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Analysis of the coxal fluid of *O. brasiliensis* tick species by analytic techniques

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Abstract: The coxal fluid of the tick species *Ornithodoros brasiliensis* (*O. brasiliensis*) was subjected to detailed chemical analysis using Instrumental Neutron Activation Analysis (INAA) and Energy Dispersive X-ray Fluorescence (EDXRF). These complementary techniques allowed the identification and quantification of key elements such as sodium (Na), chlorine (Cl), potassium (K), and zinc (Zn), along with others like sulfur (S), phosphorus (P), and iron (Fe). The study demonstrated the consistency between the two analytical methods and revealed significant data about the tick's physiological adaptations related to fluid excretion and blood meal processing. Additionally, the absence of heavy metals in the analyzed samples was confirmed. Understanding the composition of coxal fluid is vital for interpreting metabolic processes and exploring its toxicological potential, given the aggressive nature of *O. brasiliensis*, known to cause severe health impacts on humans and animals. This research contributes to advancing knowledge about tick biology and supports the development of public health measures against tick-borne hazards.

Keywords: *Ornithodoros brasiliensis*, coxal fluid, INAA, EDXRF, elemental analysis.

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Análise do líquido coxal da espécie de carrapato *Ornithodoros brasiliensis* por técnicas analíticas

Resumo: O líquido coxal do carrapato Ornithodoros brasiliensis (O. brasiliensis) foi analisado detalhadamente por meio das técnicas de Análise por Ativação Neutrônica Instrumental (INAA) e Fluorescência de Raios-X por Energia Dispersiva (EDXRF). Essas técnicas complementares permitiram a identificação e quantificação de elementos essenciais como sódio (Na), cloro (Cl), potássio (K) e zinco (Zn), além de outros como enxofre (S), fósforo (P) e ferro (Fe). Os resultados mostraram a consistência entre os métodos analíticos empregados, além de confirmarem a ausência de metais pesados nas amostras analisadas. A caracterização do líquido coxal é fundamental para o entendimento dos processos metabólicos e para avaliar seu potencial toxicológico, considerando que O. brasiliensis é uma espécie agressiva, com impactos graves à saúde humana e animal. Este estudo fornece informações importantes para compreender a biologia do carrapato e apoia medidas de saúde pública relacionadas a riscos associados a doenças transmitidas por carrapatos.

Palavras-chave: *Ornithodoros brasiliensis*, líquido coxal, INAA, EDXRF, análise elementar.

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1. INTRODUCTION

Ticks are one of the most important groups of hematophagous parasitic arthropods of domestic animals, wild animals and humans. They are mites belonging to the class Arachnida, subclass Acari and order Ixodida. In all their active stages they are harmful to the host, through the skin lesion caused by the bite and the transmission of a variety of pathogenic microorganisms. It is a group with around 954 described species, the Order Ixodida comprises three current Families, Ixodidae (736 spp.), Argasidae (218 spp.) and Nuttalliellidae (monospecific) [1-4].

In ixodidae (hard ticks), water vapor absorption occurs when the ticks are in a free-living phase (not parasitizing), and excess fluid is excreted to concentrate the ingested blood. This is performed by the salivary glands [4]. Anatomically, this organ consists of three types of acini in females and four in males [5-6]. On the other hand, in argasids (soft ticks), the coxal glands perform this function, excreting excess fluid (coxal fluid) to concentrate the ingested blood. The coxal gland functions as an ultrafiltration system, retaining most of the hemolymph proteins and other organic compounds while allowing the removal of salts and water, thus maintaining osmotic balance given that one of the characteristics of this tick family is rapid engorgement (the act of feeding on blood) within around 30 minutes, whereas ixodid ticks remain attached to the host's skin for days, and anatomically, the salivary gland in argasid ticks has only two acini [4, 7-9]. Evidence has shown the presence of microorganisms, such as rickettsiae, in coxal fluid samples [10], suggesting that this route may serve as a pathway for microbial transmission in ticks [11]. Additionally, it has been observed that coxal fluid can modulate the proliferation or inhibition of certain microorganisms [12].

Ornithodoros brasiliensis Aragão, is an argasid tick that is very aggressive towards humans, causing severe pain, fever and an intense inflammatory response, forming deep wounds at

the site of the bite. Symptoms can also include malaise, headache and even the development of toxic tick syndrome, notably, in a study conducted by Ramirez et al., 2011 [13], specimens of *O. brasiliensis* infected with *Rickettsia belli* were detected, becoming a public health problem in southern Brazil [14], and in dogs paralysis of the lower limbs has been reported, followed by death [15]. Due to the severity of the problems resulting from its bite, it is essential to highlight the importance of the various studies already conducted on this species [16-20]; however, data on the elemental composition of the coxal fluid are still lacking.

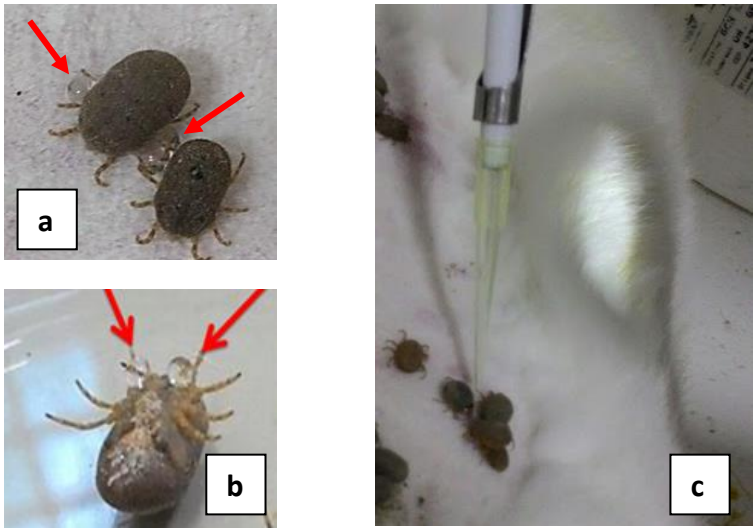
Since, the number of accidents caused by this tick has increased. These facts have stimulated the investigation of their secretions for the understanding of the many physiological processes. In this investigation, coxal fluid samples were investigated by Instrumental Neutron Activation Analyses (INAA) and Energy Dispersive X-Ray Fluorescence (EDXRF) techniques aiming to get a detailed description of its elemental composition. This knowledge contributes for tick coxal fluid characterization, for interpretation of the metabolic processes as well as to evaluate its toxicological potential.

2. MATERIALS AND METHODS

The colony of O. brasiliensis ticks is maintained in B.O.D. (Biochemical and Oxygen Demand) incubators in the Parasitology and Entomology Laboratory at the Butantan Institute, under controlled temperature (21°C ± 1°C) and humidity (RH > 85% ± 10%). The ticks were fed on New Zealand rabbits (Oryctolagus cuniculus) following the protocol submitted to the Ethics Committee of the Butantan Institute (CEUAIB N°4130270323) in the Parasitology Laboratory's animal facility at the Butantan Institute (IBu). The rabbits' backs were shaved, followed by sedation with ketamine (30-40 mg/kg), administered intramuscularly in the cranial region of the biceps femoris. After sedation, the ticks were transferred to the shaved area in groups, where they remained until fully engorged, which

took approximately 40 minutes. It is known that Argasidae ticks naturally secrete coxal fluid during or after engorgement (Figure 1.a and 1.b). The coxal fluid was collected using a micropipette and immediately placed in 1.5 ml microtubes (Figure 1.c), frozen (on dry ice) and lyophilized. The collection (2 ml) was performed monthly for 3 months. Duplicate samples (70–100 mg) were prepared.

Figure 1: Collection of coxal fluid: Excess secretion from the coxal gland during (a) and after feeding (b), and collection of the coxal fluid using a micropipette (c). (Red arrows indicate secreted coxal liquid).



2.1 EDXRF analysis

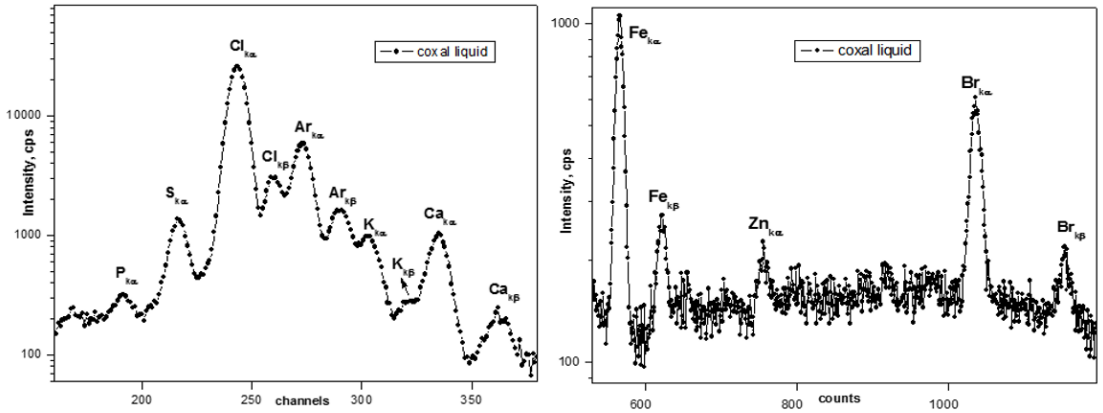
For EDXRF analysis, the samples (~20 mg) were placed inside a sample holder (with 6.3 mm in the diameter) and excited and measured using a XRF spectrometer (X-123 SDD, Amptek®, Figure 2). The instrumental measurement conditions are shown in Table 1 and a representative X-ray spectrum of the coxal fluid sample is given in Figure 3a and 3b.

Figure 2: X-ray Spectrometer, X-123SDD (Amptek®)

Table 1: EDXRF measurements conditions

Parameters	Conditions
X ray	Ag target
Voltage	30 kV
Fixed Current	5 μA
Atmosphere	Air (without vacuum)
Detector	Si Drift (SDD)
Counting Time	300 s
Emission line: Element (k_α , in keV)	P(2.0); S(2.3); Cl(2.6); K(3.3); Ca (3.6); Fe(6.4); Zn(8.6); Br(11.9)

Figure 3: X-ray spectrum of the coxal fluid sample.



2.2 INAA analysis

In the INAA analysis, sample and standard solution are irradiated simultaneously. Each sample was weighed (80–100 mg) and sealed into a polyethylene capsule and irradiated in the IEA-R1 nuclear reactor at IPEN-CNEN/SP (3.5– 4.5 MW, pool type). The elements Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, S, and Zn were activated using the thermal neutrons. Potential interference from $^{27}Al(n,\gamma)$ ^{28} Al and ^{27} Al (n,p) ^{28} Al nuclear reactions in P concentration results, by ^{31} P(n, α) ^{28} Al, was checked comparing the INAA results with EDXRF, using k_α line from P (2.015 keV). The instrumental measurement conditions are shown in Table 2. The elements Na, Mg and Mn were determined only by INAA. The measurements of the gamma induced activity of the samples and standard were carried out using an ORTEC Model, GEM- 60195 detector, and an ORTEC 671 amplifier coupled to an MCA ORTEC 919E connected to a PC. The concentration of each element in each sample was obtained by using in-house software.

Table 2: INAA measurements conditions

Element (isotope, $T_{1/2}$)	Eγ keV	Ti: Td: Tc
Br (Br^{80}, 16 min)	616	300 s: 60 s: 900 s
Ca (Ca^{40}, 9 min)	3098	300 s: 60s: 600 s
Mg (Mg^{27}, 9 min)	842 and 1012	300 s: 60s: 600 s
K (K^{42}, 12 h)	1525	300 s: 1h: 4h
Mn (Mn^{56}, 2.6 h)	846 and 1810	300 s: 1h: 4h
Cl (Cl^{38}, 37 min)	1642	60 s: 60 s: 600 s
Na (Na^{24}, 15 h)	1368	60 s: 60 s: 600 s
P (Al^{28}, 2 min)	1778	60 s: 60 s: 300 s
S (S^{37}, 5 min)	3104	60 s: 60 s: 300 s
Fe (Fe^{59}, 44.5 d)	1099	8 h: 7–10 d: 8–24 h
Zn (Zn^{65}, 244 d)	1116	8 h: 7–10 d: 8–24 h

Ti: irradiation time; **Td:** decay time; **Tc:** counting time

3. RESULTS AND DISCUSSIONS

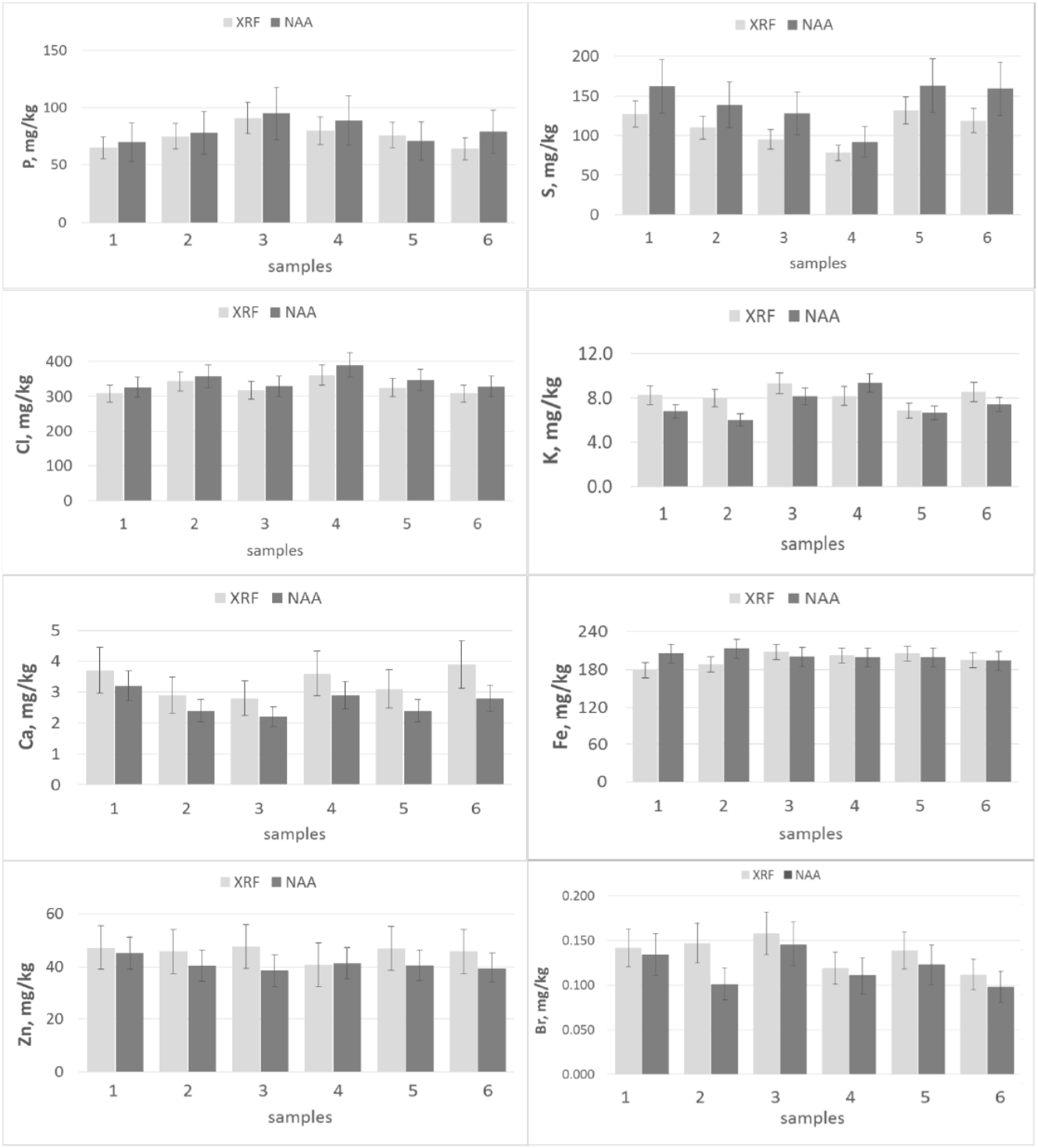
The elements concentrations determined in the coxal fluid samples are presented in Table 3 by the mean values (MV) with associated error, represented by one standard deviation (1SD). For visualization, in Figure 4, concentration results are presented for both techniques.

Table 3: Element concentrations in coxal fluid of *O. brasiliensis* species by INAA and EDXRF techniques

Elements	INAA *Min/Max*	EDXRF *Min/Max*
n=6	**MV ± 1SD**	
Br, mg/g	0.119 ± 0.017 0.098/0.145	0.136 ± 0.016 0.112/0.158
Mg, mg/g	1.55 ± 0.38 0.98/1.94	ND
Mn, µg/L	2. 13 ± 0.77 1.02/2.88	ND
Na, mg/g	297 ± 25 244/323	ND
K, mg/g	7.4 ± 1.1 6.0/9.4	8.2 ± 0.7 6.8/9.3
Cl, mg/g	346 ± 22 326/389	327 ± 19 308/361
P, mg/g	80 ± 9 70/95	75 ± 9 64/91
Ca, mg/g	2.7 ± 0.3 2.2/3.2	3.3 ± 0.4 2.8/3.9
S, mg/g	141 ± 25 92/162	110 ± 19 78/132
Fe, mg/g	202 ± 6 194/213	196 ± 9 179/208
Zn, mg/g	40.8 ± 2.1 38.5/45.1	45.6 ± 2.3 40.7/47.5

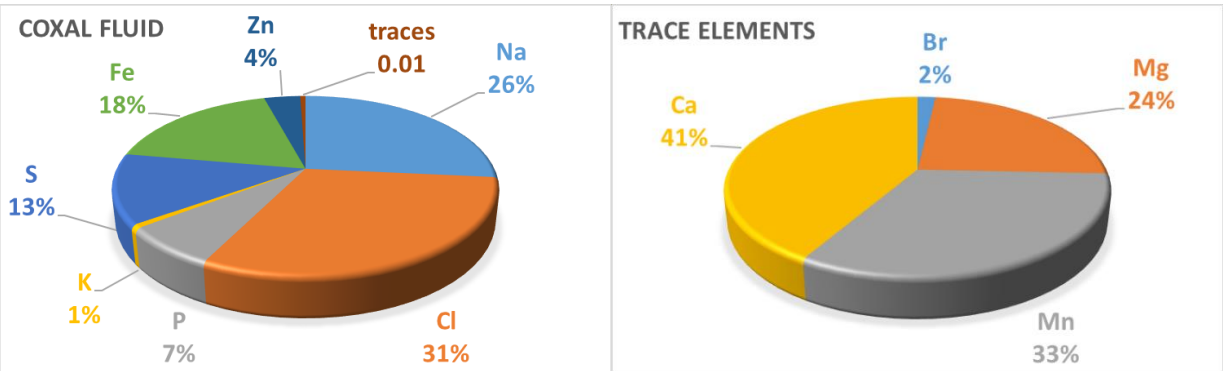
n: number of samples; **MV**: mean value; **SD**: Stander Deviation; **ND**: not determined; **Min**: minimum; **Max**: maximum.

Figure 4: Comparison of elemental concentration for coxal fluid samples by X-ray Fluorescence (XRF) and Neutron Activation Analysis (NAA)



The student's t-test was applied for results comparison between EDXRF and INAA techniques. The results for Br, P, Cl, K, Ca, Fe, and S were considered statistically equal ($p > 0.05$). Yet, according to Figure 5, using data from NAA, the presence of heavy metals was not observed.

Figure 5: The concentration of elements (expressed as a percentage) in the coxal fluid of the tick species *O. brasiliensis* by INAA



4. CONCLUSIONS

In this study, the investigation of the elemental coxal fluid of *O. brasiliensis* tick species was performed by Neutron Activation Analysis (NAA) and X Ray Fluorescence (XRF) techniques. These techniques are in good agreement and present a detailed description of the elemental composition. The knowledge of these data contributes to the physicochemical and biological characterization of the tick's coxal fluid, to the interpretation of metabolic processes, as well as to the assessment of its toxicological potential.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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