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Research

Biochemical Reference Intervals in Four Snakes of the Genus Bothrops (Serpentes: Viperidae) from Argentina

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ABSTRACT

Bothrops species comprise a species complex of terrestrial snakes distributed among all Americas which appear to be more important as a public health problem and the knowledge of their health status is necessary in captive condition.

Objectives

Establish the biochemical reference intervals from this snakes.

Subjects and Methods

Blood samples were collected by venipuncture of the ventral tail vein from 50 healthy specimens each Bothrops ammodytoides, Bothrops alternatus, Bothrops diporus and Bothrops jararacussu, in captive conditions in Argentina. Normal biochemical reference values were established for the following: total proteins, albumin, globulins, uric acid, urea, glucose, triglycerides, chloride, cholesterol, total lipids, calcium, phosphorus, magnesium, GOT (AST), GPT (ALT), and alkaline phosphatase (ALP) by UV light spectrophotometric techniques whereas potassium and sodium, were analyzed by flame spectrophotometry.

Results

Values are presented as medium standard± deviation and focused in the acid uric concentration.

Conclusion

The results were compared with published data for other south and Central American crotalids and can be used in conservational or veterinary purposes. The final recommendations are the need to perform a periodic biochemical analysis focused on the metabolism of uric acid as the main component of nitrogen metabolism in captive snakes.

***Keywords**: Blood, Biochemistry, Bothrops species, Captivity.*

INTRODUCTION

The Bothrops species comprise a complex of terrestrial snake species distributed throughout the Americas that seem to be more important as a public health problem. On the other hand, the knowledge of the state of health of the captive snakes is necessary, since these snakes are kept in captive conditions to obtain poisons destined to the manufacture of specific antivenoms to treat the intoxication. Very often, in order to reach a conclusive diagnosis, a diagnostic tool is required, such as the hemato-biochemical analyzes of the blood. In addition, with the increasing recognition of the appearance of new diseases that affect reptiles and the important role of all those haematological and biochemical investigations in diagnosis, there is an urgent need to develop new studies based on associated haematological abnormalities to the presence of different diseases in reptiles. Serum biochemical analysis is an equally important

tool for evaluating the health of animals from the capture of wildlife and those kept as captives for longer. These studies help to understand the functional capacity of several target organs, in turn acting as specific markers in the diagnosis of a pathological condition and in the monitoring of the physiological status of captive snakes and also those that are at liberty. Most of the available reference ranges are reported from other countries, where the samples come from captive animals that live in altered environments that can significantly change biochemical values. Therefore, this study was carried out with the objective of establishing reference intervals of hematological and biochemical parameters for four Bothrops species that live in Argentina; Bothrops ammodytoides, Bothrops alternatus, Bothrops diporus and Bothrops jararacussu and focus the study on the metabolism of uric acid as the main factor capable of altering the health status of animals.

MATERIALS AND METHODS

Fifty adults from captive individuals of Bothrops ammodytoides (Figure 1), Bothrops alternatus (Figure 2), Bothrops diporus (Figure 3), and Bothrops jararacussu (Figure 4) each were studied. All the specimens fed spontaneously on living prey, one adult mouse (20-25 g) per week and received filtered water ad libitum. All the samples were obtained in 2017. Blood sampling was performed by removing the snakes from the boxes using a metallic hook and firmly restraining the head and the rest of the body by two operators (Figure 5). The use of anesthetics or sedative agents, known to induce significant alterations in the hematological values[1,2] was avoided. Blood (2.0 ml) was collected by a third operator by venipuncture of the caudal vein using disposable 21G-needles fitted to sterile 2.5 ml syringes.[3,4]

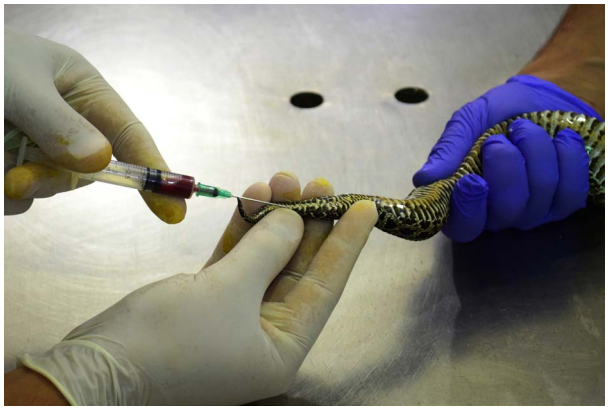
Figure 1. Bothrops ammodytoides

Figure 2. Bothrops alternatus

Figure 3. Bothrops diporus

Figure 4. Bothrops jararacussu

Figure 5. Venipucture of caudal vein



The blood samples was transferred to clean glass tubes and the serum was obtained and were stored at 5°C until their process.

In this serum sample, total protein was determined spectrophotometrically at 540 nm by the Biuret method. Albumin was measured at 600 nm after complex formation with bromocresol purple. Globuin concentration was calculated as total protein concentration minus albumin concentration. Uric acid was determined after deproteinisation with tungstic acid and calcium carbonate by treatment with uricase and peroxidase, which results in quinoneimine dye. Glucose was measured by the enzymatic method of hexoquinase/glucose

6-phosphate dehydrogenase. Urea was determined spectrophotometrically at 505 nm after reaction with indophenol blue. Triglycerides were measured at 540 nm after reaction with quinoneimine dye. Cholesterol was determined spectrophotometrically by the 4p-benzoquinone reaction. Phosphorus was determined at 630 nm after formation of phosphomolybdate; calcium was measured at 570 nm after reaction with cresophthalein-complexone; chloride was measured at 460 nm after complex formation with sulphocyanide; magnesium was measured at 520 nm after complex formation with magon. Potassium and sodium were measured in plasma by flame spectrophotometry. Alanine aminotransferase (ALT) and asparate aminotransferase (AST) activities were determined kinetically by the decrease in absorbance at 340 nm resulting from oxidation of NADH and finally, lactate dehydrogenase(LDH) Based on the following reaction scheme: LDH Pyruvate + NADH + H + L-lactate + NAD. The reactive were provided by Wiener Laboratories (Argentina). Values obtained were submitted to parametrical statically analysis using a software SPPS Statistic 17.0.

RESULTS

The different determinations performed in serum, are shown in Table 1. Values are presented as mean± Standard deviation.

Table 1. Blood Biochemistry Reference Values from Bothrops species

Determination	Units	Bothrops ammodytoides	Bothrops alternatus	Bothrops diporus	Bothrops jararacussu
Total proteins	grs/l	4.08±2.82	3.78±1.22	3.60±1.04	4.72±2.34
Albumin	grs/l	2.96±1.04	2.15±1.16	2.08±0.43	1.99±054
Globulines	grs/l	1.11±0.42	1.62±0.21	1.57±0.31	*2.73±1.07*
Glucose	mg/dL	32.32±4.67	28.83±3.61	29.19±2.79	31.53±3.26
Uric Acid	mg/dL	8.34±2.12	5.98±1.92	5.53±1.25	6.21±1.85
Urea	mg/dL	10.69±2.64	9.85±2.23	6.07±2.04	6.2±1.79
Cholesterol	mg/dL	270.91±16.74	231.28±14.93	259.87±15.29	232.4±14.08
Triglycerides	mg/dL	151.38±16.87	175.87±16.98	167.132±18.04	221.37±24.83
Phosphorous	mg/dL	5.82±1.56	3.93±0.97	3.84± 1.60	4.71±1.21
Calcium	mg/dL	29.96±2.43	14.03±2.16	13.41±1.28	16.32±2.01
Magnesium	mmol/L	6.27±1.97	4.99±0.63	3.72±0.21	3.53±0.98
Sodium	mmol/L	149.12±8.69	149.7±5.18	153.8±4.71	146.17±4.25
Chloride	mmol/L	112.02±14.42	107.6±14.94	107.5±15.67	121.45±13.23
Potassium	mmol/L	5.46±0.36	4.70±0.25	4.89±1.17	4.84±0.27
AST	UI/L	33.34±3.21	23.78±6.26	22.67±1.96	32.91±11.23
ALT	UI/L	16.84±1.71	23.78±6.26	22.67±2.96	32.91±12.23
LDH	UI/L	334.21±121.71	342.22±134.28	329.04±114.35	332,29±116.87
AST: Aspartate amino transferase; ALT: Alanine aminotrasnferase; LDH: Lactate deshydrogenase					

DISCUSSION

Our findings in biochemical metabolites from Bothrops species maintained in captive condition show the same range of variation found in other South American Bothrops and Crotalus species. The concentration of total proteins, albumin, and globulins in these four Bothrops studied fall within the range of variation described for Caudisona durisa, Bothrops asper and Crotalus simus.

With the logical differences due to the different processing techniques used to process the serum samples, the values that are detailed in table 1 show little differences with respect to species such as Crotalus durissus terrificus, Crotalus sinus, Bothrops asper, Bothropoides jararaca, B jararacussu studied in different countries of Central and South America and reported by other authors.[3,5-8] In our animals, the samples were analyzed using commercial kits provided by a local laboratory, while other authors mentioned the use of autoanalyzers.[8] This variable can cause differences in the interpretation of the findings, especially when compared with the UV or flame spectrophotometry processing of the samples.[2,4] The differences observed within the same species and the same genus imply how important the appropriate use of similar analytical procedures are as a way to eliminate the appearance of values outside the scale in a reference sample.[9,10]

Also, the sanitary status of the animals kept in captivity constitutes an important variable when analyzing the results obtained. Indeed, comparing our findings with other authors who have worked with recently captured species, statistically significant differences are found in some components[3,11] and these differences could be due to the nutritional status of the snakes, assuming that those that remain in captivity for a prolonged period receive a more controlled diet and their health programs include the administration of vermifugous .

One of the most interesting findings is the high uric acid concentration found in Bothrops ammodytoides, which is significantly higher than those described in the other Bothrops species and other south American crotalids. Interestingly, a similarly high uric acid concentration was reported for Cerastes cerastes and Cerastes vipera,[12] two viperid species which also inhabit dry regions with a high daily temperature oscillation. However, a high uric acid concentration may not represent an adaptive response to a 'dry habitat', since the B. ammodytoides specimens used in this study were kept in an environment of controlled temperature, received food monthly and had free access to water for, at least 6-months.

Further studies are required in order to ascertain whether factors like age and sex as well as seasonal variations produce statistically significant variations in the blood biochemical parameters in this autochthonous crotalids.

CONCLUSION

Data in this paper can be a useful tool in establishing normal biochemical values for captive Bothrops spp and for comparison with the free-ranging population for conservational or veterinary purposes.

In addition, the proper establishment of the reference intervals of plasma biochemistry values is an important part and a reference tool for the so-called "*viper snakes*" used for research purposes either by biologist or veterinarians in wildcaught animals.

CONFLICTS OF INTEREST

We hold that we have no conflict of interests with any consistency.

REFERENCES

1. Nardini G, Leopardi S, Bielli M. (2013). Clinical Hematology in Reptilian Species. *Vet Clin North Am Exot Anim Pract*. 2013; 16(1): 1-30. doi: 10.1016/j.cvex.2012.09.001

2. Knotek Z, Knotkova Z, Hrdá A. Clinical Haematology and Plasma Chemistry in Reptiles. 5th ed. Southern European Veterinary Conference (SEVC) Sep. 20 to Oct. 2. Barcelona, España. 2011.

3. Troiano JC, Gould E, Althaus RL, et al. Blood Biochemical profile of the south american rattlesnake (Crotalus durissus terrificus) in captivity. *J Ven Animals and Toxins*. 2001; 7(2): 183-189. doi: 10.1590/S0104-79302001000200004

4. Martínez-Silvestre A, Lavin S, Cuenca R. La bioquímica sanguínea en clínica de reptiles. *Consulta Difus Vet*. 2013; 200: 31-40.

5. Vieira DS, Rodrigues TS, de Miranda RL, et al. Determinação das concentrações plasmáticas de proteínas e metabólitos de cascavéis em cativeiro. *Pesq Vet Bras*. 2014; 34(1): 39-42 .

6. Glaser V, Boni AP, da Silva Pitz H, Coelho de Albuquerque C, Bertarello Zeni AL. Parametros hematologicos e Bioquimicos de Bothropoides jararaca e Bothrops jararacuçu (Ophidia –Viperidae) mantidas em cativerio. *ArchVet Sciences*. 2013; 18(3): 68-74. doi: 10.5380/avs.v18i3.30088

7. Troiano JC, Vidal JC, Gould E, et al. Haematological and blood chemical values from Bothrops ammodytoides (Ophidia -Crotalidae) in captivity. *Comp Haem Int*. 1999; 9: 31-35.

8. Bandeira da Silva W, Machado Soares R, Machado C, et al.(2010) Bioquímica plasmática de cascavéis (Caudisona durissa LINNAEUS, 1758) em cativeiro. *Ciência Rural*. 2010; 40(12), 2510-2514. 2010. doi: 10.1590/S0103-84782010001200012

9. Dessauer H. Chemistry of the reptilian blood. In: Gans C (Ed). *Biology of the Reptilia*. 3 ed. New York, USA: Academic Press.1982.

10. Silva LF, Riani-Costa CC, Ramos PR, Takahira RK. Seasonal influence on biochemical profile and serum protein electrophoresis for Boa constrictor amarali in captivity. *Braz J Biol*. 2011; 71(2): 517-520.

11. Gómez A, Arroyo C, Astorga W, Chacón D, Rodríguez S, Jiménez, M. (2016) Hematological and biochemical reference intervals for Bothrops asper and Crotalus simus (Serpentes: Viperidae), maintained in captivity for venom extraction. *Comp Clin Pathology*. 2016; 25(3): 615-623. doi: 10.1007/s00580-016-2240-2

12. Al-Badry KS, Nuzhy S. Haematological and biochemical parameters in active and hibernatings sand viper. *Comp Biochem Physiol*. 1983; 74A(1): 137-141. doi: 10.1016/0300-9629(83)90724-7