








Conference Report

# Immersing in a Neglected Problem Inside the Amazon Jungle: Abstracts of the 1st International Symposium on Research in Venomous Animals, Manaus, Brazil <sup>†</sup>

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**Abstract:** The Amazon represents the world's greatest biodiversity and covers different countries in South America, including Brazil with the largest area. The interaction between the resident population of this area and its ecosystem is responsible for an intense exposure to venomous animals, which are considered an important health issue in the region. Therefore, the 1st International Symposium on Research in Venomous Animals, held from 2 May to 5 May 2023 in the city of Manaus in the state of Amazonas in Brazil, aimed to address animal envenomation aspects in the Brazilian Amazon and the exchange of ideas and knowledge between research groups from around the world. The event was attended by graduate students as well as renowned researchers from the Americas, Europe, and Asia. The event presented a multidisciplinary and translational approach in the area of toxinology, including themes such as clinical envenomation (clinical management, pathophysiology, and public policy strategies), preclinical studies, biological aspects of venomous animals' venoms (venom composition/variation and geographical distribution), interdisciplinary studies on health anthropology and economics, and bioprospecting aspects (toxins with therapeutic and/or technological potential).

**Keywords:** health education; healthcare access; public health; science outreach; scientific methods; snakebites; venomous animals

## 1. Introduction

In nature, there is an encounter that can be unpleasant for both parties involved: the interaction between snakes and humans. As humans, we can only speculate, but it takes a great deal of misfortune for a snake to cross paths with a human. Usually, any such encounter carries the looming threat of a fatality, either for the human or the snake. It

would be unfortunate for the snake to waste its venom on an inedible prey. Some of these snakes will face a lifetime in captivity. Likewise, it is a stark reality that some humans also pay for this encounter with terrible pain, permanent disabilities, or even their lives [1–3].

The 1st International Symposium on Research in Venomous Animals took place in Manaus, Brazil, in the heart of Amazonia, from 2 May to 5 May 2023. The event was organized at a jungle hotel located just 40 minutes from Manaus by road and a quick 10-minute boat ride on the Ariáú River. Our primary goal for this symposium was to facilitate the exchange of ideas and knowledge between our research group in Manaus and eminent researchers from around the world working in the field of toxinology (Table 1). We aimed to foster new collaborations, strengthen existing partnerships, and encourage insightful discussions that can lead to innovative solutions and breakthroughs.

**Table 1.** Researchers and respective talks at the symposium.

Speaker and Affiliation	Talk Title
José María Gutiérrez Instituto Clodomiro Picado, San José, Costa Rica	The need to promote transdisciplinary research in the field of snakebite envenoming
Flávio Santos Dourado Ministério da Saúde, Brasília, Brazil	Epidemiological overview of snakebites in Brazil
João Ricardo Nickenig Vissoci Duke University, Durham, NC, USA	Using artificial intelligence and secondary data to address healthcare resource allocation in remote settings: the Amazon forest case
Nicholas Casewell Liverpool School of Tropical Medicine, Liverpool, UK	Novel therapeutics for snakebite
Paulo Bernarde Universidade Federal do Acre, Cruzeiro do Sul, Brazil	Venomous snakes and snakebites in the western Brazilian Amazonia
Charles Gerardo Duke University, Durham, NC, USA	Snakebite envenoming outcome measures: assessing efficacy of novel SBE therapeutics
Naira Ayvazyan Orbeli Institute of Physiology of NAS RA, Yerevan, Armenia	ABO blood group: the neglected player in toxinology and beyond
David Laloo Liverpool School of Tropical Medicine, Liverpool, UK	The future of snakebite care—how can we do better?
Michael Abouyannis Liverpool School of Tropical Medicine, Liverpool, UK	Clinical development of orally administered snakebite therapeutics
Sean Bush American Society of Toxinology, Auburn, CA, USA	Dr. Venom Saves the World
Marcelo Santoro Instituto Butantan, São Paulo, Brazil	Thrombocytopenia and coagulopathy in Bothrops envenomations: associated or independent events?
Juliana Zuliani Fiocruz Rondônia, Porto Velho, Brazil	A C-type lectin induces NLRP3 inflammasome activation via TLR4 interaction in human peripheral blood mononuclear cells
Sakthivel Vaiyapuri University of Reading, Reading, UK	Challenges and successes in mitigating snakebite burden in rural India
Lúcia Faccioli São Paulo University, São Paulo, Brazil	From SRS to understanding the role of eicosanoids in scorpionism: a journey through the world of lipids
Anna Tupetz Duke University, Durham, NC, USA	Alternative approaches to qualitative data to understand post-discharge challenges in SBE patients

Manaus, a metropolis located in the heart of the Brazilian Amazon, is home to a diverse array of venomous animals, making it an ideal location for this symposium. Moreover, the Amazon region is renowned for its cultural richness and is home to many riverside communities and indigenous peoples that possess invaluable knowledge and skills. The symposium featured presentations by masters and doctoral students, showcasing their ongoing research projects at various stages of development. The students had the opportunity to receive guidance and feedback from experienced senior researchers. Students who recently joined the research group conducted many of the studies presented. As a result, the questions were not yet well formulated and the methodologies were yet to mature, aligning precisely with one objective of the symposium: to foster growth and maturation in these aspects.

The idea for a research group on snakebites, scorpion stings, and other venomous animals, had a date and a place: 30 September 2013, at a workshop held in Manaus that was organized by the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), a reference center dedicated to treatment of and research on tropical diseases in the Brazilian Amazon, and the Instituto Butantan, the major antivenom manufacturer in Brazil. The workshop also featured representatives of health departments of Amazonian states, other antivenom producers, universities, reference hospitals, and the Ministry of Health to identify research bottlenecks and enhance implementation of collaborative work and multicenter studies resulting in the integration of services, research institutions, and health professionals. The group highlighted the importance of international collaborative endeavors toward the control of these neglected health problems through partnerships, particularly with other Amazonian countries. On that date, two distinguished researchers from Instituto Butantan were present in the event: Dr. Ana Maria Moura-da-Silva and Dr. Fan Hui Wen. They played a crucial role in our decision to create a clinical research group on animal envenomations based in the Amazon.

In 2015, Dr. Moura-da-Silva invited us to join the team of a large project that marks this beginning of this story. It was called PROCAD and was funded by the Coordenação para o Aperfeiçoamento do Pessoal de Nível Superior (CAPES), which is linked to the Brazilian Ministry of Education. PROCAD was aimed at strengthening graduate programs in the Amazon, with emphasis on snakebites. It was a partnership between the graduate programs in tropical medicine of the Universidade do Estado do Amazonas with the FMT-HVD, the toxinology program of the Instituto Butantan, and the environmental sciences program of the Universidade Federal do Tocantins. Subsequently, the engagement of Universidade Federal do Acre, led by senior herpetologist Dr. Paulo Bernarde,, further enriched our collaborative effort. Through this joint work, the group gained national visibility by publishing many scientific articles and via active participation in prestigious congresses derived from this partnership. In Manaus, the group has already graduated six PhDs and 25 masters in science. Currently, 18 PhD candidates and 28 master's students are under supervision.

Over this time, we have evolved especially in the interdisciplinary understanding of the laboratory and clinical characteristics of snakebites [4–6], in socioanthropological research with indigenous and riverine populations [7–10], and in studies involving managers and health professionals [11–14]. Some clinical studies, especially testing interventions aimed at local complications of envenomations, are currently underway. More recently, we have ventured into the science of implementation with colleagues at Duke University [10,11,15–17]. A laboratory arm stood out for conducting studies on the pathophysiology of snakebites [18–22]. In partnership with the Ministry of Health, Fundação de Vigilância em Saúde do Amazonas, Instituto Butantan (the organizations responsible for indigenous health) and Duke University, our priority today is the execution of a plan for the decentralization of antivenom treatment to health units in indigenous and riverine areas. We have already demonstrated that difficulties in terms of access were the great bottleneck in mitigating the consequences of snakebites in the Amazon [8,11,23]. We have shown that our proposed solution is not only cost-effective but also acceptable amongst managers, health professionals, indigenous healers, and victims of snakebites [7,11]. However, it still requires a great deal of effort and funding to expand that idea.

## 2. Abstracts

A total of 29 student presentations were delivered during the symposium, and the corresponding abstracts are showcased below.

*Abstract 2.1. Evaluation of the Late Occurring Sequelae of Snakebites Caused by the Genus Bothrops in Children at a Reference Hospital in the Brazilian Amazon*

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The genus *Bothrops* is the main cause of snakebites in Brazil. From 2015–2021, the incidence rate of these bites was ~5.5 cases per 100,000 hab/year in children < 14 years of age. Although bites in children do not occur frequently, when they do occur, they are more serious and seem to be due to the low body mass that children have. Snakebites can generate physical disabilities after hospital discharge; however, patients are not always followed up on an outpatient basis. This study evaluated the late sequelae of snakebites caused by *Bothrops* in children treated at a reference hospital in the Brazilian Amazon. This was a cross-sectional, retrospective, and descriptive study with a quantitative approach. The population of this study consisted of residents of the state of Amazonas who suffered a *Bothrops* bite as children (<13 years old) and were hospitalized at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT/HVD) between January 2009 and December 2022. Data collection was divided into three stages: first—medical record, using the patient’s sociodemographic and clinical data; second—telephone contact, using the number obtained from the medical record; and third—performance of the musculoskeletal assessment, which was performed at the participant’s residence or at FMT/HVD and was divided into two phases: first phase—metropolitan region of Manaus; and second phase—other municipalities. The evaluation form consisted of anamnesis and examination of the musculoskeletal structures, which evaluated the patient’s previous history, pain, swelling, deformities, range of motion, muscle strength, sensitivity, and neuromuscular reflexes. A total of 218 children were analyzed; most of them were boys, and the average age was 9.8 years. Most were from the interior of the Amazonas state and from a rural area. A total of 23% of the cases performed some type of pre-hospital treatment after the bite. The most affected limb was the foot, and the most common local manifestations were pain, edema, a burning sensation, and bleeding. A total of 23.4% of cases had complications, which were mostly classified as moderate. Of the children, 23 underwent the musculoskeletal assessment; most of them were male with a current mean age of 13.04 years, and most were from the interior and rural areas. A total of 13% of the 23 performed some type of pre-hospital treatment after the bite; the most affected limb was the foot; and the main local symptoms were pain, edema, redness, and a burning sensation. A total of 26.1% had complications; most were classified as moderate. Of those evaluated, 69.6% had subsequent complications; 70% had intermittent pain at the site/in the member and the presence of a scar; 30% had edema; and 17.4% had deformities and changes in tactile, thermal, and vibratory sensitivity. Conclusion: Preliminary data suggested that children who underwent pre-hospital procedures had more complications. However, due to the low number of cases, the evaluated children who had some type of sequelae could not be associated with pre-hospital conduct. In addition, many of the children had complications that have persisted since the time of the bite, especially pain, for which it is still possible to identify the cause of this symptom. New data could confirm these trends, as we are still in phase 1.

**Keywords:** serpents; incapacity; Amazon; children; disability

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), Call number 008/2022 KUNHÃ.

*Abstract 2.2. Pharmacovigilance of the Use of Antivenom in Patients with Snakebite Treated at a Reference Hospital in the Brazilian Amazon*

Dessana Francis Chehuan <sup>1,2</sup>, Érica da Silva Carvalho <sup>1,2</sup>, Bianca Leite Pereira <sup>2</sup>, Fan Hui Wen <sup>3</sup>, Wuelton Monteiro <sup>1,2</sup> and Jacqueline Sachett <sup>1,2</sup>

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In Brazil, 265,643 bites or stings by venomous animals were reported on the SINAN system in 2018, of which 28,961 (10.9%) were due to snakes. In the Brazilian Amazon, the species *Bothrops atrox* is the snake of greatest medical importance, accounting for 90% of envenomations. Studies conducted on snakebites treated in health units in the state of Amazonas showed that 86% were males with a productive age of 15 to 59 years (72.1%) and that 88.5% of patients were bitten in the lower limb. Few records were found on the occurrence of adverse reactions presented by patients after the administration of heterologous serum. Neutralizing serums, also called antivenoms, are neutralizing antibodies produced in horses that have been used effectively for more than a century. Adverse reactions caused by antivenoms include IgE-mediated and non-IgE-mediated anaphylactic reactions; pyrogenic reactions, and serum sickness. These data are inserted in the activities and competencies of pharmacovigilance and also contemplate adverse reactions related to the use of serums. The objective of this project was to study the adverse reactions associated with the use of antivenom in patients treated at a reference hospital in the city of Manaus, Amazonas. This is a descriptive, quantitative, observational study in which the presentation of adverse reactions associated with the use of antivenom in patients diagnosed with snakebite and treated at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado located in the city of Manaus, Amazonas, will be evaluated. Patients with a history of snakebite treated by the Emergency Care Service of the referred unit will be selected for the period between August 2023 and August 2024. Data regarding the signs and symptoms related to the administration of the antivenom will be used. This study will classify the adverse reactions as follows: early adverse reactions that appeared in the first 24 h after administration of serum and late adverse reactions that appeared 5 to 24 days after administration of the serum. At 30 days after the bite, telephone contact will be made to assess the presence of signs and symptoms of adverse reactions. The sample will be composed of all the patients who were treated with antivenom at the health unit during the aforementioned period. The average annual attendance of patients affected by venomous animals is 200 envenomations/year. Pharmacovigilance is an activity included in the service of the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, and this research will evaluate the data of this activity via the institutional database. Perspectives: Identify the main adverse reactions associated with the use of antivenom. Categorize, according to the Adverse Reactions Report Form of the Butantan Institute, the early and late adverse reactions, update possible reactions not yet identified and analyze the profile of patients who used antivenom and who presented adverse reactions correlated to previous drug or food allergy. Compare snakebite groups and monitor patients who had adverse reactions.

**Keywords:** snakebites; antivenom; pharmacovigilance

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.3. Evaluation of the Efficiency of Inflammation Neutralization Induced by Bothrops Atrox Venom by Metalloprotease Inhibitors/Chelating Agents: Preclinical Assays Using in Vitro and Ex Vivo Models*

David Jose Estrada Reyes <sup>1,2</sup>, Kamila Freitas da Silva <sup>3</sup>, Ana Moura-da-Silva <sup>4</sup>, Wuelton Monteiro <sup>1,2</sup>, Jacqueline Sachett <sup>1,2</sup> and Marco Sartim <sup>2,5</sup>

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*Bothrops atrox* represents an important aggressor that is responsible for most of the snakebite envenomations (SBEs) in the Brazilian Amazon. Among venom toxins, metalloproteinases (SVMPs) are the main components and are responsible for most of the effects observed after envenomation. Antivenom therapy is the only specific treatment for envenomations, but it has limited efficiency against the local damage caused by the venom. Drug-repositioning strategies, which consist of identifying novel treatment indications for clinically tested drugs, have brought new perspectives to improve the treatment of SBEs, and SVMP inhibitors have presented an important role. Therefore, this project evaluated the neutralization efficiency of metalloproteinase inhibitors (batimastat and marimastat) and chelating agents (dimercaprol) against *B. atrox* venom (BaV) in in vitro and ex vivo preclinical models. The study evaluated caseinolytic activity as well as inflammatory response in leukocyte culture in vitro and from whole blood collected from *Bothrops* snakebite patients before antivenom therapy that were treated at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado in Manaus, AM. Initially, we focused on the metalloprotease activity on casein and incubated different concentrations of the drugs batimastat, marimastat (0.1–100  $\mu\text{M}$ ), and dimercaprol (5–2000  $\mu\text{M}$ ) individually with BaV and in combination with anti-*Bothrops* serum. Via this first test, the ideal concentrations of the inhibitors, the proportions of the antivenom–inhibitor mixtures, and the conditions were established to evaluate the neutralizing potential of the inhibitor/chelating agents in the in vitro and ex vivo inflammation inhibition assays. The inhibitory concentration ( $\text{IC}_{50}$ ) of the inhibitors for BaV metalloproteinase activity on casein was 59.02  $\mu\text{M}$  for batimastat, 32.97  $\mu\text{M}$  for marimastat, and 604.5  $\mu\text{M}$  for dimercaprol. The anti-*Bothrops* serum alone had a 53.11% inhibition of the caseinolytic activity of the *B. atrox* venom, whereas the combinations of the anti-*Bothrops* serum with the inhibitors presented an added pharmacological inhibitory effect. This was the first study to test matrix metalloproteinase inhibitors and chelating agents in *Bothrops atrox* venom, and our observations in this early stage of the investigation indicated that the drugs used can inhibit the metalloproteinase activity of the venom and also indicated their reuse in therapy. Therefore, more in-depth studies are still needed to determine to what extent these drugs can inhibit the inflammation produced by the venom and the mechanisms of action to neutralize it. With this, clinical trials would be justified in order to use them as a complement to antivenom therapy in patients bitten by snakes.

**Keywords:** antivenoms; *Bothrops*; matrix metalloproteinase inhibitors; metalloproteinase; snakebites

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.4. Efficacy of Intervention at the Site of Bothrops Envenomations Using Ice and Hot Packs: An Open, Randomized Clinical Trial*

Mailma Costa de Almeida <sup>1,2</sup>, Kathleen Maclenny Pereira Carvalho <sup>1,2</sup>, Yasmim da Silva Mendes <sup>1,2</sup>, Débora Nery Oliveira <sup>1,2</sup>, Dessana Francis Chehuan <sup>1,2</sup>, Érica da Silva Carvalho <sup>1,2</sup>, Wuelton Monteiro <sup>1,2</sup> and Jacqueline Sachtet <sup>1,2</sup>

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Treatment with antivenom is the main therapy for *Bothrops* envenomations and the only form of treatment that can neutralize the effects at the systemic level; however, local

damage continues as a result of the inflammatory effects related to the action of the venom. The use of thermotherapy as an adjuvant treatment to improve inflammation and pain has been used empirically. The aim of the study was to compare the effectiveness of cold compresses versus hot compresses on pain in *Bothrops*-envenomated patients. A randomized trial was carried out between June 2022 and February 2022 with patients from the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado. The study distributed 45 patients into three groups: group A—cold compress; group B—hot compress; and group C—control. Groups A and B had compresses applied for 20 min twice a day at the site of the envenomation during periods of 24, 48, and 72 h to the extent of the inflammation shown by thermography, and group C was the control that received only the conventional treatment. Statistical analyses were performed using the R software in the IDE R studio environment. This study was approved by the REC under number 5.312.617-2022 and Rebec U1111-11691005. In the interim analysis, the level of creatine kinase (CK) obtained in the hot compress group was an average of 161 U/L, that for the ice pack group was 209 U/L, while the control group had an average of 291 U/L. Regarding the outcomes of inflammatory signs, the partial results of this clinical trial showed a 50% decrease in pain on the third day for the group that received a hot compress, a 44% decrease for the group that received a cold compress, and a 40% decrease for the control group. Circumference measurements of the edema decreased by 57% in the group that used a hot compress, by 53% in the group that used cold compresses, and by 43% in the control group. As for the extent of edema at the end of the third day, the cold compress group had an average circumference of 34 cm, the hot compress group had an average of 36 cm, while the control was 54 cm. The effects of thermotherapy at the site of *Bothrops atrox* envenomations were positive in improving inflammatory aspects and pain in patients when compared to the control group. The hot compress was more positive in terms of pain relief and edema extension, while the cold compress was more positive in relation to the decrease in edema extension. Both therapies showed more positive results in relation to the control group.

**Keywords:** thermotherapy; snakebite; local treatments; clinical trials

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.5. Contributions of Interviews and Playful Interaction with Children Who Were Victims of Snakebites and Their Family Member or Accompanying Guardian from the Reconstruction of Their Therapeutic Itineraries*

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Snakebites represent a serious problem for public health, especially in developing countries with tropical regions, such as Brazil and its Amazon region, due to the frequency with which they occur and their associated morbidity and mortality. Among the factors associated with the severity of this type of accident are: age < 15, a rural area as the place of occurrence, and the search for medical assistance taking greater than 6 h after the bite. It is noted that although bites and stings by venomous animals are not frequent in children, when they occur, they are more serious than in adults. It is a challenge to carry out research involving this population due to its peculiarity. Children can give accurate information about their experiences; however, it is necessary to involve them actively by using different techniques and resources, such as playful interaction. The concern regarding how and when patients seek health services to resolve their demands has become increasingly present in studies on planning, organization, and evaluation of health care services. Given this, the objective of this study is to describe the therapeutic itinerary of

children who were victims of snakebites in the Brazilian Amazon. This is a descriptive study with a qualitative approach with children that suffered a snakebite and their family members or guardians accompanying them at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD). Data collection will occur until theoretical saturation of the interviews is obtained. This research was approved by the Ethics Committee of the FMT-HVD under approval number 5,286,460. Due to the ethical need to adapt the data-collection technique to the age group of the children and the sociocultural context, we will use ludic resources in data collection. The interviews with the children will take place in three stages, all recorded in audio and video. The “opening” stage, through initial questions, will reveal the circumstances of what happened and contextualize it. In the “uptake” stage, the researcher will ask the child to create a drawing of the moment of the snakebite and the route taken to achieve treatment. Finally, in the “narrative immersion” stage, questions will be asked regarding the elements of the drawing, which will allow the child to reflect and further develop the story told. We will also associate it with some cards containing figures from the itinerary, like a story in a comic, thus encouraging the children to reconstruct their therapeutic itinerary from the moment of the bite until their arrival at FMT-HVD. Accompanying family members or guardians will also be subjected to a recorded semi-structured interview. Sociodemographic and epidemiological information will be collected from the electronic medical record for subsequent descriptive analysis. Content analysis of the thematic type of each interview will be carried out. There are few studies that addressed these difficulties when the snakebite involved children. A direct approach with children in the investigation of the therapeutic itinerary can bring something new in comparison to the narrative of adults and even generate important political and social gains. In this sense, it is expected that by verifying the efficiency of health care for children that are victims of snakebite in the state of Amazonas, we will discover the main obstacles involved in the access to antivenom in this population as well as the peculiarities in the guidelines received for cases in locations with difficult access.

**Keywords:** therapeutic itinerary; snakebites; child; interview; antivenom

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.6. Evaluation of the Profile of Circulating Microvesicles in Victims of Bothrops Bites in the Brazilian Amazon*

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In the Brazilian Amazon, *Bothrops atrox* is the main cause of reported snakebites, and its venom can bring about pathophysiological changes such as bleeding, pain, and edema formation. In the immune response process after envenomation, the action of the venom's components can lead to an increase in the concentration of microvesicles (MVs). MVs are vesicles derived from the plasmatic membrane that are released by different cells of the organism. Their presence in biological fluids can be associated with the severity of pathological processes, and they can act by modulating the inflammatory response to the bite. However, the mechanisms associated with the development of this response and the prediction of its severity has been little explored. Thus, the objective of this study



is to characterize the profile of circulating MVs in victims of a *Bothrops* snakebite and identify the biomarkers that predict severity in these patients. This is an observational, longitudinal, and prospective study that will be carried out at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) with a sample composed of individuals who suffered *Bothrops* bites and sought care at the foundation. These will be clinically evaluated using parameters such as the presence of acute inflamed reaction with signs of bleeding or coagulation. Pregnant women, indigenous people, participants under the age of 18, or individuals who report having a previous history of inflammatory disease such as autoimmune diseases or immunodeficiency will not be included in the study. The study will also have a control group of healthy individuals who have not previously suffered a snakebite, and these subjects will be obtained in partnership with the HEMOAM. Peripheral blood sample collections will be performed at three moments: before the application of serum therapy and after serum therapy (24 h and 48 h). The characterization of microvesicles will be conducted via the flow cytometry technique with a CytoFLEX cytometer (Beckman Coulter) at FMT-HVD. This equipment allows the detection and characterization of MVs on a scale of 100–900 nm using Gigamix synthetic calibration microspheres. These microspheres have defined sizes (100 nm, 160 nm, 200 nm, 240 nm, 300 nm, and 500 nm, 900 nm) that will be used as a reference for the characterization of the MVs. The assay will follow the GIPB Microvesicles Characterization Protocol (Integrated Biomarker Research Group—René Rachou CPqRR/FIOCRUZ—Minas Research Center) containing monoclonal antibodies conjugated to fluorochromes for identification of the following cells: neutrophils, endothelial cells, erythrocytes, T cells, platelets, and monocytes. As far as we know, there are no data in the literature regarding the profile of circulating MVs involved in the immune response of patients who were victims of *B. atrox* snakebites. The study is expected to expand the network of knowledge about the mechanisms involved in the immune response that develops after a *Bothrops* envenomation and elucidate the participation of microvesicles in this response, a field that is still little discussed. We hope to develop a panel of immunological biomarkers that predict severity, thus reducing the length of hospital stay for patients and improving treatment.

**Keywords:** extracellular vesicles; *Bothrops atrox*; immune response; biomarkers

**Funding:** FAPEAM; CAPES; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

*Abstract 2.7. Validation of the Minimum Requirements for Snakebite Antivenom Treatment and Its Application in Indigenous Health Units*

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Snakebites represent a major public health problem worldwide. In Brazil, the highest incidence is reported in the Amazon region, where, due to the type of snakes that inhabit the region, the highest rates of complications and lethality of these cases are recorded. Difficulties in accessing health services that provide adequate antivenom treatment and first aid are aggravating factors. Among the most affected populations, we can highlight the riverine populations and indigenous communities. In this context, the mortality rate is significantly higher in indigenous villages (1.4%) compared to non-indigenous villages

(0.5%). One of the ways to enable access to the treatment after a snakebite is to make antivenom available in strategic places that are close to communities and villages. For this, it is necessary to evaluate the infrastructure of these strategic points for the supply of antivenom, which is the only effective drug. The aim of this study was to elaborate and validate the minimum requirements for the provision of antivenom treatment for application in indigenous health units in Brazil. An instrument in the form of a checklist was prepared to assess the minimum requirements for the availability of antivenom treatment. The items were prepared by a team of researchers from CEPCLAM, Butantan, and Duke University and sent in the form of a survey to judges with expertise in the area. The items were classified by the judges as “essential”, “non-essential”, “desirable”, and “undesirable”. As for the types of units, these were divided into three types: Type 1—the injured person receives treatment with antivenom and is referred to the regional hospital; Type 2—the injured person receives treatment and is only referred to the hospital in moderate or severe cases; and Type 3—units that have the necessary structure to assist at any level of severity. After the elaboration, the checklist was applied in 14 indigenous units (two in each of the seven DSEIs in the state of Amazonas). The checklist had 69 items to be validated in its first version; in the final version, it had 80 items. In the units of Type 1, a considerable portion of the items evaluated obtained a desirable classification, in contrast to the units of Types 2 and 3, in which the items with an essential classification predominated. After the application of this instrument in the indigenous health units, it was possible to observe the infrastructure of the units such as the energy supply, means of refrigeration, and distance to the reference hospital. It is important to note that after the evaluation, all units were classified as Type 1. Among the essential items most lacking in the units, we can highlight an armrest for phlebotomy and a domestic refrigerator (both 50%). In relation to consumables, more than 50% of items were present in all units, which demonstrated that these units were better equipped with consumable items than with medicines and equipment. Regarding medicines, the lack of adrenaline stood out, since it was present in only 50% of the units. Conclusion: In this study, the existence of necessary items for the effective and safe treatment of snakebite was evidenced in addition to stipulating the necessary items for each type of unit to be validated. This validated instrument represents a set of strategies to be developed for the treatment of snakebites in a decentralized manner. Enabling access to antivenom for the most vulnerable people is part of the WHO’s set of goals to bring equal health to all. Consequently, it was possible to highlight the current lack of resources and infrastructure in the indigenous health units.

**Keywords:** snakebite; antivenom; health service; indigenous population health; health decentralization

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*Abstract 2.8. Effectiveness of Red and Infrared Low-Intensity Laser for a Reduction in Local Manifestations in Bothrops Envenomations in the Brazilian Amazon: A Randomized Clinical Trial*  
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Snakes of the genus *Bothrops* are responsible for more than 90% of the envenomations in the Amazon and cause significant clinical manifestations that can lead to disabilities or even amputations. Although antivenom has proven efficacy, its action is predominantly systemic with reduced local neutralization due to the rapid development of pathological changes after the envenomation. Regarding local treatment strategies that are adjuvant to antivenom, laser (Light Amplification by Stimulated Emission of Radiation) has been gaining prominence due to its promotion of tissue repair, reduction in inflammation, and analgesic effect. Preclinical studies have demonstrated the positive effects of laser therapy in reducing the local pathological effects caused by *Bothrops* venom and accelerating tissue regeneration related to myotoxicity. In addition, a pilot clinical trial with the use of lasers in *Bothrops* snakebite injuries demonstrated their efficacy in reducing edema, pain, and myonecrosis. Based on this first human trial, we intend to investigate the effectiveness of a low-intensity laser in order to confirm current findings and verify other local clinical outcomes in a design with a more robust sample. The objective of this study is to evaluate the effectiveness of red and infrared low-intensity laser in the recovery of acute local manifestations in patients who are victims of *Bothrops* bites. This is a randomized, double-blind, clinical trial to evaluate the effects of low-intensity laser therapy in patients who were victims of *Bothrops* envenomation and subsequently treated at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado. This is a phase 2b (therapeutic) clinical trial that will be conducted with a robust sample of patients and inclusion criteria already defined in a phase 2a study carried out previously. Based on the previous study, the sample calculation showed that the sample should contain a total of 90 patients, 45 in each group (control and intervention). In the first phase (T1), the control group will receive the specific antivenom treatment and will undergo the laser protocol but with the device turned off. The intervention group will receive the laser protocol within 30 min after antivenom infusion at a dose of 4 j/cm<sup>2</sup> to the extent of the inflammation shown by thermography. This laser therapeutic scheme will be performed in the same manner at 24 h (T2) and 48 h (T3). On the third day, if the patient is clinically stable, they may be discharged from the hospital, and their return for reassessment will be scheduled on the seventh day after hospital admission at the physician's discretion. At all stages, clinical and laboratory evaluations will be performed. Two independent typists will enter the information into the Research Electronic Data Capture (REDCap) program. The primary efficacy analysis will be performed on all participants who complete follow-up. Statistical analyses will be performed using the R software, and the significance level of the tests will be 0.05. This study seeks to demonstrate the therapeutic efficacy of the use of low-level laser on local clinical manifestations in patients with *Bothrops* envenomation and confirm current findings in a design with a more robust sample. The aim is to establish a clinical protocol for the use of laser as an adjuvant in the treatment to minimize local damage and reduce inflammatory effects, thus leading to faster recovery and prevention of complications such as local necrosis. We also intend to check the possibility of other local clinical outcomes, such as the prevention of secondary bacterial infection.

**Keywords:** clinical trial; low-level light therapy; snakebites; *Bothrops*

**Funding:** CAPES; Programa Inova Fiocruz—Inovação na Amazônia.

*Abstract 2.9. Variability of the Venom of Crotalus Durissus Ruruima Snakes: A Proteomic and Biological Analysis*

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Venomous snakes present medical importance due to the occurrence of envenomations in humans in addition to the great pharmaceutical interest regarding their toxins. Indeed, snake venoms have a wide variety of compounds that are mostly represented by proteins and that can cause several biological alterations and pathological events. In Brazil, rattlesnakes are represented by the species *Crotalus durissus*, with *Crotalus durissus ruruima* (Cdr) being a unique subspecies that inhabits the state of Roraima and is responsible for *Crotalus* bites in the northernmost region of Brazil. The aim of the project is to comparatively analyze the proteome of Cdr venoms collected in the state of Roraima and evaluate the venoms' biological aspects and the neutralizing effects of its antivenom. A total of 22 specimens of Cdr will be collected from natural environments in the municipalities of the state of Roraima: Normandia, Bonfim, Cantá, Pacaraima, Amajari, and Boa Vista. Proteomics will be carried out at the University of São Paulo's Center for Research Facilities via chromatographic profiling of each venom associated with the "shotgun" technique. Analysis will be performed using Mascot and X! Tandem software. For the biological activities, the action of the venoms on hemostasis will be evaluated through plasma coagulation and factor X activation approaches. The myotoxic activity of the venoms will also be assessed in vitro using myotube cell culture and the murine myocyte cell line C2C12. The study intends to verify the venom variability in the Roraima's rattlesnake subspecies as well as to evaluate their effects on biological activities. In addition, the study aims to verify whether the antivenom used to treat rattlesnake envenomations in Brazil can recognize and neutralize the venom of Cdr in Roraima. Thus, this study should contribute to the better management of *Crotalus* bite victims in the Amazon region.

**Keywords:** rattlesnake; proteome; snakebite; coagulation; myotoxicity

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*Abstract 2.10. Production of an Educational Tool for First Aid in Bites and Other Injuries by Venomous Animals in a Riverine Community in the Brazilian Amazon*

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The Brazilian Amazon is made up of diverse ethnic and cultural groups that are the result of historical events related to colonization and immigration, which have led to the miscegenation of peoples with different customs, religions, sociocultural relations, and traditional medicine that have become intertwined and are passed on from generation to generation by their descendants. Venomous animals are characterized by having the ability to produce and inoculate their venom through stings, bites, hair, nematocysts, etc., whether for capturing prey or defense against predators. When in contact with the human immune system, their venom can cause systemic and local alterations that can lead to sequelae and even death. As a result of the large number of cases and their frequency as well as difficulties in accessing rapid treatment, rural populations are the main victims of accidents related to venomous animals. In view of the above, the objective of this study is to carry out the construction and adaptation of an educational tool together with the community for first aid involving accidents with venomous animals and to identify the main needs that emerge in this specific population. This is a theoretical-methodological study. The development of the educational tool will be based on a didactic theory focused on historical-critical pedagogy, through which knowledge is seen as a social construction

in accordance with pre-existing cultural and economic concepts in the social group in which it is applied. The study will be carried out in a riverine community located in the city of Tabatinga in the interior of the state of Amazonas. The construction of the educational tool will occur based on previously elaborated steps and methods, including interviews and educational workshops, so that its elaboration is carried out jointly and is adapted to the needs of the target population. It is known that there is a deficit between what is scientifically produced and what actually reaches the target populations in several studies. This research is expected to provide access to information and health education for this riverine community in addition to allowing the exchange of scientific and cultural knowledge about the practices carried out in the location.

**Keywords:** educational technology; venomous animals; first aid

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

*Abstract 2.11. Knowledge of Students from the Higher School of Health Sciences at the Amazonas State University Regarding Accidents with Venomous Animals*

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The training of health professionals has become the object of frequent reflections. Training of health professionals is extensive, which makes it difficult to address diseases rarely seen in urban areas, such as NTDs. In the state of Amazonas, accidents with venomous animals stand out due to their high incidence and high rates of morbidity and mortality. Accidents involving venomous animals have never been dealt with in isolation at this university. Therefore, this study aims to offer a course on accidents caused by venomous animals and identify gaps in the knowledge of students before and after the course at ESA-UEA. This was a quasi-experimental study of the before-and-after type with equivalent groups of students from the UEA nursing, medicine, and dentistry courses enrolled in the course Special Topics of Accidents by Venomous Animals. We carried out the educational intervention with a class of 50 enrolled students. A semi-structured questionnaire was applied before the start of classes and after completion of the course that addressed the following variables: accidents by venomous animals (AVAs); epidemiology of AVAs; clinical/laboratory manifestations and classification of the AVA case; immediate care for AVA victims; care for AVA victims in the hospital environment; antivenom management; conduct when faced with AVA complications; and cross-cultural approaches. Of the 50 students, only 41 were included and evaluated in this research, all of whom were regularly enrolled from the 5th to the 10th semester of the nursing course at ESA-UEA. In the pre-test, the mean number of correct answers was 40%, errors—25%, and no answer—35%. After the course, these averages increased to 88%, 10%, and 2%, respectively. Of the variables in the pre-test, AVA epidemiology had a higher average of correct answers (71%), clinical/laboratory manifestations and classification of the AVA case had a higher average of errors (33%), and antivenom management had a higher average for no answer (54%). In the post-course test, the same was evidenced but with an increase in the frequency of correct answers to 100% and a reduction in the frequency of incorrect answers to 22% and no answer to 4%. The average number of correct answers for students enrolled in the 8th to 10th semester was 43% in the pre-test, while for students from the 5th to the 7th semester it was 22%; in the post-course test, it was 86% and 82%, respectively. The data analyzed before and after carrying out the educational intervention showed a significant change that showed growth in the knowledge of the students regarding accidents involving venomous animals. The students' knowledge base was necessary to build critical reasoning based on clinical and epidemiological evidence; however, it was non-specific for each group

of diseases. Therefore, it is necessary to address the specific content in a disaggregated way for better assimilation regarding the approach to care and management of the disease. This course has already been requested for inclusion in the medicine and dentistry courses at ESA-UEA.

**Keywords:** venomous animals; schools for health professionals; neglected diseases; Amazonas

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.12. When Evolutionary Biology Meets Toxinology: Phylogenetic and Phylogeographic Variation in Toxins of Amazonian Leaf Frogs (Anura, Phyllomedusidae)*

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Bioactive peptides in epidermal secretions of South American anurans have been researched for decades, and several have been identified, isolated, and characterized. The first bioactive peptide isolated was from a species of the genus *Phyllomedusa*, which is currently considered a rich source of biologically active peptides. Due to the evolutionary adaptations of the immune system against microbial invasions, these tree frogs use epidermal secretions as a defensive strategy against potential pathogens and predators. The tegumentary secretion of the species *Phyllomedusa bicolor* has been used by indigenous peoples from Western Amazon in shamanic healing and purification rituals known as Kambô. However, this practice has expanded to urban centers as an alternative treatment to conventional medicine despite the recognition of cryptic diversity organized into at least two lineages that correspond to Eastern and Western Brazilian Amazon. Therefore, the chemical composition of these secretions may reflect geographic and evolutionary distances even at the intraspecific level. In this sense, this study aims to test the general hypothesis that the interspecific and intraspecific variations of the peptidome of the epidermal secretion of Amazonian species of phyllomedusid tree frogs are related to the evolutionary relationship between species and populations, respectively. We intend to analyze the intraspecific and interspecific variations of the peptidome of the epidermal secretion of the leaf frogs *P. bicolor*, *Callimedusa tomopterna*, and *Phyllomedusa vaillantii*. After collecting the individuals, we will manually extract the epidermal secretion and remove the skin through an inguinal incision, after which the skin will remain frozen at  $-80^{\circ}\text{C}$ . Peptidome analyses will be performed through the evaluation of the chromatographic profile and via liquid chromatography–mass spectrometry of the secretion of each individual. The dried skin will be used in cDNA analyses, fractionation, and identification of compounds. The cDNA analyses will be performed using the simple reverse transcriptase, in which the PCR products will be purified, cloned, and sequenced. The fractionation and identification of compounds will be performed via extraction with boiling deionized water followed by lyophilization of the supernatant, which will be dissolved with aqueous trifluoroacetic acid (TFA) and applied in a reverse-phase HPLC system and then in MALDI-TOF MS. To analyze the interspecific variations, we will test for the phylogenetic signal of the peptidome in relation to evolutionary distances. For intraspecific variation, we will obtain three data sets from each population (the genetic distance (16S DNA segment), geographic distance, and peptidomic distance) and then test their correlations via partial Mantel tests.

We expect to find the presence of both phylogenetic and phylogeographic signals in the peptidome of these phyllomedusid species. We will also attempt to isolate, identify, and characterize new peptides as well as identify different peptides expressed by the cDNA analyses that were not present in the skin secretion. The results of the present study may have strong implications for the understanding of toxin evolution, bioprospection of drugs, and adoption of public health policies related to these medically important and increasingly popular frog species.

**Keywords:** *Callimedusa tomopterna*; cDNA; Kambô; *Phyllomedusa vaillantii*; peptidome  
**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.13. Evaluation of Neuromusculoskeletal Sequelae of Victims of Snakebites in the Amazonas State—A Case Series*

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Snakebites are an important public health problem in some regions of the world due to their severity and frequency. In the Amazon region, accidents involving snakes of the genus *Bothrops* are predominant. Snakebites are classified as mild, moderate, or severe depending on the clinical manifestations presented by the victims. Despite the low mortality rate, due to the venom's multisystemic actions, there is a possibility of short and/or long-term sequelae, whether anatomical or functional. The aim of this study is to estimate the incidence of snakebite victims with neuromusculoskeletal sequelae and assess their functionality and quality of life. This is a descriptive study of a series of cases with victims of snakebites treated at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) in the state of Amazonas in which the assessment will be carried out at least three months after the bite. The patient will be invited to participate in the study immediately after hospital discharge and will be asked for their telephone number or that of a family member or acquaintance for later contact. The study will use forms for collecting clinical and epidemiological data from the Idoctor<sup>®</sup> records and instruments for analysis of functionality and quality of life using WHODAS 2.0 (Version 36). The patients will also undergo a neuromusculoskeletal evaluation that considers muscle strength; range of joint motion; osteotendinous reflexes; and tactile, thermal, pain, vibratory (exteroceptive), and proprioceptive sensitivity. So far, 17 patients have been evaluated—12 men and 5 women, representing 71% and 29%, respectively. The highest incidence of snakebites occurred in the rural area (86%), with 24% occurring in the urban area. In descending order, the most prevalent previous conditions and comorbidities were: systemic arterial hypertension—SAH (22%), diabetes mellitus (9%), previous snakebite (9%), congestive heart failure—CHF (4%), childhood paralysis (4%), and ankylosing spondylitis (4%). Regarding the classification of the bite, four were classified as mild (23.5%), seven as moderate (41.2%), and six as severe (35.3%). Of these, the most incident complication was secondary infection with seven cases (41.2%) followed by compartment syndrome with one case (0.6%). On the other hand, muscular strength, range of motion, and musculoskeletal reflexes were reduced in six (35.3%), nine (52.9%), and five (29.4%) patients, respectively. Concerning tactile, thermal, pain, kinetic-postural, and vibratory sensitivity in the patients, these were decreased in nine (52.9%), four (23.5%), two (11.8%), one (5.9%), and one (5.9%), respectively. Snakebites occur mainly in men in the active working age group. After a snakebite, there is a high chance of developing complications such as secondary infections and compartment syndrome. There is also a great risk of suffering from loss of muscle strength and decreased range of motion in the affected limb and mainly loss of tactile and thermal sensitivity, although there are chances of developing alterations in pain, kinetic-postural, and vibratory sensitivity, leading to a decrease in the quality of life of these patients.

**Keywords:** snakebites; quality of life; disabilities; public health; musculoskeletal pain

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*Abstract 2.14. Riverine Voices: Development of a Community-Based Health Education Intervention in the Brazilian Amazon*

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Most of the people infected by snakebite envenomations (SBEs) are invisible and politically voiceless (riverine and indigenous populations), so it is up to researchers to give voice and space to these people so that the problems are addressed from their perspective. The WHO intends to reduce the burden of SBEs by 2030, for which it recommends that projects are carried out that aim to educate the community about the risk and prevention of SBEs through community engagement. Thus, the objective of this study was to develop an educational intervention in health using participatory community-based research in the Brazilian Amazon. This study was carried out in Tabatinga, in the interior of the state of Amazonas, in a riverine community called Limeira. This was a community-based study that used the triangulation of qualitative and quantitative data within the intervention-mapping model (IMM). The IMM is a planning framework for interventions that provides a systematic decision-making process for developing interventions. This framework relies on participatory research methods to ensure that the action matches the context and priority needs of the population. IMM has six steps that culminate in an intervention; first—careful description of the problem; second—definition of performance objectives and changes (stage changed by the team); third—discussion of initial ideas about the program; fourth—production of the material; fifth—event planning; and sixth—planning process evaluation. Currently the project is between stages 4 and 5. First stage: in the quantitative approach, 42 people were interviewed and questionnaires were applied regarding accidents involving venomous animals. There were frequent accidents with spiders, stingrays, scorpions, snakes and bullet ants. Although 36 of the participants reported contact with snakes, SBEs were rare. In the qualitative approach, 32 people participated, and participant observations, individual interviews and focus groups were carried out. During data analysis, several health problems were identified as more frequent and important when compared to SBEs, namely: accidents with sharp objects; fractures and sprains, drowning, accidents with venomous animals, viruses, domestic accidents and seizures. Second stage: the problems encountered were discussed with the community in a community assembly to define what was considered a problem, priorities, and solutions; In the third stage, fundamentals of education as a practice of freedom by Paulo Freire and learning, unlearning, and relearning by Vygotsky were chosen to conduct the construction of the intervention. SBEs are not the only problems faced by riverine populations; there are several other problems. However, due to the inclusion of community assemblies, the action will reach the target population. The main solution suggested was the creation of a group of local first aiders for cases of serious accidents. The mapping process is long; however, it has proved to be significant for the construction of the intervention. Through this study, it is possible to show that future interventions with Amazonian populations must consider the local context and other problems so that the actions have good acceptability.

**Keywords:** riverine; snakebite envenomation; mapping intervention; base community; educational intervention

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).



*Abstract 2.15. Compartment Syndrome Associated with Snakebite in the Brazilian Amazon*

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Snakebites are considered a serious public health problem in tropical countries, with higher morbidity and fatal case rates occurring in poor, underdeveloped, rural, and remote tropical forest areas. In Latin America, there are four genera of venomous snakes of greater medical importance, namely: *Bothrops*, *Crotalus*, *Micrurus*, and *Lachesis*. According to data from the Information System of Notifiable Diseases, between 2007 and 2016 in the Brazilian Amazon, 15,443 cases of snakebite were recorded. Local complications such as necrosis, blisters, and specifically compartment syndrome are less common, though they are worrisome, and immediate treatment is required. This is a cross-sectional, descriptive, and quantitative study that is being carried out in public hospitals in the city of Manaus in the state of Amazonas, which is the reference center for cases of compartment syndrome caused by snakebite. The population consists of all patients that were victims of snakebites who evolved with compartment syndrome according to their hospital records between the years 2012 to 2023 through the retrospective analysis of the medical records. Initially, data collection was performed in medical records with an instrument that is easy to fill out and using a descriptive analysis. Compensation of frequency, proportions for quantitative variables, and calculation of the mean and standard deviation were performed for quantitative variables. Statistical tests were used to define the association between clinical and epidemiological variables through logistic regression, a Chi-squared test, and Fisher's exact test with 95%CI and  $p < 0.05$ . To date, 30 patients who developed compartment syndrome associated with a snakebite were included. These were aggregated from records from three health units that treat envenomations. According to the data collected, a higher frequency of compartment syndrome could be observed as well as fasciotomy surgeries in victims who had common characteristics of being male, aged between 0 and 18 years, and transferred from rural areas of the state of Amazonas to these health units without pre-existing comorbidities and that did not receive treatment in an extra-hospital environment. Regarding the time to care, an average of 15 h was calculated between the snakebite occurrence and hospital care for those that evolved to these severe cases. All included patients used serotherapy, and the bite was by a snake of the genus *Bothrops*. So far, the partial results have confirmed the profile of the snakebite patient that was already presented in other studies when referring to sex, age, and place of origin. However, the time to care, although also presented as crucial for the prognosis of these cases by other studies, is presented in this study as an important factor for the worsening of the clinical scenario of these patients and its consequences in the short and long term. Even using serotherapy and without performing treatment with traditional medicine, this sample evolved to a clinical picture of severity and in some cases with permanent local sequelae.

**Keywords:** snakebite; fasciotomy; compartment syndrome; venomous animals

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.16. Identification of Lipid Components and Biological Activity of Bothrops atrox and Crotalus durissus ruruima Venom*

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Snakebites are a public health problem in many tropical and subtropical countries that cause numerous deaths annually and generate permanent disabilities in many patients. Snake venoms are complex mixtures of different classes of substances that are responsible for the clinical manifestations of envenomation. Since protein toxins predominate in snake venoms and are the focus of many studies, few studies have focused on their other constituents. Thus, this study aimed to identify the low-molecular-weight components present in *Bothrops atrox* and *Crotalus durissus ruruima* venoms and evaluate the inflammatory and hemostatic activities of these components. The venoms were obtained in partnership with the Museu da Amazonia and collaborators from Roraima. The metabolite extraction step was performed only with *B. atrox* venom to standardize the methodology. The pool of *B. atrox* venom was subjected to extraction with solvents of different polarities and submitted to solid-phase extraction using normal-phase and reverse-phase columns. The identification of low-molecular-weight constituents was performed based on analyses using (liquid chromatography–high-resolution mass spectrometry (LC-HRMS). The MS/MS data obtained was loaded on the Global Natural Product Social Molecular Networking (GNPS) platform using the classical mode to construct the molecular networks. Subsequently, the venoms will be submitted for lipidomic analysis. In addition, the evaluation of the inflammatory activity triggered by the identified constituents will be performed in cell culture in vitro and in vivo in mice. The hemostasis activity will be evaluated via coagulation assays. Nineteen distinct fractions of different polarities were obtained from *B. atrox* venom and analyzed using LC-MS, and it was possible to detect the presence of several low-molecular-weight compounds in the range of 100–1200 Da. In our initial studies, through the construction of molecular networks on the GNPS platform, it was possible to identify some classes of compounds, such as lipids and lipid-like molecules, benzenoids, organic acids and derivatives, organoheterocyclic compounds, phenylpropanoids and polyketides, carbohydrates, and carbohydrate conjugates. In addition, through the manual analysis of MS/MS spectra, it was possible to verify several compounds with possible structural similarities that are yet to be properly identified through comparison with the databases. The identification of small molecules in venoms is an important strategy for understanding the relevance of these constituents in the human envenomation process. The results achieved showed the presence of other classes of compounds that have not yet been studied in *Bothrops atrox* and *Crotalus durissus ruruima* venoms and that need attention, particularly to more fully understand their functions in the aspects of inflammation and alteration of hemostasis observed in *Bothrops* and *Crotalus* envenomations.

**Keywords:** *Bothrops atrox*; *Crotalus durissus ruruima*; metabolome; inflammatory response; hemostasis activity

**Funding:** PRO-ESTADO—FAPEAM, PCTI—EMERGESAÚDE/AM (FAPEAM), and CAPES.

*Abstract 2.17. Evaluation of the Immunological Memory of Snakebite Victims from the Yanomami Indigenous Reserve, Brazilian Amazon*

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The frequency of morbidity and mortality of snakebites in tropical countries make them a public health problem, which unfortunately is one that is still neglected. Brazil has more than 250 species of snakes (70 venomous), with 20,000 to 30,000 cases of snakebite

per year. Even though they are little studied in the Amazon, we know that the majority of snakebites are caused by snakes of the *Bothrops* genus in rural and/or remote areas. This makes it necessary to carry out studies in regions like indigenous reserves in the Brazilian Amazon. From this perspective, we will perform a survey of the data regarding the snakebites that occurred in Yanomami indigenous reserves (YIR) and of the humoral immune memory the Yanomami have developed for snakebites. The Yanomami are a group of hunter-horticulturists from the Amazon rainforest who dwell on both sides of the frontier between Venezuela and Brazil. We will study only the Yanomami from the Brazilian side. The specific objectives are: (1) Determine the clinical-epidemiological profile of the snakebites in the YIR; (2) map the critical snakebite zones in the YIR; and (3) verify whether the Yanomami population has the specific humoral immune memory for the snakebites. To determine the clinical-epidemiological profile of snakebites in the YIR, we will use secondary data from the Distrito Sanitário Especial Indígena Yanomami (DSEI-Y) from 2017-2022 and compare them with the data of health departments from the states of Amazonas and Roraima and from SINAN. Using this, we will map the critical zones of the YIR. In these areas, we will begin to search for indigenous people who might have humoral immune memory for snakebites. Yanomami victims and non-victims of snakebites of either sex will be included in the research. The informed consent form will be translated to Yanomami, and an interpreter will participate. The number of Yanomami with "natural" antibodies and who might have acquired immunity after snakebites is unknown. Therefore, the "snowball" method will be used as a strategy during the investigation. As a control group, in addition to the Yanomami who were not snakebite victims, we will also use samples from Yanomami snakebite victims and non-Yanomami non-victims found in the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado database. Additionally, we will document wounds with photos and/or videos. The evaluation of the presence of humoral immunity will be conducted using the ELISA test. We will analyze the data qualitative and quantitatively. With support from FUNAI and SESAI, we will carry out prevention and first aid campaigns in the Yanomami communities with the highest incidences of snakebite in order to clarify doubts, mitigate the number of snakebites in those communities, and promote the decentralization of the distribution of antivenom. After the completion of the research, we will publish at least one paper clarifying the presence or not of humoral immunity to snakebites in the Yanomami tribe. Additionally, we will realize snakebite prevention campaigns in the target population. We will also elaborate a booklet in Portuguese, English, and Yanomami, to publish the results of the research in a simple way as a benefit to the Yanomami population and the population in general. The production of a documentary about the Yanomami and their coexistence with the snakes could also occur.

**Keywords:** indigenous; immunity; snakes

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

*Abstract 2.18. Knowledge of Nursing Professionals about Snakebites in a Reference Unit in the Brazilian Amazon*

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The Brazilian Amazon has the highest incidence of snakebite envenomations in the country. Antivenom is the only resource capable of reversing the effects of the enveno-

mation and should be administered within a short period of time. In addition, before administering antivenom therapy, it is essential to identify the species of snake responsible for the bite in order to perform the correct treatment. Therefore, the health team must be prepared to use this information in the care of patients who are victims of snakebites. This study aimed to evaluate the knowledge of the nursing staff regarding snakebites at a reference unit in the state of Amazonas. This was quasi-experimental research typified as before and after that was based on the intervention of a training course conducted with the nursing staff that work at Fundação de Medicina Tropical Doutor Heitor Vieira Dourado. The nursing staff of the hospital were invited to complete a questionnaire regarding the management of snakebites. The nurses from all the sectors were included in the research, and employees who were on leave or vacation during the collection period were excluded and not trained at a later date. The questionnaire was developed by the research group SAVING with questions based on the Guide for the Treatment of Snakebites (also developed by the group). The guide was also a reference for the training course on management of snakebites, which was the educational intervention performed. The same questionnaire was reapplied to assess the knowledge gained after the intervention. A total of 198 pre-tests were answered by the nursing staff. The professionals were distributed in the sectors of Emergency Care, Medical Clinic 1 and Medical Clinic 2, Dermatology, Pediatrics, Adult ICU, Pediatric ICU, Male Ward, and Female Ward. The first part of the training course was attended by 88 professionals and the second part by 128 professionals. The number of correct answers was higher in questions about the route of administration of antivenom (83%), pre-hospital care at the site of the bite (77%), and the species responsible for most bites (72%). The lowest number of correct answers was for questions about tests that detect AKI (1%), manifestations at the bite site (2%), and complications resulting from the bite (5%). The post-test was answered by 121 professionals. The correct answers were mostly related to questions regarding the practices carried out in the routine of the hospital nursing professionals; however, it is essential to expand knowledge beyond just technical knowledge to reduce the chances of complications and prevent sequelae in the patient. Continuous education and training of professionals in the management of the victim of a snakebite is a strategy that results in improvements and greater safety in the practices of patient care for victims of snakebite.

**Keywords:** snakebites; public health professional education; health knowledge

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

*Abstract 2.19. Evaluation of the Anti-Inflammatory and Anticoagulant Potential of Crotoxin Isolated from the Venom of *Crotalus durissus ruruima* in an in Vitro and in Vivo Model of Disseminated Intravascular Coagulation*

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Sepsis is an important public health problem in Brazil and has one of the highest mortality rates in the world, with more than 185,000 cases per year and a lethality of over 50%. Sepsis is a generalized infection related to several pathological events such as exacerbated inflammation and disseminated intravascular coagulation (DIC), which is a complex coagulopathy that leads to a procoagulant response and blood incoagulability associated

with a thrombotic event. The available pharmacological therapies are limited in effect and only work for isolated events such as inflammation, infection, or clotting independently. In addition to conventional drug therapy being limited for sepsis treatment, there have been few studies that investigated drug efficacy with both anti-inflammatory and anticoagulant activities, thus justifying the search for new pharmacological options. Crotoxin (CTX) is present in the venom of the rattlesnake *Crotalus durissus* and its subspecies, and previous studies have already demonstrated its anticoagulant and anti-inflammatory activity. However, none have involved the CTX of the Amazonian rattlesnake *Crotalus durissus ruruima*. Thus, our aim is to evaluate the anti-inflammatory and anticoagulant potential of CTX isolated from the venom of the Amazonian rattlesnake *Crotalus durissus ruruima* and its therapeutic efficacy in in vitro and in vivo DIC models. The venom will be collected from a *Crotalus durissus ruruima* kept in the serpentarium of the Museu da Amazônia (MUSA), in Manaus, Amazonas (license 79102-1 SISBIO). CTX will be isolated from crude venom using high-performance liquid chromatography (HPLC). The molecular characterization of the sample will be performed using mass spectrometry (MS). In vitro assays will be performed to evaluate the toxin's anticoagulant action in human plasma and its ability to inhibit factors Xa and IIa of the coagulation cascade. The anti-inflammatory/anticoagulant crosstalk will be verified in human peripheral blood mononuclear cells (PBMCs) stimulated with lipopolysaccharide (LPS). An enzyme-linked immunosorbent assay (ELISA) will be performed for the detection and quantification of inflammatory mediators such as cytokines IL1b, IL-6, and TNF, in addition to factor III (tissue factor) of the coagulation cascade. The in vivo assays will be carried out with mice of the Balb/c lineage in the experimental model of DIC induced by LPS in order to analyze the ability of CTX to reverse coagulation and inflammation alterations in these animals. For data analysis, we will use GraphPad Prism software version 5.01 and a Student's *t*-test followed by Turkey's post-test and two-way ANOVA followed by the Bonferroni post-test. Differences with  $p < 0.05$  will be considered statistically significant. We expect to isolate the CTX from *C. d. ruruima* venom in order to evidence its anticoagulant and anti-inflammatory activities for the treatment of sepsis-induced DIC. We expect that the results of the present study will support the development of new therapeutic strategies in the treatment of sepsis aimed at inflammatory and coagulation disorders.

**Keywords:** crotoxin; *Crotalus durissus ruruima*; anti-inflammatory; anticoagulant; sepsis

**Funding:** FAPEAM (010/2021) CT&I.

*Abstract 2.20. Description of Habitat Usage by the Amazonian Scorpion Tityus metuendus in Urban Deforested Areas of Manaus, Amazonas State*

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Stings by scorpionids are a public health problem in many parts of the world and around two million cases occur every year. For the Amazon region, although this number is lower (sixty thousand/year), they still cause on average eighty deaths every year. Their treatment causes an overload of patients in the public health system that absorbs resources for other more serious diseases. The Amazon region has a high incidence of stings by scorpionids, and these occur principally in devastated areas. *Tityus metuendus* is the species that causes the most stings, since it is the most abundant in the region. Studies on the spatial ecology of scorpionids are scarce, which makes it difficult to answer the question regarding why scorpionids leave their natural habitat and enter anthropic habitats. In this project, we intend to understand the spatial ecology of *Tityus metuendus* in order to understand the movement of these animals between different habitats. First, we will analyze the FMT

database of patients that have reported scorpion stings that have occurred during the past 30 years. The entomology department at FMT gathered this data. After geolocating the addresses where the accidents occurred, we will plot them on the city map in order to determine the proximity to natural vegetation. The data will be divided into batches of 5 years to be plotted on a satellite picture for that time period, since city vegetation cover has changed as the city has developed. In the second part of the project, we will attach radio transmitters to scorpions and track them for 24 h. In parallel, we will collect information such as vegetation type, temperature, humidity, type of invertebrates found in the area used as prey, and other characteristics to describe the habitat where the animal is found. As this is a descriptive study of the area and usage, the number of individuals will be from 6 to 10. The project is divided in two parts: in the first part, analyses of the database in which scorpion stings are registered will be made; from these, we expect to produce seven maps (one map for every five years' worth of data) showing points where the sting occurred. We hypothesized that these points will be in close proximity to natural vegetation and display a trend in which an individual scorpion exits its habitat, probably searching for some resource that is scarce due to anthropomorphic changes. The second part of the project will complement the analyses of the database, since it will describe the usage of the area with a polygon that will represent the usage of the area and thus describe the movement of each individual. The polygon will incorporate other data gathered during the tracking, and this data will help to create a picture of the area and the resources available to the individuals. Through this study, we expect to understand why scorpions leave their natural habitat, since this causes an increase in the rate of encounters with humans. If we can understand why this happens, public health officials can try to avoid these encounters and reduce the rate of stings that occur.

**Keywords:** spatial ecology; *Tityus metuendus*; habitat usage; radio tracking; anthropomorphic changes

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

#### *Abstract 2.21. Psychosocial and Economic Impacts on Snakebite Victims*

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Snakebites represent an important health problem since they affect approximately 2.7 million people around the world each year, in addition to the estimated 81,000 to 138,000 deaths caused by them. In terms of physical sequelae, the consequences of snakebites affect about 400,000 people a year around the world, who may as a result suffer from muscular atrophy, loss of sensitivity, loss of mobility, and amputation. There are little data on the psychological feelings of individuals affected by snakebites, and such an investigation is necessary since these accidents are surprise events and bring a real possibility of death or physical disability, which are factors that can be precursors of anxiolytic states and depression. This study collected qualitative and quantitative data and was composed of individuals residing in the Brazilian Amazon who were victims of snakebites (ICD-10 X20) and were treated at the Tropical Medicine Foundation Dr. Heitor Vieira Dourado (FMT-HVD). The sample for the quantitative stage covered all patients who fit these criteria within a year of patient recruitment. In the quantitative stage of the study, possible psychological impacts associated with snakebites were investigated, for which psychological scales were used to assess signs and symptoms related to anxiety, depression, stress, and psychosomatic symptoms. The Revised Impact of Events Scale (IES-R); the Depression, Anxiety and Stress test (DASS-21); and the Goldberg General Health Inventory (QSG)

were used as instruments; these have already been validated in Brazil. An in-depth interview based on a semi-structured guide was used as a tool for collecting the qualitative data. A total of 24 patients were investigated (21 male and 3 female). The average age of participants was 35; 19 (80%) lived in urban areas and 5 (20%) lived in rural areas. In all, 20 participants (83%) presented alterations in some domain, while 4 (17%) presented no alterations. A total of 16 participants (67%) presented symptoms of anxiety, 18 (75%) of PTSD, and 13 (54%) of depression. A greater psychological impact was observed in patients who suffered snakebites classified as severe and with long-term consequences. Participants reported impairment in carrying out subsistence activities, financial loss, and feeling worthless after the bite, and they also reported episodes of intrusion, hypervigilance, and changes in their behavioral pattern. The sequelae caused by snakebites can be factors that modify an individual's dynamics within their social context and their relationship with subsistence activities and can cause feelings and sensations that are still little described in the literature. Investigating the environmental, psychological, social, and psychological impacts caused by snakebites is essential in order to offer a more complete theoretical basis for analyzing and understanding reality in the same way that it can support reflections and more assertive interventional actions in the care and follow-up of patients.

**Keywords:** snakebite; psychological impacts; sequelae

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.22. Health Surveillance and Quality of Data Regarding Snakebites in the Brazilian Information System for Communicable Diseases*

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Snakebite envenomations (SBEs) are a frequent and severe problem in underdeveloped and developing countries. In Brazil, SBE cases are monitored by the Ministry of Health through the Notifiable Diseases Information System (SINAN). SINAN stores clinical and epidemiological information about SBEs in Brazil, thus making it easy to access data for research purposes. Epidemiological studies are essential for improving treatment conditions and strategies for victims. This study aimed to evaluate the quality of antivenom (AV) administration in SBE cases reported to SINAN from 2007 to 2020. This study utilized a database obtained from the Department of Informatics of the Brazilian public health system. To ensure data quality, the available information was processed in R studio software (version 4.2) and evaluated for completeness, reliability, inconsistencies, and validity. Completeness was measured as the proportion of records with non-empty, non-null, and non-ignored values. Reliability was evaluated by comparing information on SBEs from the SINAN and SIM systems provided by the Ministry of Health. The study also created new variables to identify inadequacies during the treatment of SBEs based on guidelines established by the Brazilian public health system. These variables included the type of AV, number of ampoules, and severity. A total of 2,422,825 accidents involving venomous animals were identified; after filtering, 400,848 cases of snakebites were found. It was observed that most SBEs were caused by *Bothrops* (287,353/400,848; 71.69%) followed by ignored report (46,878/400,848; 11.69%), *Crotalus* (31,290/400,848; 7.81%), non-venomous (21,359/400,848; 5.32%), *Lachesis* (10,677/400,848; 2.66%), and Elapidae (3291/400,848; 0.82%). Completeness revealed that of the 77 variables, 5 were below 80% highlighted for death data (74.18%). Inconsistencies were detected in the local or systemic manifestation/complication variables, with higher values in the northern (0.4%) and northeastern

(0.4%) regions. The evaluation of accessibility showed a higher concentration of deaths registered in SINAN (1615) than in SIM (1389). Through the ratio of records between SINAN and SIM, deaths were distributed in the midwest at 249/198 (125%), the northeast at 549/465 (118%), the north at 555/478 (116%), the southeast at 85/79 (107%), and the south at 177/169 (104%). Inconsistencies were associated with a greater number of death reports on SINAN (1615) when compared to SIM (1389). AV administration in doses lower than what is recommended was higher in bites by *Lachesis* (7146/10,092; 71%) in a greater proportion in the southeastern region (13/13; 100%) followed by Elapidae (1895/3030; 63%) in the south (169/237; 71%), *Crotalus* (13,312/29,596; 45%) in the north (1571/2581; 61%), and *Bothrops* (37,173/272,388; 14%) in the northeast (10,001/56,722; 18%). The high dosages were predominantly in the regions where *Bothrops* occur (47,989/272,388; 18%), mainly in the northeast (13,022/56,722; 23%) and the south (6390/28,271; 23), and in *Crotalus* (3815/29,596; 13%) and Elapidae (71/3030; 2.3%), both with more occurrences in the northeast (1604/10,866; 15%; 62/1584; 3.9%). The quality of information is crucial for monitoring snakebite envenomations (SBEs), and prevention strategies, antivenom decentralization, and proper victim management cannot be neglected. With this information, it is possible to identify therapeutic failures in AV administration. Underdosage may occur due to shortages of AV at access points, and both inadequacies could also be related to a lack of adequately trained professionals, especially in regions where SBEs are frequent and where there is no health care available. The results presented by this research may aid the search for intervention strategies in SBE management and treatment in Brazil according to the specific needs of each region.

**Keywords:** snakebite; quality; antivenom

**Funding:** FAPEAM, CNPq, and CAPES.

*Abstract 2.23. Injuries Caused by Aquatic Animals and Their Social Impacts on Fishers in the Brazilian Amazon: A Mixed-Methods Study*

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At approximately 39,885, Amazonas is the Brazilian state with the third highest number of registered fishers, which describes the great economic importance of this activity in the state. There have been few studies in the country that described the physical, economic, and social impacts of accidents in this population. Therefore, this study aims to describe the socioeconomic impacts and experiences with accidents caused by aquatic animals and the possible barriers that exist in the life of fishers and fish traders. This will be a descriptive and observational study with a quali-quantitative approach in which the intention is to observe, collect information, and investigate the social impacts and perceptions of fishers and fish traders in the cities of Manaus, Ipixuna, Tabatinga, Iranduba, Barreirinha, Eirunepé, Careiro da Várzea, Presidente Figueredo, Barcelos, and Manacapuru. Data collection will take place at three different moments: first, the application of a sociodemographic questionnaire on exposure to accidents, history of accidents and their physical and social impacts; second, in-depth interviews and focus groups will be carried out; and the third will be the application of a generic instrument for assessing health and functionality (i.e., the World Health Organization Disability Assessment Schedule (WHODAS)). Subsequently, the data will be transcribed and entered into the MAXQDA software for reflective thematic analysis according to Braun and Clarke. Partial data obtained in the community of Limeira in Tabatinga revealed that riverine fishers carry out the activity as a means of subsistence and for the commercialization of their catches. During the analysis, it was observed that the main accidents to which fishermen are exposed are drowning, accidents with sharp objects, and accidents with animals (fish and stingrays); among the main species



involved were surubi bico-de-pato (*Sorubim lima*), mandim (*Pimelodus pohli*), and pirabutão (*Conorhynchos conirostris*; *Pinirampus*). The accidents occurred in the low-water period of the river. Accidents were mostly treated at home, and only in cases considered serious (spur perforation) and in cases of accidents with children was medical attention sought; this was due to economic barriers and difficulties involving transport. There is a need for studies that describe ichthyism and its impacts on socioeconomic, social, life, and work aspects, as well as care taken after accidents, in individuals who subsist on fishing activities. It is essential to promote the description of the problem so that public policies can be created for this population.

**Keywords:** ichthyism; impacts; fishers; quali-quantitative research

**Funding:** CAPES; FAPEAM.

*Abstract 2.24. The Collection of Animals of Medical Interest (ANIME) at FMT-HVD: Characterization of Snakes in the Collection for an Understanding of Snakebites in the State of Amazonas*

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Biological collections are fundamental for the conservation of biodiversity and the promotion of science and to help us to understand diverse aspects of the panorama of snakebites. Like the Instituto Butantan, the Fundação de Medicina Tropical Doutor Vieira Dourado (FMT-HVD) is considered a reference for the treatment of and research on snakebites in the Brazilian Amazon and has its own collection of snakes. It is called the Collection of Animals of Medical Interest (ANIME), and it includes a wide range of materials. In addition to records on the history of snakebite in the Amazon, it also supports educational activities. The ANIME collection has grown over time; in addition to the animals that cause accidents, some specimens have been donated or exchanged between researchers from FMT-HVD and other institutions. This project aimed to identify and characterize the snakes deposited in the Collection of Animals of Medical Interest (ANIME) and relate them to the cases treated at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD). For this, we began screening the specimens that compose the collection, inventorying and routinely maintaining them, identifying species, classifying them according to ontogenic stage and sex, matching specimens involved in accidents to medical records provided by FMT-HVD, and searching the geographic location of all specimens to understand the spatial relationship of snakes involved in envenomations in humans. So far, we have evaluated a total of 974 specimens, including 4 amphisbaenians and the remaining 970 snakes. These animals are distributed in a high taxonomic richness that includes 10 families, 45 genera, and 84 species. Among these records, the family Viperidae is one with the highest number of specimens ( $n = 409$ ). The most abundant species is *Bothrops atrox* ( $n = 363$ ). Most of the animals are from Manaus ( $n = 570$ ). Regarding the specimens involved in snakebites, 182 animals of 35 different species were involved in envenomations, with *B. atrox* ( $n = 96$ ) being the most frequent cause of snakebites. In summary, the Collection of Animals of Medical Interest (ANIME) compiles biological and historical data on snakebites in the Amazon and helps to increase knowledge on the subject. In addition, the collection has a didactic collection aimed at welcoming visitors, supporting lectures, and assisting in training courses on the identification of venomous snakes, which is a great achievement and gives this collection a significant role in the environmental education of society.

**Keywords:** Amazonia; biodiversity; medical importance; scientific collection; snakes  
**Funding:** FAPEAM; CAPES; CNPq/MCTI/FNDCT n. 39/2022.

*Abstract 2.25. Classification of Dermatological Lesions in Victims of Bothrops Envenomations in the State of AMAZONAS*

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*Bothrops* envenomations have pronounced case and death rates in the Amazon. The main dermatological local complications are pain, edema, erythema, ecchymosis, necrosis/myonecrosis, blisters, secondary infections, and compartment syndrome, which help in classifying the severity of envenomations, since severity is based on the evident clinical manifestations. Such events, when evolved, aggravate the clinical picture and increase the risk of death among those affected. The observation of different specific tissue alterations in envenomed patients can serve as a strategy for classifying dermatological lesions based on the construction of a classification that makes it possible to measure the severity of the damage caused by the toxins present in the venom in addition to predicting subsequent clinical complications. This process can be carried out by means of measuring instruments widely used in health care. Based on this, this study intends to contribute information about the essential characteristics of the types of tissue injuries resulting from the action of the toxins of the *Bothrops* snake venom. This will help, in a complementary way, in the classification of severity; the early diagnosis of complications such as amputations, secondary infections, and compartment syndrome; and the treatment of victims, in addition to serving as a basis for future studies involving the subject. This is a methodological, observational, and descriptive study with a series of cases that will be carried out with victims of *Bothrops* envenomation treated at the Tropical Medicine Foundation Doutor Heitor Vieira Dourado. The methodological strategies that will be addressed in this work have the purpose of building and validating an instrument for classifying dermatological lesions resulting from *Bothrops* envenomations. In the literature, no exclusive method was found that fully covered the proposed theme. Initially, definitions of essential terms for the study will be suggested based on previous work, clinical experience, and the visualization of pre-existing photographs of skin lesions of victims of *Bothrops* envenomations who were admitted to the hospital. There will be the construction and validation of a proposed scale that will be evaluated by judges with experience in snakebites and skin lesions. In this step, component items of each type of snakebite lesion will be evaluated based on the dermatological perspective. Then, photographs of snakebite lesions from previous studies at the study site will be used for the application of the instrument after validation by the judges. Finally, patients will be recruited to verify the instrument's responsiveness in the hospital routine. The results obtained during all stages will be analyzed using statistical methods with the aid of the R program. It is expected that we will obtain a tool for standardizing the diagnosis and targeting the treatment of victims. Neglected tropical diseases, such as snakebites caused by *Bothrops*, disproportionately affect low-income populations that have little access to education and health, such as riverine and indigenous populations. With the implementation of this instrument in the hospital routine, remote areas of the state of Amazonas will be able to adhere to the improvements that the study will provide, since it is an efficient tool that will be easy to handle and quickly understood by professionals.

**Keywords:** study validation; snakebite; *Bothrops*; Amazonas

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*Abstract 2.26. Identification of Bacteria Present in the Oral Cavity of Bothrops atrox and in Secondary Infections of Bothrops Snakebite Victims in the Municipality of Manaus and in the Interior of Amazonas State*

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The bite of *Bothrops atrox* causes wounds as a result of the perforations of the animal's fangs for venom inoculation that generate acute local inflammation. In fact, the inflammation is caused by a set of fractions of *Bothrops* venom and the pathogenic microorganisms present in the snake's prey. Currently, in the Amazonas state, secondary infection is prevalent in 8.3% of *Bothrops* bites treated in the interior of Amazonas state and in 39% of patients treated at the Fundação de Medicina Tropical Doutor Vieira Dourado in Manaus (FMT-HVD). Therefore, the early diagnosis of microorganisms that cause secondary lesions is crucial for the proper treatment. Moreover, inadequate or excessive use of antibiotics can lead to the development of bacterial resistance, making treatment more difficult and prolonged. Thus, the present work aims to carry out the identification of the bacteria present in the oral cavity of the *Bothrops atrox* snake and in the secondary infections of patients in the Amazonas state through metagenomic sequencing. This will be an observational study with the follow-up of victims of snakebites treated at the FMT-HVD during the period between January 2023 and December 2025 that evolve to the formation of a secondary infection at the site of the bite. Due to the high cost of the metagenomic sequencing of the samples, we will delimit the sample size for convenience; therefore, we expect to use 10 patients who evolve to secondary infection and 10 snakes involved in the snakebite, and 50% of patients will be from Manaus and 50% will be from the interior of the state. It is important to point out that on average, 45% of victims bring the snake to the health unit. After collecting the samples, the total bacterial DNA will be extracted, amplified, and sequenced; based on the data obtained by sequencing of the 16s rRNA gene, the bacteria present in the oral cavity of *Bothrops atrox* and secondary infections in victims of *Bothrops* bites will be identified. The frequency of the quantitative variables and proportions will be calculated; for the qualitative variables, these will be categorized, and the calculation of averages will be performed. In addition, statistical tests using bioinformatics tools will be carried out to evaluate the generated and exported sequences obtained from the raw samples obtained after sequencing via FastQC version 0.11.4. To determine the presence or absence of statistically significant differences in the microbial populations of the oral cavity of the snake and the secondary lesion, we will use the non-parametric Kruskal–Wallis test via the statistical software package GraphPad Prism 6. The accepted statistical significance will be  $p < 0.05$ . In this project, we aim for the first time to characterize the bacterial microbiota of the oral cavity of *Bothrops atrox* and the bacterial species present in secondary infections of patients treated at FMT-HVD using next-generation sequencing of specific regions of the 16S rRNA gene. With this, we hope to demonstrate a wide variation in bacterial species and classify them as Gram-negative or Gram-positive pathogenic or opportunistic pathogenic bacteria. In addition, we intend to verify whether there is a difference in the oral microbiota of the snakes from Manaus and the interior of the state due to their habitat and geographic region.

**Keywords:** metagenome; *Bothrops atrox*; secondary lesion; microbiota

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

*Abstract 2.27. Identification and Evaluation of Biological Activities of Non-Polar Compounds from the Epidermal Secretion of Amphibians of the Family Phyllomedusidae (Amphibia, Anura) in the Brazilian Amazon*

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Bioactive compounds from wild plants and animals are not only used in traditional medicines but also are increasingly more valued as raw materials in the preparation of medicines, since several drugs have already been produced based on these compounds. The skin secretions obtained from amphibians has been extensively studied for health applications, as they have been reported to be a rich source of several antimicrobial peptides that are efficient against multiresistant strains of bacteria, fungi, protozoa, and viruses. Despite the large number of anurans from different genera that are found in South America, a lot of attention is given to the study of those belonging to the family *Phyllomedusidae*, even though countless other species from this large family have already been identified. This project proposes to study non-polar compounds of the cutaneous secretions of the species *Phyllomedusa camba*, *Phyllomedusa vaillantii*, and *Phytocopus palliatus*, which are species that inhabit environmental protection areas in the state of Acre, and evaluate the biological activities associated with cell activation and coagulation. The target species of the study are generally found in primary and secondary forest environments during the nocturnal periods of the rainy season. The anurans collected will be temporarily taken to the herpetology laboratory at the UFAC, where they will be kept in adequate recipients. The skin secretions of these animals will be extracted by the morning of the next day. The animals will be separated according to species and submerged separately in a beaker containing deionized water and pressed gently and manually. The aqueous extracts obtained via skin scraping will be kept in 50 mL centrifuge tubes, lyophilized, and stored in a freezer at  $-20^{\circ}\text{C}$ . The crude samples will be transported on dry ice to the Laboratório de Inflamação e Imunologia das Parasitoses (LIIP) and the Central para Identificação e Quantificação de Lipídios (Ceqil), both at the FCFRP-USP. Non-polar fractions will be obtained from the samples for lipidomic and metabolomic analysis using an LC-MS/MS high-resolution system. The biological effects will be evaluated in a culture of human leukocytes stimulated or not by LPS, by the non-polar fraction, or by both for further quantification of markers. At the same time, at the Instituto de Pesquisa Clínica Carlos Borborema at the Fundação de Medicina Tropical Heitor Vieira Dourado, the nonpolar fractions will be evaluated in vitro for their ability to activate/inhibit/modulate cell functions and coagulation. Additionally, we will evaluate the pro- or anticoagulant potential in human plasma using factors II and X from the coagulation cascade. With this study, we expect to expand the knowledge regarding the venom of anurans with a focus on compounds still little studied like molecules with a non-polar disposition. Therefore, in non-polar fractions, it is expected to identify new or even already known molecules in addition to understanding the relevance of these non-polar compounds in cell activation/inhibition/regulation and in physiological dysfunctions caused by envenomations. It is also expected that the bioprospection of the non-polar fraction might lead to the discovery of new compounds with biotechnological

potential and/or therapeutic properties for inflammatory or degenerative disorders and/or coagulopathies, which may result in advances in the bioeconomy of the Amazon.

**Keywords:** envenomations; venoms; anurans; blood coagulation tests

**Funding:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

*Abstract 2.28. Study of Thrombotic Microangiopathy Markers in Victims of Bothrops Envenomations in the Brazilian Amazon*

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*Bothrops atrox* envenomation can cause coagulation disorders that are responsible for bleeding and thrombotic events. Among the events of the coagulopathy caused by the bite, thrombotic microangiopathy (TMA) is characterized by the deposition of microthrombi in the circulation, causing hemolytic anemia and damage to organs such as the kidneys. Although cases due to *Bothrops* envenomation have been reported in Brazil, no study in the northern region of Brazil has been conducted to investigate TMA associated with envenomations. Considering the importance of TMA in the pathophysiology of snakebites, the present work evaluated the hematological, biochemical, and coagulation alterations associated with TMA in patients who were victims of *Bothrops* envenomations and were treated in a reference unit in the Brazilian Amazon. This was an observational, descriptive and longitudinal study that was carried out with patients who were victims of *Bothrops* bites and treated at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) between July 2021 and November 2022. Venous blood samples were collected from patients who agreed upon admission to participate in the study (T0—pre-antivenom therapy), 24 h (T1), 48 h (T2), 72 h (T3), and 7 days after serum administration (T4). The diagnosis of TMA was characterized by a decrease in hemoglobin values (less than 13.5 g/dL for men and 11.5 g/dL for women), thrombocytopenia (platelets < 150,000/mm<sup>3</sup>), and the presence of schizocytes ( $\geq 1\%$  per field in the blood smear in 1000 red blood cells) observed at some point during the hospitalization. Eighty patients were included via the study eligibility criteria, of which ten patients presented TMA (12.5% frequency in all male patients). Patients with a history of hypertension prior to the *Bothrops* envenomation were also at greater risk for developing TMA. The TMA was also a factor for the longer length of hospital stay, with an average of 11 days. Local bleeding represents a risk factor for the development of TMA, and ecchymosis was the most common sign among patients with TMA (30%). It was also possible to observe that the presence of schizocytes was increased since admission among patients with TMA (60%), indicating that the search for schizocytes is a possible predictor marker of TMA in *Bothrops* envenomations. Regarding secondary damage, it was noted that 100% of patients with TMA developed a secondary infection. Acute kidney injury (AKI) was also observed in 40% of patients with TMA, showing a high rate in patients with TMA. For the first time, TMA was characterized in victims of *Bothrops* envenomations in the Brazilian Amazon who were treated at the FMT-HVD in Manaus. Important findings on risk factors such as secondary infection, AKI, and bleeding disorders were found. Future studies will be carried out in order to better understand the pathophysiological mechanism of TMA in *Bothrops* bites and possibly identify novel predictive factors and/or prognostic markers of TMA in victims of *Bothrops* bites in the region.

**Keywords:** thrombotic microangiopathy; blood disorders; acute kidney injury; snakebite; *Bothrops atrox*

**Funding:** Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

*Abstract 2.29. Knowledge and Practices of Self-Care of Munduruku Indigenous People Regarding Snakebites*

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Differentiated attention to biomedical or epidemiological issues should be rethought for populations with unique cultural contexts so that conflicts and ethnic damage are avoided. In this case, concepts of health, disease, life, death, and how they are seen in the environmental space are included in this idea. The distribution of Munduruku indigenous peoples occurs in regions of Pará, Mato Grosso, and Amazonas, which are distinct regions that have a uniqueness that marks their social process due to the environment and cultural process in which indigenous lineages are concentrated. In thinking about this scenario and the different occurrences of morbid snakebites in indigenous people, we adopted the concepts of Menendez (2003, 2005), who considered understanding the modalities of health care of social groups, biomedicine, and traditional practices involving religion, healers, plants/herbs, and shamanic rituals and other existing care so that, based on this, the articulation between the models of care and the strengthening of differentiated attention can occur. The general objective of this proposal is to narrate the knowledge and self-care practices of Munduruku indigenous people regarding snakebites. This is a qualitative, ethnographic study, as it permits the understanding of the vision of the subjects included in the study and the researcher's observation of the facts and the habitat or natural environment of the study participants. The interlocutors selected for the study were indigenous people of the Munduruku tribe, who live between the municipalities of Nova Olinda do Norte and Borba, AM. Two categories of analysis will be considered. The first, called Knowledge of Experts, which will be composed of chiefs, shamans, prayers, healers, curators, and spiritualists or "sacaca"; the second, titled Knowledge of Munduruku family members, will be contemplated by indigenous leaders, elders, and other members of the ethnic group over 18 years old. The study will be conducted using in-depth interviews with open questions and a field diary to register the findings during the research and from participant observation. For data analysis, content analysis was chosen, as it will allow the construction of thematic categories. In association with the triad of methods chosen for the ethnographic approach, the technique of iconography will also be used in this study; it will be constructed in the following ways: by an indigenous artist based on the researcher's photographic record during their stay in the field; and by the interviewees who accepted the proposal to draw the meaning of snakes, their importance for their ethnicity, and daily activities and the context of self-care in the face of snakebites in Munduruku indigenous people. This study intends to reflect on the knowledge and self-care practices of Munduruku indigenous people regarding snakebites that occur in the state of Amazonas and seeks to identify the care adopted during or after the snakebite treatment and the process in which specialists and family actors of this tribe participate. Based on this, it will propose new strategies and discussions that preserve the cultural genetics of indigenous people, which include self-care practices, appreciation of knowledge, effectiveness of traditional knowledge, and the union of this practice with the biomedical model.

**Keywords:** indigenous health; self-care practices; snakebites

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