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Inorganic elements in blood of mice immunized with snake venom using NAA and XRF techniques

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Abstract Brazil has the greatest diversity of snakes in the world and a large portion of them are venomous. Nowadays, Instituto Butantan (research center, at Brazil) produces various types of antivenom to meet the large number of incidences. In this investigation, mice were immunized with different species of *Bothrops* snake venom to evaluate the inorganic elements concentration in their blood by using NAA and XRF techniques. The results were compared with the control group (mice not immunized) and with human estimative. The data allows to evaluate the toxicity of these elements, important for clinical screening of patients submitted to immunological therapy.

Keywords Snake venom · NAA · XRF · Mice · Blood · *Bothrops* snake

Introduction

Venomous snakes of the genus *Bothrops* are responsible for 80 % of the snakebites in Brazil [1]. The investigation of blood in mice immunized against *Bothrops* snake venoms are significant in the development of immunological therapies. The *Bothrops* snake venoms have high prevalence in distinct regions of the Amazon in the Brazilian territory. Instituto Butantan (IBu, São Paulo city, Brazil) produces several types of antivenoms including anti-*Bothrops* serum. This antivenom preparation requires a series of steps, including tests on animal models, which allows us to verify its effectiveness before applying to envenomed patients. The need to perform these evaluations in blood is because of small changes in inorganic elements which leads to the intoxication process of victims of poisonous animals. The elements Na and K are found in the highest concentrations of *Bothrops* snake venoms while Br, Ca, Cl and Fe are slightly low [2]. One of the major clinical manifestations of poisoning is the consumptive coagulopathy by depletion of clotting factors [3]. The second is the activation of fibrinolysis accompanied by bleeding on skin and/or mucous membranes, which can cause intracerebral hemorrhage and acute renal failure [4]. In both cases, the Ca blood evaluation is essential in order to prevent a disorder characterized by prolonged bleeding after the snake bite. In the absence of medical care, which requires the administration of antivenom, the poisoning can cause death to the patient in days. According to World Health Organization (WHO) the poisoning from poisonous animals have been treated carelessly (neglected tropical disease) in developed countries leading to significant number of deaths. The situation in Brasil has been monitored in recent decades by SINAN—Sitema de Informação de Agravos e Notificação (Information System for Notifiable Diseases) [5] and currently 400 accidents occur every month. The accidents with snake bites are over 20,000 per year with a mortality rate of ~10 % [6]. Another important aspect to highlight is related to the snake venom composition. Investigations showed that the *Bothrops* venom of certain species, according to geographic distribution, can vary significantly within the genus *B. atrox* (known jararaca-do-norte). In Northern region (Rio Negro) this snake poison is 30 times more potent than that found in the

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Southeast region [7]. These findings emphasize the need for analysis of biological materials involved in the investigation of antigens for the production of antivenom sera. Particularly, investigations of animal model blood immunized with snake venom are important to compare with human data for future clinical screening of patients before undergoing immunological therapy. Since 2009, we have investigated the behavior of ions and metals in blood of animal models immunized with snake venoms (mice, rabbits and horses) using INAA and EDXRF techniques. These investigations have introduced improvements in the production of sera (antivenoms) [8–12]. Now, we intend to investigate the blood of mice immunized with *Bothrops* snake venoms, the first step in the antibodies production. In this investigation mice genetic modified, for high antibody-producer (H_{III} specie) [13], were immunized with different species of genus *Bothrops* from Brazilian Northern region. In order to evaluate the inorganic elements concentration in their blood INAA and EDXRF techniques were used. These results may incorporate changes in the *Bothrops* sera production process performed at Instituto Butantan that is always meeting the standards of good manufacturing practice and good laboratory practices.

Experimental

For this study, biological samples of 26 adult mice (4 month old) genetically modified (H_{III}) were used. These mice were divided into six groups: one control group (CG) and five groups of immunized mice with snakes venoms (G*i*, $i = 1, 2, 3, 4$ and 5) (Table 1).

Mice immunization

Each mouse (from G*i* groups) receives an injection of snake venom; about 10 μg of poison was subcutaneously injected. After few days (at least two), the antibodies against the venoms were produced [7].

Sample preparation

After the immunization period, the blood collection was performed without the use of any anticoagulant agent. The blood (~0.3 mL) was collected by the retro-orbital venous plexus from each mouse. The blood (100 μL) was deposited immediately after the collection onto a filter paper (Whatman 41). All samples were prepared in duplicates and stored, separately, in plastic bags.

Irradiation procedure

The NAA was carried out at the nuclear reactor IEA-R1 (3.5–4 MW, pool type) at IPEN. Each sample was irradiated for few minutes in a thermal neutron flux (ranged from 4.6×10^{12} to 6.8×10^{12} cm^{-2} s^{-1}). The measurements of the gamma induced activity of the samples were carried out using a high-purity Germanium detector with 60 % relative efficiency at the 1332 keV gamma ray from ^{60}Co. The concentrations were obtained by using in-house software [15]. Calibration standards obtained from high purity metals and salts were used. For ^{38}Cl ($T_{1/2} \sim 37$ min, 1642 keV) and ^{24}Na ($T_{1/2} \sim 15$ h, 1368 keV) determination an irradiation time of 2 min and decay time of 10 min, followed by 5 min of counting time were used. For ^{80}Br ($T_{1/2} \sim 16$ min, 616 keV), ^{49}Ca ($T_{1/2} \sim 9$ min, 3084 keV), ^{27}Mg ($T_{1/2} \sim 9$ min, 1012 keV), ^{42}K ($T_{1/2} \sim 12$ h, 1525 keV) and ^{37}S ($T_{1/2} \sim 5$ min, 3104 keV) samples and standards were irradiated for 5 min. After a decay time of 1 min they were counted for 20 min for Br, Ca, Mg and S determination and followed by 4 h of counting time for K. The Fe analysis was performed using MINI X-Ray spectrometer from Amptek, with Ag X-ray tube. The characteristics X-ray fluorescent intensity from K_α line (6.4 keV) was measured with a Si Drift detector (25 $mm^2 \times 500$ μm) with Be window (12.5 μm). The samples (the same used in NAA measurements) were irradiated for 5 min using 30 kV and 5 μA excitation. The quantitative analysis was performed using WinQxas software [16]. Using this procedure Cl (K_α line 2.6 keV) was also determined. For analytical–quality control standards of IAEA-

Table 1 Samples of whole blood from mice (H_{III} specie) immunized with different *Bothrops* snake venom

Groups	*Bothrops* snake venom
Control, $n = 6$	Not immunized
G1, $n = 4$	*B. atrox* from Alto Rio Negro
G2, $n = 4$	*B. atrox* from Maranhão
G3, $n = 4$	Venom mixture I (G1, *B. jararacussu*, *B. alternatus*, *B. moogeni* and *B. neuwiedi*)
G4, $n = 4$	Venom mixture II (G1, G2, *B. alternatus*, *B. moogeni* and *B. neuwiedi*)
G5, $n = 4$	*B. taeniata* and *B. viperideae*

B. taeniata is an arboreal species of *Bothrops* and had been assigned to the genus *Bothriopsis* in the past [14]

n, Number of samples; *B. atrox*, the abbreviation *B.* from *Bothrops* is used to refer to specie

A-13 Animal Blood and certified reference material NIST SRM 1577b—Bovine liver were used.

Results and discussion

The evaluation by *Z*-score test ($|Z < 2|$) indicate the adequacy of the methods (Table 2). The results are the mean of three replicate analyses. The results show good precision and the relative standard deviations were lower than 10 % for most of elements. The blank filter paper was also analysed and some impurities such as Na and Cl were identified in very low concentration (0.066–0.082 g kg^{-1} for Cl and 0.031–0.040 g kg^{-1} for Na). So, they do not interfere when compared with the concentrations in the whole blood.

The concentration of the Br, Ca, Cl, Fe, K, Mg, Na and S for all mice groups are showed in Table 3. In this table the human being data (HG) were also included for comparison aiming to check the similarities. The Student's *t* test was applied to check the statistical differences.

The variability for all elements investigated (Br, Ca, Cl, K, Fe, Na and S) in the same group of immunized mice (G*i*, $i = 1, 2, 3, 4$ and 5) are presented in Fig. 1 emphasizing high variability for Br, Na and S within the same group. In Fig. 2, a comparison of whole blood concentrations between control group and immunized mice is presented. In this figure, the results for each element concentration for all immunized groups (G*i*, $i = 1, 2, 3, 4$ and 5) were normalized in relation to the concentration value obtained for the CG (mice not immunized). In Fig. 3, a comparison between whole blood concentrations of all mice groups with human being data is presented. In this figure, the results for each element concentration for all mice groups were normalized in relation to the estimate value obtained for human group (HG).

Immunized group and control group

According to Table 3, the immunized groups formed for the most mixing poisons (G3 and G4) are those with changes for all investigated elements when compared to the control group (Fig. 2). The immunized group G3 presented the highest value of Br (2.25 ± 0.91 mg L^{-1}) while the G4 was the lowest one (1.29 ± 0.41 mg L^{-1}). Besides that, the high relative standard deviation (27 %) also reflects its highest variability within the same group (Fig. 2).

For Ca, G1 and G4 differed significantly from the others immunized groups ($p < 0.05$). The concentration values ranged from 240 ± 14 mg L^{-1} (G1) to 103 ± 6 mg L^{-1} (G4). A comparison with CG (201 ± 10 mg L^{-1}) shows that a good agreement with G2, G3 and G5 while for G1 is near of the upper limit for a confidence interval of 95 % (181–221 mg L^{-1}). However, for G4 there is no compatibility, even considering ±3 standard deviations (85–121 mg L^{-1}). In addition, the results of Ca are in

Table 2 *Z*-score values using NAA and XRF for IAEA-A-13 Animal Blood and NIST SRM-1577b Bovine liver

Elements	This work Mean ± 1 SD	Reference values Mean ± 1 SD	RSD (%)	R_E (%)	|Z Score|
Br (g kg^{-1})	0.0223 ± 0.0019[a]	0.0220 ± 0.0024[c]	8.5	1.4	0.2
Ca (mg kg^{-1})	292 ± 21[a]	286 ± 54[c]	7.2	2.1	0.1
	0.119 ± 0.012[a]	0.116 ± 0.004[d]	10.1	2.6	0.8
Cl (g kg^{-1})	13.61 ± 0.55[a]	13.40 ± 1.07[c]	4.1	1.6	0.2
	12.58 ± 0.80[b]	13.40 ± 1.07[c]	6.4	−6.1	0.8
	2.76 ± 0.21[a]	2.78 ± 0.06[d]	7.6	−0.7	0.3
Fe (g kg^{-1})	2430 ± 151[a]	2400 ± 144[c]	6.2	1.3	0.2
	2301 ± 132[b]	2400 ± 144[c]	5.7	−4.1	0.7
	0.190 ± 0.019[a]	0.184 ± 0.015[d]	10.0	3.3	0.4
K (g kg^{-1})	2.55 ± 0.46[a]	2.50 ± 0.35[c]	18.0	2.0	0.2
Na (g kg^{-1})	13.19 ± 0.54[a]	12.60 ± 1.01[c]	4.1	4.7	0.6
	2.41 ± 0.15[a]	2.42 ± 0.06[d]	6.2	−0.4	0.2
S (g kg^{-1})	6.61 ± 0.74[a]	6.50 ± 0.52[c]	11.2	1.7	0.2
	7.93 ± 0.49[a]	7.85 ± 0.06[d]	6.2	1.0	1.3

SD Standard deviation, *RSD* relative standard deviation, R_E relative error

[a] NAA

[b] XRF

[c] IAEA-A-13 Animal Whole Blood

[d] NIST SRM-1577b Bovine Liver Powder

Table 3 Element content (mean value ± 1 SD) of whole blood mice samples

Groups	Br^{b} (mg L^{-1})	Ca^{b} (mg L^{-1})	$Cl^{b,c}$ (g L^{-1})	Fe^{c} (mg L^{-1})	K^{b} (g L^{-1})	Na^{b} (g L^{-1})	S^{b} (g L^{-1})
G1 ($n = 4$)	1.91 ± 0.06	240 ± 14	2.52 ± 0.13[b] 2.46 ± 0.22[c]	371 ± 26	1.99 ± 0.21	2.15 ± 0.42	1.06 ± 0.38
G2 ($n = 4$)	1.33 ± 0.03	188 ± 11	2.55 ± 0.22[b] 2.71 ± 0.29[c]	362 ± 44	2.07 ± 0.13	2.02 ± 0.04	0.88 ± 0.15
G3 ($n = 4$)	2.25 ± 0.91	182 ± 14	2.09 ± 0.25[b] 2.01 ± 0.34[c]	336 ± 9	1.61 ± 0.21	1.79 ± 0.02	0.88 ± 0.47
G4 ($n = 4$)	1.29 ± 0.41	103 ± 6	2.23 ± 0.18[b] 2.39 ± 0.20[c]	371 ± 26	1.78 ± 0.16	1.95 ± 0.04	1.09 ± 0.33
G5 ($n = 4$)	1.33 ± 0.64	201 ± 12	2.43 ± 0.37[b] 2.67 ± 0.33[c]	417 ± 33	1.63 ± 0.18	2.07 ± 0.48	0.65 ± 0.11
Average ($n = 20$)	1.62	183	2.36[b]	371	1.82	1.99	0.91
RSD (%) ($n = 20$)	27.2	27.4	8.5	7.9	11.5	8.1	9.8
CG (n = 6)	2.07 ± 0.07	201 ± 10	2.50 ± 0.35[b]	274 ± 49	2.01 ± 0.12	1.58 ± 0.31	0.67 ± 0.16
[range][a]	[1.93–2.24]	[181–221]	[1.80–3.20][b]	[176–372]	[1.77–2.25]	[0.96–2.20]	[0.35–0.99]
HG [range][a]	[<13.2][d]	[98–482][e]	[2.01–3.33][d]	[284–492][f]	[0.87–1.75][d]	[1.06–1.78][d]	[0.04–1.28][g]

The range for the control group (CG) and human being group (HG) were also included for comparison

n Number of samples

[a] confidence interval of 95 %

[b] NAA

[c] XRF

[d] Reference [17]

[e] Reference [18]

[f] Reference [19]

[g] Reference [20]

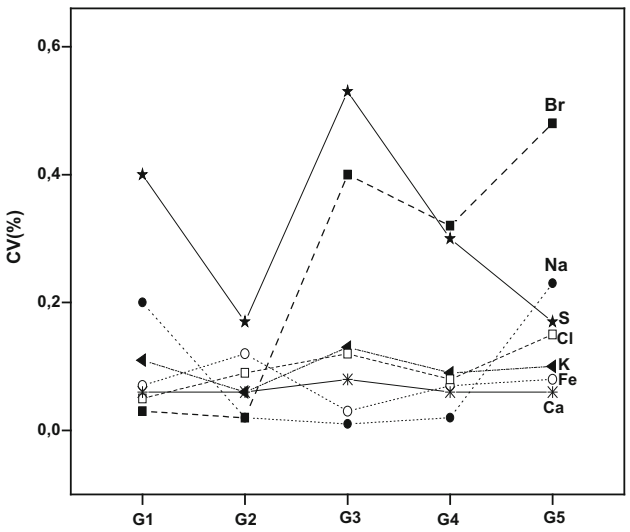

Fig. 1 Coefficient of variation (CV) of Br, Ca, Cl, Fe, K, Na and S concentrations in whole blood samples of immunized mice (G*i*, $i = 1$, 2, 3, 4 and 5)

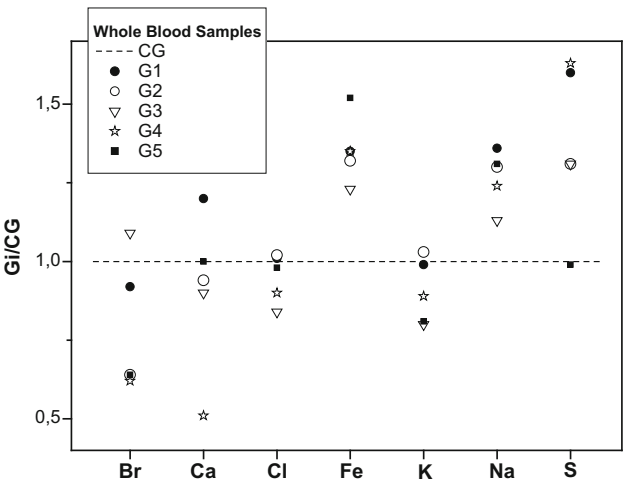

Fig. 2 Comparison of whole blood Br, Ca, Cl, Fe, K, Na and S concentrations between: mice immunized (G*i*, $i = 1$, 2, 3, 4 and 5), and mice not immunized (CG)

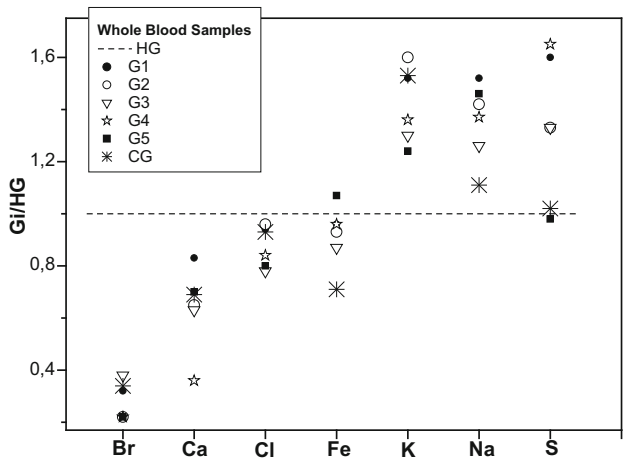

Fig. 3 Comparison of whole blood Br, Ca, Cl, Fe, K, Na and S concentrations between: mice groups (CG and G*i*, $i = 1,2,3, 4$ and 5) and human group (HG)

reasonable agreement with data from previous study [8]: the concentration ranged from 93 ± 7 mg L^{-1} to 246 ± 15 mg L^{-1} with a relative high standard deviation (31 %) which agree with the present data (27 %).

Related to Cl and K concentration no alteration was observed when compared with the CG. All immunized groups are in agreement with the control group with a confidence interval of 68 % (2.15–2.85 g L^{-1} for Cl and 1.89–2.13 g L^{-1} for K). The results of Cl and K are in agreement with other published data [8], which showed average and standard deviation of 2.34 ± 0.18 g L^{-1} for Cl, and 1.89 ± 0.10 g L^{-1} for K. Yet, according to Table 3, the results of Cl from both techniques (INAA and EDXFR) are in reasonable agreement.

For Fe, 80 % of the data from G*i* are near to the upper limit of CG, for confidence interval of 95 % (176–372 mg L^{-1}) usually adopted for checking the clinical status of the organisms, only for G2 (362 ± 44 mg L^{-1}) there is an agreement considering a 68 % of confidence interval (225–323 mg L^{-1}). These results suggest that blood must be monitored for Fe concentrations on immunological therapies.

For Na and S the results in Table 3 revealed high concentration for almost all groups; the exception is for S in G5. For Na, G2, G3 and G4 results are near to the upper limit of CG for a confidence interval of 95 % (0.96–2.20 g L^{-1}). Only for G1 and G5 there are compatibility with a 68 % of confidence interval (1.06–1.78 g L^{-1}) due the highest variability within the same group (2.15 ± 0.42 g L^{-1} and 2.07 ± 0.48 g $L^{-1,}$ for G1 and G5, respectively). Other published data also shown Na concentration in immunized groups with a higher average value (2.00 ± 0.15 g L^{-1}) [8]. Considering that, Na is majority in blood and in *Bothrops* snake venoms [8, 10, 11] which it must be monitored in victims of poisoning because small variations can be lethal. Related to S, the relative standard deviation reflects its variability within the same group (Fig. 2) for G1 (1.06 ± 0.38 g L^{-1}), G3 (0.88 ± 0.47 g L^{-1}) and G4 (1.09 ± 0.33 g L^{-1}). A comparison with CG (0.67 ± 0.16 g L^{-1}) shows that all immunized groups are in agreement for a confidence interval of 68 % (0.51–0.83 g L^{-1}).

Control group and human group

Related to the mice control group the concentration values for Ca (201 ± 10 mg L^{-1}), Cl (2.50 ± 0.35 g L^{-1}), Na (1.58 ± 0.31 g L^{-1}) and S (0.67 ± 0.16 g L^{-1}) are in agreement with human being estimative considering a confidence interval of 68 % (191–211 mg L^{-1} for Ca; 2.15–2.85 g L^{-1} for Cl; 1.27–1.89 g L^{-1} for Na and 0.51–0.83 g L^{-1} for S), while for Br (2.07 ± 0.07 mg L^{-1}) and Fe (274 ± 49 mg L^{-1}) only if considering the confidence interval of 95 % (1.93–2.24 mg L^{-1} and 176–372 mg L^{-1}, respectively). However, for K (2.01 ± 0.12 g L^{-1}) the comparison with human range (0.87–1.75 g L^{-1}) [16] emphasizes an increase. Consequently, there is an agreement only if the confidence interval adopted is 99 % (0.65–1.97 g L^{-1}).

Immunized group and human group

When the comparison is performed between immunized mice and human estimative (Fig. 3), there is a systematic behavior in blood levels: an increase for K, Na and S and a decrease for Br (at least, three times less), Ca, Cl and Fe. Considering that, K and Na are the majority of elements in blood and in *Bothrops* snake venoms ($\sim$g L^{-1}) these elements in blood must be monitored in case of accident. Ca is also important once stringent variations may interfere with the blood coagulation [3, 4]. Another element with significant differences in blood levels is S. It is not evaluated by clinical tests but high concentration in blood can lead to the development of cardiovascular diseases [21]. In addition, it is important to emphasize that the results for the immunized mice groups formed for the most mixed poisons (G3 and G4), are those with changes for all investigated elements, suggesting that the production of a single serum requires monitoring of these elements in mice immunized blood.

Finally, when the comparison is performed between CG and the two groups of *B. atrox*, from Alto Rio Negro (G1) and from Maranhão (G2), it is observed that there is a significant decrease ($p < 0.05$) in G2 for Br and an increase for Na and S ($p < 0.05$). Furthermore, when the comparison performed with human being estimative, there is compatibility only for Cl and Fe (Fig. 3).

Conclusion

The whole blood concentrations of Br, Ca, Cl, Fe, K, Na and S for mice H_{III} and mice H_{III} submitted to difference immunization (*Bothrops* venom) were determined using INAA and EDXFR analytical techniques. These procedures are complementary and both techniques shown to be appropriate for blood analysis. The results of mixing poisons for the immunized mice groups (G3 and G4) are those with changes for all investigated elements. Although there is a systematic increase of K, Na and S in the immunized mice blood levels, none of the results undertakes the serum production by toxicity. However, the variability of the elements investigated in the same immunized group (mainly Br, Ca, Na and S) suggests that they may be monitored in blood in immunological therapies because small variation in blood of patients may cause severe adverse reactions. Moreover, the control group results provide parameters to interpret future investigations of the behavior of these elements in whole blood when the immunization is performed with other *Bothrops* species. Finally, it is important to emphasize that the data from immunized mice groups contribute for the validation process of the anti-*Bothrops* serum, nowadays in preclinical tests.

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References

1. MINISTÉRIO DA SAÚDE (2001) Manual de diagnóstico e tratamento de acidentes por animais peçonhentos, 2ª ed. Fundação Nacional de Saúde, Brasília
2. Jiménez-Porras JM (1970) Biochemistry of snake venoms. Clin Toxicol 3(3):389–431
3. Global Resource for Advancing Cancer Education (2015).http://cancergrace.org/cancer-101/2009/12/22/cancer-and-clotting-protecht. Accessed 05 Feb 2015
4. Rosa RR, Vieira EG, Villarroel MS, Siracura YQ, Iizuka H (1981) Análise comparativa entre os diferentes esquemas de imunização empregados na produção de soros antiofídicos pelo Instituto Butantan. Memórias do Instituto Butantan 45:259–270
5. SINAN/SVS/MS (2015) Boletim Epidemiológico Eletrônico, Junho 2009. http://sinan.saude.gov.br/. Accessed 12 April 2015
6. Butantan Institute (2015). http://www.butantan.gov.br/Paginas/default.aspx. Accessed 05 Feb 2015
7. Furtado MFD, Cardoso ST, Soares OE, Pereira AP, Fernandes DS, Tambourgi DV, Sant'Anna OA (2010) Antigenic cross-reactivity and immunogenicity of *Bothrops* venoms from snakes of the Amazon region. Toxicon 55:881–887
8. Zamboni CB, Metairon S, Suzuki MF, Furtado MF, Sant'Anna OA, Tambourgi DV (2009) Quantitative evaluation of blood elements by neutron activation analysis in mice immunized with *Bothrops* snake venoms. J Radioanal Nucl Chem 282:37–39
9. Baptista TS, Zamboni CB, Medeiros JAG, Freitas MG, Marcelino JR (2009) Blood evaluation of Cl and Na concentration in crioulo breed horses using NAA: comparison with human levels. AIP Conf Proc 1139:160–161
10. Baptista TS, Zamboni CB, Kovacs L, Freitas MG, Marcelino JR (2010) Blood levels of zinc in creole horses used in sera production. AIP Conf Proc 1265:407–410
11. Baptista TS, Zamboni CB, Marcelino JR (2011) Analysis of Cl and Na hyper immune sera by NAA. AIP Conf Proc 1351:332–335
12. Baptista TS, Redígolo MM, Zamboni CB, Sato IM, Marcelino JR (2012) Comparative study of inorganic elements determination in whole blood from Crioula breed horse by EDXRF and NAA analytical techniques. J Radioanal Nucl Chem 291:399–403
13. Sant'Anna OA, Mouton D, Ibanez OM, Bouthillier Y, Mevel JC, Reis MH, Biozzi G (1985) Basal immunoglobulin serum concentration and isotype distribution in relation the polygenic control of antibody responsiveness in mice. Immunogenetics 22:131–139
14. Wolfgang W, Salomão MG, Quijada-Mascareñas JA, Thorpe RS (2002) Biology of the Vipers. Origin and evolution of the South American pit viper fauna: evidence from mitochondrial DNA sequence analysis. Eagle Mountain Publishing, Eagle Mountain, pp 111–128
15. Medeiros JAG, Zamboni CB, Zahn GS (2006) Software para realização de análises hematológicas utilizando processo radioanalítico. In: Conference proceedings of 39° Brazilian Congress of Clinical Pathology/Medicine Laboratorial CD ROM
16. Ressler T (1998) WinXAS: a program for X-ray absorption spectroscopy data analysis under MS-Windows. J Synchrotron Radiat 5:118–122
17. Oliveira LC, Zamboni CB, Mesa J (2006) Quantitative estimation of Br, Cl, K and Na in sample blood by NAA. J Radioanal Nucl Chem 269:541–545
18. Zamboni CB, Oliveira LC, Kovacs L, Metairon S (2012) Ca and Mg determination from inhabitants of Brazil using neutron activation analysis. J Radioanal Nucl Chem 291:389–393
19. Zamboni CB, Metairon S, Rizzutto MA, Bernades S (2014) The use of portable X-Ray fluorescence spectrometry (PXRFS) for clinical practices. Conf Proc X LASNPA 1:1–6
20. Oliveira LC, Zamboni CB (2013) Sulfur determination in blood from inhabitants of Brazil using neutron activation analysis. AIP Conf Proc 1529:73–75
21. Ingenbleek Y, Kimura H (2013) Nutritional essentiality of sulfur in health and disease. Nutr Rev 71(7):413–432