The purpose of this study was to assess the intraobserver variation of various echocardiographic measures in standardbred trotters. Serial echocardiographic examinations were carried out on eight standardbred mares by one ultrasonographer for 5 separate days. During each examination, five nonconsecutive cardiac cycles (frames) were recorded and an average obtained for each individual measure. Various echocardiographic measures were obtained by use of two-dimensional (2-D), M-mode, color flow Doppler and pulsed wave Doppler echocardiography. The total variation in the echocardiographic measurements was split into three levels: the variation between horses, the day-to-day variation within individual horses, and finally the variation within horse on the same day of examination (intercardiac cycle variation). The intraclass correlation coefficient (ICC) was calculated for each measure. The ICC represents the variability of the measurements because of differences between the horses. In general the 2-D, M-mode and color flow Doppler measures had higher ICC values (ICC from 0.63 to 0.95) than the pulsed wave Doppler measures (ICC from 0.24 to 0.46), and the former measures were more repeatable than the pulsed wave measures. Exceptions to that were left ventricular free wall in diastole, the pulmonary artery in systole and the left ventricular mass, which all had low repeatability (ICC from 0.22 to 0.49). The results were used to calculate the relative differences that must be detected to diagnose a statistically significant change between two measurements in an individual horse. Differences from 4.2% to 21.8% must be achieved to document significant changes between serial measurements. A general tendency is that the color flow and pulsed wave Doppler measures require a larger relative difference (11.4–21.8%) between the measures to point out statistically significant cardiac changes than the 2-D and M-mode measures (4.2–13.9%).

Key words: echocardiography, horse, intraobserver variation, repeatability.

Introduction

Echocardiography is a fundamental technique for evaluation of cardiac function and morphology in the horse. Two-dimensional (2-D) and M-mode echocardiography can be used for measuring the size of the cardiac structures and for quantification of functional parameters. For evaluation of the valve function, color flow Doppler can be used in combination with 2-D echocardiography.1 It is also possible to assess the velocity of blood flow in the aorta or the pulmonary artery (PUL) by use of pulsed wave Doppler. These echocardiographic techniques can be used for diagnosis of cardiac disease in horses,2–7 and recently they have been used to assess sequential changes in cardiac parameters in response to training.8,9 Although the above methods have been used widely, repeatability (intraobserver) and/or reproducibility (interobserver) variation have rarely been assessed.10–15 Intraobserver variation lies in results obtained by the same operator. This variation is separated into two components in the present paper: variation between different cardiac cycles obtained from a horse on the same day (intercardiac cycle variation) and variation between examinations on different days from the same horse (day-to-day variation). In addition to intraobserver variation, significant differences have been observed by assessments performed by different operators.14,16 In daily clinical work, as well as in prospective clinical trials, it is essential to be aware of the intraobserver as well as the interobserver variation to ensure correct interpretation.

We found no information on the variation in color flow Doppler imaging of valvular regurgitation in the horse. Furthermore, the variation between the cardiac cycles within the horses on the same day of examination (intercardiac cycle variation) has not been calculated.

The aim of the present study was to describe intercardiac cycle variation as well as day-to-day variation for various echocardiographic measures in standardbred trotters. These results are used for calculation of the relative minimum differences that must be observed to diagnose a significant change between two measurements in the same horse.
Materials and Methods

The study was carried out at The Department of Large Animal Science, Large Animal Hospital, The Royal Veterinary and Agricultural University, Copenhagen, Denmark. Eight standardbred trotter mares owned by the University were used.

Horses

The mares were between 3 and 8 years of age and ranged in body weight from 477 to 540 kg. All mares were accustomed to the surroundings and the echocardiographic procedure. During examination the horses were restrained in stock in a quiet room and received no tranquilizers or sedatives. The parasternal area of the thorax caudal to the elbows was shaved. The same investigator who had approximately 1 ½ years experience in echocardiography performed all examinations. Each horse was examined on 5 different days within a period of 2 weeks.

Echocardiography

Echocardiography was carried out by using a Vingmed Vivid 3 ultrasound system with a 1.5 MHz phased array transducer with harmonic imaging. A base-apex ECG was superimposed for timing and appropriate sections of the scans were recorded on Super VHS videotape. Cardiac cycles of all echocardiographic views were stored digitally on the ultrasound machine. During each examination, five nonconsecutive cardiac cycles (five frames) were measured and an average obtained for each individual measure. Care was taken to ensure that each of the selected frames was of good quality and separated by at least five to ten heartbeats. Images were only recorded when heart rate was consistently below 45 b.p.m.

Measurements

Long axis aorta (AO) was obtained from the right parasternal long-axis 2-D view. The diameter of the aorta was measured in late systole at the sino-tubular junction using the leading edge method.

Long axis PUL was obtained from the right parasternal angled 2-D view. The diameter of the PUL was measured in late systole just beyond the insertion of the pulmonary valves using the inner-edge method.

The left ventricular internal diameter (LVID), the thickness of the interventricular septum (IVS) and the left ventricular free wall (LVFW) were measured from the short-axis view of the left ventricle (LV). This was done using M-mode, the curser being placed on a 2-D image of the LV obtained from the right parasternal window. Care was taken to place the M-mode curser so that the plane of section was perpendicular to the IVS and the curser bisected the ventricle. M-mode echocardiograms were recorded between the mitral valve and papillary muscles at the level of the mitral valve apparatus. Using the M-mode display, the image was chosen where a short border of the opened anterior mitral valve leaflet was seen during the beginning of ventricular filling. The leading edge methodology was used in all M-mode measurements. Care was taken not to include the septomarginal trabecule located in the right ventricle when measuring the IVS. End diastolic measurements were timed with the point of the Q-wave on the simultaneously recorded ECG. End-systolic measurements were made at the time of maximal IVS thickness. LVID in systole (LVIDs) and diastole (LVIDd), the interventricular septal thickness in systole (IVSs) and diastole (IVSd) as well as the LVFW thickness in systole (LVFWs) and diastole (LVFWd), were obtained from the same image. An estimate of the left ventricular mass (LV mass) was calculated by the following formula:

\[
LV \text{ mass} = 1.04 \times \left( \frac{LVIDd + LVFWd + IVSd}{LVIDd} \right)^3
\]

RVT is expressed as a fraction.

The tricuspid, mitral, aortic, and pulmonary valves were all examined using color flow Doppler to determine insufficiency (regurgitation jet). All valves were identified in 2-D and the color flow Doppler was used to examine the valve area. If a jet was present, the imaging plane was angled to show the maximal size of the jet. No zoom feature was used. Signals were ignored if they were of very short duration and immediately after valve closure. These signals were attributed to either valve closure artifact or simply the movement of blood in front of closing valve leaflets. The tricuspid valves were examined in the right parasternal long-axis and long-axis tipped view. The pulmonic valves were examined in the right parasternal angled view, and the mitral valves in the left parasternal long-axis view and in the long-axis apical view. Finally, the aortic valves were examined in the left parasternal long-axis view and in the five-chambered view. Based on the area of the jet in comparison with the approximated size of the atrium for the mitral and tricuspid regurgitation, four groups were defined: “Very small jet” (<10% of the area of the atrium), “small jet” (>10–30%), “medium jet” (>30–50%), and “large jet” (>50%). For the aortic and pulmonary regurgitation the maximal jet diameter just below the aortic/pulmonic valves was measured.

The right- and left-ventricular outflow tracts were examined by pulsed wave Doppler to determine the blood flow velocity. To optimize the accuracy of the velocity

*Vingmed GE Medical Systems, Glostrup, Denmark.
Data Handling and Statistical Analysis

The echocardiographic measures were derived after the examination by the operator. Possible outliers were tested using Cochran’s test (ISO standards, 1995) for each horse and echocardiographic measure. The standard deviation between measurements within the same day and horse was calculated for each of the 5 days. The standard deviation between measurements for 5 days for the same horse was then tested using Cochran’s test statistics based on the maximum standard deviation within each horse divided by the sum of the squared standard deviations for the same horse. Further analyses were performed with (1) the original observations and with (2) the possible outliers deleted, respectively. Whenever Cochran’s test was significant, all observations for the same horse, day and echocardiographic measure were deleted in (2).

To quantify the variation in the echocardiographic measures between horses ($H_{ij}$), days ($D_{ij}$), and frames ($e_{ijk}$), an analysis of variance was performed. Variation between horses, variation between days (within the same horse), and variation between cardiac cycles (within day and horse) were included as random effects in the model. The variation between cardiac cycles indicates the difference between the five separate cardiac cycles obtained in each horse within a few minutes in a single examination.

The intraclass correlation coefficient (ICC or $\xi$) was estimated as a measure of repeatability and was defined as the proportion of the total variance because of the between-horse variance. The average of the five cardiac cycles was used to compute ICC ($\xi$) in the present study with results in other studies. The total variance was defined as the sum of the between horse variance ($\sigma_H^2$) and between day within horse variance ($\sigma_d^2$). The two components of variance ($\sigma_H^2$ and $\sigma_d^2$) were estimated using an analysis of variance and the model is given by

$$X_{ij} = \mu + H_i + e_{ij},$$

where $X_{ij}$ is the echocardiographic measure on horse $i$ at day $j$, $H_i$ is the random effect of horse $i$, $i=1, \ldots, 8$, $H_i \sim N(0, \sigma_H^2)$, $e_{ij}$ is the random effect of day $j$ within horse $i$, $j=1, \ldots, 5$, $e_{ij} \sim N(0, \sigma_d^2)$.

The ICC ($\xi$) is estimated as

$$\xi = \frac{\sigma_H^2}{\sigma_H^2 + \sigma_d^2} \quad \text{ (model 2)}.$$

An exact 95% confidence interval (CI) of the ICC was estimated.\(^{23}\)

The ICC takes values in the interval $[0; 1]$. The more repeatable a measurement is, the closer ICC will be to 1. It can be tested whether the ICC (i.e., the between horse variance, $\sigma_H^2$) is significantly different from 0 using the one-way analysis of variance (model 1).

The 95% CI for a single echocardiographic measurement is given by

$$X \pm 1.96 \times \sigma_e,$$

where $X$ is the measurement and $\sigma_e$ is the square root of the random variation.

The relative minimum difference that is to be archived to prove a change is given by

$$1.96 \times \frac{\sigma_e}{X} \times 100\%.$$

The analyses of variances were performed using PROC MIXED in SAS version 8.2. A 5% significant level was used.

Results

The mares had no murmur or arrhythmia detected on auscultation. Two of the horses were more alert and it was difficult to keep the heart rate constantly below 45 b.p.m. true resting values were hard to obtain. Therefore more cardiac cycles were disregarded for these two mares since heart rate was above 45.

The measurements were obtained by both 2-D, M-mode, and Doppler recordings, which for each variable included 200 measurements (25 measurements per horse). However, because of technical problems in storing five of the images, only 195 observations were made for AV peak and AVVTI. Only examinations with a detectable regurgitation jet were reported in the color flow Doppler study. In total, 145 jets were observed.

Cochran’s test for outliers identified 23 possible outliers within 23 horse–day combinations. The ICC was calculated for all echocardiographic measures with and without these possible outliers. A maximum change below 2% was seen for all ICC, except for two. However, the ICC for these two measures were below 50% and the differences were not considered relevant. In the following, all observations have therefore been used.
The descriptive analysis of 2-D, M-mode, and Doppler measures are shown in Table 1. The coefficient of variation ranged from 4.8% to 34.4% with the Doppler variables having the highest values. Only the descriptive analysis of the color flow Doppler measures of aortic regurgitation is shown in Table 1. The color flow Doppler measures of atrioventricular regurgitation will be described later.

The total variation in echocardiographic measurements was divided into three levels (Table 2): the variation between horses, the day-to-day variation (within horses) and the variation within horse on the same day of examination (intercardiac cycle variation). In general, the variables can be categorized into two groups. One group includes measures where more than 50% of the total variation is because of the difference between horses. The measures included in this group are AO, IVSd, IVSs, LVIDd, LVIDs, LVFWs, RWT, and AO jet. The remaining group contains measures where day-to-day and the intercardiac cycle variations contribute to the largest part of the variation. PUL, LVFWd, LV mass, AV peak, AVVTI, PV peak, and PVVTI belong to this category. The intercardiac cycle variation for the PUL, IVSd, LVFWd, AV peak, and AVVTI accounted for 25% or more of the total variation and a large difference was observed for the five measurements performed on a single examination. The ICC represents the proportion of the total variance that arises because of variability between individual horses (Table 3). ICC was above 0.60 for AO, IVSd, IVSs, LVIDd, LVIDs, LVFWs, RWT, and AO jet with the differences between the horses as the largest source of variability. The pulsed wave Doppler measures LVFWd, and LV mass have ICCs below 0.60.

The relative minimum difference that represents a significant change between two or more examinations in serial measurements is shown in Table 3. A general tendency is that the color flow and pulsed wave Doppler measures require a larger relative difference (11.4–21.8%) between the measures to point out statistically significant cardiac changes. In contrast, the minimum relative difference is 4.2–13.9% for the 2-D and M-mode measures.

No regurgitation jets were identified at the pulmonic valves using color flow Doppler. Four horses had signs of aortic regurgitation, two had mitral regurgitation, and two had tricuspid regurgitation. The size of the mitral and tricuspid regurgitations (in common termed atrioventricular regurgitations) was predominantly classified as “very small” occupying less than 10% of the atria. The repeatability of the atrioventricular regurgitations within the same examination was good. If the jet was present, it was possible to diagnose the jet repeatedly. Furthermore, the semiquantified size of the jet remained relatively constant.
2-D IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall in diastole; LVFWs, left ventricular free wall in systole; LV, left ventricle.

Table 3. The Distribution of the Variation between Horses and Day-to-Day Variability

<table>
<thead>
<tr>
<th>Echocardiographic Measure</th>
<th>Horses</th>
<th>Days</th>
<th>ICC (2)</th>
<th>P-value</th>
<th>95% CI (2)</th>
<th>95% CI for a single measure</th>
<th>Relative minimum difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO (cm)</td>
<td>0.081</td>
<td>0.022</td>
<td>0.79</td>
<td>0.038</td>
<td>0.55; 0.95</td>
<td>± 0.29</td>
<td>4.2</td>
</tr>
<tr>
<td>PUL (cm)</td>
<td>0.028</td>
<td>0.064</td>
<td>0.31</td>
<td>0.102</td>
<td>0.04; 0.72</td>
<td>± 0.50</td>
<td>9.0</td>
</tr>
<tr>
<td>M-mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>0.023</td>
<td>0.006</td>
<td>0.79</td>
<td>0.038</td>
<td>0.56; 0.95</td>
<td>± 0.15</td>
<td>5.9</td>
</tr>
<tr>
<td>IVSs (cm)</td>
<td>0.055</td>
<td>0.010</td>
<td>0.85</td>
<td>0.035</td>
<td>0.66; 0.96</td>
<td>± 0.19</td>
<td>5.5</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>0.397</td>
<td>0.087</td>
<td>0.82</td>
<td>0.037</td>
<td>0.61; 0.95</td>
<td>± 0.58</td>
<td>5.0</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>0.297</td>
<td>0.077</td>
<td>0.79</td>
<td>0.038</td>
<td>0.56; 0.95</td>
<td>± 0.54</td>
<td>7.1</td>
</tr>
<tr>
<td>LVFWd (cm)</td>
<td>0.006</td>
<td>0.020</td>
<td>0.22</td>
<td>0.142</td>
<td>0.0; 0.65NS</td>
<td>± 0.28</td>
<td>11.6</td>
</tr>
<tr>
<td>LVFWs (cm)</td>
<td>0.063</td>
<td>0.036</td>
<td>0.63</td>
<td>0.047</td>
<td>0.34; 0.89</td>
<td>± 0.37</td>
<td>10.8</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>44645</td>
<td>46205</td>
<td>0.49</td>
<td>0.061</td>
<td>0.19; 0.83</td>
<td>± 421.31</td>
<td>13.9</td>
</tr>
<tr>
<td>RWT</td>
<td>0.001</td>
<td>0.0003</td>
<td>0.82</td>
<td>0.037</td>
<td>0.60; 0.95</td>
<td>± 0.04</td>
<td>8.2</td>
</tr>
<tr>
<td>CF Doppler</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO jet (cm)</td>
<td>0.096</td>
<td>0.005</td>
<td>0.95</td>
<td>0.113</td>
<td>0.88; 0.99</td>
<td>± 0.14</td>
<td>15.0</td>
</tr>
<tr>
<td>PW Doppler</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV peak (m/s)</td>
<td>0.0023</td>
<td>0.0027</td>
<td>0.46</td>
<td>0.066</td>
<td>0.16; 0.81</td>
<td>± 0.10</td>
<td>11.4</td>
</tr>
<tr>
<td>AVVTI (cm²)</td>
<td>1.875</td>
<td>4.825</td>
<td>0.28</td>
<td>0.114</td>
<td>0.02; 0.70</td>
<td>± 4.31</td>
<td>14.6</td>
</tr>
<tr>
<td>PV peak (m/s)</td>
<td>0.002</td>
<td>0.005</td>
<td>0.24</td>
<td>0.131</td>
<td>0.0; 0.67NS</td>
<td>± 0.14</td>
<td>17.1</td>
</tr>
<tr>
<td>PVVTI (cm²)</td>
<td>5.322</td>
<td>8.839</td>
<td>0.38</td>
<td>0.082</td>
<td>0.09; 0.77</td>
<td>± 5.83</td>
<td>21.8</td>
</tr>
</tbody>
</table>

The intraclass correlation coefficient (ICC, 2) is an estimate of repeatability of a measurement.13 Further, the 95% CI for a single measurement and the relative minimum difference are presented. The data are derived from eight standardbred trotter mares examined on 5 separate days. NS, ICC (2) not significantly different from zero; AO, axis aorta; PUL, pulmonary artery; IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVFWd, left ventricular free wall in diastole; LVFWs, left ventricular free wall in systole; LV, left ventricle.

However, the day-to-day variation was poor, especially for the atrioventricular regurgitations, because in only one of four horses was it possible to detect the jet on all five examinations. Pooling all 100 observations where an atrioventricular regurgitation was assumed to be present, jets were only detected in 60% of the examinations. The aortic regurgitation tends to be much more constant. The repeatability of a single examination was in agreement with the atrioventricular regurgitations. The quantification method tends to be highly repeatable (Tables 2 and 3). Also, the day-to-day variation was better than the atrioventricular regurgitations, since the jets were detected in 85% of the total 100 observations in which a jet was assumed to be present.

**Discussion**

In previous studies in humans and horses, the ICC was used as an estimate of repeatability of a measurement.13,24 A very repeatable measurement will have an ICC close to 1.0. In the present study, the ICC was above 0.60 for AO, IVSd, IVSs, LVIDd, LVIDs, LVFWs, RWT, and AO jet and these measures were repeatable with differences between horses as the major source of variation. The pulsed-wave Doppler measures and the PUL, LV mass, and LVFWd were less repeatable with ICC values ranging from 0.22 to 0.46. These results are in agreement with another study13 where high and low repeatability was found for the same measures, respectively.

The ICC represents the fraction of variation in measurements because of differences between horses. In general, this implies that if a large difference is observed for a measurement between individual horses, the ICC will be higher than if the groups of horses are more homogeneous. In humans,24 the ICC was considerably higher than in the present study. This was probably because of the fact that higher variation was observed between the humans examined. In the present study, and in a prior study,13 the horses were homogenous with minimal differences between individual horses. The purpose in the present study was to assess the reliability of echocardiographic measures in standardbred trotters for detecting serial changes in response to race training in a large-scale epidemiological study. Use of a relatively homogenous group of horses was necessary to show the limitation of the method. For a more general evaluation, selection of a more heterogeneous group (i.e., two Shetlands ponies, two standardbred trotters, two warmblood horses, and two shire horses) would ensure significant difference in the measurements between the horses, and result in higher repeatability.

In the present study we decided to split the intraobserver variability into two components; the day-to-day variability and the intercardiac cycle variability. To assess intercardiac variability it is essential to be careful in the selection of separated, nonconsecutive cardiac cycles, which is a
broader representative for the individual horse. In contrast to studies where consecutive frames are selected, we recorded cardiac cycles over a period of approximately 2 min and in the subsequent data analysis; the selected frames were divided by at least five cardiac cycles. The purpose of this procedure was to ensure selection of independent cardiac cycles and avoid bias.

Two-Dimensional and M-Mode Measures

The diameter of the aorta measured in the present study was highly repeatable (ICC = 0.79) with the largest variation because of differences between horses, which is similar to other studies. On the other hand, the diameter of the PUL was not repeatable (ICC = 0.31) with the largest variation because of day-to-day variation within horses. The maximal diameter of the PUL in the right parasternal angled view is difficult to obtain, which could be the reason for the low repeatability. A better view may be the right parasternal long-axis aorta view where a short-axis view of the PUL is located below the aorta. Values for AO and PUL in the present study were approximately 1 cm smaller than in previous studies. This may be because of the fact that the horses in the present study were less trained in comparison with the racehorses used in earlier studies, or from differences in obtaining the AO and PUL measures.

M-mode measurements of the LV are commonly used in the diagnosis of dilated cardiomyopathy as well as for estimation of the size of the LV (LV mass). For both purposes it is essential to place the M-mode curser through the short-axis view of the LV to get a perpendicular intersection of the IVS and LVFW. Slightly oblique cursor positioning can influence these measurements, especially for smaller structures such as LVFW. In some views, the IVS can be covered by the septomarginal trabecule on the right ventricular side. In the present study, the septomarginal trabecule was not included in the measurement; this results in smaller values than in previous studies. In addition, we tried to measure closer toward the mitral valve apparatus than described previously. This measure may contribute to the different mean values. The largest variation of the M-mode studies was because of variation between horses (Table 2). One exception was LVFWd where the variation mainly was caused by intercardiac cycle and day-to-day variation. This was surprising since this measure seemed easy to record. The variation probably reflects the small magnitude of the measure, making it more prone to errors. In addition, a slightly oblique curser position in the short axis view of the LV will have a marked effect on LVFW thickness.

An estimate of the left ventricular size is important in evaluating cardiac adaptation to exercise. However, the formula is adapted from humans and is complicated, involving the sum of three cubic variables. One of the variables is LVFWd, which does not appear to be repeatable. Low repeatability for this measure is particularly problematic for use of this measure in the third power. The relative minimum difference for LV mass was 13.9%, which means the left ventricular size needs to change more than 13.9% to be considered a significant effect of training. On the other hand, the RWT, which is an index to characterize sports-specific cardiac adaptations in human athletes, is much more reliable and only an 8.2% relative difference is needed between serial measurements (Table 3).

Color Flow Doppler Measures

Color flow Doppler is an informative diagnostic tool for detection and evaluation of regurgitant flow. The method is sensitive for detection of valvular regurgitation in horses. Different methods have been described to assess the severity of valvular insufficiency. These methods relate the severity of insufficiency to the regurgitant jet length, width, or area. Also, jet area related to atrial size (atrioventricular regurgitation) or jet diameter related to left ventricular outflow tract (aortic regurgitation) has been used in dogs and human. However, success in imaging regurgitant jets is affected by many instrumental as well as physiologic factors, e.g., acoustic impedance, frame rate, gain setting, topography, loading conditions, heart rate, and jet orientation. Thus it can be difficult to classify the severity of the jets quantitatively and a semiquantitative grading scale is probably better.

Although no murmurs were detected on auscultation before the echocardiographic examination, five of the eight horses had one or more regurgitant valves. This incidence of valvular regurgitation is in agreement with previous color flow Doppler studies of normal horses. For the tricuspid and mitral regurgitant jet, four groups were defined as described previously. Atrioventricular regurgitations were observed in four horses and categorized as “very small” (group 1). Surprisingly, only 60% of the jets persisted during the 5 days. This probably relates to changes in the physiologic factors such as heart rate and loading conditions, which differ within each horse on different days. To quantify aortic regurgitation we measured the maximal diameter of the jet just below the valves. In humans, the highest repeatability was achieved by measuring the ratio between the jet diameter and diameter of the left ventricular outflow tract. We did not measure the diameter of the left ventricular outflow tract and only the diameter of the jet was registered. Four horses had aortic regurgitation. During the five examinations, 85% of aortic regurgitations persisted during all 5 days; this was more consistent than the atrioventricular regurgitations. The diameter of the aortic regurgitation had high repeatability (ICC = 0.95) (Table 3), which makes it a good parameter for quantifying aortic insufficiency. The study indicates...
that day-to-day variation within horses in detecting small regurgitations is significant, and especially the atri-ventricular regurgitations should be interpreted with caution. It would be useful to test this in more horses with both smaller and larger regurgitant jets. The repeatability would likely be higher if horses with pathologic murmurs were examined, where larger regurgitant jets would be present.

**Pulsed-Wave Doppler Measures**

The repeatability of pulsed-wave measures in horses has been evaluated infrequently.\(^{13,15}\) Measurements of blood flow velocity in the right and left ventricular outflow tract are difficult in horses. To estimate velocity accurately, it is essential that the transmitted ultrasound wave is parallel to the direction of the blood flow. The velocity will be underestimated if an angle occurs between flow and the ultrasound wave.\(^{31}\) In dogs and humans an apical five-chamber view from the left sternum is used for pulsed-wave Doppler studies of the aorta, and a right parasternal short axis view is used for the PUL, which enables optimal alignment with the blood flow.\(^{31,32}\) Good alignment is very difficult to obtain in horses, and consequently blood flow velocity is typically underestimated. The peak velocity in the PUL in this study was lower in comparison with some previous studies,\(^{12,13}\) but in agreement with others.\(^{15}\)

Flow velocity integrals (VTI) are variables that should be directly proportional to stroke volume.\(^{24}\) The area under the flow velocity curve represents the distance a volume of blood travels; it is used with the area of the vessel to calculate stroke volume. The mean values of AVVTI and PVVTI are similar to previous studies.\(^{12,13}\) In general the pulsed wave Doppler measures are not very repeatable (ICC < 0.46). This agrees with a former study,\(^{13}\) but the intraclass coefficient of correlation in the present study is overall lower than in the previous study.\(^{15}\) The reason for this may be different operator experience, probes, and views used in the two studies.

In conclusion, we assessed sources of variation regarding selected echocardiographic measures in the horse. In general 2-D and M-mode measures were more repeatable than the pulsed-wave Doppler measures. Not all small regurgitant jets were repeatable from day to day. This is important relative to studies where valvular competence is evaluated. The variation between measurements was divided into three components. The intercardiac cycle variability was relatively high. This increases the need for several measurements during the same examination. The frames should probably not be consecutive but separated by several cardiac cycles to reflect real repetition. Differences between two examinations in serial measurements have to be larger than 4.2–21.8% to document true changes, with the most reliable results in the 2-D and M-mode measurements. This will likely be larger if more operators are participating in performing the examinations.

**REFERENCES**


