ORIGINAL RESEARCH

Comparison of the Repeatability of Echocardiographic Measurements from Different Modes and Views in Horses of Various Breeds and Sizes

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ABSTRACT

The objective of this study was to compare the repeatability of echocardiographic measurements obtained from different echocardiographic modes and views in healthy adult equids of various sizes, breeds, and thorax shapes. Ten equids (body weight: 120–662 kg; age: 1–26 years) from various breeds, free of cardiac disease, were used in this study. Each animal was submitted to a standardized echocardiographic and Doppler protocol 3 times at 1 day interval. This protocol included the measurements of left and right ventricular, aortic, pulmonary, and left atrial parameters obtained from different views using the bidimensional (2D) or the motion (M) modes, and the measurement of several parameters of blood flow obtained from the pulsed wave Doppler mode. Repeatability of each measurement was estimated on the basis of the residual variance using a linear model and the coefficient of variation of repeated measurements. A two by two comparison of the repeatability of measurements performed in different views was performed using the residual variances in a variance ratio *F* test. Results showed that repeatability of echocardiographic or Doppler measurements in equids of various sizes, breeds, and thorax shapes are comparable to previously reported results in thoroughbred and standardbred horses. Left ventricular morphologic parameters showed a good repeatability in the classic M-mode right parasternal short axis view at the level of the chordae tendineae, but the 2D-mode right parasternal long axis four-chamber view appeared to offer an interesting alternative measurement. This latter view also allowed obtaining the most repeatable measurement of right ventricular internal diameter. The left atrial diameter was maximal and most repeatable in the 2D-mode left parasternal long axis four-chamber angled view, and the repeatability of the aortic diameter was best in the 2D-mode right parasternal long axis five-chamber view. Finally, aortic systolic time intervals were more repeatable when measured from the Doppler mode as compared with the M-mode. In conclusion, repeatability of echocardiographic measurements in horses could be optimized after the following protocol: (1) M-mode right parasternal short axis view at the level of chordae tendineae to measure left ventricular morphologic parameters, and 2D-mode right parasternal long axis four-chamber as an alternative view; (2) 2D-mode right parasternal long axis five-chambers to measure the aortic diameter; (3) 2D-mode left parasternal long axis four-chambers angled view to measure the left atrial diameter.

Keywords: Echocardiography; Repeatability; 2D-mode; M-mode; Doppler measurements

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INTRODUCTION

Echocardiography and Doppler echocardiography are routinely used in cardiology as they allow a noninvasive assessment of cardiac morphological dimensions and functional indices.[1] Many reports have demonstrated their usefulness as diagnostic and prognostic tools for most congenital and acquired cardiac diseases in the equine species.[1-6] Moreover, echocardiography has been used in several studies to assess the changes induced by training[7-9] or drug administration[4,10-13] in this species.

Regardless of the target species, to allow an accurate evaluation of cardiac dimensions and functional indices, measurements obtained by echocardiography have to be first demonstrated to be repeatable and accurate in this species.[14-16] Accuracy is difficult to assess because there is no other gold standard for cardiac measurements in vivo that could be referred to, and postmortem measurements are not truly representative of in vivo morphologic measurements.[17-19] Consequently, the reliability of echocardiographic or Doppler echocardiographic measurements is often based on evaluation of their repeatability.[20] However, there is no clear consensus on how to assess the reliability of echocardiographic variables.[16]

Despite the wide use of echocardiography and Doppler echocardiography in horses, repeatability of these techniques has been poorly documented in this species. Moreover, most of the studies that were performed to test the intra and interobserver repeatability of equine echocardiographic measurements were performed in thoroughbreds and standardbreds.[4,6,14-16,21-25] It has been established that echocardiographic views are easier to obtain in these breeds than in more broad chested breeds in which the images are of poorer quality.[21] Furthermore, these studies focused on echocardiographic views most commonly used in dogs or human patients. No other views, potentially more adequate for the equine, have been tested in these studies. In addition, the results of the previous repeatability studies performed in horses were sometimes conflicting, with some of the tested parameters, especially Doppler parameters, showing a high variability,[6] and thus questioning the accuracy of those parameters.

Finally, several authors compared the mean value of echocardiographic parameters when measured from different views.[4,14,26] The results of those studies were conflicting: some authors showed significant differences when the parameters were measured in those different views, whereas other studies showed no significant differences between the different views.

The purpose of this study was to test and compare the intraobserver repeatability and the mean values of echocardiographic and Doppler echocardiographic measurements obtained from different modes (M and 2D-mode) and views (long and short axis, right and left parasternal) in healthy adult equids of various size, breeds, and thorax shape.

MATERIALS AND METHODS

Animals

Ten equids (5 mares and 5 geldings), including one donkey, two standardbreds, one pony, and six half-bloods were used in this study. Ages ranged from 1.5 to 25.6 years (Mean $\pm$ SD: 10.10 $\pm$ 7.6 years) and weights from 120 to 662 kg (Mean $\pm$ S.D: 459.8 $\pm$ 128.8kg). All the studied animals were free of cardiac disease or any pathologic condition, with a potential repercussion on heart size or function. They were hospitalized in the equine teaching hospital of the University of Liège for a problem without repercussion on cardiac function (ie, ophthalmic or dermatologic problem, lameness, or recurrent colic). Normal clinical examination, normal preliminary electrocardiographic, qualitative echocardiographic, and Doppler echocardiographic evaluation were prerequisite to be included in the study.

Each animal was submitted to the echocardiographic protocol three times at 1 day interval. Before imaging, the animals were shaved between the third and fifth intercostal space just caudal to the triceps muscle mass, from 3 to 5 cm below the olecranon to 5 to 10 cm above it. The shaved areas were then copiously rinsed with water and acoustic coupling was obtained using ultrasound gel.

Imaging Technique

An ultrasound system (System five, GE Vingmed medical systems, General Electrics, Belgium) equipped with a 2.5 MHz phased-array sector scanner and bidimensional (2D), time motion (M), color mapping, and spectral Doppler modes were used for this study. The ultrasound machine had an integrated electrocardiogramme (ECG) function, in which the ECG traces were displayed simultaneously with the images. A base-apex bipolar DII lead system was used, with electrodes applied over the left jugular furrow and the ventral portion of the left abdomen over the girth place.

During the investigation, the forelimb of the investigated hemithorax was pulled as forward as could be tolerated by the horse. All images or loops of images were recorded on a disc driver (Echopac, General Electric, Belgium) for subsequent analysis.

Terminology and image orientation were those recommended by the echocardiographic committee of the specialty of cardiology (American College of Veterinary Internal Medicine).[27] Imaging planes were selected according to the identification of intracardiac landmarks as previously reported in horses.[4,21,24,28] For Doppler-mode views, alignment with blood flow was initially assessed from a 2D ultrasound image to minimize beam angulation.[6] Accurate alignment with blood flow was assumed when the audible signal was clear and the spectral envelope of the Doppler waveform was complete.[22] The sample volume was set at 5 mm for all Doppler measurements.

Animals were first imaged from the right hemithorax by placing the ultrasound beam in the fourth or fifth intercostal space, perpendicular to the thorax wall, just dorsal to the olecranon. The position of the beam was adjusted to obtain and record a 2D-mode right parasternal long axis (LAx) four-chamber reference view, with the interventricular septum orientated as horizontal as possible.

In this view, the motion mode (M-mode) was then selected and the cursor was placed perpendicular to the interventricular septum and the left ventricular free wall at level of the chordae tendineae to obtain and record an M-mode right parasternal LAx four-chamber reference view.

Starting from the 2D-mode LAx four-chamber reference view, the beam was rotated clockwise with slightly cranial and dorsal direction to obtain and record a 2D-mode right parasternal LAx five-chamber view showing the left ventricle and the left ventricular outflow tract, where the aorta was positioned perpendicular to the axial beam.

In this view, the M-mode was then selected to produce and record an M-mode right parasternal five-chamber view of root of the aorta by placing the cursor perpendicular to the wall of the aorta in the direction of the largest part of the left atrium.

Restarting from the 2D-mode right parasternal LAx four-chamber reference view, the beam was then rotated in a clockwise direction toward the olecranon to produce and record a 2D-mode short-axis (SAx) view of the left ventricle at the level of the chordae tendineae where the interventricular septum, the right and left ventricle, and the left ventricular free wall were bisected at right angles and the left ventricle was circular. In this view, the chordae tendineae were clearly visible whereas papillary muscles and the mitral valve leaflets were not visible.

In this view, M-mode was then selected to obtain the M-mode SAx view of the left ventricle at the level of the chordae tendineae by placing the cursor at the angle through the left ventricle, dividing the left ventricle in symmetric halves.

From the 2D-mode right parasternal SAx view of the left ventricle at level of chordae tendineae, the beam was pivoted dorsally and cranially toward the base of the heart at the level of the aortic root that appeared circular at the centre of the image. This view was recorded, and therefore the M-mode was selected to obtain and record the M-mode right parasternal SAx view at level of the root of aorta by dividing the aorta in symmetric halves by the cursor.

Restarting from the 2D-mode right SAx view at the chordal level, the beam was then pivoted dorsally in the direction toward the base of the heart and rotated slightly clockwise or anticlockwise until a clear 2D right parasternal image of the base of the heart at the level of the pulmonary valve was obtained and recorded.

The Doppler pulsed-wave mode was then selected, and the gate placed just distal to the pulmonary valve to record the pulmonary Doppler outflow velocity spectrum.

Restarting from the 2D-mode right parasternal LAx four-chamber reference view, the transducer was then directed as ventrally as possible to obtain the angled view of the right ventricle inflow tract. In this view, pulsed-wave Doppler mode was selected and the gate was placed so that it was at the valve tips in systole and between the valve leaflets in diastole. This allowed obtaining and recording the tricuspid inflow velocity spectrum.

Animals were then examined from the left hemithorax. The beam was placed in the fourth or fifth intercostal space perpendicular to the hemithorax wall just dorsal to the olecranon and was adapted to produce and record a 2D-mode left parasternal LAx four-chamber view, where the interventricular septum was as horizontal as possible. In this view, the M-mode was then selected to obtain and record an M-mode left parasternal LAx four-chamber view of the left ventricle. To obtain this view, the cursor was placed perpendicular to the interventricular septum and the left ventricular free wall at the level of the chordae tendineae, between the mitral valve leaflets tips and the left ventricular papillary muscles.

From the 2D-mode left parasternal LAx four-chamber view, the beam was then rotated in a clockwise direction to produce and record a 2D-mode SAx view of the left ventricle at the level of the chordae tendineae where the interventricular septum, right ventricle, left ventricle, and free wall of the left ventricle were bisected at right angles and the left ventricle was circular. From this view, an M-mode left parasternal SAx view of the left ventricle was obtained and recorded by placing the cursor perpendicular to the interventricular septum and the left ventricular free wall and dividing the left ventricle in symmetric halves.

Coming back to a 2D-mode left parasternal LAx four-chamber view, the beam was then moved ventrally and caudally to produce and record a 2D-mode left parasternal LAx angled view of the left ventricle where the largest diameter of the left atrium was obtained.[16] Then, the Doppler pulsed-wave mode was selected and the gate was placed at level of the mitral valve tips in systole and between the mitral valve leaflets in diastole to record the mitral inflow velocity spectrum.

From the left parasternal LAx angled view, the beam was then rotated anticlockwise until the aortic root was visible, allowing recording a 2D-mode left parasternal apical five chamber view. Then, the Doppler pulsed-wave mode was selected and the gate was placed just distal to the aortic valve for recording the aortic outflow velocity spectrum.

Echocardiographic Measurements

All measurements were the mean for three cardiac cycles. Measured cardiac cycles were not necessarily consecutive. Echocardiographic examinations and measurements were always performed by the same investigator (A.A.H.).

All echocardiographic measurements were made at end-diastole and end-systole. Diastolic measurements were made at the onset of the QRS complex or at largest left ventricular dimension. Systolic measurements were made at smallest left ventricular dimension (2D-mode) or peak downward point of septal motion (M-mode).[6] All measurements were made using the leading edge to leading edge method.[21,29,30] Because the heart rate influences the repeatability of echocardiographic measurements,[19] all measurements were obtained when the heart rate was below 45 beats/min.

2D-mode Measurements. The right and left ventricular internal diameter (RVID and LVID, respectively), and the interventricular septum and left ventricular free wall thickness (IVS and LVFW, respectively) were measured in the 2D-mode at end-diastole and end-systole from the right and left parasternal LAx four-chamber view at the level of an imaginary line perpendicular to interventricular septum and left ventricular free wall between the mitral valve and papillary muscles.

The RVID, LVID, IVS, and LVFW were also measured in the 2D-mode at end-diastole and end-systole from the right and left parasternal SAx view at the level of an imaginary line that divided left ventricle into symmetric halves. The left atrium internal diameter (LA) was measured in the 2D-mode at end-diastole from the right parasternal LAx four-chamber reference view and from the left parasternal LAx angled view of the left ventricle.[8]

The aortic internal diameter (Ao) was measured in the 2D-mode at end-diastole from the following views:

- The right parasternal LAx left ventricular outflow (five-chamber) view. In this view, a line connecting the annulus was made and measured (Ao_{valve}), and the sinus of Valsalva was measured at its largest dimension (Ao_{sinus}). The ascending aorta was measured during systole at its narrowest dimension distal to the sinus, at the sino-tubular junction (Ao_{min}).
- The right parasternal SAx view at the level of the aortic root, using an imaginary vertical line perpendicular to the aortic walls and dividing the aortic root into symmetric halves.
- The left parasternal apical five-chamber view, at the level of sinus of Valsalva.

The pulmonary artery diameter was measured in the 2D-mode at end-diastole and at its largest diameter from the right parasternal image of the base of the heart at the level of the right ventricular outflow tract. The left ventricular internal and external area (LVIA and LVEA, respectively) were measured using planimetry in the 2D-mode at end-diastole and end-systole from the right and left parasternal SAx view of the left ventricle at the chordal level.

M-mode Measurements. The RVID, LVID, IVS, and LVFW were measured in the M-mode at end-diastole and end-systole from the right and left parasternal LAx four-chamber views and from the right and left parasternal SAx views at the chordal level. Ao was measured at end-diastole in the M-mode from the right parasternal LAx five-chamber view and from the right parasternal SAx view at the level of the aortic root. The LA was measured in the M-mode at end-diastole from the right parasternal LAx five-chamber view. The pre-ejection period and the ejection time of the aortic flow (PEP and ET, respectively) were measured from the M-mode right parasternal LAx five-chamber view of the aortic valves from the onset of QRS-complex to the opening of the aortic valves, and from the opening to the closure of the aortic valves, respectively.

Doppler Measurements. From the mitral and tricuspid velocity spectral recordings, the peak velocity of blood flow during the rapid ventricular filling (Peak E) and the atrial contraction (Peak A) were measured by placing the cursor at the apex of the maximal upward motion of blood flow. From the aortic and pulmonary velocity spectral recordings, the peak velocity of blood flow was measured by placing the cursor at the apex of the maximal downward motion of blood flow. The area under the velocity waveform was measured by tracing the modal velocity (represented by the brightest line in the spectral Doppler waveform) envelope of the Doppler signal.

The ET was measured from the onset to the end of the spectral waveform. The time to peak was measured from the onset of the Doppler waveform to the start of the maximum velocity plateau. The PEP was measured from the onset of the QRS complex to the onset of the spectral waveform.

Statistical Analysis

Repeatability of each measurement was estimated on the basis of the residual variance in a linear model including the effect of the animal, of the day of measurement, and of the interaction between animal and day of measurement when those factors were significant. For the parameters that were measured in different views, the repeatability of the measurement performed in each of the views was compared two by two using a variance ratio F test. Additionally, for each of the parameters measured in different views, the repeatability of the measurements obtained in M-mode were compared with those performed in 2D-mode using a variance ratio F test. Moreover, the coefficient of variation (CV) obtained for each parameter performed in each view was calculated. The degree of repeatability based on the obtained CV was defined as CV $<$ 5%: very high repeatability; 5% $<$ CV $<$15%: high repeatability; 15% $<$ CV $<$25%: moderate repeatability; CV $>$ 25%: low repeatability.[16]

RESULTS

The comparison of the repeatability of the 2D and M-mode echocardiographic parameters obtained in the different tested views is shown in Table 1. On the basis of the CV, most of the measurements showed a very high repeatability (CV $<$ 5 %), and some of them a high repeatability (CV between 5% and 15 %).

The RVID showed more repeatability when measured from the right LAx 2D-mode view than in the other views and less repeatability when measured from the left M-mode views than from the right views. Most of the left ventricular

Table 1. Comparison of the mean ± SE value, of the coefficient of variation (CV), and of the residual variance error (VE) of morphological echocardiographic parameters measured from different views three times at 1 day intervals by a single observer in 10 equids free of cardiac disease and of various breeds

Parameter	View	Symbol	Mean ± SE	CV (%)	VE
RVIDd (cm)	Right, LAx, 2D	1	4.80 ± 0.06	2.9	0.019[a,b,c,d,e,f,g]
	Right, LAx, M	2	4.70 ± 0.06[f]	4.4	0.042[e,g]
	Right, SAx, 2D	3	4.64 ± 0.06[f]	5.0	0.054[g]
	Right, SAx, M	4	4.38 ± 0.06[a,b,f,h]	5.2	0.057[g]
	Left, LAx, 2D	5	3.23 ± 0.06[a,b,c,e,f,h]	6.2	0.041[e,g]
	Left, LAx, M	6	3.43 ± 0.06[a,b,c,f,h]	8.4	0.083
	Left, SAx, 2D	7	4.95 ± 0.06	4.2	0.044[e,g]
	Left, SAx, M	8	2.88 ± 0.06[a,b,c,d,e,f,h]	11.7	0.112
LVIDd (cm)	Right, LAx, 2D	1	10.91 ± 0.05	1.1	0.016
	Right, LAx, M	2	10.73 ± 0.05	1.3	0.019
	Right, SAx, 2D	3	10.07 ± 0.05[a,d,e,f,g,h]	1.4	0.019
	Right, SAx, M	4	10.19 ± 0.05[a,d,e,g,h]	1.2	0.016[g]
	Left, LAx, 2D	5	10.39 ± 0.05[a,g,h]	1.1	0.013[g]
	Left, LAx, M	6	10.37 ± 0.05[a,g,h]	1.2	0.017
	Left, SAx, 2D	7	10.26 ± 0.05[h,a,g]	1.3	0.019
	Left, SAx, M	8	10.70 ± 0.05[h]	1.5	0.025[d]
LVIDs (cm)	Right, LAx, 2D	1	6.53 ± 0.05[a]	1.8	0.013[a,b,d,e,f]
	Right, LAx, M	2	6.78 ± 0.05	2.7	0.033[d]
	Right, SAx, 2D	3	6.35 ± 0.05[a,h]	3.3	0.045
	Right, SAx, M	4	6.25 ± 0.05[a,h]	2.4	0.023[b,d,e,f]
	Left, LAx, 2D	5	6.21 ± 0.05[a,b,h]	3.6	0.050
	Left, LAx, M	6	6.22 ± 0.05[a,b,h]	3.1	0.038
	Left, SAx, 2D	7	6.01 ± 0.05[a,b,c,d,e,g,h]	3.3	0.039
	Left, SAx, M	8	6.26 ± 0.05[a,h]	2.8	0.031[d]
IVSd (cm)	Right, LAx, 2D	1	2.45 ± 0.02[a,b,c,f]	2.6	0.004[a,b,c,d,e,g]
	Right, LAx, M	2	2.67 ± 0.02[b,f]	4.2	0.013
	Right, SAx, 2D	3	3.02 ± 0.02	2.8	0.007[a]
	Right, SAx, M	4	2.67 ± 0.02[b,f]	3.6	0.009
	Left, LAx, 2D	5	2.42 ± 0.02[a,b,c,f]	3.6	0.008[a]
	Left, LAx, M	6	2.36 ± 0.02[a,b,c,f,h]	4.3	0.010
	Left, SAx, 2D	7	2.86 ± 0.02[b]	2.5	0.005[a,c,e,g]
	Left, SAx, M	8	2.25 ± 0.02[a,b,c,d,e,f,h]	4.4	0.010
IVSs (cm)	Right, LAx, 2D	1	3.68 ± 0.02[a,b,c,e,f]	2.6	0.009[a,c,d]
	Right, LAx, M	2	4.03 ± 0.02[b,f]	3.0	0.015
	Right, SAx, 2D	3	4.11 ± 0.02[f]	2.7	0.012
	Right, SAx, M	4	4.05 ± 0.02[f]	3.2	0.017
	Left, LAx, 2D	5	3.63 ± 0.02[a,b,c,e,f,g]	3.3	0.014
	Left, LAx, M	6	3.78 ± 0.02[a,b,c,f]	2.7	0.010[c]
	Left, SAx, 2D	7	4.20 ± 0.02	2.3	0.009[a,c,d]
	Left, SAx, M	8	3.73 ± 0.02[a,b,c,f]	2.9	0.011
LVFWd (cm)	Right, LAx, 2D	1	2.00 ± 0.02[a,b,f]	4.1	0.007[c,f,g]
	Right, LAx, M	2	2.11 ± 0.02[b,f]	4.5	0.009
	Right, SAx, 2D	3	2.23 ± 0.02[f]	3.1	0.005[a,c,d,e,f,g]
	Right, SAx, M	4	2.00 ± 0.02[a,b,f]	5.9	0.014
	Left, LAx, 2D	5	1.93 ± 0.02[a,b,c,e,f,g,h]	4.7	0.010
	Left, LAx, M	6	2.05 ± 0.02[a,b,f]	4.3	0.008[c,f]
	Left, SAx, 2D	7	2.36 ± 0.02	4.8	0.013
	Left, SAx, M	8	2.00 ± 0.02[a,b,f]	5.2	0.011

(*Continued on next page*)

Table 1. *Continued*

Parameter	View	Symbol	Mean ± SE	CV (%)	VE
LVFWs (cm)	Right,LAx,2D	1	3.27 ± 0.04[a,c,d,e,f,g]	3.9	0.016[a,d,g]
	Right, LAx, M	2	3.54 ± 0.04	4.9	0.030
	Right, SAx, 2D	3	3.25 ± 0.04[a,c,d,e,f,g]	3.9	0.016[a,d,g]
	Right, SAx, M	4	3.48 ± 0.04	4.2	0.022
	Left, LAx, 2D	5	3.48 ± 0.04	5.2	0.032[c]
	Left, LAx, M	6	3.53 ± 0.04	4.3	0.023
	Left, SAx, 2D	7	3.42 ± 0.04[a,e]	2.5	0.007[a,b,c,d,e,g,h]
	Left, SAx, M	8	3.49 ± 0.04	4.5	0.025
LVIAd (cm^2)	Right, SAx, 2D	1	71.11 ± 0.60	4.8	11.86
	Left, SAx, 2D	2	77.31 ± 0.60	4.5	12.27
LVIAs (cm^2)	Right, SAx, 2D	1	28.53 ± 0.46	6.6	3.76
	Left, SAx, 2D	2	32.78 ± 0.47	6.1	4.05
LVEAd (cm^2)	Right, SAx, 2D	1	178.38 ± 1.22	4.1	52.70
	Left, SAx, 2D	2	190.73 ± 1.22	3.6	46.42
LVEAs (cm^2)	Right, SAx, 2D	1	170.02 ± 0.93	3.2	30.21
	Left, SAx, 2D	2	175.40 ± 0.93	3.6	40.98
LA (cm)	Right, LAx, 2D	1	8.81 ± 0.09[a,b]	1.5	0.019[b]
	Left, LAx, 2D	2	10.32 ± 0.09[b]	0.8	0.007[b,h]
	Right, LAx, M	3	5.76 ± 0.09	8.2	0.222
Ao (cm)	Right, LAx, 2D (Ao_{valve})	1	5.39 ± 0.04[a,c,d,e,f]	1.3	0.005[b,c,e]
	Right, LAx, 2D (Ao_{sinus})	2	7.57 ± 0.04[c,e]	1.1	0.007[b,c,e]
	Right, LAx, 2D (Ao_{min})	3	5.43 ± 0.04[a,d,e,f]	1.9	0.010[c]
	Right, LAx, M	4	8.75 ± 0.04	1.6	0.020
	Right, SAx, 2D	5	7.60 ± 0.04[c,e]	1.0	0.006[b,c,e]
	Right, SAx, M	6	8.68 ± 0.04	1.2	0.010[c]
	Left, LAx, 2D	7	6.56 ± 0.04[a,c,d,e]	1.2	0.006[b,c,e]
Pu (cm)	Right, SAx, 2D		5.65 ± 0.13	3.1	0.020

s and d, measurement at end-systole and end-diastole, respectively; RVID and LVID, right and left ventricular internal diameter, respectively; IVS and LVFW, interventricular and left ventricular free wall thickness, respectively; LVIA and LVEA, internal and external area of the left ventricle, respectively; LA, internal diameter of the left atrium; Ao and Pu, aortic and pulmonary internal diameter, respectively; Ao_{valve}, Ao_{sinus}, and Ao_{min}, internal diameter of the aorta at the level of the valve annulus, of the Valsalva sinus and of the sino-tubular junction, respectively; Right and Left, right and left parasternal view respectively; LAx and SAx, long and short axis view, respectively; M, time-motion mode; 2D, bidimensional mode.
[a,b,c,d,e,f,g,h] = VE significantly lower than VE obtained for the measurement of the parameter in the view symbolized by the corresponding number for this parameter, variance ratio F test, $P \leq 0.05$.

morphometric parameters showed a significantly higher repeatability when measured from the right LAx 2D-mode view than in the other views. The variability of LVIA and LVEA was not statistically different when measured from the right or from the left. The LA showed more ($P <$ .05) repeatability when measured from the left LAx 2D-mode view than from the other views. The repeatability of Ao measurement was, in general, very good as demonstrated by a low CV. However, it showed less ($P <$ .05) repeatability when measured from the right LAx 2D-mode view at the level of the sino-tubular junction or from the right LAx or SAx M-mode views than in most of the other views.

When pooling the results of the left ventricular morphometric parameters obtained from M or 2D- mode, the IVS and LVFW showed a significantly higher repeatability when measured from the M-mode than when measured from the 2D-mode.

The repeatability of the Doppler parameters is shown in Table 2. On the basis of the CV, all measurements showed a high repeatability (CV between 5% and 15%), except for aortic area under the velocity waveform that showed a CV just above this limit (15.6%).

The comparison of the repeatability of the aortic ET and PEP obtained from the Doppler aortic flow tracings and from the right parasternal LAx and SAx M-mode views are shown in Table 3. Regardless of the view used, the CV of those measurements ranged from 6.0% to 8.2%, thus showing a high repeatability. However, the PEP showed a significantly lower repeatability when measured from the right LAx M-mode view rather than from the right SAx M-mode view or from the blood flow Doppler view.

Table 2. Comparison of the coefficient of the mean ± SE value, of the coefficient of variation (CV), and of the residual variance error (VE) of Doppler parameters measured three times at one day interval by a single observer in 10 equids free of cardiac disease and of various breeds

	Parameter	Mean ± SE	CV (%)	VE
Tricuspid	Peak A (m/sec)	0.403 ± 0.005	9.9	0.002
	Peak E (m/sec)	0.535 ± 0.007	10.1	0.002
Pulmonary	V_{max} (m/sec)	0.681 ± 0.008	10.0	0.002
	VTI (cm)	28.598 ± 0.692	13.4	11.01
	TTP (sec)	0.292 ± 0.006	10.3	0.0002
	ET (sec)	0.511 ± 0.005	9.9	0.0003
	PEP (sec)	0.095 ± 0.001	14.9	0.00005
Mitral	Peak A (m/sec)	0.391 ± 0.007	10.1	0.001
	Peak E (m/sec)	0.538 ± 0.011	12.5	0.002
Aortic	V_{max} (m/sec)	0.683 ± 0.015	13.4	0.002
	VTI (cm)	28.031 ± 0.615	15.6	6.737
	TTP (sec)	0.265 ± 0.006	14.2	0.002
	ET (sec)	0.524 ± 0.005	6.0	0.001
	PEP (sec)	0.080 ± 0.0006	6.2	0.00004

Peak A and peak E, peak velocity of the mitral or tricuspid blood flow during the atrial contraction and the rapid ventricular filling, respectively; V_{max}, peak velocity of the aortic or pulmonary blood flow; VTI, area under the velocity waveform of the aortic or pulmonary blood flow; TTP, ET and PEP, time to peak, ejection time and pre-ejection period of the aortic or pulmonary blood flow, respectively.

Table 3. Comparison of the coefficient of the mean ± SE value, of the coefficient of variation (CV), and of the residual variance error (VE) of the aortic flow ejection time (ET) and pre-ejection period (PEP) measured from different Doppler and echocardiographic views three times at one day interval by a single observer in 10 equids free of cardiac disease and of various breeds

Parameter	View	Symbol	Mean ± SE	CV (%)	VE
ET (sec)	Doppler	1	0.524 ± 0.005	6.0	0.0013
	Right, LAx, M	2	0.483 ± 0.005[a]	6.4	0.0013
	Right, SAx, M	3	0.469 ± 0.005[a]	6.2	0.0017
PEP (sec)	Doppler	1	0.080 ± 0.0006	6.2	0.00005[b]
	Right, LAx, M	2	0.076 ± 0.0008	7.5	0.00007
	Right, SAx, M	3	0.078 ± 0.0008	8.2	0.00004[b]

Right and Left, right and left parasternal view respectively; LAx and SAx, long and short axis view, respectively; M, time-motion mode.
[a] Mean ± SE significantly lower than Mean ± SE obtained for measurement of parameter in the right, LAx, M-mode view or in the right, SAx, M-mode view respectively.
[b] VE significantly lower than VE obtained for the measurement of the parameter in the right, LAx, M-mode view or from the right, SAx, M-mode view, respectively, variance ratio F test, $P \leq 0.05$.

The comparison of the mean values of morphological echocardiographic measurements obtained from different echocardiographic views and modes is shown in Table 1. Most of the right and left ventricular parameters showed significantly different values according to the mode and view from where they were measured, with the exception of the systolic and diastolic measurements of LVIA and LVEA that were not statistically different when measured from the right or from the left parasternal view. The mean value of LA measurement was significantly higher when it was obtained from left hemithorax than from right hemithorax and from the 2D-mode rather than from the M-mode. The mean values of Ao measurement showed significant differences according to the view and the level from which it was measured. It was significantly higher when measured from the M-mode as compared with corresponding 2D-mode views. The comparison of the mean values of aortic PEP and ET measurements obtained from Doppler aortic flow trace and from the two right parasternal M-mode tested views is shown in Table 3. The mean value of those parameters was significantly longer when measured from the Doppler aortic flow trace than from the M-mode views.

DISCUSSION

Repeatability of echocardiographic measurements depends on several technical, physiologic, and examiner-dependent factors.[4,6,15,16,24,25] Technical factors include the quality and the accuracy of the equipment, the minimal resolution of measurements provided by the software, the distance of the measured structure from the transducer, and quality of the images recording system and of the calibration used to perform the measurements.[16,24,25] However, with recent equipment in digital format, the latter factor (calibration error) is avoided.[16] Physiologic factors mainly include hemodynamic (mostly heart rate) variability that is dependent on the behavior of the horse and its habituation to the echocardiographic examination. The thorax conformation and bodily condition are also important physiologic factors that influence the quality of the images.[6,15,24,25] Examiner-dependant factors include the quality of the imaging planes and the adherence of the operator to the imaging standardization and measurements guidelines. This is highly dependent on the experience of the operator.[6,15,21,24,25,31]

The present study was performed with a good quality machine, with a digital recording allowing obviation of calibration errors. However, the animals were not accustomed to echocardiographic examination and had varying body size and thorax conformation, and the images and measurements were made by an examiner with a limited (1 year) experience of echocardiography in the equine species. Those conditions reflect every day conditions encountered in most clinical cases examined by echocardiography.

There is no consensus on the method that should be used to evaluate the reliability of echocardiographic measurements.[16] In the present study, the intraobserver day-to-day variability of the measurements has been tested and both the CV produced by repeated measurements and a comparison of the variance produced in different views using an *F* test were used. The CV of repeated measurements has been previously used by several authors in the equine species,[6,14-16,21,24,25,31] whereas the comparison of the variance is an original approach of evaluating the repeatability.

On the basis of the CV, the repeatability of most of the morphological echocardiographic parameters performed in the present study in equids of various breeds and thorax shapes was high to very high, whatever the mode and view that was used to perform the measurements. The CV was closely comparable if not better than in the previous studies.[6,14-16,21,25,31] This suggests that even in horses with a cardiac window of a lower quality than in standardbreds and thoroughbreds, it is possible to obtain repeatable echocardiographic measurements when a standardized cardiac imaging is used. However, even if the CV of the echocardiographic measurements was low, it was not the same for all measured parameters, as previously reported by several authors.[6,14,15,21,25] Moreover, the variability of most of the measurements showed significant differences between the different views used to measure it. To our knowledge, this is the first study that statistically compared the repeatability of echocardiographic measurements performed from different views in the equine species.

For most of the left ventricular morphologic parameters, the quality of the images obtained from the left hemithorax in this study were of poorer quality than those obtained from the right hemithorax, as previously reported.[21] The M-mode right parasternal SAx view at the level of the chordae tendineae, which is considered as the reference view to perform the measurements, produced good repeatability as compared with the other tested views, but was not the view giving the best repeatability for most of those parameters. Interestingly, the 2D-mode right parasternal LAx view was the view giving the best or one of the best repeatability for several left ventricular parameters measurements. This view could thus be a good alternative for left ventricular measurements in horses.

In contrast, the comparison of the mean value of the left ventricular parameters obtained in the different tested views evidenced significant differences. This is contrary with previous studies performed in horses or donkeys that showed no significant differences when left ventricular measurements were performed from the M-mode rather than from the 2D-mode, or from the right rather than from the left hemithorax,[4,21,26] but in accordance with another study that evidenced LVID measurements differences when measured from the left or from the right side.[4] The significant differences between LVID measurements from different views obtained in the present study could be due to the higher number of measurements performed in the present study. Differences in LVID measurements between views could be due to differences in the plane or axis used, or slightly different cursor's positioning between views.

Measurement of RVID is known to be less repeatable than left ventricular measurements in horses,[14,21] and this was also the case in the present study. This could be due to the noncircular shape of the right ventricle, presence of trabecular structures in its wall, and difficulties in obtaining a perpendicular position of the right ventricle in the recorded images.[21] Indeed, because the echocardiographic studies usually focus on the left ventricle rather than on the right ventricle, most of the landmarks for standardization of the echocardiographic views depend on specific positions of left ventricular structures such as the interventricular septum, the left ventricular free wall, the chordae tendineae, or the papillary muscles.[4,21,28]

The CV of RVID measurement during diastole has been reported to range from 4.6% to 23.8%.[14,21] In the present study, the CV ranged from 2.9% to 5.22% in the right parasternal views, and from 4.2% to 11.7% in the left parasternal views, and its measurement was statistically more repeatable in the 2D-mode right parasternal LAx view than in the other views. It could thus be advocated to measure RVIDd in this latter view in the equine species.

Previous studies reported a CV of aortic measurements in standardbred and thoroughbred horses ranging from 1.7% to 6.0%. However, the view used to measure this parameter was variable; some authors used a right parasternal SAx view and others a right parasternal LAx view, and some used the 2D-mode whereas others used the M-mode.[6,14,15,21,24,25] In the present study, measurement of the aortic diameter was highly repeatable whatever the view used to measure it, but the views associated with the best repeatability were the views performed in the 2D-mode. This is in agreement with the study of Patteson et al who also suggested performing aortic measurements in the 2D-mode. M-mode measurements could be more variable because they are dependent on the level at which the cursor crosses the aorta, and because there are marked differences between the diameter of the aorta at its base, at the sinus of Valsalva, and at the level of the sino-tubular junction.[4] This is supported by the results of the present study where significant differences between the mean values of the aortic diameter at the base, the sinus of Valsalva, and the level of the sino-tubular junction were obtained. The timing of aortic measurement was also not standardized in the previous studies, end-systolic and end-diastolic measurement both being performed depending on the authors. In this study, the measurements were performed at end-diastole to obviate the lung interference that is known to occur during mid systole.[4]

The repeatability of the pulmonary artery measurements obtained in the present study was very good (CV 3.1 %). This is in contrast with previous studies that reported larger day-to-day variation in the measurement of this parameter (CV ranging from 5.9% to 15%) that has been attributed to the difficulties in imaging the maximal pulmonary artery diameter.[6,15,24] The use of a machine with higher quality and resolution in the present study as compared with previous studies could have helped to minimize this problem.

The repeatability of LA measurement has not been previously extensively studied in horses, but most of the authors recommend measuring this parameter from an M-mode right parasternal SAx view at the level of the base of the heart or from a 2D-mode left parasternal LAx four chambers view.[4,21] However, other authors recommend measuring that parameter from a 2D-mode right parasternal LAx four chambers view.[16,24] M-mode measurement of LA is known to give a lesser spatial and landmarks resolution than does the 2D-mode, and is highly dependent on the cursor placement, which could produce the measurement of the left atrial appendage rather than of the left atrial body.[4,16] Moreover, in this view, the M-mode cursor is usually placed perpendicular to the aortic root, but this positioning is often not perpendicular to the left atrium, which also induces an error in the LA measurement. In the present study, the M-mode measurements of LA also produced a significantly higher variability of the measurement of LA than the 2D-mode views. Schwarzwald et al[16] hypothesized that the 2D-mode right parasternal window could be of better value in measuring LA than the left parasternal window because this latter view does not allow imaging the entire left atrium and provides anatomic landmarks of poorer quality for measurements, which could lead to a higher variability in its measurement and to an underestimation of the maximal LA. However, in the present study, the 2D-mode left parasternal LAx angled view yielded a significantly higher repeatability of LA measurement than the right parasternal LAx view, and was not associated with an underestimation of its measurement as demonstrated by the mean value of this parameter that was significantly higher in the 2D-mode left parasternal LAx view than in the corresponding right parasternal view. Those results could be due to the fact that the LA was not measured in a classic four chamber view, but rather in angled view of the left ventricle and atrium, and that this view was adjusted to obtain the largest diameter of the left atrium. In this view, the anatomic landmarks of the left atrium are easier to obtain than in the classical four chambers view. The 2D-mode left parasternal angled view could thus be the best view to measure the LA in the equine species.

The repeatability of pulsed-wave Doppler measurements has infrequently been studied in the equine species. In the few studies performed on this field, repeatability of those measurements was lower than the repeatability of morphologic echocardiographic parameters.[6,15,16,24] In the present study, this was the case as well. The obtained CV for Doppler measurements ranged from 6% to 15.6%, which is in agreement with previously reported results.[6,15,16,24] For some authors, such a variability is low enough to warrant Doppler measurements in clinical and experimental studies,[15,16,24] but according to other authors, only large relative differences could be detected by those measurements.[6]

The poor repeatability of Doppler measurements is mainly because of the difficulties encountered in horses to obtain a correct (parallel) alignment between the Doppler beam and the valvular blood flows, especially for the transmitral and transaortic blood flow.[6,16,24] The high physiologic variability of stroke volume and heart rate in the conscious horse during the echocardiographic examination could also largely account for the high variability of Doppler measurements in this species.[15] The technical, physiologic, and examinator-dependent factors that have been mentioned concerning echocardiographic measurements are also important contributing factors to repeatability of Doppler measurements.

The repeatability of Doppler measurements was not compared between different views in this study because nowadays, there is a rather clear consensus on the views that have to be used in the equine species to minimize the angulation effect. Only the repeatability of aortic PEP and ET measurements were compared in this study because they can be obtained from an M-mode or from a Doppler-mode view. Measurements of those parameters showed a better repeatability and higher mean values in the Doppler mode than in the M-mode, which suggests a better reliability of the Doppler mode to measure those parameters.

In conclusion, results of repeatability of echocardiographic and Doppler echocardiographic measurements obtained in this study in equids of various sizes, breeds, and thorax shapes were comparable with previously reported results in thoroughbred and standardbred horses and suggest that for some parameters, the repeatability can be optimized using specific views:

1. M-mode right parasternal SAx view at the level of chordae tendineae to measure left ventricular morphologic parameters, and 2D-mode right parasternal LAx four-chamber as an alternative view.
2. 2D-mode right parasternal long axis five-chambers to measure the aortic diameter.
3. 2D-mode left parasternal long axis four-chambers angled view to measure the left atrial diameter.

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