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Echocardiographic reference ranges for sedated healthy peccaries (*Tayassu tajacu*, Linnaeus, *1758*)

[*Valores de referência para catetos saudáveis sedados*]

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ABSTRACT

Peccaries are wild mammals belonging to the *Tayassu* genus that are found almost everywhere in the Americas and have demonstrated great potential as an experimental model for scientific investigations. Twelve healthy adult animals were sedated to perform echocardiographic examinations in B, M and Doppler mode. The variables that exhibited statistically significant correlation coefficients with weight were LVFWd, LVIDd, LVIDs, E wave, A'RV, MAM, and TAPSE. The HR exhibited a negative relationship with the IVRT. The LA variable showed a positive correlation with the AO. The MAM exhibited correlations with the LVIDd and LVIDs. The TAPSE showed positive correlations with the E'RV and A'RV. The present study provides the first reference values for echocardiographic measurements in B, M and Doppler modes from peccaries anesthetized with ketamine and midazolam. Echocardiography was easy to perform in collared peccaries, and the collected data revealed values that can aid in their clinical management and conservation.

Keywords: cardiology, wild animals, diagnostic imaging, heart, hemodynamics

RESUMO

Catetos são mamíferos selvagens, pertencentes ao gênero Tayassu, *encontrados em quase toda a América, os quais têm se destacado como modelos experimentais. Doze animais adultos saudáveis foram sedados para a realização de exames ecocardiográficos em modos B, M e Doppler. As variáveis que apresentaram coeficientes de correlação estatisticamente significativos em relação ao peso foram: LVFWd, LVIDd, LVIDs, onda E, A'RV, MAM, TAPSE. O HR mostrou correlação positiva com LA/AO, onda E, onda A, MAM, TAPSE e E/IVRT, e negativa com IVRT. Os valores de AVmax mostraram correlações positivas com onda E e onda A'. A variável LA mostrou correlação positiva com AO e correlação negativa com IVSd. A EF apresentou forte correlação com a relação de ondas FS, E/A, com A', E'RV. FS apresentou correlação positiva com a relação de onda E/A. O MAM mostrou correlação com LVIDd e LVIDs. TAPSE mostrou correlações positivas com E'RV e A'RV. O presente estudo forneceu os primeiros valores de referência para medições ecocardiográficas em modos B, M e Doppler de catetos anestesiados com ketamina e midazolam. O exame ecocardiográfico em catetos foi de fácil execução e os dados encontrados evidenciaram valores que podem auxiliar no seu manejo clínico e conservação.*

Palavras-chave: cardiologia, animais silvestres, diagnóstico por imagem, coração, hemodinâmica

INTRODUCTION

Peccaries (*Tayassu tajacu*, Linnaeus, 1758) are wild mammals belonging to the Tayassuidae family and *Tayassu* genus. They are found naturally in almost all of the countries of the Americas and are rustic animals that easily adapt to captive breeding due to their omnivorous food habits and satisfactory reproductive indexes (Cabrera and Yepes, 1940). Due to these characteristics and because they are good

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experimental models for evaluating the evolution of diseases, peccaries are a good alternative model for scientific investigations (Bezerra *et al*., 2014).

Echocardiography is a non-invasive and sensitive imaging method used to obtain cardiovascular hemodynamic data. Studies have demonstrated strong correlations between parameters obtained with echocardiography and those obtained with more expensive methods, such as magnetic resonance imaging (Shukan and Hitt, 2011).

Advanced echocardiography techniques have been incorporated into veterinary medicine in experiments using different animal species (Chetboul *et al*., 2005; Lee *et al*., 2007). These studies have demonstrated numerous similarities in the parameters of cardiac function between animals and humans.

Peccaries can only be safely handled after the administration of chemical restraint, and all hemodynamic changes promoted by anesthetic or sedative agents should be considered (Bertozzo *et al*., 2008). Moreover, to date, no study of the hemodynamic profile of this species as evaluated by Doppler echocardiography has been reported.

Ketamine is widely used in the chemical restraint of wild animals because of its low lethality and high margin of safety (Green *et al*., 1981). The combination of ketamine and midazolam promotes adequate myorelaxation, thus reducing muscular hypertonicity and promoting tranquilization, hypnosis and amnesia, and possesses an anticonvulsive activity (Valadão, 2002). This combined anesthetic protocol is commonly used in procedures with small animals and has been demonstrated to be a good option for work with wild animals (Diniz *et al*., 2017).

The aim of this study was to characterize the echocardiographic values of measurements of the cardiac chambers and variables related to the cardiovascular hemodynamics of peccaries (*Tayassu tajacu, Linnaeus, 1758*) after sedation with a combination of ketamine hydrochloride and midazolam.

MATERIAL AND METHODS

Twelve peccaries from the Center for the Study and Preservation of Wild Animals (NEPAS/CCA-UFPI) of the Federal University of Piauí, Teresina-PI were used. The animals were randomly grouped in bails (10x5x3m) and were distributed in two groups consisting of 6 males and 6 females each. All animals were adults with a mean age of three years and weights ranging from 15 to 20kg. The animals were fed twice daily *ad libitum* with a commercial diet for domestic pigs that was based on soybean meal and contained 18% crude protein and various vegetables as a source of fiber, energy, minerals and water.

The protocols used in this study were approved by the Committee on Ethics in Animal Experimentation - EAA/UFPI (N° 0117/2015) and were authorized by the Ministry of the Environment through the System of Authorization and Information on Biodiversity (SISBIO) of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA; N^{o}. 26101-1).

The animals were submitted to clinical, hematological and biochemical examinations (Almeida *et al*., 2011). Auscultation of the pulmonary fields and cardiac sounds was performed to identify valve insufficiencies and cardiac rhythm disturbances. This examination was followed by an electrocardiographic examination (Tilley, 1992) and an echocardiographic screening (Boon, 2011).

The peccaries were previously prepared with a 12-hour solid fast and a 4-hour water fast. Later, the animals were captured using restraint nets (dip nets) and physically restrained with leather gloves for the application of the anesthetic drugs.

Sedation occurred due to the intramuscular application of the combination of 15mg/kg ketamine hydrochloride (5%) and 1.0mg/kg midazolam. Approximately 5 to 10 minutes after the application, the anesthetic action was observed, and the procedures to perform the echocardiographic examinations were initiated. The protocols produced an average anesthetic time of 30 to 40 minutes in all animals, and there was no need to reapply the drugs during the examinations.

Transthoracic echocardiography with continuous monitoring was performed using an M-Turbo system 5 (FUJIFILM® SonoSite, Washington

21919, USA) equipped with a 4.0–8.0MHz phased-array transducer (Px10, FUJIFILM® SonoSite, Washington 21919, USA). The hair was clipped between the right fourth and sixth intercostal spaces, and coupling gel (Mercur®, São Paulo, Brazil) was applied to this thoracic area.

The echocardiographic examinations were performed, and the standard measurements were collected according to previously established protocols for small animals (Boon, 2011). The left ventricular free-wall and interventricular septal thicknesses in diastole and systole (LVFWd, LVFWs, IVSd, IVSs), left ventricular end-diastolic and end-systolic diameters (LVIDd and LVIDs), and the diameters of the aortic root 2D (AO) and left atrium (LA) were measured in the parasternal right projection. The shortening fraction (FS) and the ejection fraction (EF-Simpson's Method) were calculated. Additionally, the mitral E-point septal separation (EPSS), the final diastolic ratio between the aorta and the left atrium (LA/AO) and the flow velocity in the pulmonary artery (Pmax) were also measured (Figure 1).

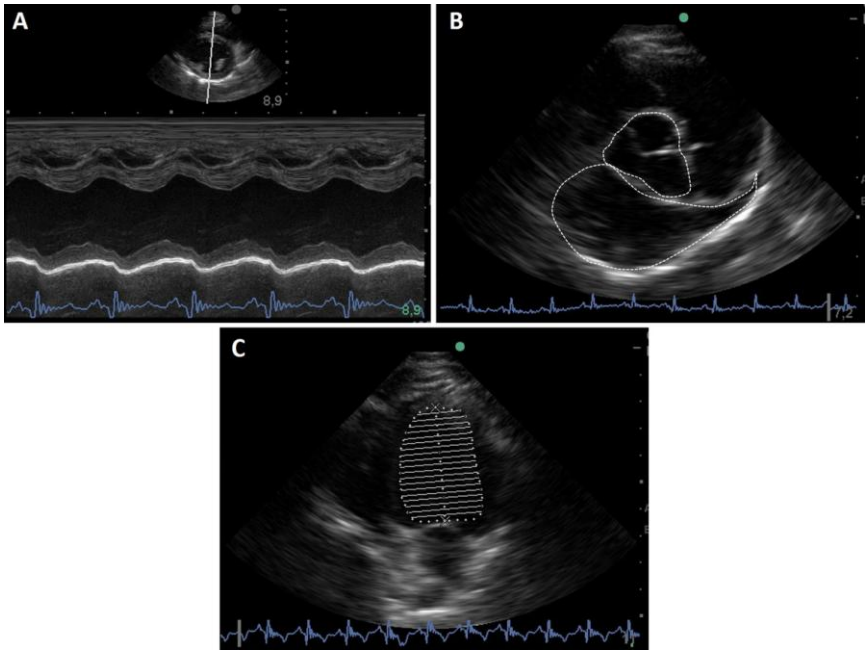

Figure 1**.** M-mode and two-dimensional (2D) echocardiographic images obtained from anesthetized peccaries (*Tayassu tajacu*, Linnaeus, 1758). (A) M-mode was used for the measurements of the interventricular septal wall, left ventricular wall, and left ventricular internal dimensions. (B) Two-dimensional right short axis view at the base of the heart used to measure the LA/AO ratio. (C) Left apical 4-chamber view for measurement of the echocardiographic end-diastolic and end-systolic left ventricular volumes using Simpson's method of discs.

The aortic velocity peak (AVmax), isovolumetric relaxation time (IVRT), mitral early diastolic flow (E Wave), mitral late diastolic flow (A wave), E/A ratio, E/IVRT ratio, mitral annular motion (MAM), and tricuspid annular plane systolic excursion (TAPSE) were calculated through the left parasternal window with the apical four-chamber view. Pulsed-wave tissue Doppler imaging (PWTDI) was then used to measure the left (E' and A' waves) and right ventricle (E'RV and A'RV; Figures 2 and 3).

The heart rate (HR) was obtained from the Doppler tracing of the pulmonary artery. Flow assessments of the mitral, tricuspid and semilunar valves, as well as the large vessels were performed using color and spectral Doppler on each individual valve. The 2D sector size was adjusted to improve image quality as well as calibrating the color gain to demonstrate excellent filling of the investigated chambers and vessels. The highest pulse repetition frequency (PRF) was used to prevent signs of aliasing during normal flows.

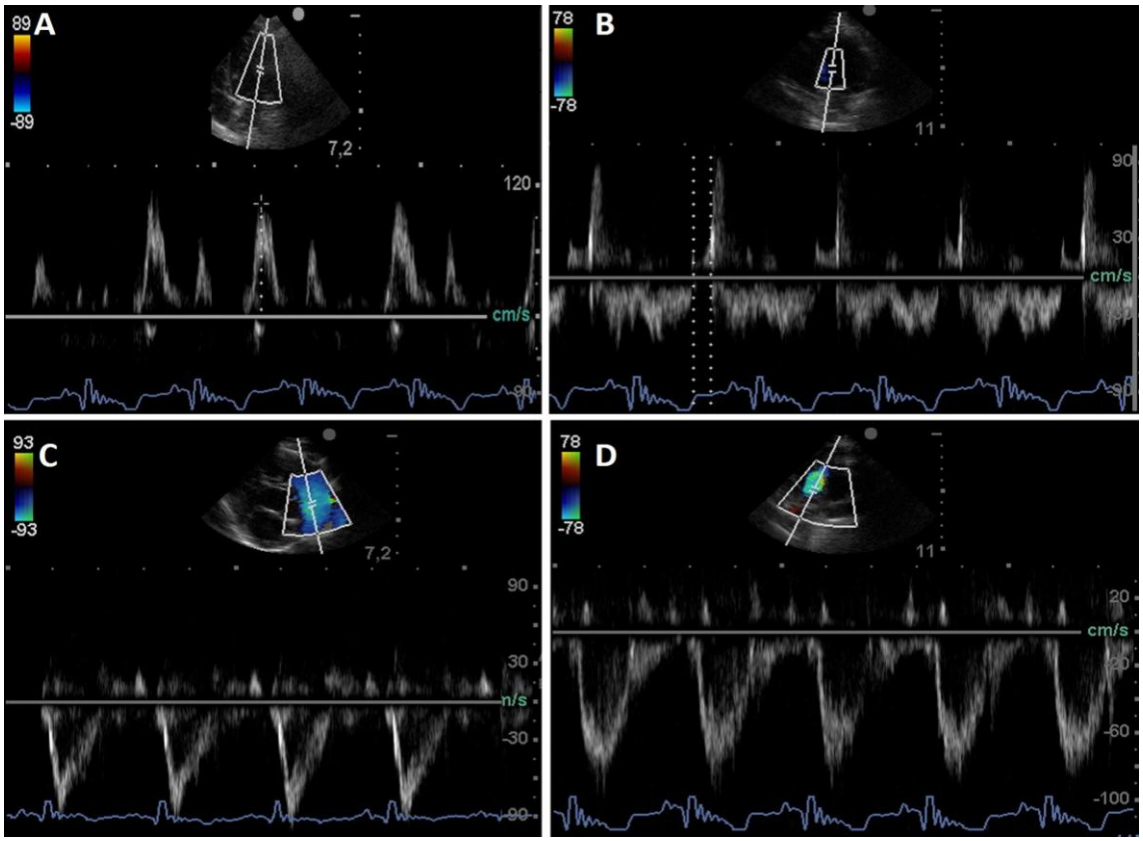

Figure 2**.** Doppler echocardiographic images obtained from anesthetized peccaries (*Tayassu tajacu, Linnaeus*, 1758). (A) Pulsed-wave Doppler inflow assessment of the mitral valve showing the early diastolic mitral inflow (E) and late diastolic mitral inflow. (B) Doppler measurements, including the peak aortic blood flow velocity and the mitral inflow, were used to measure the isovolumic relaxation time (IVRT) obtained from the left apical 5-chamber view. Pulsed Doppler assessment of the pulmonary (C) and aortic (D) flow velocity curves.

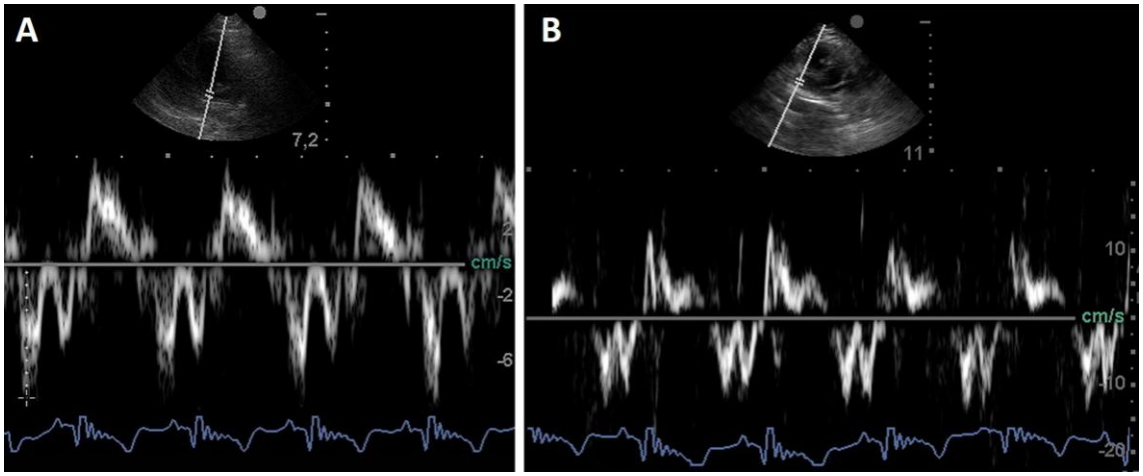

Figure 3**.** Pulsed-wave tissue Doppler imaging (PWTDI) mode images obtained from anesthetized peccaries (*Tayassu tajacu,* Linnaeus, 1758). (A) PWTDI from the apical 4-chamber view sampling the septal mitral annulus. (B) Myocardial velocity curve from the tricuspid annulus.

Graphpad Prism 7 software was used to analyze the data and to perform non-parametric Wilcoxon-Mann-Whitney (Mann-Whitney U) tests to verify the existence of differences between the variables according to the sex of the animals. The Spearman rank correlation coefficient was used to assess dependence. In these tests, 0.05 was considered as the level of significance.

RESULTS

Table 1 presents the echocardiographic parameters of the studied group of animals. Comparisons of the means between the males and females did not indicate significant differences (P> 0.05, P= 0.337). Thus, the other statistical treatments were based on the total sample of 12 animals. The variation reference intervals were determined by the 95% tolerance interval calculations and were designed to cover 99% of all future events. The variables that exhibited statistically significant correlation coefficients with respect to weight are presented in Table 1. The other variables that exhibited correlations are described as follows: The HR was negatively correlated with the IVRT (*r*= -0.46). The LA variable was positively correlated with the AO (*r*= 0.57). MAM was correlated with the LVIDd (*r*= 0.41) and LVIDs (*r*= 0.32). The TAPSE was positively correlated with E'RV (*r*= 0.4) and A'RV (*r*= 0.43).

Table 1. Normal values for bidimensional echocardiographic variables in B, M and Doppler modes as observed in healthy peccaries that were anesthetized with ketamine and midazolam

Variable	Mean	SD	Confidence interval	*r*	*p value*
BW (Kg)	17.89	3.37	14.52-22.26	-	-
AO (cm)	1.47	0.13	1.34-1.68	0.078	0.806
LA (cm)	2.01	0.2	1.81-2.30	-0.221	0.611
LA/AO	1.37	0.09	1.28-1.48	0.080	0.832
IVSd (cm)	0.49	0.03	0.46-0.55	0.398	0.343
IVSs (cm)	0.94	0.05	0.89-1.01	0.042	0.821
LVFWd (cm)	0.66	0.02	0.64-0.75	0.572[a]	0.041
LVFWs (cm)	0.83	0.04	0.79-0.92	0.342	0.279
LVIDd (cm)	2.60	0.6	2,0-3.32	-0.639[a]	0.042
LVIDs (cm)	1.92	0.43	1.49-2.41	-0.610[a]	0.047
EF (%)	53.00	2.30	50.57.2	0.183	0.586
FS (%)	26.00	1.70	24.3-28.32	0.038	0.863
HR (bmp)	105.00	11.00	94-221	-0.353	0.234
EPSS (cm)	0.57	0.05	0.52-0.65	0.241	0.470
E wave (cm/s)	89.6	1.43	88.17-92.04	-0.609[a]	0.048
A wave (cm/s)	74.9	1.22	73.68-77.15	-0.123	0.807
AV max (cm/s)	124.4	3.7	120.7-129.3	0.181	0.488
Pmax (cm/s)	78.2	2.3	75.9-82.7	-0.237	0.451
IVTR (ms)	65.00	3.20	61.8-69.4	0.165	0.479
E' wave (cm/s)	8.76	2,11	6.65-11.32	-0.446	0.329
A' wave (cm/s)	5.67	1.29	4.38-6.97	0.032	0.829
E'-RV (cm/s)	11.35	2.41	8,94-13.81	-0.285	0.327
A'-RV (cm/s)	6.11	1.53	4.58-7.98	-0.497[a]	0.048
MAM (cm)	0.37	0.03	0.34-0.47	-0.538[a]	0.042
TAPSE (cm)	0.51	0.12	0.39-0.64	-0.573[a]	0.04
E/A	1.65	0.15	1.50-1.80	-0.210	0.667
E/IVRT	1.10	0.09	1.01-1.22	-0.422	0.173

SD, standard deviation; *BW*, body weight; *AO*, diameters of the aortic root 2D; *LA*, left atrium; *LA/AO*, ratio between the aorta and the left atrium; *IVSd*, interventricular septal thicknesses in diastole; *IVSs*, interventricular septal thicknesses in systole; *LVFWd*, left ventricular free-wall in diastole; *LVFWs*, left ventricular free-wall in systole; *LVIDd*, left ventricular end-diastolic diameters, *LVIDs*, left ventricular end-systolic diameters; *EF*, ejection fraction; *SF*, shortening fraction; *HR*, heart rate; *EPSS*, mitral E-point septal separation; *E wave*, mitral early diastolic flow; *A wave*, mitral late diastolic flow; *AVmax*, aortic velocity peak; *Pmax*, flow velocity in the pulmonary artery; *IVRT*, isovolumetric relaxation time; *E' wave*, Pulsed-wave tissue Doppler imaging of the early diastolic phase from left ventricle; *A' wave*, Pulsed-wave tissue Doppler imaging of the late diastolic phase from left ventricle; *E'-RV*, Pulsed-wave tissue Doppler imaging of the early diastolic phase from right ventricle; *A'-RV*, Pulsed-wave tissue Doppler imaging of the late diastolic phase from right ventricle; *MAM*, mitral annular motion; *TAPSE*, tricuspid annular plane systolic excursion; *E/A*, ratio of E wave and A wave; *E/IVRT*, ratio of E wave and IVRT.

DISCUSSION

Echocardiographic measurements were obtained following previously established protocols that ensure safe measurements and diagnostic imaging and have been performed in other studies of wild species, such as wild dogs (Garcia *et al.*, 2016).

In veterinary medicine for domestic animals, echocardiographic parameters are well documented, and standardized values are available for several species including canines, felines, equines and domestic pigs (Lee *et al*., 2007; Boon, 2011). However, few studies have examined the hemodynamics and cardiac functions of wild animals (Cetin *et al*., 2005; Mantovani *et al*., 2012; Diniz *et al*., 2017). This is the first study to report measurements of the echocardiographic variables of chemically restrained peccaries (*Tayassu tajacu*).

The HR values found in this study (105±11bpm) were similar to the findings for peccaries under inhalation anesthesia (100.2±9.98bpm) (Oliveira *et al*., 2014) and lower than those of physically restrained peccaries (129.7±13.79bpm) (Schilling; Stone, 1969). In relation to smaller wild species, these findings were lower than those of the agouti (*Dasyprocta prymnolopha*; 142-149bpm) (Diniz *et al*., 2013) and pacas (*Cuniculus paca;* 150±2.81bpm) (Uscategui *et al.,* 2016) under different anesthetic protocols and lower than the values for non-anesthetized guinea pigs (*Cavia porcellus;* 288.4±49.7bpm) (Botelho *et al*., 2016). The HR is inversely proportional to the size of the animal and is thus higher in smaller species (Schmidt-Nielsen, 1999).

Regarding the interventricular septum (IVS) measured in M-mode, the present study demonstrated that the diameters of the IVS of peccaries in both diastole and systole are greater than those observed in maned wolves (*Chrysocyon brachyurus:* IVSd: 0.20±0.03; IVSs 0.15±0.08) (Estrada *et al*., 2009) and New Zealand rabbits (IVSd: 0.25±027) (Silva *et al*., 2011).

The measurements obtained for the LVFWs of peccaries in diastole and systole were higher than those observed for hedgehogs (LVFWd: 0.16±0.01; LVFWs: 0.23±0.02) (Black *et al*., 2011) and guinea pigs (LVFWd: 0.22±0.10; LVFWs: 0.28±0.13) (Cetin *et al*., 2005). However, these values were lower than those observed in maned wolves (LVFWd: 0.80±0.12; LVFWs 1.13±0.12) (Estrada *et al*., 2009) and in large breeds dogs (LVFWd; 0.58-1.20; LVFWs: 0.93-1.69) (Cornell *et al*., 2004).

Regarding the LVID, the values obtained for the diameters in diastole and systole (LVIDd: 2.60±0.6; LVIDs: 1.92±0.43) were lower than those obtained for domestic pigs (LVIDd: 4.13±0.22; LVIDs 2.5±0.13) (Lee *et al*., 2007) and maned wolves (LVIDd: 3.14±0.31; LVIDs: 2.06±0.16) (Estrada *et al*., 2009). However, these values were higher than those found in smaller mammals, such as cats (LVIDd: 1.45; LVIDs: 0.69) (Schober; Maerz, 2005), guinea pigs (LVIDd: 0.68±0.11; LVIDs: 0.43±0.05) (Cetin *et al*., 2005) and New Zealand rabbits (LVIDd: 1.21±1.90; LVIDs: 0.67±1.90) (Silva *et al*., 2011).

In the present study, several variables exhibited statistically significant correlation coefficients in relation to weight (LVFWd, LVIDd, LVIDs, E wave, A'RV, MAM, and TAPSE). Corroborating the findings of this study, similar relationships have been described between body weight and LVIDd, LA, AO and SIV in dogs of different breeds (Pellegrino *et al*., 2007). Additionally, positive correlations of body weight with LVIDd, IVS, LVFW, LA and AO were observed by Bradley and Ross (1987) in sheep.

The LA/AO ratios of the peccaries (1.37±0.09) were similar to the normal findings for other domestic and wild mammals, such as domestic pigs (1.42), minipigs (1.40) (Lee *et al*., 2007), dogs over 20kg (<1.6) (Cornell *et al*., 2004) and maned wolves (1.39±0.21) (Estrada *et al*., 2009). However, the diameters of the left atrium and the aorta (AE: 2.01±0.2; AO: 1.47±0.13) were smaller than those of domestic pigs (LA: 3.01±0.17; AO: 2.18±0.12) and minipigs (LA: 2.76±0.19; AO: 1.96±0.10) (Lee *et al*., 2007). The positive correlations between weight and cardiac dimensions are observed because the diameter of the aorta and the LA proportionally increase with the animal's size (Hanton *et al.*, 1998).

The EPSS values of peccaries (0.57±0.05cm) were similar to those of wines (0.56±0.03cm)

and minipigs (0.56±0.04cm) (Lee *et al*., 2007) and were within the normal range for dogs (≤0.77cm) (Boon *et al.*, 2011). These results are consistent with the literature because most reports suggest that normal EPSS values are less than 0.6cm for most domestic and wild species (O'Grady *et al*., 1986; Diniz *et al*., 2017).

The mean FE found for peccaries (53±2.30) indicated the preservation of systolic function and was below the standard normal values for domestic pigs (65.47±5.17), minipigs (58.40±8.18) (Lee *et al*., 2007) and maned wolves (62.91±13.50) (Estrada *et al*., 2009).

No statistical correlation was found between the FS (%) and weight, as has been described in dogs and humans (O'Grady *et al*., 1986; Feigenbaum, 1994). For peccaries (26±1.70), this variable presented values similar to those found in minipigs (24.35±0.68) (Lee *et al*., 2007) and lower than those found in domestic pigs (35.95±4.15) (Lee *et al*., 2007). A depressant effect of ketamine is associated with a decrease in FS when it is used as the sole agent or in anesthetic combination in cats and mice (Roth *et al.*, 2002).

The Doppler echocardiographic evaluation revealed a maximal aortic velocity (AVmax; 124.4±33.7cm/s) that was greater than the flow velocity in the pulmonary artery (Pmax; 78.2±2.3cm/s), as has been reported for domestic pigs (AVmax: 87±0.04; Pmax: 72±0.06) and minipigs (AVmax: 84±0.06; Pmax: 66±0.02cm/s) (Lee *et al*., 2007). The E wave exhibited a mean velocity peak (89.6±1.43cm/s) greater than that of the A wave (74.9±1.22cm/s), and this pattern has also been reported for dogs of different breeds (Boon, 2011), minipigs (Lee *et al*., 2007) and maned wolves (Mantovani *et al*., 2012). These data allowed the evaluation of the diastolic function of the animals in this study as described for sedated minipigs (Lee *et al*., 2007) and maned wolves (Mantovani *et al*., 2012) that have been submitted to similar protocols (Lee *et al*., 2007).

TDI has been demonstrated to be a sensitive technique for the quantitative evaluation of segmental myocardial movement (Wess *et al*., 2011) and is reproducible for examinations of basal cardiac function (D'Hooge *et al*., 2000)) in peccaries. In humans, TDI is useful for the differential diagnosis of heart diseases, the follow-up of cardiac transplantation and the evaluation of left and right ventricular function (Vinereanu *et al*., 2001). The mean velocity peak of the E' wave of peccaries (8.76±2.11cm/s) was greater than that of the A' wave (5.67±1.29), as has been observed in anesthetized cats (E': 7.2±2.5, A': 4.7±1.5) (Borlini *et al*., 2009) and dogs (E': 6.9; A': 7.4) (Dickson *et al*., 2017). Similar to LV TDI, the RV exhibited a negative diastolic velocity curve composed of the E'RV (11.35±2.41) and A'RV (6.11±1.53) waves. These characteristics have been reported for dogs of different breeds (Chetboul *et al*., 2005). The TAPSE demonstrated positive correlations with the E' RV (r= 0.4) and A' RV (r= 0.43), and these correlations are explainable as the result of normal RV functioning during systole, which influences the RV TDI values measured adjacent to the tricuspid annulus (Kjaergaard *et al*., 2009).

The IVRT was found to be consistent and to exhibit little variability between the studied animals. The values found for this variable (65±3.20m/s) were similar to findings that have been reported for humans (65±20ms/s) (Graziosi, 1998).

Tachycardia and sympathetic stimulation may exacerbate the IVRT, reduce diastolic filling time and accelerate the early LV diastolic elastic recoil. The partial drainage of the LV caused by chronic mitral valvular insufficiency, along with the activation of the sympathetic system, leads to LV hyperkinesia, an increase in HR and a reduction in the IVRT (Pipers *et al*., 1981). Indeed, although in the present study the IVRT values were within the normal limits, they exhibited a negative correlation with the HR (r= -0.46).

In this study, the MAM (0.37±0.03) was correlated with the LVIDd (r= 0.41) and LVIDs (r= 0.32). These values were lower than those found in cats (0.47±0.09) (Schöber *et al*., 2005) and dogs of different sizes (38-49) (Schöber *et al*., 2001), suggesting that these results may be well correlated with LV function.

Some limitations of this study should be considered: although these examinations have been performed by an experienced operator, we believe that the small number of animals may be responsible for the deviation found within each

variable. In addition, the action of the studied drugs, which although well characterized in domestic species, still have few references to its application in wild species. Nevertheless, in the authors' knowledge, this is the first study regarding the echocardiographic assessment of peccaries, allowing the preliminary acquisition of knowledge of the hemodynamic effect of drugs most commonly used for sedation and anesthesia in this species.

CONCLUSION

The present study provided the first reference values for B, M and Doppler mode echocardiographic measurements of peccaries (*Tayassu tajacu*) anesthetized with ketamine and midazolam. Echocardiography of collared peccaries was easy to perform, and the collected data revealed values that can aid in their clinical management and conservation.

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