BOTHROPS LEUCURUS WAGLER, 1824 (SERPENTES; VIPERIDAE): NATURAL HISTORY, VENOM AND ENVENOMATION

BOTHROPS LEUCURUS WAGLER, 1824 (SERPENTES; VIPERIDAE): HISTÓRIA NATURAL, VENENO E ENVENENAMENTO

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***Bothrops leucurus* has a large distribution in the Brazilian coast, from the State of Maranhão until Espírito Santo, where it occurs in a variety of forested and anthropogenic habitats. It may be found in humid, sub-humid, dry and semi-arid environments. It is terrestrial and medium-sized (250-1840mm). Preys are mostly rodents for adult snakes and frogs/lizards for juveniles, indicating an ontogenetic shift in diet. Data about the reproductive biology showed that B. leucurus is a viviparous species, pregnancy (four months) and birth occurs during the winter and summer seasons, respectively and litter medium size is 19 young. Its venom is characterized by a high fibrinolytic, proteolytic, hemorrhagic and edematogenic activity and a low coagulant activity and it has an important capacity for getting myonecrosis. The *B. leucurus*' poison has a capacity of inhibiting the neuromuscular transmission in an irreversible and dose-dependent way, because of its post-junction actions, and in low concentrations it has a pre-synaptic action. This snake is responsible for most of the ophidic accidents in Bahia, Brazil. The envenomation is characterized by local (pain, edema, erythema and ecchymosis), hemorrhagic, coagulation, digestive (vomit, nausea) and urinary (oliguria, anuria, haematuria) symptoms, besides headache, dizziness, hypotension, bradycardia, clouded vision and trembling.**
Key words: *Bothrops leucurus*. Natural History. Venom. Envenomation. Snakes.

Bothrops leucurus a une suffisante distribution dans la côte brésilienne, de l'État du Maranhão au Espírito Santo, où se produit dans une variété d'environnements des florestée et des antropizée. Cette espèce peut être trouvée dans des lieux de climat humide, sub-humide, sèche et demi-sèche. C'est terrestre et de dimension de moyenne (250-1840mm). Les proies préférentielles des adultes sont les rongeurs et les grenouilles/lézards des juvéniles, indiquant un changement ontogenetique dans le régime. Des données sur la biologie reproductive démontrent que B. leucurus est vivipare, l'accouplement se produisent dans l'hiver, la grossesse dure environ quatre mois et la naissance se produit dans l'été, quand les femelles arrêtent dans moyenne 19 fistons. Le venin est caractérisé par haute activité fibrinolitique, proteolique, miotoxique, hemorragique et edematogenique et une basse activité coagulante. Son poison est capable d'inhiber la transmission neuromuscular, avec blocus irréversible et dose-dependente, dû les actions pós-juncionais et dans de basses concentrations a action pre-sinaptique. C´est responsable de la majorité des accidents dans la Bahia, Brésil et l'empoisonnement est caractérisé par des manifestations locales (douleur, edema, eritema, equimose) et émeutes hémorragiques, de la coagulation, digestifs (nausées, vomis) et urinaires (oligurie, anurie, hematurie), outre céphalée, vertige, hipotension, bradicardie, vision boueuse et des tremblements.
Mots-clés: Bothrops leucurus. Histoire Naturalle. Venin. Empoisonnement. Serpents.

Brazil has the richest fauna and flora of all Central and South Americas featuring the 5th biggest abundance of reptiles in the planet, with more than 680 described species and an endemism of 37% [43]. The Serpents, in special are represented by 365 species grouped in 10 families [48] being the country´s most representative Squamata group. From the total amount of Brazilian serpent species, 28 belong to the Viperidae Family (Crotalinae), among them *Bothrops leucurus*, object of this work. According to Greene [31], among the living serpents, the viperidae crotalines comprehend approximately 75% of the serpents from the Viperidae family and 6% of the total amount of all the serpent species in the planet. They live in a variety of biomes and habitats on the three continents, in mild and tropical regions, with size varying from 50cm to about 4 meters long. Despite its great medical importance, data about the Viperidae's natural history and ecology, or even about the neotropical region's rich serpent fauna are scarce or inexistent and the biology of most species is still unknown [47]. We still do not appropriately know how some species live and researches suggest that this ignorance is due to the serpents' fugitive and secretive way of life, that makes it hard to observe them for long periods of time. Greene [31] discussed that the natural history of *Bothrops* might be relatively homogeneous when compared to other crotalines such as *Trimeresurus*. The present scarcity of behavioural and ecological information, however, makes further generalization impossible.

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Our aim is to survey the natural history, venom and envenomation of the jararaca pitviper, *B. leucurus*. This review is based on our studies over the past fifteen years, as well as on publications and data supplied by my students working on these snakes. Our data are deriving from field studies, in captivity in the scientific vivarium from the NOAP/UFBA – Ophiology and Venomous Animals Regional Center from the Federal University of Bahia, registered at IBAMA (Brazilian Institute of Natural Resources) under the number 1/29/2000/000076-8 and the analysis of 2.217 specimens in herpetological collections from 17 Brazilian scientific institutions.

B. leucurus (Figure 1), when young is known as jararaca-do-rabo-branco (white tail jararaca) and/or caiçaca, and when adult is known as malha-de-sapo, cabeça de capanga, jararacuçu, jaracuçu, jararacuçu-de-quatro-ventas, caiçaca, patrona and/or, simply, jararaca. The epithet is taken from the Greek *leuco*, meaning "white" and *oura*, meaning "tail", in reference to the pale tail of the specimen described by Wagler, which was a juvenile [5].

Figure 1. *Bothrops leucurus*. Photos by Breno Hamdan.

Wucherer [54] examined 40 specimens of this species in Bahia, in 1863, when he identified them as *Craspedocephalus atrox*. The legitimacy of the species was questioned by many authors, who created a big taxonomic confusion for many years, but it was determined as authentic by authors various[4,44,51,52,53]. This is due to the fact that *B. leucurus* is polymorphic, showing a great variety of coloration pattern, with geographic, ontogenetic and sexual variation. An example of this was the synonymization of *B. pradoi* with *B. leucurus* by Puorto *et al.*[41], who declared that the characters conventionally used to differentiate these species are inadequate, since the spots on the supralabial scales are related to an ontogenetic variation, besides the geographic variation between populations on the north and on the south of Bahia. We believe that Mata Atlântica's juveniles usually have a big pigmentation on the sides of the head, as a cryptic coloration defense strategy on the Mata's undergrowth.

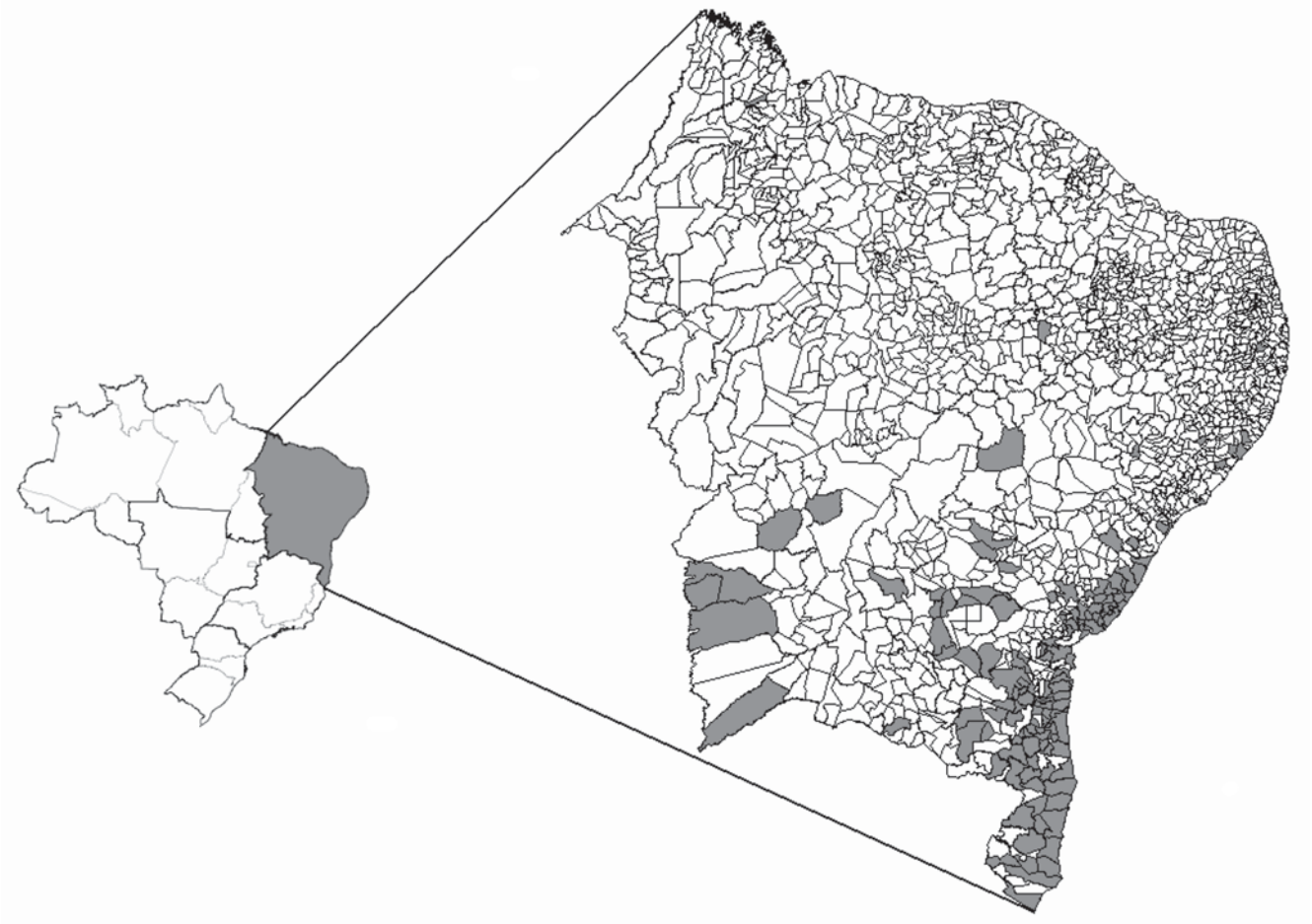
Distribution and Habitat

Melgarejo [32] considers this serpent to be the most important viperidae on the Atlantic zone of Brazil's Northeast Region and points out its incidence from the north of the Southeast Region (Espírito Santo) to all the Northeast of Brazil. The species' distribution is the widest, stretching to the north of the State of Maranhão, south of Ceará, and in Bahia it occurs at Chapada Diamantina and Chapadão Ocidental de São Francisco (Figure 2), corroborated by previous works[16,18,39], that already registered this species' distribution from the Northeast to the Southest regions of Brazil, at the Tropical Atlantic Morphoclimatic Domain or Atlantic Forest realm.

B. leucurus occurs in humid climate (60 - 20mm), humid to sub-humid climate (20-0mm), dry to sub-humid climate (0-20mm) and even semi-arid environments. Specimen were registered in Bahia, in environments with temperatures between 17º and 29ºC and preferrably in places with milder temperatures, averages from 23º to 25ºC and rainfall over 1.600mm [18]. Its vertical distribution zone goes from sea level up to 1200 m (Chapada Diamantina, Bahia) [18], although Campbell & Lamar [5] claim that this species is distributed at sea level between 300-400m.

It is common in the forested areas of the Northeast and adapts well in urban environments, including in peridomicile environments. Ulloa *et al.*'s *work* [49] registered this species' incidence also in the Caatinga region of Bahia, with prevailing vegetation of secondary Caatinga, highly impacted by anthropic action. This incidence indicates that *B. leucurus* is increasing its geographic distribution to areas with different pedoclimatic and vegetational characteristics, raiding in a different biota, probably favored by deforestation an by its big ecological plasticity. Also at Pernambuco State, *B. leucurus* was found on a Mata Atlântica's remanescent, located at the semi-arid region at Serra dos Cavalos, Caruaru [8, 13]. The data above contradict Greene & Campbell's [41] statement that list *B. leucurus* as a potentially vulnerable species due to its supposed distribution restricted to small fragmented areas.

Figure 2. Distribution of *Bothrops leucurus* in northeast Brazil. Designer by Yukari Mise. Source: NOAP/UFBA.



Feeding Habits

All serpents are carnivores and ingest their preys whole [30]. Juveniles and adults exhibit a significant difference in size, what could lead to an ontogenetic difference in diet, since many species of Viperidae serpents prefer anurans and lizards when young and birds and mammals when adults. This family's juveniles can perform the caudal luring when capturing ectothermic preys, losing this aspect as it grows old, when the diet changes to endothermic preys. Nevertheless, some male sub-adults and adults keep this conspicuous coloration on the tail. The event of ontogenetic shifts in diet and the caudal luring are well documented on the Viperidae family, which venom has an important role on the capture of preys, being suggested that its variation could be caused by differences in the feeding habits of juveniles and adults [1]. Information on feeding habits of most snakes has been collected mainly from examination of preserved snakes; direct observations and field studies are scarce. Nevertheless, food items for several crotaline species are known with detail, although Neotropical pitvipers remain poorly studied [47]. Here we presented data on feeding habits of *B. leucurus* including prey items and caudal luring [14, 23].

Ninety-three specimens of *B. leucurus* (68 males and 25 females) are examined, received and/or collected in different regions of the State of Bahia, Brazil, on the period from 1982 to 1996, through NOAP/UFBA and through Bahia Anti-venom Information Center (CIAVE), from State Health Bureau. Two serpent size classes were considered: juvenile (smaller than 600 mm SVL – Snout Vent Length) and adults (equal or bigger than 600 mm SVL). The stomach and bowels were open to search for eaten feeding items. Each item was identified to the possible taxon. The overlap of feeding niche between juveniles and adults and males and females was estimated using the niche symmetry coefficient [42]. Through MICROSTAT computer program, using the Exact Fisher Test, the significant differences between the diets of juveniles and adults and males and females were evaluated [23]. From the 93 serpents analyzed, 38 (40.9%) had a full stomach and in only 22 (23.7%) of them, it was possible to identify the feeding item. *B. leucurus* fed in higher proportion on lizards (*Cnemidophorus* sp, *Phyllopezus* sp, *Phyllopezus pollicaris*, *Tropidurus* sp, *Tropidurus hispidus* e Teiidae), followed by rodents (Muridae), and anurans (Hylidae). There was a diet overlap between juvenile and adult *B. leucurus* (Ojk= 0.71) and an ontogenetic variation

on the feeding pattern was observed, where the juvenile ingested preferably lizards (pi = 0.64, $p< 0.05$) and the adults preyed more on rodents (pi = 0.60, $p< 0.05$), confirming previous works on other species of Viperidae serpents [23].

Martins *et al.* [30], referred that published data on feeding habits of 22 *Bothrops* species indicate that most species are diet generalists, at least some show an ontogenetic diet shift (i.e., from ectothermic to endothermic prey), and most ambush prey from a coiled posture. All the species of the *B. atrox* group (*B. atrox, B. moojeni* and *B. leucurus*) presented generalist diets, ontogenetic change in prey type [37]. Sazima [47] too observed these changes on *B. jararaca*. Andrade & Abe [31] suggest that prey immobilization and death are the main roles of juvenile venom, whereas a digestive role becomes more important as snakes mature and their prey becomes larger. *B. leucurus* juveniles performed the caudal luring when capturing ectothermic preys, observed in animals in captivity. The males exhibit a white or yellow tail tip, losing this aspect as it grows, although some male sub-adults and adults keep this conspicuous coloration on the tail. It was not possible to observe if this happens when the diet shifts to endothermic preys, but it could be an indication. Caudal luring in snakes occurs in species of at least five families, including several species of pitvipers. This behaviour is frequently associated with feeding on ectothermic prey, especially anurans. For *Bothrops*, caudal luring is known to occur in *B. atrox, B. bilineatus, B. jararaca, B. jararacussu*, and *B. moojeni* [1,31,47], and suggested for *B. asper, B. leucurus*, and *B. neuwiedi* based on the possession of a distinctly coloured tail tip in juveniles [31].

Overlap of diet between males and females was observed (Ojk= 0.89) and there was no variation on the feeding pattern ($p< 0.05$), because males and females did not significantly differed in relation to the eaten feeding items. Similar data were obtained by Oliveira [37] that studied the diet of different populations of the *B. atrox* group (*B. atrox, B. moojeni* and *B. leucurus*) and concluded that these species have generalist diet and did not differ significantly between mature males and females in relation to the ingestion of ectothermic and endothermic prey. All species presented generalist diets and ontogenetic change in prey type. *B. moojeni* feeding based on field studies and analysis of 207 preserved specimens and demonstrated that females consumed more endothermic prey than males, and this difference probably reflects on size differences [36].

Reproduction

Reproductive data are available on only a few of the more than 20 species occurring in Brazil, such as *B. jararaca, B. moojeni, B. atrox, B. neuwiedi, Bothrops neuwiedi pubescens* [10], *B. leucurus* and *B. erytrhomelas* [21].

Lira da Silva *et al.* [21] reported for the first time data about the *B. Leucurus* reproductive biology, defining it as a viviparous serpent, having the autumn (September and October) as the favorable time for courtship, and the summer (January and March), the favorable time for the birth of the hatchlings. The minimum period of pregnancy is of 147 days (about 5 months), when about 12 hatchlings are born per litter, with average size and weigh of 27 cm and 7 g respectively, and with a sex proportion of 40% females to 60% males.

A study about the *B. Leucurus* reproductive biology [38], referring to the postnatal attendance of 11 litters obtained in captivity at NOAP/UFBA, including in regards to the growth curve. The author observed that the preferable time for mating and/or ovule maturation was the winter months, when the pregnancy was observed in 42% of the females. The minimum period of pregnancy was of 124 days and the preferable time for births comprehended the summer months (72%). Average 19 hatchlings were born per litter, being 55.99% males, 26.79% females and 17.22% indefinite. There was no sexual variation on the size and weigh of the hatchlings at birth (males 26.77cm/6.61g and females 26.53cm/6.93g). There was difference in weigh, but not in final size between males and females. At one year old, the animals doubled their sizes (independent of sex), at two years old they grew 50%, at three, 20% and at four, they practically stopped growing. The weigh gain was bigger and varied between the sexes on the first, third and fourth years of life, but not on the second. For *B. leucurus*, maturity was reached at, at least, 2.7 years old. However, due to the fact that males and females of same age were put to mate at the same time, we cannot specify if males reach sexual maturity earlier than females in this species. On the first year of life, 66% of males and 54% of females died; on the second year, the mortality was 64% males and 47% females. In reverse, on the third year of life, 25% of males and 33% of females died. On the fourth year of life, there was an increase on the mortality rate of males to 33%, while only 16% of females died. These pieces of information helped explain why more males are born than females, making us believe that this happens to guarantee that some males will reach the reproductive age [38].

Venom

Little is known about the toxinology of *B. leucurus* when compared to other *Bothrops* species. In a study of the biological activities of South American snake's venoms, Sanchez *et al.* [45] showed that the lethality (i.c.v., i.v. and i.p.), as well as the coagulant, edematogenic, hemorrhagic, and necrosis-inducing activities of *B. leucurus* venom were similar to those of several other *Bothrops* species, including *B. jararaca*.

In 1996, Lira-da-Silva *et al.* [28] determined the biological activities of the *B. leucurus* venom. The following activities were analysed: coagulant on human plasma, edema-forming by intraplantar injection, haemorrhagic and necrotizing *in vivo* and fibrinogenolytic by analysing blood coagulation time. Among the tested activities, the fibrinogenolytic one appeared to be the most significant (DMF = 4.25mg/mouse) followed by the edema-forming (ED30 = 0.5μg/mouse), haemorrhagic (11.02±4.87U/mg) and necrotizing (3.02±0.65 U/mg) activities. The coagulant activity one appeared to be the

least significant (DMC = 85.98mg/L), different from the obtained by Sanchez *et al.* [45] (1.4mg/L) for the same species. The pharmacologic study of the *B. leucurus* venom was performed by Lira-da-Silva in 2001 [15], with the objective to characterize the biochemical and immunibiological activities of this venom, as a means to evaluate the degree of serum protection given by the anti-venoms produced in Brazil and anti-venom specific for *B. leucurus*, produced at Instituto Malbrán. It was used venoms from two different geographic regions of the State of Bahia (Metropolitan Region of Salvador – MRS –and South/Southeast Region of Bahia – SSR) and from males and females, in order to establish possible geographic and sexual variations. Therefore, it was determined the chromatographic profile and the electropherotype of this samples, toxicity, haemorrhagic, necrotizing, pro-coagulant on bovine fibrinogen and myotoxic activities. The immunochemistry characterization was made by ELISA and Westernblot test and it was performed the neutralization of the lethal power, haemorrhagic, necrotizing, pro-coagulant and indirect haemolytic activities of the venom in face of antibothropic-crotalic serums (SABC) from Instituto Butantan (IB), Instituto Vital Brazil (IVB) and Fundação Ezequiel Dias (FUNED). The results show that the electropherotype of the *B. leucurus* venom is characterized by the presence of 4 bands and was similar on the different samples analyzed, except the female venom that had band 2 a little faded (almost absent) and a band (3) very clear. The chromatographic profile showed the presence of 7 peaks and the venom's components started to elute around 8 mL, with a slight difference on the profiles of the different samples used. There was regional variation, but not sexual variation, on the toxicity of the *B. leucurus* venom, where the SSR serpents' venom was almost twice more toxic than the venom from MRS animals. There was regional and sexual variation, on the biological activities of the *B. Leucurus* venom, since the SSR serpents' venom showed bigger haemorrhagic, necrotizing and myotoxic activities than the MRS serpents, however, the coagulant activity of the latter was bigger than the first. On the other hand, the females' venom showed bigger coagulant activity than the males, considering that the other biological activities were not tested for these venoms yet. The myotoxic activity of this species appeared proportionally bigger in relation to *B. moojeni*, equivalent to *B. asper* and lesser than the activity showed by *B. neuwiedi*, all from the same taxonomic group (ATROX group) and phyllogenetically close. Practically no regional variation was observed for this activity.

The ELISA studies showed that all samples had antibody titers in face of all antiserums tested [15]. However, the titration was bigger in face of specific anti-*B. leucurus* serum for all the samples, except MRS, where this serum titration was equivalent to the SABC from IB. Among the unspecific serums (SABC) the one that showed bigger titration was the IB's, followed by the SABC from FUNED and the IVB. There was variation on the neutralizing capacity of the different serums facing the venom samples used and the SABC/IB showed bigger antibody titration in face of the males venom and MRS' venom and lesser facing the females' venom and SSR' venom. The SABC/FUNED and IVB were better recognized by the male serpents' venom, MRS' venom and SSR's than by the females' venom. The Westernblot studies showed that this species' venom had crossed reaction both facing anti-*B. leucurus* serum and to SABC from IB and that the reaction was more intense when the specific serum was used. We deduce that all the anti-venoms were able to neutralize the haemorrhagic, necrotizing and hemolytic activities of the 2 samples of *B. leucurus* venom, despite the regional variation observed at this serum neutralization. The SABC/IB was more efficient to neutralize the haemorrhagic and indirect hemolytic activities than the FUNED and IVB's serums. We consider to be premature the idea that the serums produced commercially could be customarily used on human envenomations by this species, without being sure that a specific serum would be actually the best. There is still the need to proceed with the tests of serum neutralization of the lethal activities and to compare the results obtained so far, with the ones obtained facing the specific anti-*B. leucurus* serum for this venom's activities. It is our intention to study the pharmacologic effect of the venom, not only to contribute for the knowledge of the envenomation physiopathology, but also to establish the lines of convergence and divergence of this species, in relation to the other Brazilian *Bothrops*, besides relating the variation of the venom with extrinsic factors such as feeding and geographic distribution.

Correia [6] realized the purification and partial characterization of two haemorrhagic toxins from *B. leucurus* venom. The hemorrhagins Bleu-H1 and Bleu-H2 were purified through three chromatographic steps: ionic exchange of 600 mg from *B. leucurus* venom, divided in four aliquots, in Mono Q column (10 x 100 mm), followed by gel filtration TSKG-3000 (7.5 mm x 60 cm) and rechromatography in Mono Q column (5 x 50 mm), obtaining yielding of 0,1% for each toxin. Dot-blot results indicated that both hemorrhagins are metalloproteases. According to the SDS-PAGE results in reducing conditions, Bleu H1 is constituted of at least six chains (66, 63, 38, 28, 19 e 17 KDa), while Bleu H2 revealed five chains (63, 41, 38, 28, 18, 16 kDa), suggesting that hemorrhagins Bleu H1 and Bleu-H2 present molecular mass superior to 278 KDa and 207 KDa, respectively. The SDS-PAGE without the presence of a reducing agent confirmed the homogeneity of Bleu-H1. The *B. leucurus* venom Minimum Hemorrhagic Dose (MHD) was 1,96 μg, and the Bleu H1 and Bleu H2 ones were 1,23 μg and 1,42 μg, respectively. Bleu H1 and Bleu H2 presented activity activator of factor X, and capacity of hydrolyzing the chromogenic substrates S-2222 and S-2238, Bleu-H2 being considerably more active than Bleu-H1. Concluding, two acid metalloproteinases of P-IV class, with low haemorrhagic activity, high activity activator of factor X and capacity of hydrolyzing the chromogenic substrates S-2222 e S-2238, called Bleu-H1 and Bleu-H2, were purified from *B. leucurus* venom.

Bello *et al.* [2] published the isolation and biochemical characterization of a fibrinolytic proteinase from *B. leucurus* snake venom. The enzyme called leucurolysin-a (leuc-a), is a 23 kDa zinc metalloendopeptidase since it is inhibited by EDTA. The proteinase showed proteolytic activity on dimethylcasein and on fibrin. Leuc-a degrades fibrin and fibrinogen by hydrolysis of the alpha chains. Moreover, the enzyme was capable of cleaving plasma fibronectin but not the basement membrane protein laminin. Antibody raised in rabbit against the purified enzyme reacted with leuc-a and with the crude venom of *B. leucurus*. *In vitro* studies revealed that leuc-a dissolves clots made either from purified fibrinogen or from whole blood, and unlike some other venom fibrinolytic metallopeptidases, leuc-a is devoid of hemorrhagic activity.
In 2007, Magalhães *et al.* [29] purified and studied the properties of a coagulant thrombin-like enzyme from the venom of *B. leucurus*. A thrombin-like enzyme named leucurobin (leuc) was purified by gel filtration, affinity and ion exchange chromatographies. Physicochemical studies indicated that the purified enzyme is a 35 kDa monomeric glycoprotein and evoked the gyroxin syndrome when injected into the tail veins of mice at levels of 0.143 mug/g mouse. The clotting effect of the enzyme was strongly inhibited by antibothropic serum. Higuchi *et al.* [11] published the purification and partial characterization of two phospholipases A2. Molecular mass for both enzymes was estimated to be approximately 14 kDa by SDS-PAGE. The PLA2 activity of both enzymes is Ca2+ dependent. blD-PLA2 did not have any effect upon platelet aggregation induced by arachidonic acid, ADP or collagen, but strongly inhibits coagulation and is able to stimulate Ehrlich tumor growth but not angiogenesis.

Sanchez *et al.* [47] presented the structural and functional characterization of a P-III metalloproteinase, leucurolysin-B (leuc-B), a 55kDa haemorrhagic metalloproteinase and Gremski *et al.* [7] studied the cytotoxic, thrombolytic and edematogenic activities of leucurolysin-a (leuc-a) from *B. leucurus* snake venom.

Estimation of the Amount of Venom Inoculated by *B. leucurus*

The amount of secreted venom, accumulated on the serpents' venom glands, and, eventually injected on the event of a bite, has been the object of studies for a long time. Studies has shown that the amount of venom released through pressure on the glands during milking, is not the same as on the spontaneous bite ("atack"). We present the study on the sexual variation of the estimation of the amount of venom of *B. leucurus* and the estimation of the amounts of venom produced on the glands and released in an "attack" for feeding, on males and females of this species, kept in captivity at NOAP/UFBA. Eleven specimens were used, 6 females (114.3cm and 258g average) and 5 males (97.6cm and 137.6g average), that were submitted to two treatment methods during the venom milking. The serpents were submitted to venom milking after "attacking" a mouse, and on the next milking (a month later), they were submitted to venom milking only. These procedures were applyed alternately from February to June/1997. This species shows the least venomous content in the glands when compared to all *Bothrops* already studied. It was observed that *B. leucurus* females injected in average, a greater amount of venom on the "attack" than males (8.75mg and 4.74mg, respectively) and also had more venom content in its glands (10.67mg and 6.34mg, respectively), corresponding to data obtained for other *Bothrops* species [24].

These data corroborate clinic-epidemiological works on this species, that showed that females cause more serious accidents than males (17.7% and 9.6%, respectively), despite having lesser toxicity (1.7 and 1.3 mg/kg, respectively), due to the bigger amount of venom they inject in one bite [14].

***B. leucurus'* buccal cavity's Aerobic Bacterial Flora and Venom (Serpents; Viperidae)**

The data presented are partial results of the preliminary characterization of the *B. Leucurus'* buccal cavity's aerobic bacterial flora and venom. This species' venom causes local signs in its victims, such as pain, edema and necrosis. The soft tissue's necrosis can favor the development of infections with the formation of tumors, possibly caused by bacteria present on the buccal cavity and venom. The objective of the work was to characterize the aerobic micro biota of the oral cavity and of the venom of *B. leucurus* serpents recently captured and kept in captivity. Experiments were realized at the Microbiology Laboratory from the Science and Health Institute at the Federal University of Bahia and at NOAP/UFBA. It was used 8 (eight) apparently healthy serpents, including 2 (two) specimen recently arrived from nature (not fed, nor venom milked) and 6 (six) kept in captivity. The oral mucosa and venom samples (extracted by pressure to the glands) were collected by sterile swabs and, from the bacterial growth; the colonies were isolated and identified. The data showed the lack of bacterial growth on the venom samples and the presence of *Pseudomonas* sp, *Salmonella* sp, *Escherichia coli* and *Klebisiela* sp on the buccal flora of the serpents recently arrived from nature. On the jararacas raised in captivity it was found the presence of *E. coli*, *Klebisiela* sp, *Proteus* sp, *Staphylococcus aureus* and *Pseudomonas* sp both on the venom and buccal flora. These differences can be connected mainly to the conditions of the captivity (terrariums' contamination, feeding, low resistance due to stress), auspicious to the development of microorganisms, besides possible contamination of the collected material [12].

Action of the *B. leucurus* Venom on the Neuromuscular Junction [15, 22, 27, 40]

Venom effects (males and females from MRS and South/Southeast region – SSR, Bahia, Brazil) were examined using isolated phrenic nerve diaphragm preparation of mice. Indirect (10 to 200 μg/mL; n=3 to 6) and direct (50 and 100 μg/mL; n=3) muscle stimulation were used according the methods of the Bülbring [3] and Vital Brazil [50], respectively [15].

Results indicated that by indirect stimulation, *B. leucurus* venom induced dose-dependent and irreversible neuromuscular blockade under concentration upper of 50 μg/mL, with exception of the male's venom (upper 100 μg/mL). It is the strongest bothropic venom studied so far. This occurred probably due to postsynaptic action (depolarizing the cellular membrane, characterized by the severe contracture in 100 μg/mL and 200 μg/mL concentrations) of the venom derived from the MRS and SSR regions and moderate contracture in 200 μg/mL concentration of the males and females venom. A males and females venom-induced effect on presynaptic activity was suggested by the marked increase in the twitch tension in 10 μg/mL concentration. The female's *B. leucurus* venom deriving from the MRS region was stronger than the male's venom from the SSR region, it caused 50% of the neuromuscular blockade ($p<0.05$) [15, 22].

With direct stimulation it was observed the reduction of the acetylcholine response in concentrations of 50 μg/mL and 100 μg/mL ($MRS_{50\mu g/mL}$=36±8.7%, $MRS_{100\mu g/mL}$=22±2.7%, $SSR_{50\mu g/mL}$=39±8.7%, $SSR_{100\mu g/mL}$=27±2.7% and $♂_{50\mu g/mL}$=33±14.4%, $♂_{100\mu g/mL}$=16±4.9%, $♀_{50\mu g/mL}$=53±21.1%, $♀_{100\mu g/mL}$=20±5.9%), and the reduction of dose-dependent and irreversible twitch tension of the muscular response. *B. leucurus* venom presented sexual and regional variability in the action on the neuromuscular junction [15].

Prianti *et al.* [40] examined the action of *B. leucurus* venom (5-100 mg/mL) on contractile responses in chick biventer cervicis preparations. Muscle damage was assessed by quantifying the release of creatine kinase (CK) and by histological analysis. *B. leucurus* venom dose-dependently inhibited the contractile responses of indirectly stimulated preparations, the maximum inhibition with 100 mg of venom/mL being 74.0±6.6% (mean±SEM) after 120 min. The venom also reduced contractures to exogenous acetylcholine (55 and 110 mM) and K^+ (13.4 mM) (85-100% reduction with 100 mg of venom/mL) and increased the release of CK (348 ± 139 U/mL in controls vs 1260 ± 263 U/mL with 20 mg of venom/mL after 120 min, $p<0.05$). The accompanying morphological changes included multivacuolated, swollen, amorphous fibers and agglutinated myofibrils. These results indicate that *B. leucurus* venom can adversely affect neuromuscular transmission and produce muscle damage in avian preparations. *B. leucurus* venom showed neuromuscular and myotoxic actions in avian nerve–muscle preparations. A similar action in mammals could perhaps contribute to the local and systemic effects seen after envenomation by this species.

Myotoxic Action of *Bothrops leucurus* Venom [15, 17, 33, 34, 40]

Viperidae snakes' venoms induce predominantly local myotoxicity, characterized by myonecrosis at the anatomical region where venom is injected, but lack systemic myotoxic manifestations. This local myonecrosis is often associated with other effects, such as haemorrhage, blistering and edema, in a complex pattern of local tissue damage. Myotoxic components, known as 'myotoxins', are molecules that induce direct cytotoxicity to skeletal muscle cells, an effect that can be studied by a variety of methodologies [9]. However, the most important and abundant muscle damaging components in animal venoms are phospholipases A2 (PLA2, EC 3.1.1.4), enzymes that catalyze the hydrolysis of the sn-2 acyl bond of glycerophospholipids, in a calcium dependent fashion, generating free fatty acids and lysophospholipids [9].

Lira-da-Silva [15] and Lira-da-Silva *et al.* [17] investigated *in vitro* the myotoxicity of *B. leucurus* venom on mouse phrenic nerve-diaphragm (PNDp) and quantified the release of creatine kinase (CK), 3h after inoculation of the venom. The preparations were incubated in Tyrode solution with *B. leucurus* crude venom (10, 20, 50,100 e 200 μg/mL), for 2 hours. For histological analysis, muscle were removed and fixed on formol 10% and processed for examination by light microscopy. The results demonstrated that *B. leucurus* venom for the preparation time required for the venom to produce 50% neuromuscular blockade and muscle degeneration were concentration-dependent. This venom induced myonecrosis characterized by histological cellular degenerative events and reveals a common series of pathological alterations which include: (1) plasma membrane disruption, (2) formation of 'delta-lesions', wedge shaped areas of degeneration located at the periphery of muscle fibers and (3) hyper contraction of myofilaments. The myotoxic action of *B. leucurus* venom does not seem to affect myogenic satellite cells, blood vessels and nerves, although some myotoxic and neurotoxic PLA2 induce degeneration of the motor nerve terminals. Similar results were observed by other authors [9].

Mise [33] and Mise *et al.* [34] investigated *in vivo* local tissue damage caused by *B. leucurus* venom assessed by quantifying the release of creatine kinase (CK) and by histological analysis. Myotoxicity was evaluated *in vivo* by intramuscular (50 μL) of *B. leucurus* crude venom (25, 50 or 100 μg/mL) over gastrocnemius muscle of right posterior limb. Control was injected with physiological saline solution. Blood was collected by orbital puncture after 1, 3, 6, 12 and 24 hours. Plasma was separated by centrifugation for subsequent determination of creatine kinase (CK) activity using CK-NAC Liquiform test (LABTEST Diagnostic). For histological analysis, muscle was removed and fixed on formol 10% and processed for examination by light microscopy. Myotoxicity of *B. leucurus* venom caused muscle damage and release of CK into blood plasma. The peak of liberation of CK was detected at 6 (1758.16), 3-6 (1183.67 – 1145.44) and 3 (1718.16) hours for 25, 50 and 100 g/mL, respectively. Morphological analysis revealed that *B. leucurus* venom affected a large number of muscle fibers as show by widespread and varying degrees of necrosis. These results too confirmed the clinical observations.

Preliminary Data About the Protective Action of *Callendula officinalis* (Asteraceae; Compositae) Facing the Myotoxic Activity of *Bothrops leucurus* Venom [33, 34]

The main objectives of these works were to verify the applicability of *Callendula officinalis* for previous and

associated treatment in bothropic accidents. The effects of *C. officinalis* were evaluated with intramuscular inoculation 20 minutes after the incubation with venom and *C. officinalis'* extract and topical treatment 20 minutes after the inoculation. The anti-miotoxicity of *C. officinalis* extract was evaluated *in vivo* by the intramuscular inoculation of 50μL of solution. Orbital punction was done in order to extract blood samples, at 1, 3, 6, 12 and 24 hours after the inoculation of the solution. The plasma was separated by centrifugation and took the upper fraction. The samples were kept at 4ºC during all the process. The CK activity was determined by CK-NAC liquiform kit. In order to make a histological analysis, the muscle was removed, fixed at formol 10%, and processed for optic microscopy. The incubation of venom with *Calendula* extract suggests the existence of any ant venom fraction in the aqueous extract of *Calendula*. Due to the results we recommend the use of extract of *C. officinalis* in the form of topical treatment previously to anti-bothropic serum treatment or may be in combination with the proper serum.

Envenomation

The data presented here are results of the investigations [14, 19, 20, 25, 26,] about descriptive studies about the clinical and epidemiological aspects of the ophidian accidents caused by *B. leucurus* in the metropolitan area of Salvador, Bahia, Brazil, from January 1982 to April 1996. These accidents were notified to the Anti-venom Information Center (CIAVE). This study is based on medical records of 126 patients and questionnaires filled by the author who interviewed 13 patients or the persons accompanying them during the prospective phase of study (from January, 1995 to April, 1996). None of the cases resulted in death.

The 126 accidents occurred mainly in the cities of Salvador (29,3%) and Camaçari (26,1%). However, the highest incidences were reported in the cities of Itanagra (119,1/100,000 inhabitants), Mata de São João (26,8/100,000 inhabitants) and Camaçari (25,7/100,000 inhabitants). The accidents occurred mainly in March, May, June, September and October. Most of them took place in the rural areas (56,7%) and during diurnal period (60.0%). Most of the patients were adult male rural workers, 10 to 49 years old, and 65.4% of the accidents occurred during work. The accidents involved the feet (65.9%) and the hands (25.4%) [14, 19, 20]. Similar data were obtained by Mise *et al.* [35] on a study that described the clinical and epidemiological characteristics of 655 cases of *Bothrops* snakebites in Bahia, Brazil (2001).

Time elapsed between the snakebite and medical care, snakebite and serotherapy, and admission to the CIAVE and serotherapy was of less than six hours in 84.2%, 87.0% and 82.6% of the cases, respectively [14, 19, 20].

Envenomation by *B. leucurus* is mainly characterized by: local disturbance manifestations: pain (80.9%), edema (79.8%), heat (26.3%), numbness (20.2%), erythema (16.7%), ecchymosis (13.2%) and plycthena (10.5%) and coagulation manifestations: period of altered coagulation and blood incoagulability (58.2%). General manifestations such as headache (14.0%) and dizziness (7.9%), vomit (4.4%) and nausea (3.5%), oliguria (3.5%), haemorrhage (3.5%), hypotension (1.8%), reduced vision acuity (1.8%) and tremblings (1.8%) were observed with less frequency [14, 19, 20].

Proportionally, female snakes were responsible for more severe accidents than male snakes. Adult *B. leucurus* accounted for local manifestations such as edema, plycthena and necrosis than young snakes. Consequently, adult snakes were responsible for more severe accidents (7.6% young and 22.4% adult snakes), and less capable of causing blood coagulation alteration according to the information about the venom action of others Bothrops species. Most of the envenomation cases were mild (52.1%) and all evolved to cure. This might have a direct relation to the time between the snakebite and the medical care, therefore the gravity of the case increased with time. The high frequency of mild cases may also be related to snake length, however the majority of the accidents were caused by young snakes. The high severity of the case was not associated to stomach content (11.1% of severe accidents by snakes with full stomach and 13.3% by snakes with empty stomach) [14, 19, 20].

On average, 7.5 ampoules/patient were used, as the majority of the patients received 1 to 4 ampoules (50.5%). They were administered, on average, 5.0 ampoules in mild cases, 7.4 ampoules in moderate cases and 8.8 ampoules in severe cases. The patients bitten by adult snakes received, on average, a higher dose of bothropic anti-venom than those bitten by young snakes, not only when anti-venom was first administered (5.6 and 7.2 ampoules, respectively) but also when anti-venom was administered additionally (1.2 and 1.6 ampoules, respectively). These results demonstrated that local manifestations commonly related to accidents involving large snakes required treatment with high dose of anti-venom [14, 19, 20]. This information has already been reported in clinical studies with other *Bothrops* species.

The frequency of reactions after the anti-venom (67.5%) may be considered one of the highest in Brazil if compared with that reported on other regions. The absence of the lethality and the high frequency of mild cases with no sequelas suggest that the treatment with bothropic antivenom effectively neutralized the action of venom, indicating the efficiency of this treatment on accidents in the metropolitan area of Salvador [14, 19, 20].

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