Cardiovascular profile of crab-eating foxes (*Cerdocyon thous*) in captivity anesthetized with ketamine and midazolam

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Short Report

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Abstract

The cardiac evaluation of wild animals is still a wide and largely unknown field for several species. Therefore, through complimentary examinations such as radiography, echocardiography and serum troponin levels, this study aimed at describing the values observed in 12 crab-eating foxes (*Cerdocyon thous*) anesthetized with a combination of intramuscular ketamine and midazolam. Through the use of this chemical restraint, a clinical, radiographic and echocardiographic (linear and indexed values in M-mode) evaluation of the 12 male specimens included in the sample group. Among the findings observed in comparison with domesticated dogs were a decrease in the dimensions of the septum, wall and cavity of the left ventricle, as well as a deceleration in the E wave (EDT), which could be correlated with the maintenance of the serum troponin dosage values (cTnI). Therefore, M-mode echocardiography has proven to be safer and comparative to other species of wild canids when performed through indexed values. In addition, when evaluating the systolic function and segmentary contractions, the anesthetic combination did not have any effects on the results of complementary examinations performed in crab-eating foxes (*Cerdocyon thous*) included in this study.

Introduction

One of the six species of wild canids living within the Brazilian territory, crab-eating foxes (*Cerdocyon thous*) have gained notoriety due to their distribution across the different biomes found in Brazil and other south American countries. This distribution may be correlated to the species' dietary and reproductive adaptation, despite the closer contact with humans (Beisiegel 1999; Jorge & Jorge 2014; Reis 2011).

Nutritionally incomplete diets that promote metabolic deficiencies may predispose these animals do acquired cardiopathies (Freeman & Rush 2007), while congenital cardiopathies may indicate endogamic mating.

But there are very few reports of cardiac evaluations in wild canids, and the number becomes even more scarce when filtering the search specifically for *Cerdocyon thous*. However, due to the similarities in the execution and interpretation of imaging examinations in wild canids and domestic dogs (Jorge & Jorge 2014), this study sought to perform and correlate complementary examinations in healthy *C. thous* in order to aid in the diagnosis and treatment of possible cardiopathies in animals living in captivity or in the wild. The examinations performed were the measurement of the systemic arterial blood pressure, a chest radiograph (VHS, VLAS and RLAD), an echocardiogram, and the measurement of serum troponin dosage (cTnI) after the application of anesthesia with ketamine (10 mg/kg) and midazolam (1 mg/kg).

Materials And Methods

Animals

This study performed the evaluation of 12 male crab-eating foxes (*Cerdocyon thous*) of varied ages weighing between 4.32 and 7.44 kg forwarded to the Wild Animal Medicine and Research Center (CEMPAS, *Centro de Medicina e Pesquisa de Animais Selvagens*) by the Environmental Police after rescue and capture missions around the city of Botucatu, Brazil. Authorization by IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) was acquired under registration SISBio 68707-1 for eight of the animals, and SISBio 51767-1 for the other four. The animals were only included in the study after the results of tests such as full blood count, blood sugar levels, kidney biochemistry (urea, creatinine, calcium and phosphorus), liver biochemistry (total cholesterol and triglycerides) and urinalysis (Jorge & Jorge 2014) were within the reference standards for the species. Radiographs of the chest and abdomen, as well as an electrocardiogram were also taken.

After dry fasting for 12 hours, the canids were restrained physically with a net and then chemically with a combination of ketamine (10 mg/kg) and midazolam (1 mg/kg) in the same syringe applied intramuscularly, with the due anesthetic monitoring of heart rate, respiratory rate, capillary refill time, coloration of the mucosae, rectal temperature and systolic arterial blood pressure through the Doppler method. After capture and sedation, the following examinations were performed: measurement of the systolic arterial blood pressure, measurement of the thoracic circumference, chest radiograph, echocardiograph and blood collection for the serum troponin dosage (cTnI) restraint during an interval of 40 to 60 minutes in average, without any complications during the anesthetic or recovery periods.

Measurement of the systemic arterial blood pressure

The systemic arterial blood pressure was measured by the Doppler Ultrasound method following the guidelines for domestic dogs, with the reference values oscillating between 110 and 140 mmHg (Acierno et al. 2018).

Chest radiograph

The animals were positioned accordingly for three radiographic projections (left and right lateral, and ventrodorsal) with a digital x-ray equipment (DR-F, GE Health Care Unit, USA) with a 150kVp/500mA high frequency generator at the Diagnostic Imaging Service of FMVZ – Unesp Botucatu.

Therefore, the Vertebral Heart Size (VHS) (Buchana & Bucheler 1995), the Radiographic Left Atrial Dimension (RLAD) (Salguero et al. 2018), and the Vertebral Left Atrial Size (VLAS) (Malcolm et al. 2018) were measured in the right lateral projection.

Echocardiogram

Based on the reference values described by Boon (2011) and with the animals positioned in right and left lateral decubitus, the echocardiogram was performed with an ultrasound device (M-turbo Sonosite - Fujifilm®) with Doppler function and a 2–8 MHz multi-frequency sectorial transducer in 2D Mode. The animals were monitored with the electrocardiogram during the echocardiographic examination.

After obtaining the dimensions of the heart, the indexed values were calculated by dividing the linear body weight values raised to a fixed exponent (with different values for the linear values), according to the values described for adult dogs (Cornell et al. 2004).

Serum troponin dosage (cTnI)

The blood was stored in individual, previously identified tubes without anticoagulant and frozen until the serum troponin dosage (cTnI) assay was performed. The assays were performed using the automated immunoenzymatic method mini-VIDAS® manufactured by Biomeriux. The technique employed was the Enzyme-Linked Fluorescent Assay (ELFA).

The clinical parameters and radiographic, echocardiographic and immunoenzymatic values from eight specimens of *Cerdocyon thous* were added to data previously collected from another four animals using the same directives, devices and inclusion/exclusion criteria mentioned in this study, totaling a sample group of 12 different *C. thous* specimens.

Statistical analysis

Descriptive statistics were calculated for the data obtained in order to obtain the mean $\pm$ standard deviation, maximum and minimum values, and confidence interval. Data normality was verified by the Shapiro-Wilks test. The aforementioned calculations were done using the software Excel and R.

Results

All experimental procedures were performed after approval in the Ethics Committee for Animal Use (CEUA, *Comit de tica no Uso de Animais*) of the School of Veterinary Medicine and Animal Science at the So Paulo State University, campus Botucatu (CEUA protocol no. 0183/2019).

Chest radiograph

For the analysis of quantitative parameters, the mean $\pm$ standard deviation, maximum and minimum values, and the confidence intervals for VHS, short and long axes, VLAS and RLAD are shown in Table 1.

Echocardiogram

The weighting the animals, measuring the heart rate (HR), systolic arterial pressure (SAP), echocardiographic measurements obtained using the Bidimensional Mode, M-Mode and the values obtained in the Doppler mode (Table 2), and the allometric measurements were calculated using the values obtained in M-Mode (Table 3). No arrhythmias were observed during the evaluation of the cardiac rhythm, and the sinus rhythm was predominant across the entire sample group.

Serum troponin

The serum troponin dosage assays of the 12 animals evaluated revealed levels below 0.01ng/ml. It was impossible to calculate the descriptive statistics and correlate the parameters since the values are within

the reference values for healthy domestic dogs (<0.01 to 0.03ng/ml) (O'Brien et al. 2006).

Discussion

Starting the clinical evaluation by weighing the animals, the mean and standard deviation for the 12 specimens of *C. thous* was 6.2 ± 0.96 kg, which is within the reference values for body mass described in the literature (4.5 to 8.5kg) (Jorge & Jorge 2014). The HR was also with the reference standards described in the literature (Camacho & Mucha 2014) for small domestic dogs, which correlate with the weight of the wild canids. On the other hand, SAP presented mean values within the reference range proposed for dogs with the Doppler method (Acierno et al. 2018) and were close to the systolic arterial pressure values measured in a population of 150 domestic dogs with the *petmap*[®] method (Tebaldi et al. 2012). However, after adding the standard deviation to the mean, the upper values exceed the acceptable threshold. These values may be correlated to increased systolic arterial pressure values in three animals included in the study, which increased the mean for the 12 animals. Therefore, we need to consider a possible discordance between systolic arterial pressure values exceeding 140 mmHg in comparison with values obtained through invasive methods (Garofalo et al. 2012).

The wild canids evaluated in this study presented VHS values within the reference standards, presenting a mean and standard deviation of 8.22 ± 0.55 vertebrae, a result that is close to the VHS values of 8.27 ± 0.48 observed in maned wolves (*Chrysocyon brachyurus*) (Estrada et al. 2009). The VHS evaluation in this study was performed by a single observer in the right latero-lateral projection, but it is important to highlight a possible interobserver difference reported in both healthy and sick dogs (1.05 ± 0.32 vertebrae) (Hansson et al. 2005).

Other innovative quantitative radiographic methods such as VLAS and RLAD are based on the specific evaluation of the left atrium, taking as reference the physiopathology of cardiac remodeling in myxomatous mitral valve disease, which is the most recurrent cardiopathy in domestic dogs. Therefore, considering reference values described in the literature of $\leq 2.3v$ and $\leq 1.8v$ respectively for VLAS and RLAD, the sample group of *C. thous* were within normality, presenting values of $1.77v \pm 0.20v$ for VLAS, and $1.25v \pm 0.18v$ for RLAD.

Starting the echocardiographic examination by positioning the transducer in the thorax of the animals, we noticed a higher degree of cardiac horizontality in *Cerdocyon thous* than in imaging examinations performed in domestic dogs, which is correlated to the different thoracic conformations in the species. However, the formation of echocardiographic images through different windows, axes and views in *C. thous* was similar to that of domestic felines. Progressing to the subjective analysis in bidimensional and colored Doppler modes, we did not observe engorged chambers, regurgitations, communications or stenoses in the 12 specimens of *C. thous* evaluated.

In the echocardiographic evaluation in M-Mode and B-Mode, the analyses were compared to the weight of the animals. When comparing only the echocardiographic findings to the values observed in the literature

for mean weight, LVd, IVSs, and the diameter of the root of the aorta obtained in bidimensional mode and M-Mode were lower in comparison to domestic dogs.

However, when we exclude weight as a variable through the allometric equation (Cornell et al. 2004), the values for left ventricular diameter in systole and diastole were lower (Table 3).

In the evaluation of the transmitral, aortic and pulmonary flows (Table 2), it is possible to note a reduction in EDT and the fusion of the E and A waves in three animals, both possibly happening due to the increased heart rate observed. Therefore, the statistical analysis of the velocity of the A wave and of the E/A ratio was calculated for nine animals, excluding those that presented the aforementioned fusion of the waves referring to the transmitral flow.

The subjective analysis of segmental contractions did not note any complications related to the action of the anesthetic agents applied and noticed that the values for left ventricular fractional shortening (LVFS) by the Teicholz method and for the ejection fraction (EF) were maintained. The evaluation of a maned wolf (*Chrysocyon brachyurus*) under the same protocol (Mantovani et al. 2012) presented similarities in the assessment of the systolic function in M-Mode, arterial flow, pulmonary flow and transmitral flow. The animal in question was described to weight 29 kg and, after adjusting the weight and the measurements obtained in the allometric scale (Cornell et al. 2004), the values were similar to those observed in this study.

Therefore, due to the absence of acquired or congenital cardiopathies in the sample group after the aforementioned complementary examinations, we understand the reason for the lack of alterations in the serum troponin dosage during the analysis, presenting values compatible with those found in healthy dogs (< 0.01 ng/ml) (O'Brien et al. 2006), which means these values could be used as a reference for *C. thous*. It is important to note the higher specificity of this test in mammals with heart disorders in comparison with birds and reptiles (Feltzer et al. 2016; Zafalon-Silva et al. 2018).

Given these results, the study may still be limited by the small sample group used. However, considering this study in a descriptive light, these results may be used as parameters for future studies dealing with exotic and wild animals, as well as in the evaluation of *Cerdocyon thous* and other wild canids in the field or in captivity, however, it is important to underline that utility of the allometric equation and the comparison of the values with wild animals of distinct body weights that belong to the same taxonomic class due to the scarcity of literature regarding cardiac evaluation in wild canids.

Declarations

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ETHICS APPROVAL: All experimental procedures were performed after approval in the Ethics Committee for Animal Use (CEUA, *Comitê de Ética no Uso de Animais*) of the School of Veterinary Medicine and Animal Science at the São Paulo State University, campus Botucatu (CEUA protocol no. 0183/2019).

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Leomil: Conceptualization oh the manuscript, Alessandra Melchert, Diogo Silva and Priscylla

Okamoto: Methodology, Vivianne Codognato: Immunoenzymatic assay, Angélica Alfonso, Alicia

Hippolito, Heloisa Coppini and Ariana Ramos: Drafting – preparation of the original draft, Miriam

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Tables

TABLE 1. Means, standard deviations and confidence intervals (CI 95%) for radiographic parameters: long axis length (cm); short axis length (cm), number of vertebrae in the long axis, number of vertebrae in the short axis, VHS, VLAS and RLAD in crab-eating foxes (*Cerdocyon thous*)

Parameters	Mean ± SD	Maximum	Minimum	CI (95%)	Reference Values
Long axis length (cm)	6.93 ± 1.57	11.5	5.10	6.1-7.81	
Short axis length (cm)	4.72 ± 0.91	7.39	4.03	4.21-5.23	
Long axis Number of Vertebrae	4.78 ± 0.65	5.5	3.00	4.42–5.14	
Short axis Number of Vertebrae	3.43 ± 0.56	5.00	2.90	3.11-3.75	
VHS	8.22 ± 0.55	8.90	7.50	7.91-8.53	≤10.5*
VLAS	1.77 ± 0.20	2.0	1.40	1.66-1.88	≤2.3**
RLAD	1.25 ± 0.18	1.5	1.0	1.15-1.35	≤1.8***

Abbreviations: cm – centimeters; VHS – Vertebral Heart Size; VLAS – Vertebral Left Atrial Size; RLAD- Radiographic Left Atrial Dimension; SD – standard deviation; CI – confidence interval. *Buchanan & Bucheler; ** Malcolm *et al.*, 2018 *** Salguero *et al.*, 2018

TABLE 2. Means, standard deviations and confidence intervals (CI 95%) for the echocardiographic parameters: Weight (kg); heart rate (bpm), systolic arterial pressure (mmHg); IVSd (cm); LVd (cm); LVFWd

(cm); IVSs (cm); LVs (cm); LVFWs (cm); LVFS (%); EF (%); LA (cm), Ao (cm), LA:Ao ratio; EPSS (cm); Pulmonary Flow (cm/s); Aortic Flow (cm/s); E-Wave (cm/s); EDT (ms); A-wave (cm/s); E/A ratio; IVRT (ms); E/IVRT ratio in crab-eating foxes (*Cerdocyon thous*).

Parameters	Mean ± SD	Maximum	Minimum	CI (95%)	Reference Values (Boon, 2011a)
Weight (kg)	6.2±0.96	7.44	4.32	5.7-6.7	5.24-7.16
SAP (mmHg)	126.92±38.97	200	90	105-149	
HR (bpm)	138.27±27.66	192	100	123-154	
IVSd (cm)	0.61±0.13	0.87	0.43	0.54-0.68	0.60-0.81
LVd (cm)	1.87±0.25	2.32	1.43	1.73-2.01	1.97-2.53
LVFWd (cm)	0.66±0.16	0.86	0.41	0.58-0.74	0.48-0.65
IVSs (cm)	0.86±0.14	1.12	0.63	0.79-0.93	0.91-1.16
LVs(cm)	1.22±0.22	1.60	0.87	1.10-1.34	1.11-1.54
LVFWs (cm)	0.83±0.12	1.06	0.65	0.77-0.89	0.81-1.03
LVFS (%)	37.14±12.54	65.3	18	30-44	30-46
EF (%)	68.08±14.7	94	41	60-76	55-75
LA (cm)	1.34±0.22	1.74	1.02	1.22-1.46	1.31-1.69
Ao (cm)	1.07±0.11	1.20	0.82	1.01-1.13	1.31-1.63
LA:Ao	1.26±0.17	1.54	0.99	1.17-1.35	≤ 1.6
EPSS (cm)	0.20±0.11	0.40	0.06	0.14-0.26	≤0.7
Pulmonary Flow (cm/s)	82.43 ± 12.43	99.30	61.10	75-89	≤ 160
Aortic Flow (cm/s)	87.09 ± 24.34	136.90	52.60	74-100	≤ 200
E-Wave (cm/s)	73.62 ± 18.21	109.90	53.40	63-83	≤ 52 - 81
EDT (ms)	36.27 ± 6.93	52.20	27.60	33-39	52 - 108
A-Wave (cm/s)	45.59 ± 12.82	66.80	20.90	38.34-52.84	45-78
E/A Ratio	1.62 ± 0.54	2.67	1.11	1.32-1.92	1 – 2
IVRT (ms)	65.42 ± 18.52	100	40	55-75	31 – 73

E/IVRT Ratio 1.26 ± 0.64 2.75 0.53 0.91-1.61 2 – 2.5

Abbreviations: HR – Heart rate; bpm- beats per minute; SAP – systolic arterial pressure; mmHg – millimeters of mercury; TC – thoracic circumference; cm – centimeters; IVSd – Interventricular septum in diastole; LVd – Left ventricular diameter in diastole; LVFWd – Left ventricular free wall in diastole; IVSs – Interventricular septum in systole; LVs – Left ventricular diameter in systole; LVFWs – Left ventricular free wall in systole; LVFS – Left ventricular fractional shortening; EF – Ejection fraction; LA – Left atrium; Ao – Aorta; LA:Ao – Left atrial-to-aortic root ratio; EPSS – E-point septal separation; cm – centimeter; cm/s – centimeters per second; ms – milliseconds; EDT – E-wave deceleration time; IVRT – Isovolumetric relaxation time; SD – standard deviation; CI – confidence interval

TABLE 3. Means, standard deviations and confidence intervals (CI 95%) for the echocardiographic parameters normalized for the body weight of the animal: IVSd (cm); LVd (cm); LVFWd (cm); IVSs (cm); LVs (cm); LVFWs (cm) in crab-eating foxes (*Cerdocyon thous*).

Parameters	Mean ± SD	Maximum	Minimum	CI (95%)	Normalized Reference Values (Cornell <i>et al.</i> , 2004)
IVSd (cm)	0.39 ± 0.09	0.58	0.27	0.34-0.44	0.29-0.59
LVd (cm)	1.09 ± 0.14	1.33	0.83	1.02-1.16	1.27-1.85
LVFWd (cm)	0.43 ± 0.10	0.55	0.28	0.38-0.48	0.29-0.60
IVSs (cm)	0.62 ± 0.10	0.78	0.43	0.57-0.67	0.43-0.79
LVs (cm)	0.69 ± 0.12	0.88	0.50	0.63-0.75	0.71-1.26
LVFWs (cm)	0.55±0.07	0.69	0.46	0.52-0.58	0.48-0.87

Abbreviations: IVSd – Interventricular septum in diastole; LVd – Left ventricular diameter in diastole; LVFWd – Left ventricular free wall in diastole; IVSs – Interventricular septum in systole; LVs – Left ventricular diameter in systole; LVFWs – Left ventricular free wall in systole; cm – centimeter; SD – standard deviation; CI – confidence interval