venom, we observed distinct peaks in the regions corresponding to disintegrins, phospholipases A_2, serine proteases, and more prominently, C-type lectins and metalloproteases (Figure 2). It is important to note that peak 11 (eluted between 60 and 70 minutes) is likely a serine protease. This peak was noteworthy for its presence in both the mother and newborn venoms, although it is less abundant in female newborns. Conversely, peak 6 (eluted between 50 and 60 minutes) generally corresponded to K49-PLA_2 and was exclusive to maternal venom. In general, the maternal venom showed a simpler pattern in the elution region, eluting PIII-class SVMPs after 80 minutes. Figure 2 reveals an overlap between peaks 1, 2, 3, 11, 13, 14, 19, and 20 in the chromatogram of the tested samples.

The MCD of the venom from newborn females (0.20 ± 0.05 µg/mL) did not differ significantly from that of the venom from male newborns (0.40 ± 0.04 µg/mL). However, the MCD of the newborn venoms showed significantly higher coagulation activity than that of the maternal venom (16.70 ± 0.93 µg/mL; $p < 0.001$).



VENOM	PEAKS (% area)																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Newborn female	1.579	2.927	1.367	1.747	0.460	0.905	13.223	3.969	2.941	5.821	2.962	0.337	0.141	19.769	4.436	9.785	1.250	2.516	3.681	5.847	4.438	1.742	0
Newborn male	1.718	2.921	1.750	1.572	0.261	0.889	14.934	3.562	2.748	4.599	3.853	0.527	0	24.764	0.816	9.980	0.952	1.985	15.949	4.541	3.533	1.591	0
Adult female	2.054	3.996	0	0	0	17.741	9.650	1.690	0.822	1.718	4.959	0.785	0	22.677	0.921	0	0	0	13.178	0	22.337	0.644	0.287

Figure 2. Chromatographic analysis of the venoms of newborn female and male *Bothrops moojeni* and their mother. The flow was stopped at 1 mL/min, and the absorbance was monitored at 215 nm throughout the process. After applying the sample, the percentage of B was maintained at 5% for 5 minutes. Then, gradients were made from 5 to 25% B for 10 minutes, from 25 to 45% for 60 minutes, from 45 to 70% for 10 minutes, and from 70 to 100% for 10 minutes. Finally, a 100% B step was conducted for 10 minutes.

The serine protease activity of the venom from newborn females (0.34 ± 0.05 Abs/min/mg) was higher than that of the venom from newborn males (0.26 ± 0.03 Abs/min/mg; $p < 0.001$). However, the serine protease activity of both sexes was lower than that of the maternal venom (0.66 ± 0.03 Abs/min/mg; $p < 0.0001$; Figure 3). The phospholipase A_2 activity of the newborn venoms was similar to that of the maternal venom (Figure 4) but significantly lower than that of the control group (*B. jararacussu*).

The lethality of newborn venoms was confirmed in all groups of inoculated mice. However, the venom from newborn females was more lethal. After 48 hours, 100% mortality was observed in mice inoculated with the venom from newborn females, whereas the mortality rate was 50% for mice inoculated with the venom of newborn males and the mother.

The intradermal injection of 2 µg/animal of *B. moojeni* venom in mice (Figure 5) resulted in the formation of a hemorrhagic halo. The venom from newborn females caused a significantly larger halo (97.90 ± 7.49 HeU) than the venom of newborn males (47.40 ± 6.91 HeU; $p < 0.05$) and the mother (24.10 ± 19.88 HeU; $p < 0.001$).

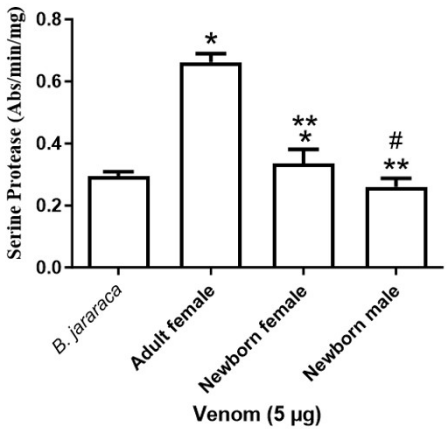


Figure 3. Serine protease activity of the venom of newborn female and male *Bothrops moojeni* and their mother. *$p < 0.001$: differences between the venom of *Bothrops moojeni* and *Bothrops jararaca*. **$p < 0.001$: differences between maternal and neonatal venom. #$p < 0.001$: differences between the venom of newborn females and males.

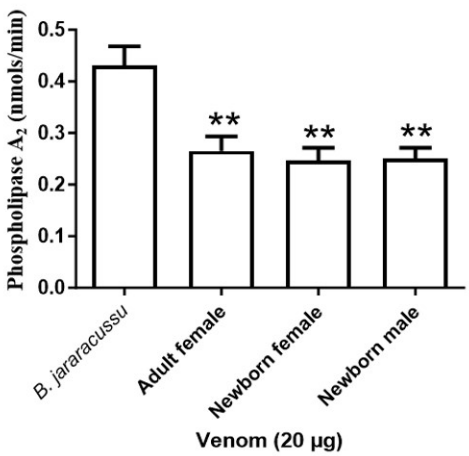


Figure 4. Phospholipase A_2 activity of the venom of newborn female and male *Bothrops moojeni* and their mother. **$p < 0.001$: differences between the venom of *Bothrops moojeni* and *Bothrops jararacussu*.

4. Discussion

The composition of snake venom has been studied using omic tools to demonstrate variations within and among populations. These variations contribute to diverse damages and symptoms observed during the clinical evolution of snakebites (Gonçalves-Machado et al., 2016; Sunagar et al., 2016; Sousa et al., 2017; Amazonas et al., 2018).

Sunagar et al. (2014) suggested that the molecular evolution of venom may have been influenced by several factors, including ontogeny and sex. This is because the protein patterns of venoms from newborns differ from those of adults. It is important to highlight that the Brazilian system for reporting snakebites does not distinguish the snakes by life stage. Consequently, analyzing epidemiological data on these aspects becomes challenging. However, knowledge of the ontogenetic variation of the venom of these animals is necessary to understand the clinical outcomes of envenomations. This knowledge is vital for assessing the effectiveness of antivenoms and prospecting molecules with potential pharmacological applications, particularly considering that bites by adult snakes lead to more necrosis than those involving juvenile snakes (Nicoleti et al., 2010; Wray et al., 2015).

This is the first study to examine sexual and ontogenetic differences in the venom of a snake species from the state of Tocantins. Our proteomic analysis revealed that the venoms of newborn male and female *B. moojeni* exhibit over 20 protein peaks in the chromatographic profile. In contrast, maternal venom exhibited 15 major protein peaks, i.e., five peaks fewer than those of the newborns. Chromatographic analysis further revealed that disintegrating enzymes, such as phospholipase A_2, serine proteases, and metalloproteases, are common to the venom of both the mother and the young.

Bothropic venom is mainly composed of classes of metalloprotease-like proteases, serine proteases, C-type lectins, and phospholipases (Sousa et al., 2013). Metalloproteases are present in the range of 20 to 110 kDa (Fox and Serrano, 2008). The 50 kDa range, found in venoms, is associated with the presence of PIII class metalloproteases (Morais et al., 2012). The 28 kDa range is associated with PI class metalloproteinases, while the 15 kDa range is associated with phospholipase A_2 (Valente et al., 2009). These findings closely align with compounds identified in regions similar to those in our study. Therefore, we conclude that we detected the same classes previously described. Furthermore, SDS-PAGE analysis showed prominent bands at 30, 90, and 12 kDa. In the venom of *B. moojeni*, researchers isolated a phospholipase in the 13.8 kDa band

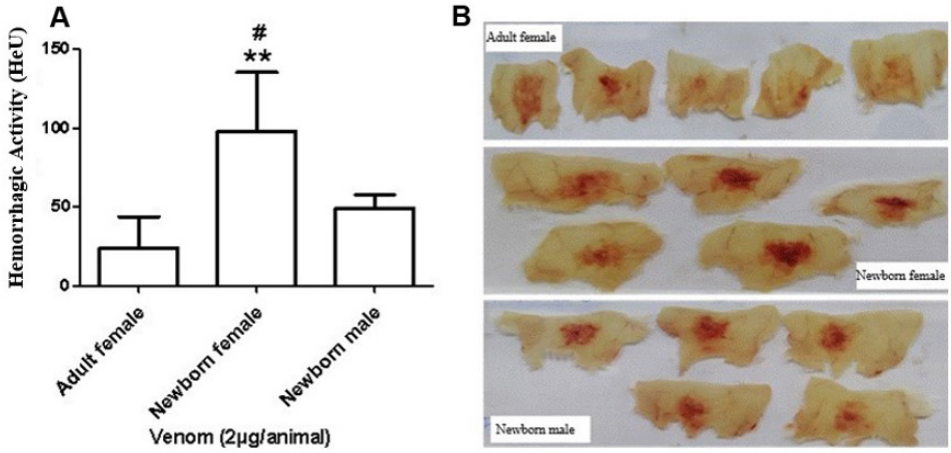


Figure 5. (A) Hemorrhagic activity of the venom of newborn female and male *Bothrops moojeni* and their mother; (B) The hemorrhagic halos resulted from the intradermal injection of 2 µg of *B. moojeni* venom. **p < 0.001: differences between maternal and newborn female venom. #p < 0.05: differences between the venom of newborn female and male *B. moojeni*.

(Silveira et al., 2013), a serine protease between 32 and 35 kDa (Oliveira et al., 2013), and a metalloprotease at 45 kDa (Morais et al., 2012).

The similarity in venom components of *B. moojeni* from Tocantins with the venom from conspecifics from other regions is relevant information. This similarity may be linked to the composition of venom in both adult and newborn snakes. Despite geographic distances and varying environmental factors, fundamental proteins such as metalloproteinases, serine proteases, and phospholipases exhibit consistent expression (Assakura et al., 1985; Amorim et al., 2017).

Similarly, variations in the protein profile between the venoms of newborns and adults also occur in Tocantins snakes. Adult snakes predominantly express the phospholipase A_2 protease, whereas newborns express different metalloproteinase isoforms, consistent with previous studies (Amorim et al., 2018; Freitas-de-Sousa et al., 2020; Hatakeyama et al., 2021).

As with the venom from young *B. jararaca* in southeastern Brazil (Zelanis et al., 2011), the venom of newborn *B. moojeni* from Tocantins also exhibits higher expression of metalloproteinases and does not differ from adults in terms of phospholipase A_2 expression. This proteomic class undergoes ontogenetic changes, transitioning from a PIII-rich profile in newborns to a PI-rich profile in adults. Simultaneously, serine proteases are produced in higher quantities in adult venom. This explanation allows us to satisfactorily understand the differences in the effects of envenomation by newborn or adult snakes.

The coagulant activity of the newborns' venom was also higher than that of the mother's venom. These results corroborate other studies conducted with *Bothrops*. Saldarriaga et al. (2003) studied the venom of *B. atrox* from the Colombian Amazon and found that the venom of newborns and juveniles is more coagulant than that of adults. Zelanis et al. (2010) studied the venom of newborn *B. jararaca* and found that the coagulant activity is ten times more potent than that of adult conspecifics. Similarly, Hatakeyama et al. (2021) reported higher procoagulant and lower proteolytic activities in the venom of neonatal *B. moojeni* (up to one year old). From the age of two, the venom starts to show greater proteolytic activity, as in adult snakes.

Disturbances in the hemostatic system have been associated with specific serine proteases found in the venom of *B. moojeni*, exhibiting a molecular weight between 28–40 kDa and increased coagulant activity (Zingali et al., 1993; Nishida et al., 1994). Serrano et al. (1993) described a 38 kDa serine protease (MSP2) in *B. moojeni* venom with coagulant activity. Oliveira et al. (2013) identified serine proteases in the venom of adults from other *Bothrops* species, with coagulant activity and molecular masses between 32 and 35 kDa.

Our results support previous studies, as protein bands ranging from 30 to 40 kDa were detected in the SDS-PAGE electrophoresis of newborn venom (Figure 1). Notably, the venom of newborn females exhibited higher activity of serine protease, greater hemorrhagic effects, and increased lethality compared to the venom of newborn males.

Metalloproteases induce changes in microvessels, increasing permeability and promoting extravasation, leading to symptoms such as edema and hemorrhage (Fox and Serrano, 2008; Markland Junior and Swenson, 2013). In *B. moojeni*, the local effects of the venom can be triggered and intensified by type A_2 phospholipases (Dennis et al., 2011; Souza et al., 2015; Mamede et al., 2016).

Collectively, our results on the activity of serine proteases, phospholipases, and metalloproteinases agree with López-Lozano et al. (2002). Their proteome analysis of juvenile, sub-adult, and adult *B. atrox* from the Manaus region revealed variations in venom composition during ontogeny, potentially influencing coagulation.

Saldarriaga et al. (2003) demonstrated that venoms from newborn *B. asper* and *B. atrox* in Colombia have greater

lethality and higher hemorrhagic and coagulant activity than adult venom. These variations in activity were attributed to an increased quantity of proteins, likely metalloproteinases. The myotoxic activity of this protein class has been previously reported in the venom of *B. alternatus* and *B. leucurus* (Costa et al., 2010; Gomes et al., 2011). This protein class is also present in the venom of *B. moojeni* from Tocantins.

Our results show higher serine protease and hemorrhagic activity in the venom from newborn females. Furthermore, the proteolytic activity of metalloproteinases seems to be more important. *Bothrops moojeni* venom is considerably more potent in inducing local effects than, for example, *B. alternatus* venom (Mamede et al., 2016). This feature has been attributed to the central role of metalloproteases and phospholipases A_2, which cause local damage, and the contribution of serine proteases that induce and exacerbate inflammation. Venoms containing a higher amount of these metalloproteinases are more lethal and exhibit more pronounced hemorrhagic activity (Massey et al., 2012).

The correlation study conducted by Souza et al. (2015) showed that the venom of *Bothrops* species from Brazil varies inter- and intraspecifically (Amorim et al., 2018), as well as ontogenetically (López-Lozano et al., 2002; Saldarriaga et al., 2003; Zelanis et al., 2010; Antunes et al., 2010). Interpopulation and interindividual variability in the composition of bothropic venom can influence the evolution of clinical conditions resulting from envenomation (Gonçalves-Machado et al., 2016; Sousa et al., 2017, 2018).

The results of Antunes et al. (2010) showed important ontogenetic changes in the venom of *B. jararaca*, particularly concerning hemostatic, hemorrhagic, proteolytic, lethal, and inflammatory activities. In this sense, the sources of venom variation demonstrated in our study are relevant to understanding snake venom and the potential clinical outcomes of human envenomation, given the diverse physiological and toxic effects these venoms trigger.

5. Conclusion

We show significant biochemical variabilities in the composition of *B. moojeni* venom. This variability interferes with the intensity and nature of biological activities and may be closely associated with sexual dimorphism, body size, and body mass. The venom of newborn females exhibited higher serine protease activity, as well as greater hemorrhagic and lethal effects than the venom of newborn males. This suggests that envenomations caused by female newborns of *B. moojeni* may be more severe. Additionally, the maternal venom exhibited higher serine protease activity. The intraspecific variation in venom composition and activity reinforces the importance of considering ontogeny and sex as variables in antivenom production. These factors are pivotal in guiding studies aimed at identifying new medicines and biotechnological tools for snakebite treatment.

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