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ANALYSES OF HEMOLYMPH FROM *AMBLYOMMA CAJENNENSE* (ACARI: IXODIDAE) USING NAA

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ABSTRACT

Instrumental Neutron Activation Analysis technique (INAA) was applied to determine the elemental composition of hemolymph from *Amblyomma cajennense* tick. This biological material came from Butantan Institute (São Paulo city, Brazil) and it was investigated using the IEA-R1 nuclear reactor (4MW, pool type) at IPEN/CNEN-SP - Brazil. The concentration values for: Br (0.0032 ± 0.0005gL^{-1}), Ca (0.104 ± 0.029gL^{-1}), Cl (4.41 ± 0.25gL^{-1}), I (76 ± 27μgL^{-1}), K (0.38 ± 0.09gL^{-1}), Mg (0.038± 0.011gL^{-1}), Na (4.30 ± 0.26gL^{-1}) and S (1.35± 0.37gL^{-1}) were determined for the first time. These data were compared with the concentration values established for Americanum and Anatolicum Excavatum tick species to clarify the ion balance in this biological material (hemolymph). This comparison suggests that Na concentration, majority in these species, has a similar behavior. These data also contribute to the understanding of hemolymph composition complementing its characterization as well as for the understanding of several physiological processes, especially those related to salivary secretion.

1. INTRODUCTION

The tick *Amablyomma cajennense* is considered one of the most important and widespread species in Brazil. It belongs to the hard tick's family (*Ixodidae)*, one of the most prevalent species in the Neotropic region [1]. In the last years several investigations have been performed in its salivary secretion. Particularly, in Brazil, the tick *Amablyomma cajennense* is target of several important research such as, investigations in the biocenology field [2,3], as the vector of diseases [4], as a source for the development of new molecules focusing on anticoagulation (Factor X activate inhibitor, platelet inhibitor) [5,6] and also as antitumor agents [7,8]. Recently, NAA technique was applied to investigate the saliva of this species [9] and now we intend to determine the elemental composition of the hemolymph this specie also using the INAA technique to complement its biochemistry characterization.

2. MATERIAL AND METHODS

The biological material came from Butantan Institute (São Paulo city, Brazil). The experiments were carried out in accordance with protocol approved by the Committee of Ethics of the Butantan Institute (548/08).

To obtain this body fluid it is necessary to keep a colony of several adult (females/males). For this investigation 30 females and 15 males were infested in domestic rabbits, *Oryctolagus cuniculus*, to be fed. After two weeks these ticks were removed and the hemolymph was collected. The hemolymph was obtained through a perforation (puncture) in the cuticle Aloscutum using a 30 gauge needle coupled to calibrated capillaries.

For this investigation the collection procedure was repeated two times. Samples of 500 µL (in duplicate) were lyophilized and transferred to a polyethylene tube (cylinder). Aliquots of standard solutions were transferred to Whatman filter paper (~1.5 cm^2), using a calibrated micropipette, and dried using an infrared lamp. Sample and standard were irradiated in the IEA-R1 nuclear reactor at IPEN/SP (2-4MW, pool type) for short time irradiation, in a thermal neutron flux of 4.8 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$, and for long time irradiation in a thermal neutron flux of $8.5 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$. Each sample was measured at least twice.

For ^{38}Cl ($T_{1/2}$=37 min, E_γ =1642 keV) and ^{24}Na ($T_{1/2}$=15h, E_γ =1368 keV) determination an irradiation time of 1 minutes followed by 4 minutes of counting time was used. For ^{80}Br ($T_{1/2}$ ~ 16 min, E_γ = 616 keV), ^{49}Ca ($T_{1/2}$ ~ 9 min, Eγ = 3098 keV), ^{128}I ($T_{1/2}$ ~ 25 min, Eγ = 443 keV), ^{27}Mg ($T_{1/2}$ ~ 9 min, Eγ = 1012 keV), ^{42}K ($T_{1/2}$ ~ 12h, E_γ = 1525 keV) and ^{37}S ($T_{1/2}$ = 5 min, E_γ = 3104 keV) sample and standard were irradiated for 4 minutes and after a decay time of 1 minute they were counted by 15 minutes for Br, Ca, Mg and S determination followed by 2 hours of counting for I and 3 hours for K. For ^{59}Fe ($T_{1/2}$ ~ 44.5d, E_γ =1099 keV and 1291 keV) determination an irradiated for 8 hours, decay time of 4 days followed by 4 hours of counting time was used. ^{81}Br ($T_{1/2}$ ~ 35h, E_γ = 776 keV) and ^{47}Ca ($T_{1/2}$ ~ 4.5 d, E_γ = 1297 keV) isotopes were also determined by long irradiation time using a decay time of 1 day and counting time of 2h.

A γ- spectrometer system with an ORTEC detector (Model GEM-60195, FWHM=1.89 keV), calibrated for energy through the measurements of standard sources of $Co^{56,60}$ and Eu^{152}, coupled to a MCA ORTEC (Model 919E) and connected to a PC, was used to measure the induced gamma-ray activity. The background radiation was reduced by employing the iron shield described by Medeiros et al [10]. The concentrations of the elements were calculated using in-house software [11]. The quality of analytical results was evaluated by analyzing the NIST 8414 Bovine Muscle Powder and IAEA A-13 Animal Blood certified reference materials. The filter paper and polyethylene tube were also analyzed using the same irradiation conditions.

3. RESULTS AND DISCUSSION

The accuracy evaluation by Z-score test ($Z < |2|$) for NIST 8414 Bovine Muscle Powder and IAEA A-13 animal blood certified reference materials indicate the adequacy of the method. Some impurities such as Al, and Ca, Cl and Na were identified in the filter paper and Mg in the polyethylene tube, but they do not interfere.

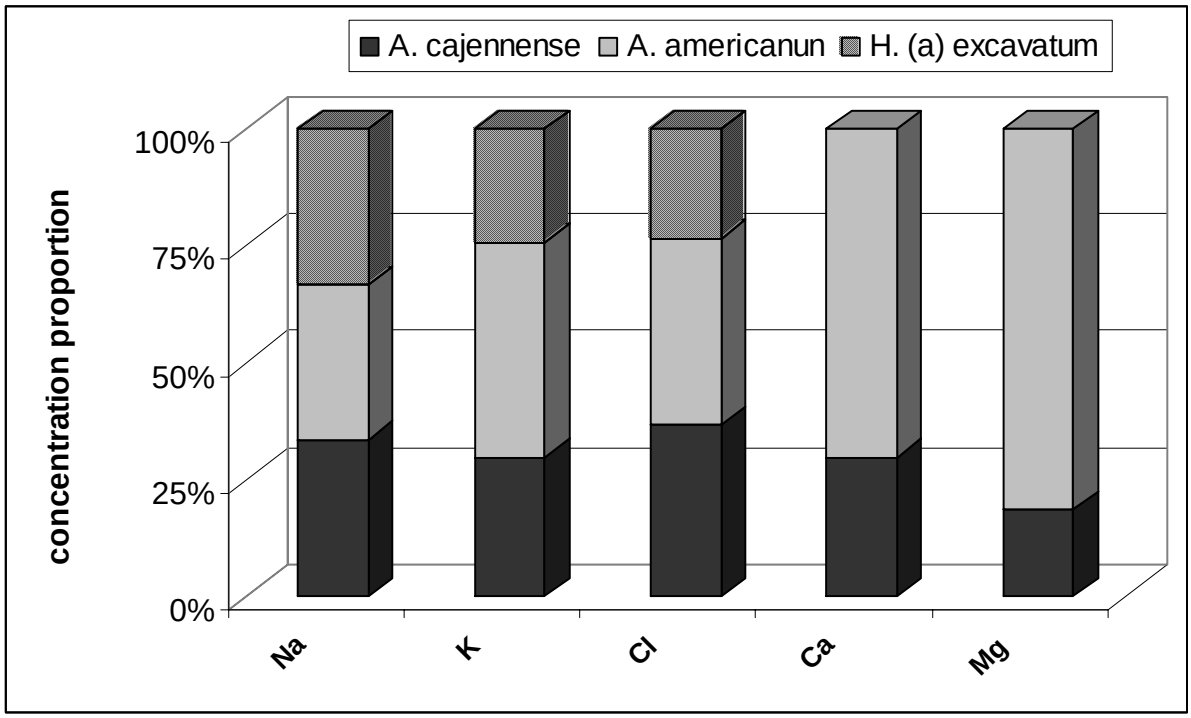
The Br, Ca, Cl, I, K, Mg, Na and S concentration values determined in hemolymph samples are presented in Table 1. The mean values with associated error, represented by one standard deviation (1σ), were compared to the values established for *A. americanum* [12] and *H. (a). excavatum (Hyalomama Anatolicum Excavatum*) [13] species from *Ixodidae* tick's family. These comparisons are summarized in Figure 1.

Table 1. Elemental concentrations in hemolymph of *Amblyomma cajennense* specie using INAA.

Elements	Mean ± 1σ	Min	Max	Confidence Interval (95%)
Br, mgL^{-1}	3.2 ± 0.5 3.0 ± 0.4[LI]	1.7	4.6	2.2 - 4.2
Ca, mgL^{-1}	104 ± 29 110 ± 29[LI] 255[c]	99	134	46 – 168
Cl, gL^{-1}	4.41 ± 0.25 4.79 ± 0.96[a] 2.80[b]	3.64	5.68	3.91 – 4.91
I, µgL^{-1}	76 ± 27	60	85	22– 110
K, gL^{-1}	0.38 ± 0.09 0.59 ± 0.12[a] 0.31[b]	0.32	0.40	0.20 – 0.56
Mg, mgL^{-1}	38.0 ± 1.1 167[c]	30.0	45.1	35.8 – 40.2
Mn, mgL^{-1}	0.21 ± 0.03	13.6	24.7	0.15– 0.27
Na, gL^{-1}	4.30 ± 0.26 4.23 ± 0.85[a] 4.29[b]	4.09	5.13	3.78 – 4.82
S, gL^{-1}	1.35 ± 0.37	0.78	1.46	0.61 – 2.09

[a] data from *A. americanum* specie [12]
[b] data from *H. (a). excavatum* specie [13]
[c] data from *A. americanum* specie [14]
[LI]: long irradiation time
min: minimum concentration value determined
max: maximum concentration value determined

Figura 1. Concentration proportions between *Ixodidae* species.



According to Table 1, the concentration value for Na obtained for *A. cajannense* ($4.30gL^{-1}$) is in agreement with the *A. americanum* ($4.30gL^{-1}$) and also with *H. (a) excavatum* ($4.29gL^{-1}$) species. Then, the high concentration of Na in hemolymph appears to be feature in these *Ixodidae* species. This behavior was also identified in investigation performed by Rehacek and Brzostowski [15] and by Tatchell [16] in others hard tick's family (*Ixodidae).* Cl is also majority in this fluid [13,15,16] and its concentration in *A. cajannense* specie ($4.41gL^{-1}$) is in agreement with data from *A. americanum* ($4.79gL^{-1}$). Related to K concentration values, there is a better agreement between results from *A. cajannense* ($0.38gL^{-1}$) and *H. (a) excavatum* ($0.31gL^{-1}$) species. For Ca and Mg the concentration results can be only compared with *A. americanum* species but they are quite different. All these comparisons are summarized in Figure 1.

Br and Ca were determined by short and long irradiation time and the results were included in Table 1. Related to Fe, although it has been identified, it was not quantified due the quantity of biological material available was not enough to produce good statistics.

The comparative analyses of hemolymph from species (*A. cajannense, A. americanum and H. (a) excavatum)* suggest that Na, K and Cl concentrations have a similar behavior.

4. CONCLUSIONS

For the first time NAA technique was applied to determine elemental composition (Br, Ca, Cl, I, K, Mg, Mn, Na and S) in hemolymph from *Amblyomma cajennense* tick. These data contribute to the understanding of it hemolymph composition, complementing its characterization, and adding expertise in several research areas such as formulation of vaccine as well as therapeutic targets.

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