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ARTICLE *in* AMERICAN JOURNAL OF VETERINARY RESEARCH · OCTOBER 2012

Impact Factor: 1.34 · DOI: 10.2460/ajvr.73.10.1560 · Source: PubMed

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Comparison of transcoelomic, contrast transcoelomic, and transesophageal echocardiography in anesthetized red-tailed hawks (*Buteo jamaicensis*)

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Objective—To assess the agreement and reliability of cardiac measurements obtained with 3 echocardiographic techniques in anesthetized red-tailed hawks (*Buteo jamaicensis*).

Animals—10 red-tailed hawks.

Procedures—Transcoelomic, contrast transcoelomic, and transesophageal echocardiographic evaluations of the hawks were performed, and cineloops of imaging planes were recorded. Three observers performed echocardiographic measurements of cardiac variables 3 times on 3 days. The order in which hawks were assessed and echocardiographic techniques were used was randomized. Results were analyzed with linear mixed modeling, agreement was assessed with intraclass correlation coefficients, and variation was estimated with coefficients of variation.

Results—Significant differences were evident among the 3 echocardiographic methods for most measurements, and the agreement among findings was generally low. Interobserver agreement was generally low to medium. Intraobserver agreement was generally medium to high. Overall, better agreement was achieved for the left ventricular measurements and for the transesophageal approach than for other measurements and techniques.

Conclusions and Clinical Relevance—Echocardiographic measurements in hawks were not reliable, except when the left ventricle was measured by the same observer. Furthermore, cardiac morphometric measurements may not be clinically important. When measurements are required, one needs to consider that follow-up measurements should be performed by the same echocardiographer and should show at least a 20% difference from initial measurements to be confident that any difference is genuine. (*Am J Vet Res* 2012;73:1560–1568)

The development of avian echocardiography as an imaging technique has considerably improved the diagnosis and management of avian cardiovascular diseases. This powerful tool can be used to noninvasively investigate cardiac function, structure, and hemodynamics and determine cardiac chamber sizes in raptorial and companion psittacine species. A standardized protocol has been developed for the performance of TE via a ventromedian approach and has been described for parrots, pigeons, chickens, crows, and raptors.[1–7]

Multiple avian case reports,[8–16] including those for birds of prey, document the use and capabilities of echocardiography in a clinical setting. Reference values have been reported in the literature for psittacine species, chickens, birds of prey (Falconiformes, Accipitriformes, and Strigiformes), and pigeons for B-mode morphometric measurements of the left and right portion of the heart and blood flow velocities recorded via pulsed-wave Doppler echocardiography.[1–7,17]

Despite its clinical usefulness, echocardiographic evaluation of birds is not without limitations. Only 2 views (longitudinal horizontal and longitudinal vertical) can be obtained in most birds when the ventromedian approach is used, compared with 13 views in dogs and cats.[18,19] The parasternal approach allows more imaging planes (as many as 8) to be obtained but can only be performed in species in which caudal extension of the ribs is limited, such as pigeons, chickens, and some raptor species.[4] Some views obtained in this manner are also redundant.[4] The inability to perform M-mode echocardiography in birds and obtain transverse

Abbreviations

CI	Confidence interval
CTE	Contrast transcoelomic echocardiography
CV	Coefficient of variation
ICC	Intraclass correlation coefficient
TE	Transcoelomic echocardiography
TEE	Transesophageal echocardiography

Received September 9, 2011.
Accepted November 23, 2011.
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views of the heart from a transthoracic approach has prevented the establishment of the same examination standards as developed for mammals.[18,19] These limitations and the lack of suitable sonographic windows are attributable to the specific topographic anatomy of the avian heart that is located in a concave indentation of the keel bone (fascies visceralis sterni pars cardiaca)[20] and surrounded by the extensive airsac system. Thus, morphometric measurements are usually performed in B-mode in birds, whereas measurements are usually performed in or complemented by M-mode in mammals because of that mode's excellent temporal resolution.[19,21] Furthermore, because of the small size of birds, the structures imaged are considerably smaller than those in most mammals, and heart rate is much faster in most avian species.

With the aforementioned considerations in mind, one may question whether measurements are reliable and useful when obtained on a small, rapidly beating avian heart from imaging planes not generally used in other species to estimate cardiac dimensions. Precise measurement of the right ventricle and atria in birds can be difficult.[3] Statistically adequate reference intervals have not been determined for parrots and raptors thus far, and echocardiographic values determined in other studies[2,4,5] are limited in usefulness for reference purposes. Regression-based prediction intervals have been described for radiographic cardiac measurements in falcons but have not been investigated for echocardiographic measurements in any avian species.[22] Moreover, the sensitivity and specificity of the standard transcoelomic examination have not been evaluated in birds with various types and severities of cardiac conditions. The only studies[23,24] on the value of echocardiographic measurements in cardiac diseases involve broiler chickens with ascites or pulmonary hypertension syndrome, which is a worldwide and important cause of mortality in commercial poultry. The ability to detect early or mild cardiac conditions via diagnostic imaging may also be poor in birds. Finally, interobserver and intraobserver reliability and variation of echocardiographic measurements have not been evaluated in birds and need to be assessed before clinical application.

To overcome some of the limitations associated with the transcoelomic approach, a TEE protocol has been developed for use in birds.[25] In this technique, the heart is approached and imaged from inside the proventriculus, providing better acoustic windows than with the transcoelomic method and limiting the interference from air sacs and bones. Five repeatable echocardiographic views are reportedly possible with 3 probe positions in TEE: caudal longitudinal, caudal transverse, middle transverse, middle-cranial longitudinal view, and cranial transverse.[25] The ability to perform M-mode echocardiography and the improved resolution of the imaging planes make the transesophageal approach promising for use in birds. Additionally, a technique for CTE in birds has been described and may improve the visualization and delineation of the cardiac chambers relative to non–contrast-enhanced sonographic methods.[26] The contrast agents used in the CTE technique are suspensions of microspheres filled with a perfluorocarbon gas that are similar in size to RBCs, which allow them to go through the pulmonary capillary bed and be visualized in both cardiac chambers.[27] However, both of these techniques are invasive, require anesthesia, are not readily available, have not been evaluated in diseased birds, and lack published reference intervals.

The objective of the study reported here was to assess the agreement and reliability of measurements obtained with 3 echocardiographic methods in anesthetized red-tailed hawks (*Buteo jamaicensis*). Our hypothesis was that low interobserver and intraobserver agreement and high variation would be present among and within the 3 techniques.

Materials and Methods

Animals—Ten red-tailed hawks (7 juveniles and 3 adults) that were admitted to the Wildlife Hospital of Louisiana for rehabilitation were used for the study. Body weight ranged from 0.82 to 1.50 kg (median, 1.25 kg). Red-tailed hawks were specifically chosen because of their availability, commonness in captivity and wildlife rehabilitation, and large size, which would allow TEE to be performed safely.

All birds were treated for trauma, and none had signs of a cardiac condition. Nevertheless, the use of hawks without underlying heart disease was deemed not necessary because the study objectives were to assess agreement and variation of echocardiographic measurements. The study protocol was approved by the Louisiana State University Institutional Animal Care and Use Committee.

Echocardiographic evaluation—Food was withheld from the hawks for 12 hours prior to echocardiography to prevent regurgitation during anesthesia and artifacts associated with ingested food material. Anesthesia was induced with 5% isoflurane via a facemask. Birds were then intubated with a 3.5-mm uncuffed endotracheal tube and positioned on a heating pad. Anesthesia was maintained with 2% to 3% isoflurane in a 1-L flow of 100% oxygen. Birds were manually ventilated every 15 to 20 seconds during the entire procedure. Heart rate was monitored via cardiac auscultation and from the live echocardiographic images. End-tidal CO_2 concentration was monitored with a mainstream capnometer. A 24-gauge IV indwelling catheter was placed in the ulnar vein of the right or left wing and secured in place with a transparent adhesive bandage.

Transesophageal echocardiographic, TE, and CTE examinations were performed in all birds in this specific order. The order was not randomized owing to contrast agent administration that could have affected findings with other methods. Cineloops of each echocardiographic view for the 3 methods were saved in the hard drive of the echocardiographic machine for measurements to be performed at a later time.

Transesophageal echocardiography was performed as described elsewhere for birds.[25] A 7-MHz pediatric multiplane transesophageal probe[a] connected to the echocardiographic system[b] was introduced into the mouth and brought to the level of the heart. The probe was directed through the crop and thoracic inlet by manual palpation. A complete TEE examination was performed, including caudal transverse, caudal longitudinal, middle transverse, cranial transverse, and middle-cranial longitudinal views.[25]

Transcoelomic echocardiography was performed in accordance with an established protocol.[3,4,6] A 4- to 12-MHz phased-array transducer probe[c] was used and placed over the abdomen in a ventromedian approach. Longitudinal horizontal and vertical views were obtained.

Contrast TE was performed as described elsewhere.[26] Ultrasonographic contrast agent (perflutren lipid microspheres[d]) was activated for 5 minutes with a mechanical activating device.[e] A 0.1-mL volume of the contrast agent was diluted with 0.9 mL of saline (0.9% NaCl) solution in a 1-mL syringe and injected IV via the indwelling catheter during a 20- to 30-second period. The catheter was then flushed with an additional 1 mL of saline solution for 20 seconds. Harmonic imaging was used during this part of the study, and low acoustic power (mechanical index, 0.2 to 0.3) was used to limit microsphere destruction. The contrast agent was visible in the left ventricular cavity for several minutes and allowed a complete transcoelomic evaluation to be performed.

Measurements—Three observers (HB, RP, and DR) with experience in avian echocardiography and diagnostic imaging obtained cardiac measurements on 3 occasions. All observers made their measurements on the same predetermined still images selected from the recorded cineloops on the basis of clarity and adequate resolution of the structures to be measured so that image choice would not be a source of variation. All still images were selected by the same observer (HB). All observers had specific guidelines for measurement and landmark identification, which were reviewed on test images prior to the study. To avoid potential confounding and carryover effects, the order of birds and the order of echocardiographic techniques within birds were randomized in a stratified approach with the aid of statistical software.[f] Additionally, within observers, each replicate was performed on a different day. The randomization pattern was the same for the 3 observers, and none had access to the other observers' measurements prior to the completion of the study.

The variables measured included the length, width, and width-to-length ratio for the left and right ventricular chambers in systole and diastole, aortic root diameter in systole, and aortic outflow velocity obtained in pulse-wave Doppler assessment. Fractional shortening for the left and right ventricles were computed as 100 × (diastole width – systole width)/diastole width. For image selection, systole was identified as the frame of a cineloop in which the left ventricular internal diameter was at its minimum. Diastolic frames corresponded to images in which the left ventricular internal diameter was at its maximum. Aortic root diameter was measured when the aortic valve was open.

For TE, ventricular length was obtained from the horizontal 4-chambered view from the apex of the ventricular cavity to the atrioventricular valve, and the ventricular width was measured at half the ventricular length (**Figure 1**). For CTE, ventricular measurements were performed on the contrast agent–filled ventricular cavities as described for the examination without contrast agent (**Figure 2**). For TEE, ventricular length was obtained from longitudinal views and the width measurements were obtained via M-mode on the caudal transverse view at the level of the widest diameter determined by scanning along

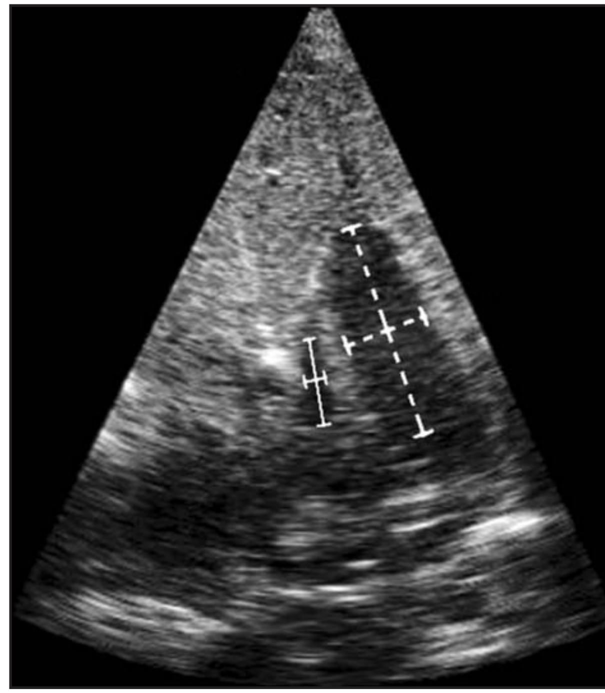

Figure 1—Transcoelomic echocardiographic horizontal view of the heart of a red-tailed hawk (*Buteo jamaicensis*) in diastole, as obtained through a ventromedian approach to transducer probe placement (frame rate, 225 Hz; depth, 4 cm). Measurement lines are shown within the left ventricle (dashed lines) and right ventricle (solid lines).

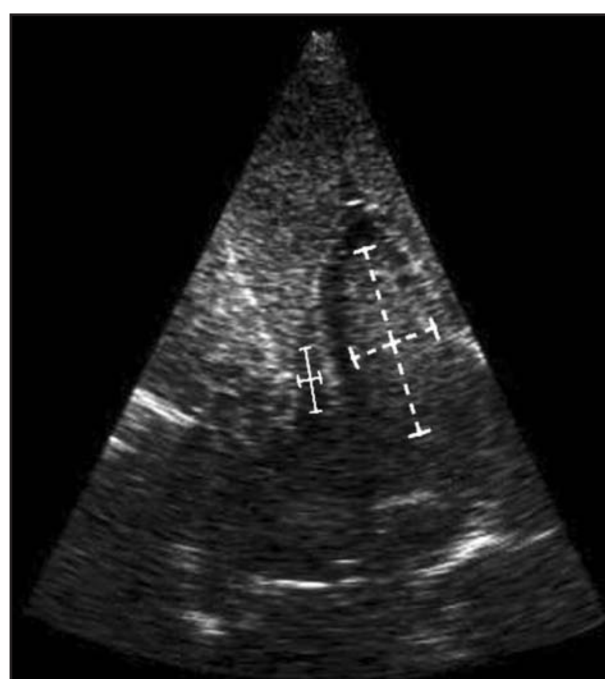

Figure 2—Contrast transcoelomic echocardiographic horizontal view of the heart of a red-tailed hawk in diastole, as obtained through a ventromedian approach to transducer probe placement (frame rate, 225 Hz; depth, 4 cm). *See* Figure 1 for remainder of key.

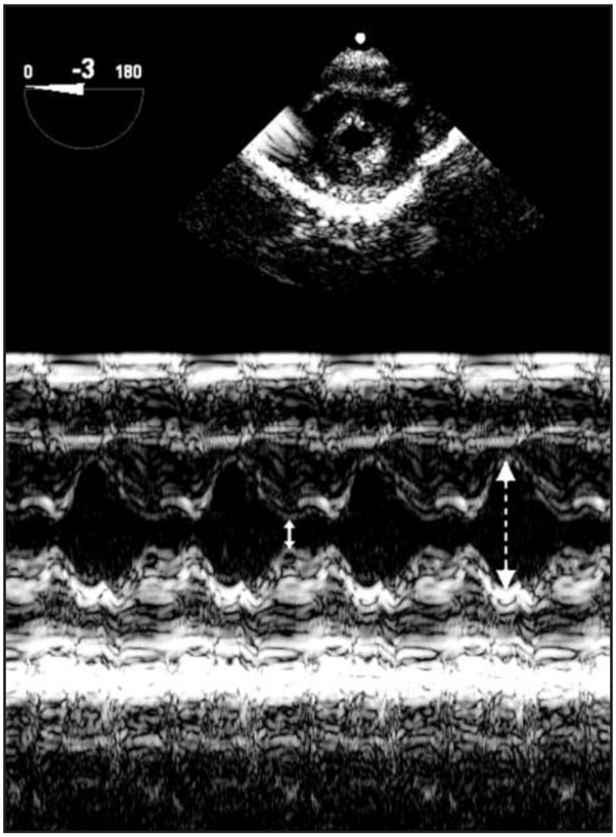

Figure 3—Two-dimensional guided M-mode image obtained via multiplane TEE from a caudal transverse view in a red-tailed hawk (frame rate, 67 Hz; depth, 3 cm). Measurement arrows are shown for left ventricular diameter in diastole (dashed arrow) and systole (solid arrow). The icon in the upper left corner indicates the degree of transducer rotation.

the long axis of the heart (**Figure 3**). Fractional shortening was determined in M-mode only. The aortic root diameter and aortic outflow velocity were determined on the cranial longitudinal view.

Variables measured were selected on the basis of reference values reported elsewhere for various avian species including raptors. Although values have been published for aortic root diameter in diastole, interventricular septum thickness in systole and diastole, atrial dimensions, and diastolic inflow for the left and right ventricles, these variables were intentionally not measured because the associated structures were not visualized or were not obtained in a consistent way across echocardiographic techniques and individual birds.

Statistical analysis—To investigate the differences among methods and potential sources of variation, a linear mixed model was fitted for each echocardiographic variable. The variables included the techniques (TE, CTE, and TEE) modeled as fixed effects and the subjects (birds) and observers modeled as factorial random effects.[28] Replicates accounted for the residual variance of the models. Competing fitted models with the various random effects were compared by the Akaike information criterion and likelihood ratio tests to assess whether birds, observers, and replicates were significant sources of variation in the models. Final models were evaluated for normality of data distribution, homoscedasticity, and noncorrelation of residuals by use of residual graphical methods and quantile plots. An ANOVA was performed with the fixed effect of the model (echocardiographic technique) to compare means. Post hoc multiple comparisons were performed with a Tukey adjustment, and intermethod differences are reported.

Table 1—Mean differences and agreement (ICC) among 3 methods of echocardiographic measurement performed in 10 anesthetized red-tailed hawks (*Buteo jamaicensis*) by 3 observers in triplicate.

	TE vs CTE		TE vs TEE		TEE vs CTE
Variable	Difference	ICC (95% CI)	Difference	ICC (95% CI)	Difference
LVLS (cm)	0.00	0.24 (0.03–0.43)*	0.32*	0.36 (0.00–0.60)†	–0.32*
LVLD (cm)	–0.05	0.28 (0.00–0.50)†	0.22*	0.22 (0.01–0.40)†	–0.27*
LVWS (cm)	–0.08†	0 (NA)	0.12*	0 (NA)	–0.20*
LVWD (cm)	–0.12*	0 (NA)	0.07[a]	0 (NA)	–0.19*
LVS ratio	–0.07	0.12 (0.00–0.32)	0.03	0 (NA)	–0.10*
LVD ratio	–0.04*	0.30 (0.10–0.47)*	0.00	0 (NA)	–0.04*
FS.LV (%)	–2.15	0 (NA)	–13. 5*	0 (NA)	11. 3*
RVLS (cm)	–0.13†	0.24 (0.00–0.46)†	0.12	0.24 (0.00–0.61)†	–0.25*
RVLD (cm)	–0.12	0.20 (0.00–0.41)†	0.15	0.38 (0.03–0.65)†	–0.27*
RVWS (cm)	–0.07*	0 (NA)	0.05	0 (NA)	–0.12*
RVWD (cm)	–0.08*	0 (NA)	0.08*	0 (NA)	–0.17*
RVS ratio	–0.09	0 (NA)	–0.03	0.58 (0.20–0.81)*	–0.06
RVD ratio	–0.02	0.21 (0.00–0.43)†	0.00	0.21 (0.00–0.54)†	–0.02
FS.RV (%)	–1.20	0.12 (0.00–0.37)	–0.85	0 (NA)	–0.32
AoS (cm)	0.01	0 (NA)	–0.20*	0 (NA)	0.21*
Ao outflow (cm/s)	8.10	NA	1.61	NA	6.49

AoS = Aortic diameter in systole. Ao outflow = Aortic outflow velocity. FS.LV = Fractional shortening of the left ventricle. FS.RV = Fractional shortening of the right ventricle. LVD ratio = Left ventricular width-to-length ratio in diastole. LVLD = Left ventricular length in diastole. LVLS = Left ventricular length in systole. LVS ratio = Left ventricular width-to-length ratio in systole. LVWD = Left ventricular width in diastole. LVWS = Left ventricular width in systole. NA = Not applicable. RVD ratio = Right ventricular width-to-length ratio in diastole. RVLD = Right ventricular length in diastole. RVLS = Right ventricular length in systole. RVS ratio = Right ventricular width-to-length ratio in systole. RVWD = Right ventricular width in diastole. RVWS = Right ventricular width in systole.

*Indicated value is significantly ($P < 0.01$) different from 0. †Indicated value is significantly ($P < 0.05$) different from 0.

To evaluate interobserver and intraobserver agreement, 2-way ICC for absolute agreement (ICC[2,3]) among results and 95% CIs were computed. Values of *P* were obtained from an *F* test for a null hypothesis of ICC = 0. Additionally, mean coefficients of variation were computed for all measurements for assessing interobserver and intraobserver variation. Agreement between TE and CTE and between TE and TEE measurements was also computed as an ICC(2,3). Agreement was considered high when the coefficient value was ≥ 0.76, medium when it was between 0.40 and 0.75, and low when it was ≤ 0.39.

Analyses were performed with statistical software.[f] Values of $P < 0.05$ were used to define a significant difference.

Results

Animals—No adverse effects were observed in the hawks during or after the examinations or contrast agent administration. All birds recovered from anesthesia without complication and were observed to be eating after the procedure.

Images—Excessive attenuation of ultrasound penetration from the accumulation of bubbles in the near field occurred during CTE soon after injection of contrast agent. Therefore, the best images were obtained several cardiac cycles after more homogeneous distribution of contrast enhancement in the left ventricle. A total of 270 measurements of 16 echocardiographic variables was obtained for each method (4,320 measurements).

Agreement—Individual birds and observers were significant ($P < 0.05$) sources of variation in the model for all variables except for right ventricular morphometric measurements, for which only the variable of birds had a significant random effect. Birds and observ-

Table 2—Interobserver and intraobserver agreement and variation in TE values obtained in 10 anesthetized red-tailed hawks by 3 observers in triplicate.

			Interobserver		Intraobserver	
Variable	Median	Range	ICC (95% CI)	CV	ICC (95% CI)	CV
LVLS (cm)	1.7	1.0–2.5	0.43 (0.08–0.69)*	15.4	0.84 (0.72–0.91)*	7.8
LVLD (cm)	2.1	1.3–3.2	0.46 (0.06–0.73)*	15.1	0.82 (0.70–0.90)*	8.2
LVWS (cm)	0.5	0.2–1.2	0.05 (0.00–0.25)	26.4	0.65 (0.46–0.8)*	17.7
LVWD (cm)	0.8	0.4–1.4	0.00 (NA)	22.5	0.74 (0.58–0.85)*	11.4
LVS ratio	0.3	0.1–0.8	0.22 (0.02–0.45)†	29.5	0.78 (0.64–0.88)*	15.5
LVD ratio	0.4	0.2–0.7	0.17 (0.00–0.40)†	23.9	0.82 (0.70–0.90)*	10.1
FS.LV (%)	39.0	2.4–64.9	0.26 (0.05–0.50)*	33.0	0.47 (0.26–0.67)*	25.8
RVLS (cm)	0.9	0.4–1.6	0.10 (0.00–0.34)	26.8	0.36 (0.12–0.61)*	20.2
RVLD (cm)	1.1	0.5–1.9	0.20 (0.00–0.43)†	20.8	0.60 (0.39–0.77)*	13.6
RVWS (cm)	0.3	0.1–0.5	0.16 (0.00–0.42)	26.3	0.41 (0.16–0.65)*	20.9
RVWD (cm)	0.4	0.2–0.7	0.29 (0.06–0.54)*	18.2	0.46 (0.23–0.67)*	16.3
RVS ratio	0.3	0.1–0.7	0.34 (0.08–0.60)*	27.1	0.82 (0.67–0.9)*	15.8
RVD ratio	0.4	0.1–0.9	0.44 (0.12–0.69)*	26.5	0.73 (0.56–0.86)*	14.9
FS.RV (%)	28.2	0.0–65.5	0.09 (0.00–0.34)	62.3	0.39 (0.14–0.63)*	59.1
AoS (cm)	0.4	0.2–0.8	0.06 (0.00–0.29)	20.2	0.08 (0.00–0.32)	18.6
Ao outflow (cm/s)	85.6	47.4–115.0	NA	2.3	NA	2.3

See Table 1 for key.

Table 3—Interobserver and intraobserver agreement and variation in CTE values obtained in 10 anesthetized red-tailed hawks by 3 observers in triplicate.

			Interobserver		Intraobserver	
Variable	Median	Range	ICC (95% CI)	CV	ICC (95% CI)	CV
LVLS (cm)	1.8	0.7–3.2	0.20 (0.00–0.45)†	24.7	0.80 (0.67–0.89)*	11.3
LVLD (cm)	2.1	0.8–3.2	0.28 (0.00–0.57)†	18.1	0.81 (0.67–0.90)*	7.8
LVWS (cm)	0.5	0.2–2.2	0.32 (0.09–0.56)*	18.4	0.14 (0.00–0.40)	16.1
LVWD (cm)	0.9	0.6–1.9	0.14 (0.00–0.39)	8.6	0.24 (0.03–0.48)†	7.8
LVS ratio	0.3	0.2–3.0	0.00 (NA)	25.5	0.00 (NA)	19.1
LVD ratio	0.4	0.2–0.9	0.30 (0.02–0.57)†	20.5	0.70 (0.53–0.83)*	8.4
FS.LV (%)	43.0	0.0–70.0	0.42 (0.19–0.65)*	34.3	0.62 (0.41–0.78)*	30.6
RVLS (cm)	1.0	0.4–1.9	0.05 (0.00–0.23)	36.6	0.56 (0.30–0.76)*	19.9
RVLD (cm)	1.2	0.4–1.9	0.00 (NA)	33.0	0.66 (0.44–0.82)*	16.3
RVWS (cm)	0.3	0.2–0.8	0.02 (0.00–0.31)	31.8	0.27 (0.02–0.55)†	23.8
RVWD (cm)	0.5	0.2–0.9	0.16 (0.00–0.45)	21.5	0.19 (0.00–0.46)†	18.9
RVS ratio	0.3	0.1–5.9	0.00 (NA)	32.8	0.02 (0.00–0.32)	27.2
RVD ratio	0.4	0.2–0.8	0.28 (0.00–0.58)†	27.8	0.71 (0.51–0.85)†	14.1
FS.RV (%)	31.1	28.8–59.6	0.00 (NA)	59.3	0.35 (0.09–0.62)*	48.3
AoS (cm)	0.4	0.2–1.0	0.00 (NA)	22.4	0.26 (0.01–0.55)†	18.4
Ao outflow (cm/s)	68.0	55.0–69.8	NA	2.0	NA	2.1

See Table 1 for key.

Table 4—Interobserver and intraobserver agreement and variation in TEE values in obtained 10 anesthetized red-tailed hawks by 3 observers in triplicate.

			Interobserver		Intraobserver	
Variable	Median	Range	ICC (95% CI)	CV	ICC (95% CI)	CV
LVLS (cm)	1.4	0.6–2.5	0.44 (0.18–0.66)*	18.1	0.81 (0.68–0.90)*	9.3
LVLD (cm)	1.8	0.3–1.1	0.61 (0.39–0.78)*	9.4	0.76 (0.60–0.87)*	6.8
LVWS (cm)	0.4	0.1–0.9	0.50 (0.39–0.78)*	38.1	0.91 (0.84–0.95)*	15.0
LVWD (cm)	0.7	0.5–1.2	0.27 (0.05–0.52)*	13.1	0.80 (0.66–0.89)*	5.4
LVS ratio	0.2	0.1–0.6	0.36 (0.13–0.60)*	32.2	0.80 (0.65–0.90)*	18.7
LVD ratio	0.4	0.2–1.1	0.13 (0.00–0.36)	17.1	0.74 (0.57–0.85)*	7.7
FS.LV (%)	50.0	7.9–90.6	0.52 (0.23–0.74)*	28.4	0.85 (0.74–0.92)*	15.6
RVLS (cm)	0.8	0.4–1.4	0.00 (NA)	39.0	0.46 (0.07–0.82)*	23.8
RVLD (cm)	1.1	0.5–1.5	0.13 (0.00–0.55)	24.8	0.66 (0.30–0.90)*	13.9
RVWS (cm)	0.2	0.1–0.7	0.18 (0.00–0.58)	28.9	0.33 (0.00–0.69)†	28.2
RVWD (cm)	0.3	0.2–0.5	0.58 (0.25–0.84)*	17.4	0.65 (0.36–0.86)*	14.3
RVS ratio	0.25	0.1–0.8	0.44 (0.05–0.80)†	39.2	0.77 (0.45–0.94)*	24.2
RVD ratio	0.3	0.1–1.1	0.33 (0.00–0.73)†	37.4	0.70 (0.36–0.91)*	18.3
FS.RV (%)	26.8	0.0–57.3	0.14 (0.00–0.56)	51.0	0.20 (0.00–0.60)	55.7
AoS (cm)	0.6	0.3–1.2	0.09 (0.00–0.37)	17.0	0.42 (0.19–0.64)*	11.1
Ao outflow (cm/s)	69.3	55.0–99.0	NA	13.2	NA	15.6

See Table 1 for key.

ers each accounted for 10% to 30% of the variation observed in the echocardiographic measurements.

Significant differences were evident among the 3 echocardiographic methods for most measurements, and the agreement was low except for the right ventricular ratio, where it was medium (**Table 1**). Measurement values obtained during contrast imaging were generally greater than with TE and TEE, and those obtained with TEE were generally lower than with TE. Values for fractional shortening of the left ventricle were significantly higher with TEE than with TE.

Interobserver agreement was generally low to medium for echocardiographic measurements, with better overall agreement obtained with TEE (**Tables 2–4**). Intraobserver agreement was generally medium to high with the exception of CTE, which had generally medium agreement. Overall, interobserver and intraobserver agreement was best for left ventricular measurements versus other measurements and for the transesophageal approach versus other methods. Interobserver and intraobserver agreement was consistently low for aortic diameter measurements. Agreement for aortic outflow velocity could not be assessed because of the number of instances in which it was not measured during the examination. However, the CV was low for this variable. Agreement results were corroborated by the high degree of variation observed (> 20%), particularly among observers.

Discussion

In the study reported here, 3 echocardiographic techniques (TE, CTE, and TEE) were used and compared in anesthetized red-tailed hawks. Significant differences were found in the means, agreement, and variation of most echocardiographic variables among techniques, supporting our research hypothesis. Within techniques, low to medium interobserver and intraobserver agreement was observed for most variables, with the exception of those pertaining to the left ventricle, for which medium to high intraobserver agreement was observed. No variable had high interobserver agreement, regardless of the techniques used. Likewise, interobserver and intraobserver measurement variation was high, with 73% and 31% of measurements having an interobserver and intraobserver CV > 20%, respectively.

The study also demonstrated slightly better reliability of the TEE results, compared with results of TE and CTE, with all left ventricular measurements obtained by the same observer showing high agreement and low variation (CV < 10%), except for the diastolic ratio. Measurements obtained for right ventricular variables and aortic diameter were consistently of low reliability across echocardiographic methods.

In human, canine, feline, and equine echocardiography, interobserver and intraobserver agreement is reportedly high for selected measurements. In studies[29–31] of adults and children, high ICCs (> 0.9) are common. Because of the complex shape of the right ventricular chamber and the challenges in obtaining standardized imaging planes and repeatable measurements, assessment of the right ventricular size is often more qualitative than quantitative.[32,33] In dogs, CVs for morphometric measurements are commonly < 10% to 15% and repeatability of the measurements is better for the left versus right ventricle and in M-mode versus B-mode.[34,35] For Doppler echocardiographic measurements, variation is often < 20%.[36] Cardiac measurements in cats are also repeatable.[37] In addition, measurements in horses are reproducible and repeatable, particularly when M-mode echocardiography is used, with high ICCs (> 0.8) and low CVs (< 15%).[38,39] Sources of measurement variation in mammalian echocardiography include the animal position, imaging plane, operator, interpreter, and heart size. In birds of prey, stress of handling and isoflurane anesthesia are known to affect the values obtained with spectral Doppler echocardiography.[40,41]

Contrast agents enhance endocardial border delineation and ventricular opacification and can improve measurement reproducibility and diagnostic accuracy as well as decrease interobserver variation in human echocardiography, particularly when poor acoustic windows are present.[21,27,42] The bubble size of the contrast agent varies between 1 and 4 μm.[43] Intraclass correlation coefficients obtained with contrast enhancement are commonly > 0.95.[42] It has been estimated that contrast agent administration gives 37% more information, enhances decision making, and shortens the time to diagnosis of cardiac disease in humans, compared with non–contrast-enhanced echocardiography.[42] Complications from the administration of ultrasonographic contrast agent have been reported to be rare in humans.[42,44]

Transesophageal echocardiographic examination improves the visualization and resolution of many cardiac structures in humans, compared with transthoracic echocardiography, and is routinely used during cardiac surgeries and interventions.[21,45,46] Reproducibility of TEE measurements are excellent in humans.[47] The larger differences observed between transesophageal measurements and measurements obtained with other techniques may be attributable to the differences in the imaging planes obtained, and these differences have also been reported in human medicine.[21]

Major complications associated with TEE are also rare in humans but may be more important in smaller species such as birds.[45,48] Death due to esophageal perforation was reported after a TEE procedure in an Amazon parrot.[25] Therefore, this technique is not recommended in smaller avian species and medium-sized psittaciformes.[25] However, the recent release of neonatal TEE transducers may allow the procedure to be performed in such birds in the future.

The overall low reliability observed in the present study was unlikely caused by the variation in the degree of competence with avian echocardiography among the 3 observers. This is because the study was designed to avoid the potential sources of variation imposed by differences in clinical experience, imaging plane selection, and measurement methods by preselecting still images and reaching a prior consensus on echocardiographic landmarks. Therefore, the overall low reliability may have been attributable to the small size and different appreciation of anatomic landmarks on low-resolution images with ill-defined edges of measured cardiac structures and to the limited use of M-mode. Cardiac size significantly affects the reproducibility of measurements of human hearts.[49] Although the use of contrast agent appeared to facilitate measurement of the left ventricular chamber in our study, it did not appear to improve the reliability of TE measurements; interobserver and intraobserver agreement was even lower, compared with results of TE. These suboptimal results were difficult to interpret but may have been influenced by the lower ultrasonographic power used during imaging and to the heterogeneity of contrast enhancement.[42] Nevertheless, the use of contrast agent may remain advantageous for the diagnosis of structural abnormalities and masses. Subjectively, endocardial borders were more visible in the hawks when this method was used, compared with when no contrast agent was used.

The higher reliability of left ventricular versus other measurements obtained with TEE may have been associated with the use of M-mode, which, with a better temporal resolution than B-mode, allows more precise measurements at the different phases of the cardiac cycle. Comparisons of reliability between TEE left ventricular width in diastole and systole and fractional shortening between M-mode and B-mode were not performed. The CV was low for aortic outflow velocity in our study (< 3% for TE and CTE), but this value must be interpreted with caution considering that it was not obtained consistently in all hawks. The range of ventricular fractional shortening was wide, with some values < 10%; however, the birds used were healthy, and the low values obtained may not have truly reflected actual fractional shortening because of the difficulties in obtaining accurate and reliable measurements in birds.

The sample size in the present study was 10 birds, which could be considered low. However, because of the high number of measurements typically obtained, studies[34–37,39] of agreement in veterinary echocardiography commonly comprise 4 to 10 subjects. In addition, sufficient power to detect differences among techniques was apparent in the high number of significant findings. Simultaneous ECG recording could have been used to better identify the frame at the onset of diastole and systole; however, this technique was deemed impractical because manual repositioning of the birds was frequent to allow adequate images to be obtained with the 3 techniques used.

Another limitation of our study was that measurement reliability was not assessed in birds with cardiac disease. Reliability of measurements in diseased birds might be higher than that in healthy birds because the examination is facilitated by the potential presence of cardiomegaly, hepatic congestion, and accumulation of fluids in the surrounding tissues that often improves the echocardiographic window.[6] Additionally, the most common echocardiographic changes diagnosed in birds are right ventricular enlargement and right atrioventricular valve regurgitation. In most situations, by the time a bird is brought to a veterinarian for evaluation, the right chamber dilation is dramatic and the right heart is larger than the left.[3,10–12,16,50] Cardiac measurements might not have any diagnostic usefulness in birds in which obvious changes are present; however, such measurements may be used to assess progression on follow-up examinations.

Echocardiographic measurements in hawks were not reliable in the present study, suggesting that some cardiac morphometric measurements may not be clinically meaningful. If measurements are to be obtained in clinical practice, one needs to consider that follow-up measurements should be performed by the same echocardiographer and a difference of at least 20% in values between measurement sessions should be used to ensure any difference is genuine. We also recommend calculating the mean of measurements from several cardiac cycles to obtain representative measurements. Furthermore, reference intervals established by 1 observer without accounting for measurement variation may not be applicable for an examination performed by another independent operator or when a different echocardiographic technique is used. These effects may be exacerbated by the use of different echocardiographic machines, use of imaging software, degree of bird distress, and use of sedatives. Given that birds weighing > 0.82 kg were used in the study, measurement reliability may be even lower in smaller avian species such as parrots. Further advances in avian echocardiography and improvement in measurement reliability will likely depend on higher resolution imaging techniques and smaller transducers.

a. S7-3t TEE, Philips Healthcare, Andover, Mass.
b. IE 33, Philips Healthcare, Andover, Mass.
c. S12-4 Sector array, Philips Healthcare, Andover, Mass.
d. Definity, Lantheus Medical Imaging Inc, North Billerica, Mass.
e. Vialmix, Lantheus Medical Imaging Inc, North Billerica, Mass.
f. R, version 2.12.1, R development core team (2011). R Foundation for Statistical Computing, Vienna, Austria. Available at: www.r-project.org/. Accessed Sep 9, 2011.

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