FULL ARTICLE

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Echocardiography of turquoise-fronted parrots (*Amazona aestiva*, Linnaeus, 1758) subjected to physical or pharmacological restraint protocols

Ecocardiografia de papagaios-de-fronte-azul (Amazona aestiva, Linnaeus, 1758) submetidos a protocolos de contenção física ou farmacológica

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ABSTRACT

The tranquilizers can influence the preload and the afterload and alter echocardiographic parameters in parrots since echocardiography in these animals invariably depends on pharmacological containment. This study aimed to determine the echocardiographic parameters of turquoise-fronted parrots (*Amazona aestiva*) under physical and pharmacological restraint using midazolam and a combination of midazolam and ketamine and evaluate these drugs as facilitators of echocardiography. Echocardiographic examinations were performed on 10 healthy parrots using three restraint protocols at 30-day washout: midazolam 1.5 mg/kg IM (PM protocol), midazolam 0.2 mg/kg with ketamine 25 mg/kg IM (PMK protocol), and physical (PRP protocol). Apical images were obtained using the horizontal ventromedian approach using B-mode. Sedation scores and duration of exams were determined. In the PMK protocol, it was observed that Vmax-Ao ($p = 0.0052$), Ao-Grad ($p = 0.0110$), LVLd ($p = 0.0219$), LALd ($p = 0.0304$), IVSs ($p = 0.0219$) and IVSd ($p = 0.0156$) was lower than the PRP protocol and equal to PM protocol. However, cardiac debit was lower than the PM protocol ($p = 0.0417$) and equal to the PRP protocol. The A-wave velocity was greater than the E-wave velocity in 80% of the birds, while 20% showed E-wave velocity greater than the A-wave velocity in the PMK and PM protocols. PMK protocol showed a higher level of sedation without resistance to manipulation. In parrots, ketamine and midazolam alter echocardiographic parameters dependent on preload and afterload, but midazolam causes minor changes. In addition, pharmacological restraint facilitates echocardiography without altering the examination time.

Keywords: Echocardiogram. Psittacines. Sedation.

RESUMO

Os tranquilizantes podem influenciar a pré-carga e a pós-carga, podendo alterar parâmetros ecocardiográficos em papagaios, uma vez que a ecocardiografia nesses animais invariavelmente depende da contenção farmacológica. O objetivo deste estudo foi determinar os parâmetros ecocardiográficos de papagaios-de-frente-azul (*Amazona aestiva*) sob contenção física e farmacológica com uso de midazolam e combinação de midazolam e cetamina, e avaliar essas drogas como facilitadoras da ecocardiografia. Exames ecocardiográficos foram realizados em 10 papagaios saudáveis utilizando três protocolos de contenção com intervalo de 30 dias: midazolam 1,5 mg/kg IM (protocolo PM), midazolam 0,2 mg/kg com cetamina 25 mg/kg IM (protocolo PMK) e físico (protocolo PRP). Imagens apicais foram obtidas a partir da janela ventromediana horizontal no modo B. Os escores de sedação e a duração dos exames foram determinados. No protocolo PMK observou-se que Vmáx-Ao ($p = 0{,}0052$), Ao-Grad ($p = 0{,}0110$), CIVEd ($p = 0{,}0219$), CIAEd ($p = 0{,}0304$), SIVs ($p = 0{,}0219$) e SIVd ($p = 0{,}0156$) foi menor que o protocolo PRP e igual ao protocolo PM. No entanto, o débito cardíaco foi menor que o protocolo PM ($p = 0{,}0417$) e igual ao protocolo PRP. A velocidade da onda A foi maior que a velocidade da onda E em 80% das aves, enquanto 20% das aves apresentaram velocidade da onda E maior que a velocidade da onda A nos protocolos PMK e PM. O protocolo PMK mostrou maior nível de sedação sem resistência à manipulação. Em papagaios, cetamina e midazolam

alteram parâmetros ecocardiográficos dependentes da pré-carga e pós-carga, mas o midazolam causa as menores alterações. Além disso, a contenção farmacológica facilita a ecocardiografia sem alterar o tempo de exame.
Palavras-chave: Ecocardiograma. Psitacídeos. Sedação.

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Introduction

Echocardiography allows the morphological assessment of the heart by enabling the visualization of several cardiac structures and providing information about the hemodynamic and cardiac function (Almeida et al., 2021). It has been a part of routine diagnostic assessment of heart disease in birds for early diagnosis since heart diseases are not uncommon in these species but diagnostic confirmation is often obtained in the post-mortem examination (Cornelia & Krautwald-Junghanns, 2022; Krautwald-Junghanns et al., 2022; Kubale et al., 2018; Oster & Pariaut, 2022; Pees & Krautwald-Junghanns, 2009).

Birds not conditioned to manipulation can exhibit a high-stress level during physical restraint. Stress generates physiological changes, altering cardiovascular parameters and consequently influencing echocardiographic assessment (Fischer et al., 2018; Schrøder et al., 2024; Straub et al., 2003). In addition, an animal's reluctance to undergo an examination under physical restraint can generate artifacts in the image, making the examination challenging to perform and interpret. Moreover, it may also lead to unreliable results (Boon, 2012).

Tranquilizers minimize stress, facilitate the handling of birds, and decrease echocardiographic image artifacts. They provide greater safety while performing painless and quick procedures such as imaging examinations (Mans, 2014; Rankin, 2015). However, it is necessary to know the cardiovascular changes caused by the drugs to avoid interference with the interpretation of the examination results (Johard et al., 2018; Sciabarrasi Bagilet & Fajardo Camps, 2020; Silva et al., 2011).

Midazolam is a benzodiazepine used for chemical restraint of birds. It does not exhibit marked cardiovascular effects and provides satisfactory sedation with good muscle relaxation (Net et al., 2019). Usually, midazolam is used in combination with ketamine to provide greater sedation. However, since ketamine is a dissociative anesthetic, it directly stimulates the cardiovascular system and may cause changes in cardiovascular parameters, such as increased blood pressure and an inotropic effect on the myocardium (Bitencourt et al., 2013; Silva et al., 2011).

Since the tranquilizers show effects that can influence the preload and the afterload, the echocardiographic parameters dependent on these variables can undergo significant changes in parrots because echocardiography in these animals depends mainly on pharmacological containment. Thus, the objective of this study was to determine the echocardiographic values of turquoise-fronted parrots (*Amazona aestiva*) under physical and pharmacological restraint using midazolam and a combination of midazolam and ketamine and evaluate these drugs as facilitators of echocardiography.

Materials and Methods

Animals

Ten healthy adult turquoise-fronted parrots (*A. aestiva*), five females and five males, were selected after a complete physical examination. Birds with clinical evidence of diseases were excluded. The mean weight of the birds was 398 ± 37.25 g. The birds belonged to a commercial breeding site in Morada do Sol, Vila Velha, Espírito Santo, Brazil.

Experimental procedures

The parrots were subjected to three restraint protocols at a 30-day washout, in which echocardiographic examinations were performed. The first restraint protocol (the PM protocol) consisted of intramuscular administration of 1.5 mg/kg midazolam. The second restraint protocol (PMK) consisted of intramuscular administration of 0.2 mg/kg of midazolam and 25 mg/kg of ketamine (Hawkins et al., 2018). The third restraint protocol (the PRP protocol) consisted of physical restraint of the birds with intramuscular administration of 0.1 ml placebo (based on the average volume of doses from the other protocols) of 0.9% saline solution.

An ultrasound device (GE LOGIQ e Vet, GE Healthcare, Chicago, IL, USA) with a cardio-sectoral transducer with a 2.5-7 MHz frequency was used for echocardiography. Apical images were obtained using the horizontal ventromedial approach (four-chamber view) with the transducer positioned caudal to the bird's sternum (Figure 1A).

In the horizontal ventromedial approach (four-chamber view) using B-mode, the following echocardiographic measurements (in cm) were evaluated: the thickness of the interventricular septum during systole (IVSs) and diastole (IVSd), the diameter of the aorta (AoD), the left ventricular internal diameter in systole (LVDs) and in diastole (LVDd), the left ventricular internal length in systole (LVLs) and in diastole (LVLd), the right ventricular internal diameter in systole and diastole, the right ventricular internal length in systole and diastole, the left atrial internal diameter in systole and diastole, and the left atrial internal length in systole and diastole (LALd) (Figure 1B).

In addition, the left ventricular shortening fraction was calculated using the formula (LVDd–LVDs/LVDd × 100). The ejection fraction of left ventricular end-systolic volume (LVEVs), left ventricular end-diastolic volume, and cardiac debit (CD) was calculated automatically by the Simpson method (Figure 1C). Left atrioventricular annular movement was determined with the cursor positioned under the insertion of the left atrioventricular valve in the free wall of the left ventricle (Figure 1D).

Left atrioventricular and right atrioventricular flow velocities, E-wave (Left atrioventricular flow E/ Rigth atrioventricular flow E) and A-wave (Left atrioventricular

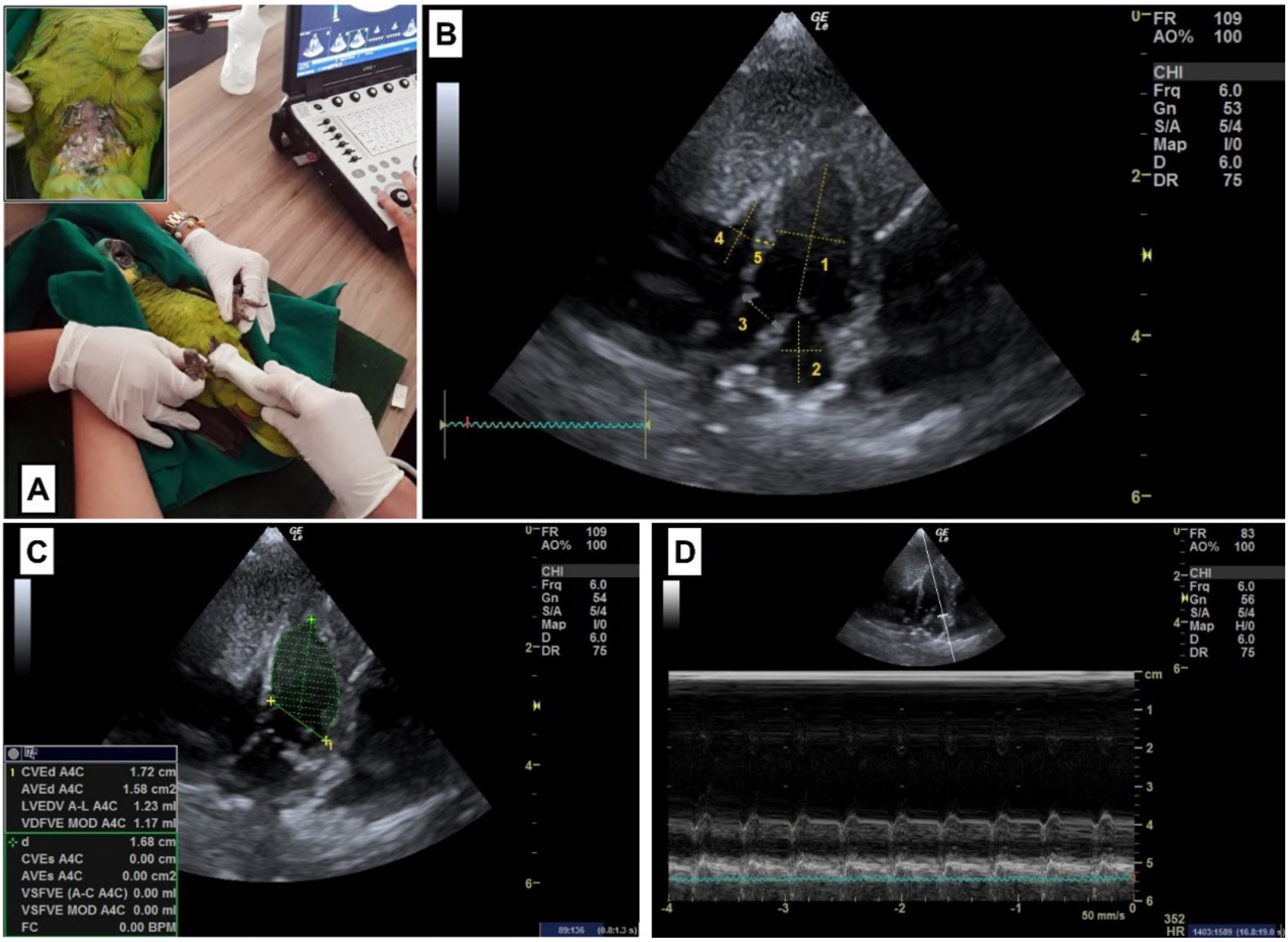

Figure 1 – Echocardiographic of *Amazona aestiva*. (A) Positioning for the horizontal ventromedian approach. Feathers were removed from the caudal area and moved to the sternum to position the transducer; (B) Measurement of cardiac chambers using the apical four-chamber image in B-mode in the horizontal ventromedian approach. The dashed transverse lines represent the diameter, and the lines in the longitudinal direction represent the length (1: left ventricle; 2: left atrium; 3: aorta; 4: right ventricle; 5: interventricular septum); (C) Use of the Simpson method in the left ventricle; (D) Measurement of the left atrioventricular annular movement.

Table 1 – Simple sedation scale

Posture	Score	Response to external stimulus	Score	Reluctance	Score
In station	0[a]	Highly responsive	0	Constant	0
Able to stand up	1	Responsive	1	Small	1
Tendency for lateral/sternal decubitus	2	Lightly responsive	2	Absent	2
Lateral/sternal decubitus	3[b]	Not responsive	3	Absent	3

[a]A score of 0 denotes no sedation; [b]A score of 3 denotes deep sedation. Adapted from Kamiloglu et al. (2008).

flow A/Right atrioventricular flow A) velocities, the ratio of the E-wave and A-wave velocities of the left atrioventricular valve (E/A Left atrioventricular flow), maximum aortic flow velocity (Vmax-Ao), and aortic flow pressure gradient (Ao-Grad) were also detected with pulsed Doppler. In the tissue Doppler, the relationship between e' and a' was studied with the cursor positioned close to the left atrioventricular annulus on the free wall of the left ventricle.

The drugs' facilitating action on the echocardiogram was evaluated 10 min after each protocol was applied using the sedation scale adapted from Kamiloglu et al. (2008), which classifies the level of sedation in birds from 0 (no sedation) to 3 (deep sedation) (Table 1).

The duration of echocardiography for each bird was also recorded to reveal whether there was a difference in the duration according to the restraint protocol. After the examination, the birds were subjected to the PM, and the PMK protocols were placed in individual cages for recovery from the anesthesia before returning to their respective enclosures.

Statistical analysis

The mean values, standard deviations, and coefficients of variation of the echocardiographic parameters were determined. Data regarding the sedation scale scores and the duration of the examination using different restraint protocols were also collected. All data were subjected to the D'Agostino-Pearson test to assess the normality. Friedman's test was used, followed by Dunn's post-hoc analysis, to compare echocardiographic parameters. The paired samples t-test was used to compare the data regarding sedation scores. Analysis of variance for paired samples was used to compare the data regarding the duration of the examination. An alpha level of 5% was considered for all statistical tests. The statistical analyses were conducted using commercially available GraphPad Prism® 7 (GraphPad Software, San Diego, CA, USA).

Results

Among the quantitative parameters, only the Vmax-Ao, Ao-Grad, LVLd, LALd, CD, IVSs, and IVSd showed a significant difference among the proposed protocols (Table 2).

Vmax-Ao (Figure 2A) in the PMK protocol was lower than in the PRP protocol ($p = 0.0052$). However, Vmax-Ao in the PMK protocol did not differ significantly from that in the PM protocol. Similar results were observed for the values of Ao-Grad ($p = 0.0110$), LVLd ($p = 0.0219$), and LALd ($p = 0.0304$) (Figure 2B, C).

The values of IVSs and IVSd in the PMK protocol were significantly lower than those in the PRP protocol ($p = 0.0219$ and $p = 0.0156$, respectively). Still, they were not significantly different from those in the PM protocol. The PMK protocol showed significantly lower CD values than the PM protocol ($p = 0.0417$). Still, there was no significant difference when the values from the PMK protocol were compared with the PRP protocol.

It was observed that 20% of the PM and PMK protocols birds showed E-wave velocities greater than A-wave velocities (left atrioventricular flow E > A pattern). However, 80% (8/10) of the birds had lower E-wave velocities for left atrioventricular flow and right atrioventricular flow than the A-wave velocities (E < A pattern) (Figure 3) regardless of the restraint protocol. The tissue Doppler (e' < a' pattern) observed the same result. Even with the high heart rate of the studied species, a fusion of left atrioventricular flow and right atrioventricular flow waves was not observed in the pulsed Doppler.

There was a significant difference in the level of sedation between the PM and the PMK protocols ($p = 0.001$) (Figure 4A). Birds that underwent the PMK protocol showed better sedation, which was associated with sternal/lateral decubitus, no reluctance, and better echocardiogram performance. Birds that underwent the PM protocol showed good reassurance and good muscle relaxation, facilitating the execution of the echocardiographic examination. However, a moderate level of resistance to restraint was observed.

The duration of the echocardiographic examination varied between 10 and 20 min in different protocols. However, no significant difference in the duration of the examination was observed among the protocols ($p = 0.741$) (Figure 4B). Notably, the performance of the echocardiogram under the PRP protocol was hampered by the birds' reluctant and aggressive behavior.

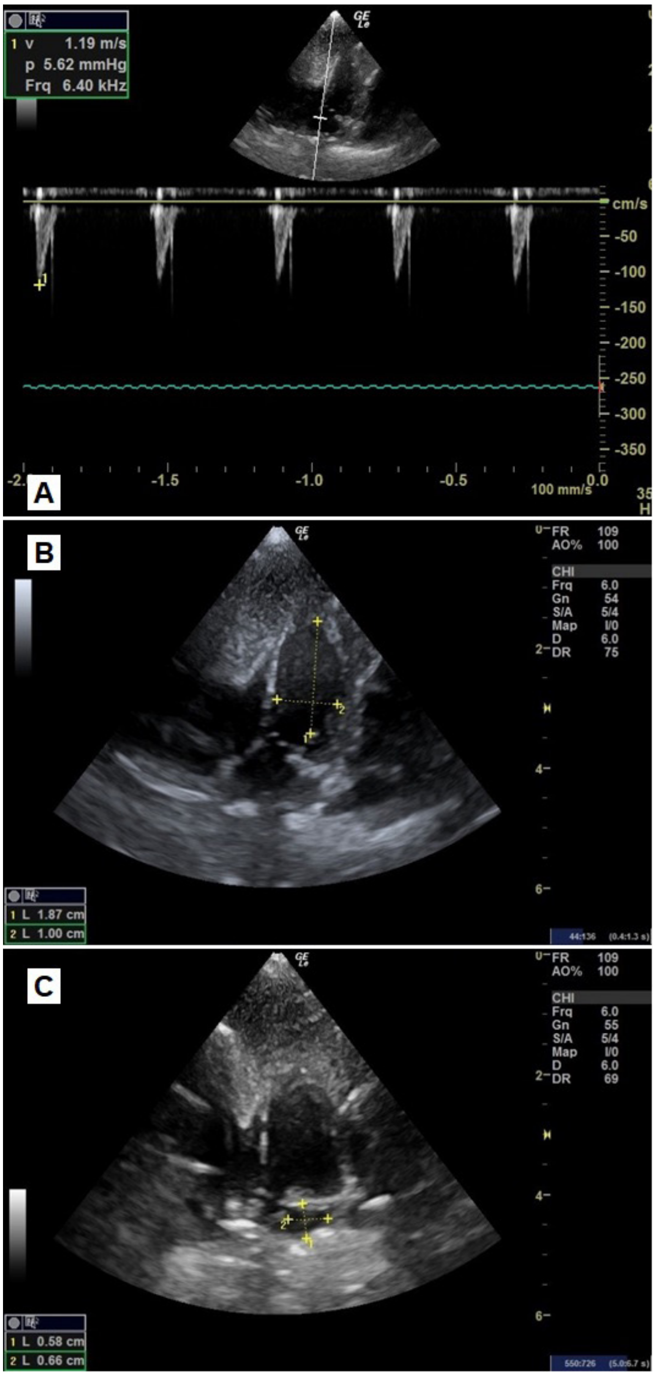

Figure 2 – Echocardiographic findings of *Amazona aestiva* subjected to chemical restraint with midazolam (0.2 mg/kg) associated with ketamine (25 mg/kg) (PMK protocol). (A) Aortic flow obtained using the horizontal ventromedian approach; (B) Left ventricular internal length in diastole (LAVd) through apical four-chamber view obtained via the horizontal ventromedian approach; (C) Left atrial internal length in diastole (LALd). Longitudinal lines represent the length, and transverse lines represent the left ventricular diameter.

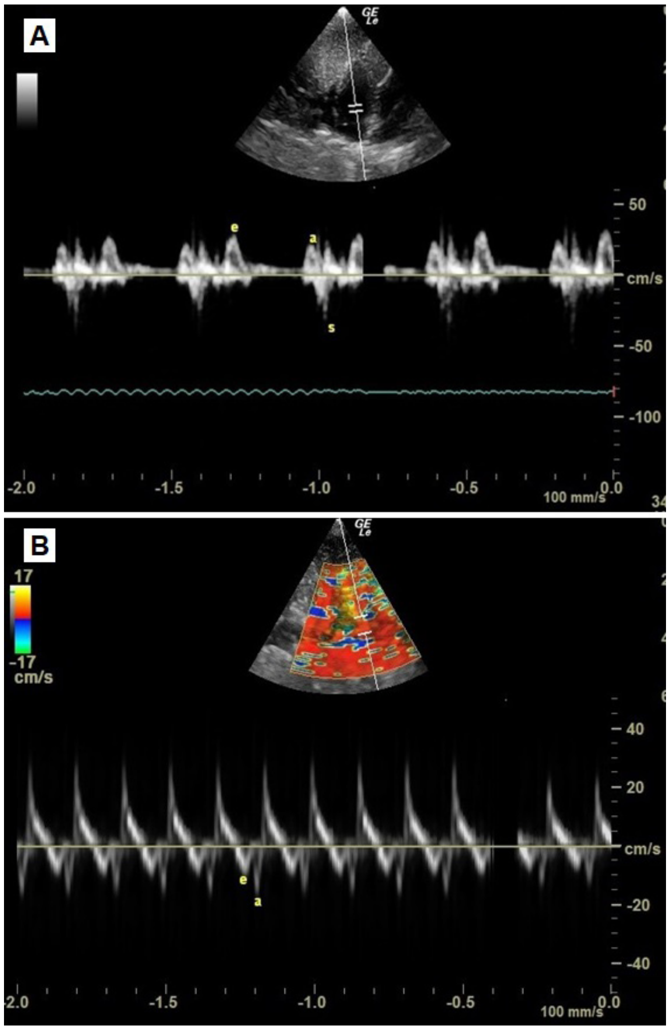

Figure 3 – Left atrioventricular flow obtained with pulsed Doppler (A) and tissue Doppler (B) using the horizontal ventromedian approach in the *Amazona aestiva* subjected to chemical restraint with midazolam (0.2 mg/kg) associated with ketamine (25 mg/kg) (PMK protocol) shows the standard E < A pattern.

Discussion

The results showed that the use of the midazolam and ketamine promotes a lower Vmax-Ao compared to the other protocols used. It is believed that the sympathomimetic effects of ketamine can lead to an increase in peripheral vascular resistance and, consequently, to an increase in the afterload (Nunes et al., 1996). This implies a greater ventricular work to eject blood through the left ventricular outflow tract, which may explain the lower aortic blood flow velocity observed in the PMK protocol. Similar results about Vmax-Ao were found in psittaciformes submitted to echocardiographic examination; however, they were found under the influence of isoflurane (Pees et al., 2005).

The Ao-Grad values in the PMK protocol were lower than those in the other protocols. An increase in the peripheral resistance results in a pressure difference between the left ventricle (LV) and the aorta (Ao), which may also suggest a decrease in the aortic pressure gradient (Boon, 2012; Howard & Detweiler, 2006). Thus, in *A. aestiva*, the effects of increased afterload and pressure difference LV-AO resulting from the increase in the peripheral resistance caused by ketamine seem to decrease the gradient of aortic

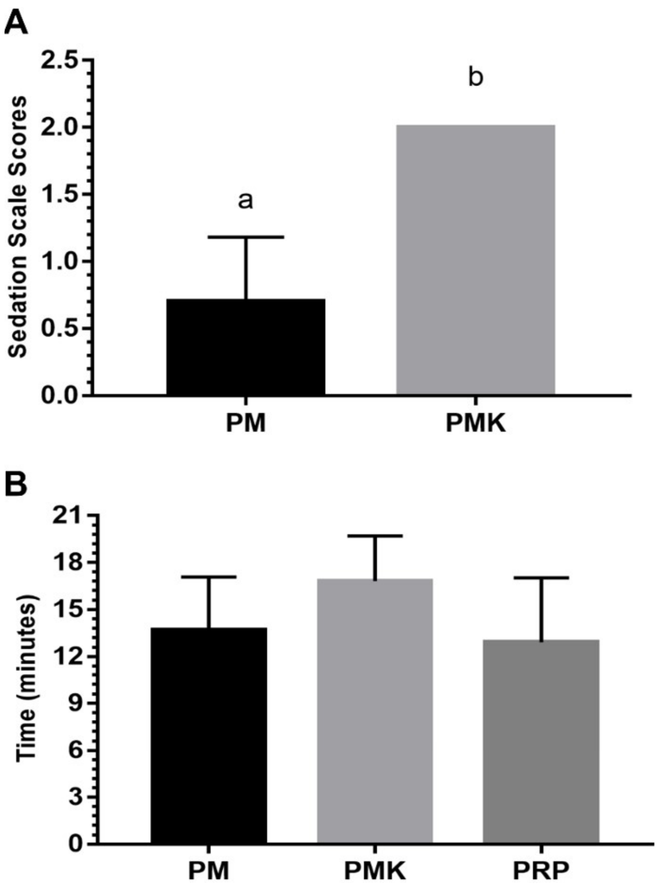

Figure 4 – (A) Sedation scores in *Amazona aestiva* subjected to IM midazolam (PM protocol) and association of IM midazolam and ketamine (PMK protocol). Mean (± SD) values followed by different letters differ significantly according to the t-test at a 5% significance level; (B) Duration of the echocardiographic examination in *Amazona aestiva* in different restraint protocols (PM: midazolam protocol; PMK: protocol with a combination of midazolam and ketamine; PRP: physical restraint protocol).

pressure and the aortic blood flow velocity. This finding may be due to the slower passage of blood through the left ventricular outflow tract, as the ventricle has to overcome more pressure during systole.

In PMK treatment, the decrease in the left ventricular end-systolic volume may be related to the lower measurements of LVLd and LALd in this protocol (Table 2). Thus, a reduction in preload is suggested, as there is a directly proportional relationship between diastolic volume and preload (Riedesel & Knight, 2006). Therefore, the changes observed in *A. aestiva* regarding the tendency to decrease LVEVs and pre-charge in the PMK protocol would imply less blood volume in the cardiac chambers during diastole, suggesting a possible explanation for the lower left ventricular and left atrial length values.

The PMK protocol resulted in lower cardiac debit (CD) values. CD is known to be directly influenced by heart rate (Riedesel & Knight, 2006; Stephenson, 2004). In the present study, there was a tendency for lower mean heart rate values in the PMK protocol, even though ketamine tends to increase heart rate due to its sympathomimetic activity (Hawkins et al., 2018). These results are consistent with a previous study in *A. aestiva* wherein birds that received ketamine exhibited lower heart rate values (Paula et al., 2013). Thus, these results may be related to the higher degree of sedation in both studies.

Birds subjected to the PMK protocol showed lower heart rates associated with lower preload and greater afterload due to increased peripheral vascular resistance caused by ketamine. This finding may also explain the lower CD values in the PMK protocol, as these variables affect ventricular work (Riedesel & Knight, 2006), resulting in lower CD values.

The E < A pattern observed for psittacines in this study is equally observed in the echocardiography of healthy pigeons (Masoudifard et al., 2016). It can be related to the higher heart rate of this species of birds.

It is known that a higher heart rate can lead to a decrease in the E wave due to shorter diastolic time and, consequently, to decreased volume in the fast-filling phase. This is associated with a greater flow during atrial contraction, leading to an increase in the A wave (Boon, 2012; Nishimura et al., 1989). The E < A pattern in the pulsed Doppler is consistent with the results observed in the left atrioventricular tissue Doppler (e' < a') in this study, and similar findings were observed in the echocardiogram of healthy pigeons. Most birds showed E < A in left atrioventricular flow and e'< a' in tissue Doppler mode (Legler et al., 2020, 2021). Thus, it can be suggested that the E < A pattern observed in this study is considered normal for *A. aestiva*, as the e' and a' waves in the tissue Doppler presented a morphological pattern similar to the pattern of the E and A left atrioventricular flow waves in the pulsed Doppler in healthy subjects with preserved diastolic function (Boon, 2012; Kibar et al., 2009; Parashar, 2011).

The E > A pattern observed in 20% of the birds may be associated with the lower transient heart rate exhibited by these birds in the PMK and PM protocols, which increased the diastolic time and, consequently, the flow velocity of the fast-filling phase (E wave) (Boon, 2012).

The non-fusion of the E and A waves in the pulsed Doppler observed in this study differs from the results observed in other bird species (Pees et al., 2004; Pees & Krautwald-Junghanns, 2005). The results observed in the present study may be related to the species' anatomical and physiological characteristics. However, no data were found in the literature to confirm this assumption.

Table 2 – Echocardiographic findings of turquoise-fronted parrots (*Amazona aestiva*, Linnaeus, 1758) according to physical restraint protocol (PRP) and pharmacological restrain protocols with midazolam (PM) and ketamine/midazolam (PMK)

Parameters	PM	VC %	PMK	VC %	PRP	VC %	p-value
HR (bpm)	256.1 ± 63.7	24.8	213.7 ± 67.2	31.5	255.6 ± 83.9	32.8	0.722
IVSs (cm)	0.287 ± 0.03	10.3	0.285 ± 0.03	10.1	0.317 ± 0.02	7.6	0.014*
IVSd (cm)	0.198 ± 0.04	19.3	0.198 ± 0.02	12.1	0.237 ± 0.03	12.9	0.017*
LVDs (cm)	0.687 ± 0.08	12.2	0.780 ± 0.14	18.0	0.777 ± 0.11	14.7	0.122
LVDd (cm)	0.934 ± 0.14	15.2	0.983 ± 0.15	15.5	1.018 ± 0.16	16.3	0.622
LVLs (cm)	1.592 ± 0.21	13.0	1.507 ± 0.21	13.8	1.572 ± 0.19	12.0	0.798
LVLd (cm)	2.007 ± 0.17	8.6	1.881 ± 0.20	10.7	2.097 ± 0.19	9.0	0.025*
RVDs (cm)	0.347 ± 0.05	15.8	0.408 ± 0.14	35.7	0.368 ± 0.12	32.6	0.928
RVDd (cm)	0.539 ± 0.07	13.9	0.514 ± 0.12	23.4	0.535 ± 0.12	23.5	0.741
RVLs (cm)	0.656 ± 0.10	16.1	0.659 ± 0.17	26.1	0.629 ± 0.20	32.1	0.975
RVLd (cm)	0.992 ± 0.17	17.6	0.856 ± 0.13	15.6	0.960 ± 0.24	25.5	0.061
LADs (cm)	0.948 ± 0.11	11.7	0.938 ± 0.11	12.1	1.112 ± 0.29	26.5	0.207
LADd (cm)	0.721 ± 0.17	14.6	0.700 ± 0.10	23.4	0.833 ± 0.13	15.5	0.054
LALs (cm)	0.737 ± 0.15	20.3	0.796 ± 0.19	24.0	0.760 ± 0.16	20.5	0.149
LALd (cm)	0.636 ± 0.25	38.9	0.502 ± 0.08	16.6	0.630 ± 0.11	17.6	0.023*
AoD (cm)	0.488 ± 0.04	7.5	0.481 ± 0.04	8.6	0.502 ± 0.03	6.0	0.341
Ao-Grad (mm/hg)	5.59 ± 2.35	42.1	4.263 ± 1.23	29.0	6.558 ± 1.65	25.1	0.014*
Vmax-Ao (m/s)	1.181 ± 0.25	21.6	1.006 ± 0.15	15.1	1.269 ± 0.17	13.3	0.005*
CD (L/min)	0.131 ± 0.03	27.3	0.089 ± 0.03	33.2	0.106 ± 0.05	50.4	0.026*
LVEVs (ml)	0.524 ± 0.12	23.0	0.442 ± 0.15	33.6	0.428 ± 0.17	40.4	0.135
LVEDd (ml)	1.146 ± 0.25	21.9	0.930 ± 0.24	25.8	1.000 ± 0.21	21.5	0.22
SF (%)	25.75 ± 8.26	32.1	20.63 ± 6.64	32.2	23.09 ± 7.94	34.4	0.497
EF (%)	51.1 ± 9.99	19.6	46.8 ± 11.6	24.7	51.4 ± 14.00	27.2	0.798
Left AV flow E (m/s)	0.347 ± 0.06	18.0	0.344 ± 0.08	23.0	0.392 ± 0.06	16.8	0.497
Left AV flow (m/s)	0.563 ± 0.20	35.3	0.529 ± 0.18	34.7	0.551 ± 0.16	29.1	0.975
E/A Left AV flow	0.760 ± 0.55	73.0	0.715 ± 0.30	40.9	0.800 ± 0.39	48.8	0.509
Rigth AV flow E (m/s)	0.275 ± 0.07	26.5	0.263 ± 0.07	28.8	0.293 ± 0.04	13.5	0.497
Rigth AV flow A (m/s)	0.5 ± 0.10	20.9	0.475 ± 0.09	19.4	0.524 ± 0.10	19.7	0.272
MAM (cm)	0.481 ± 0.04	8.4	0.422 ± 0.06	13.7	0.483 ± 0.07	14.1	0.098

*Significant difference according to the Friedman test post hoc Dunn's multiple comparisons tests ($p < 0.05$). VC%: variation coefficient. HR: heart rate; IVSs: interventricular septum thickness in systole; IVSd: interventricular septum thickness in diastole; LVDs: left ventricular internal diameter in systole; LVDd: left ventricular internal diameter in diastole; LVLs: left ventricular internal length in systole; LVLd: left ventricular internal length in diastole; RVDs: right ventricular internal diameter in systole; RVDd: right ventricular internal diameter in diastole; RVLs: right ventricular internal length in systole; RVLd: right ventricular internal length in diastole; LADs: left atrial internal diameter in systole; LADd: left atrial internal diameter in diastole; LALs: left atrial internal length in systole; LALd: left atrial internal length in diastole; AoD: aorta diameter; SF: shortening fraction; EF: ejection fraction; Left atrioventricular flow E, rapid filling of the left ventricle; Left atrioventricular flow A, slow filling of the left ventricle; Left atrioventricular flow E/A: Left atrioventricular flow E/Left atrioventricular flow A ratio; Rigth atrioventricular flow E: rapid filling of the right ventricle; Rigth atrioventricular flow A: slow filling of the right ventricle; Ao-Grad: aortic flow pressure gradient; Vmax-Ao: maximum aortic flow velocity; CD: cardiac debit; LVEVs: left ventricular end-systolic volume; LVEDd: left ventricular end-diastolic volume; MAM: Left atrioventricular annular movement.

The cardiovascular changes, especially the hemodynamic changes resulting from the use of ketamine, do not explain the lower values of IVSs and IVSd in the PMK protocol. In addition, no data were found in the literature to elucidate the reason for this species' lower thickness of the interventricular septum. Thus, further studies are needed to understand the changes related to these parameters.

The use of electrocardiography concomitant with echocardiography may be a limitation in avian echocardiography since greater sedation is necessary to immobilize the birds. Moreover, the examination time is increased substantially to achieve an acceptable electrocardiographic tracing. Additionally, the need for excessive use of alcohol between the electrodes and birds' body surfaces to obtain better tracing can decrease body temperature, leading to significant hypothermia in birds.

Birds that underwent the PMK protocol showed a higher level of sedation. Similar results were observed in a study with parakeets (Melopsittacus undulatus) that underwent the same drug combination, wherein almost all birds presented a level of sedation equivalent to the score 2 observed in the present study (Trevisan et al., 2016). Another research involving parrots used the same chemical restraint protocol and observed that some birds exhibited sedation equivalent to score 2. In contrast, some of them exhibited sedation equivalent to score 1, possibly due to the lower dose of ketamine (15 mg/kg) (Hawkins et al., 2018).

Parakeets (Melopsittacus undulatus) subjected to IM midazolam (5 mg/kg) alone exhibited a lower degree of sedation even with a higher dose of the drug (Silva et al., 2017). The sedation was equivalent to the score 1 from the present study, which was consistent with the lower sedation levels observed in the PM protocol in the present study.

The drugs' characteristics may justify the results mentioned above. Ketamine is a dissociative anesthetic responsible for the state of catalepsy. This implies a higher level of sedation, especially in combination with a benzodiazepine such as midazolam, resulting in greater muscle relaxation and a higher level of sedation (Rankin, 2015). Thus, the higher level of sedation exhibited by the birds that underwent the PMK protocol in the present study may be due to the association of ketamine with midazolam due to its pharmacological characteristics.

There was no significant difference in the duration of the examination among different protocols. A similar result was observed in a study involving dogs that underwent echocardiographic examinations with and without reassurance. However, in dogs, there was a tendency for a decrease in the examination time with an increase in the degree of sedation (Santos et al., 2018), which is different from the result in the present study. The duration of the examination tended to be shorter in the PRP protocol and higher in the PMK protocol in the present study. However, no studies have been performed in birds comparing the duration of the examination using different chemical restraint protocols for echocardiographic examination.

Thus, the time taken for echocardiography in birds was not associated with the level of sedation but with the operator's experience. Since the PRP protocol was the last to be performed, experience and agility in equipment handling were acquired and adapted to the birds' acoustic window.

Conclusion

In *Amazona aestiva*, the physical or pharmacological restraint allowed echocardiographic examination. Still, the use of midazolam alone caused minor changes in the echocardiographic parameters, and this is indicated as a pharmacological restraint method in this species while performing echocardiographic examination.

The combination of ketamine and midazolam caused significant changes in the echocardiographic parameters dependent on the preload and afterload, affecting this species' interpretation and cardiological diagnosis. Midazolam, with or without ketamine, facilitates echocardiographic examination. However, their combination results in a greater degree of sedation.

Both protocols do not interfere with the duration of the echocardiographic examination.

Conflict of Interest

The authors do not have any conflicts of interest to disclose.

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experimental procedures were reviewed and approved by the Animal Ethics and Utilization Committee in accordance with the Protocol 015/2018. In addition, authorization was obtained from the Chico Mendes Institute for Biodiversity Conservation – ICMBio, Brazil (No. 66631) to perform the experiment. The responsible for the animals signed a Term of Free and Informed Consent, authorizing their participation in the study.

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