

Assessment of systolic left ventricular rotation in horses with two-dimensional speckle tracking echocardiography

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Summary: The current study evaluated rotational deformation of the equine left ventricle by two-dimensional speckle tracking on the chordal level (MV), the papillary muscle level (PM) and, for the first time, at the apical level (AP). Sixteen healthy horses were examined. Data was acquired on three consecutive days by two observers. A similar systolic rotational motion at the three myocardial levels was identified, however with different amplitudes. As seen from the base, the motion starts with an initial (or early systolic) clockwise rotation followed by a counter-clockwise rotation. There was a significant difference between the rotational amplitudes at each level ($p < 0.0001$). An increase in left ventricular rotation amplitude from the chordal to the apical level was found. As seen from the base, there is an initial clockwise rotation (positive rotation), followed by a dominant counter-clockwise rotation (negative rotation) at each level. The coefficient of variation on the papillary muscle level (PM) was the lowest compared to the MV and AP scan level. In conclusion, the left ventricular rotational (LVrot) motion can be evaluated non-invasively using two-dimensional speckle tracking at three different scan levels. Further studies are necessary to confirm our results. Additionally, this method should be evaluated in horses with cardiac dysfunction, at rest and after exercise.

Keywords: horse, echocardiography, speckle tracking, myocardium, systole, ventricular rotation

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Introduction

Rotational deformation (twist) of the left ventricle (LVrot) plays an important role with respect to LV ejection and filling (Rademakers et al. 1992). During the cardiac cycle, there is a systolic twist and an early diastolic untwist of the LV about its long axis because of oppositely directed helical-oriented myofibers (rotation). This wringing motion (twist) has been shown to be a key factor of the normal systolic and diastolic myocardial function in both dogs and humans (Chetboul et al. 2008, Helle-Valle et al. 2005, Notomi 2005 et al., Opdahl et al. 2005, Park et al. 2008, Zocalo et al. 2007).

The magnitude and characteristics of this rotational deformation have been described in different clinical and experimental studies in humans (Kim et al. 2007, Shaw et al. 2008) and dogs (Kim et al. 2007). It is well established that left ventricular rotation (LVrot) or twist is sensitive to changes in both regional and global left ventricular function (Edvardsen et al. 2006, Han et al. 2007). Therefore, assessment of LVrot represents an interesting approach for quantifying left ventricular function. So far, two-dimensional (2D) speckle tracking (2DST) has been used in 2 studies with horses but the rotational deformation of the LV was measured at two different scan levels only (Declodt et al. 2012, Schwarzwald et al. 2009). The aim of the present study was to evaluate three different scan planes for the measurement of LVrot motion using 2DST in healthy horses. A second aim was to assess the repeatability of the measurements

at each of these 3 levels to determine whether one of the levels would give more accurate measurements.

Material and methods

Study population

A total of 16 warmbloods (9 geldings, 7 mares; age range 3–10 years; body weight range 523–645 kg) were examined. All horses belonged to the Bavarian state stud farm of the Bavarian State Research Centre for agriculture at Schwaiganger.

All subjects were kept in stalls and used for sport riding (show jumping, dressage). They were considered healthy based upon a physical examination, cardiac auscultation, surface electrocardiogram (ECG) and 2D, M-Mode and colour flow ECG studies. None of the horses received any medical treatment for two months prior to examination. Animal handling and care was performed following the guidelines of the local ethics committee.

Echocardiography

Study subjects remained unsedated in their stalls restrained by grooms familiar to them. The ECG examination was performed using a portable ultrasound system^a and a phased

array transducer^b with a frequency of 1.5/3.1 MHz. Surface ECG was obtained simultaneously.

The imaging depth ranged between 26 and 30 cm, and the sector width was reduced one step from its maximum width to achieve a frame rate of at least 55 frames per second in the 2D Imaging Mode. At least three representative, consecutive cardiac cycles were recorded in each view and stored as cine-loops in digital raw data format for offline analyses. Cycles immediately after sinus pause or 2nd degree atrioventricular block were excluded. Three consecutive cardiac cycles of scan planes described below were evaluated by one well-trained observer and stored for offline analyses^c.

Conventional echocardiography

Standard 2D, M-Mode and colour-flow Doppler echocardiography of right and left parasternal views were performed to assess heart dimensions and valvular insufficiencies (Stadler, D'Agostino et al. 1988).

Two-dimensional speckle tracking

Raw data recorded during the routine echocardiograms was analysed using a dedicated software package^c. Therefore, three cardiac cycles were chosen and the mean of the three measurements was used for further analyses.

Firstly, the time of the aortic valve closure, which was used to delineate the end of ventricular systole during 2DST analyses, was measured manually (tAVC). It was defined as the time interval between the beginning of the electrocardiographic S wave and the closure point of the aortic valve identified on a long axis M-Mode recording of the aortic valve.

The LV rotational deformation was measured at three different positions (Fig. 1). The first scans (1) derived from the short axis images recorded at the chordal level (MV) were centered to show the complete round LV in a short axis and the chordae leading to the valve leaflets as echodense spots. The second scan plane (2) named the papillary muscle (PM) level, showed the LV at a level where the PM could be seen during the complete systole and diastole. The third scan plane (3) called the apical (AP) level, was defined as the deepest possible short axis view where the ventricular lumen could be depictable as a round shape and the PM was not visible anymore in the diastole (Fig. 1). At least one complete cardiac cycle of adequate quality had to be available on each recording to be included in the analyses.

The 2DST variables were measured as follows, as described by Schwarzwald et al. (Schwarzwald, Schober et al. 2009): firstly, the appropriate short axis image was selected and the "Q-Analyses" module was started. A single heart cycle was selected using the cine-loop mode. Afterwards, the "2D Strain" module was started. The short axis grey scale loops were analysed using the "SAXMV", "SAX-PM" and "SAX-AP" option.

The region of interest was determined by tracing the endocardial border of the LV at the end of systole defined as the

time of the aortic valve closure (tAVC, manually measured in M-Mode). The tracing was started at the mid-septum and proceeded in a counter-clockwise direction. The region of interest was then adjusted so that the entire myocardial thickness was covered throughout the cardiac cycle. The speckle tracking analysis started automatically.

The software algorithm automatically divided the myocardium into six segments of equal size, performed the speckle tracking analysis and provided confirmation of adequate tracking for each segment (Fig. 2).

The segments were preselected by the software based on regional wall motion analysis standards developed for human patients, which were not adjusted for use in the horse.

"Sept" and "AntSept" indicate the interventricular septum, "Ant" and "Lat" the caudal LV free wall, and "Post" and "Inf" the cranial LV free wall.

The quality of the tracking was visually assessed by the operator during motion playback. If necessary, the line tracing the endocardium was readjusted and the speckle tracking analysis was repeated until adequate segmental tracking was confirmed by the software. Six curve profiles were then obtained, corresponding to the average of each of the six myocardial segments (Fig. 3). The measurements for rotation (degree) were performed on the "Results" screen of the 2D Strain software module, using the default settings for special smoothing, temporal smoothing and drift compensation. The mean of the six segmental measurements was calculated to obtain indices of average rotation.

Reliability of echocardiographic variables

All horses underwent repeated examinations by two experienced examiners, according to imaging guidelines determined previously. For the analyses of the intra-observer and inter-observer variability the measurement of 8 horses were used and the 8 horses were examined 3 times a day and also at 3 consecutive days from both examiners.

Statistical analysis

Statistical analysis was performed using PASW Statistics 17.0^d and data were expressed as mean (M) and standard deviation (SD), minimum and maximum and the range.

The normality of the data was tested using a Kolmogorov-Smirnov statistic and all data were normally distributed. The variability was expressed by the coefficient of variation: (SD/mean)*100. The degree of variability was defined as follows: CV 5 to 15% low variability, CV between 15 and 25% moderate variability and CV over 25% high variability (Schwarzwald, Schober et al. 2009). A paired Student's t-test was used to compare LVrot at the different segments at each level. To compare mean values of the different scan levels a 1-way analysis of variance was performed. When significant, post hoc comparisons were performed using the Bonferroni correction method. The level of significance was defined as $p < 0,05$.

Results

Conventional echocardiography

Standard 2D, M-Mode and colour-flow Doppler echocardiography of right and left parasternal views showed normal heart dimensions and function and none of the horses had clinically relevant valvular insufficiencies.

Two-dimensional speckle tracking

Most echocardiogram from the 16 horses were of adequate quality to be included into the study. However, some had to be eliminated as shown in table 3.

In all segments a similar systolic rotation (twisting) motion could be measured but with different amplitudes. As seen from the basis, the motion starts with an initial (or early systolic) clockwise rotation (positive orientated rotation angle) followed by a counter-clockwise rotation (negative orientated rotation angle) (Fig. 3; Table 2). All six segments analysed showed a similar progression with decreasing amplitude from MV to AP level. The highest levels were seen in all segments at the position "inf" (AP with $-15,12^{\circ} \pm 3,28^{\circ}$; PM with $-15,12^{\circ} \pm 3,28^{\circ}$; MV level with $-11,00 \pm 3,13$). There was a significant difference between the rotation angel at each level ($p < 0.0001$) (Fig. 4).

The segments showed different performance in rotation, especially at the MV level and the coefficient of variation differs between the scan levels (Table 1a, b).

Discussion

The aim of our study was to describe the rotational myocardial deformation (rotation, twist) of the equine heart on three different scan levels. Compared to previous studies (Declodt

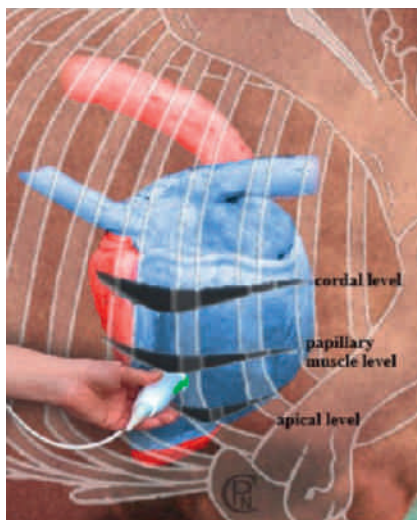


Fig. 1 The three scan levels from the right parasternal short axis view starting from the heart basis with the chordal level (MV), followed by the papillary muscle level (PM) and finally the apical level (AP). | Die drei Schallebenen von der rechten, parasternalen kurzen Achse: Mitralklappen(MV)-, Äquatorial(PM)- und Apikal(AP)-Ebene.

2012, Schwarzwald et al. 2009), we include in particular also the AP level and evaluate the optimal scan plane to achieve reliably data.

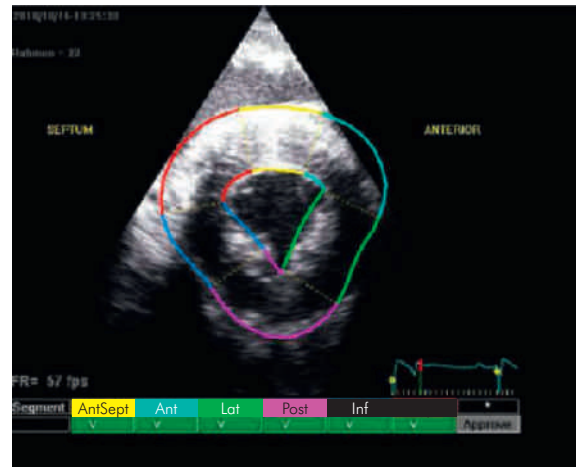


Fig. 2 The analysing screen. The region of interest was determined by tracing the endocardial border of the LV at the end of systole. The segments were preselected by the software based on regional wall motion analysis standards developed for human patients, which were not adjusted for use in the horse. "Sept" and "AntSept" indicate the interventricular septum, "Ant" and "Lat" the caudal LV free wall, and "Post" and "Inf" the cranial LV free wall. All 6 segments were of adequate tracking quality and marked with a green "V". | Bildschirmansicht bei der Untersuchung. Die für die Untersuchung entscheidende Region wurde durch Verfolgung der endokardialen Grenze des LV am Ende der Systole aufgefunden. Die Segmente wurden mittels Software, welche für die Standards der Bewegungsanalyse der verschiedenen Herzwandregionen für Humanpatienten entwickelt und nicht ans Pferd angepasst wurden, präselektiert. „Sept“ und „AntSept“ geben das zwischenventrikuläre Semptum an, „Ant“ und „Lat“ benennen die freie Wand des kaudalen LV. „Post“ und „Inf“ dagegen meinen die freie Wand des kranialen LV. Alle 6 Segmente wiesen eine adäquate Auffindequalität auf und sind mit einem grünen „V“ markiert.

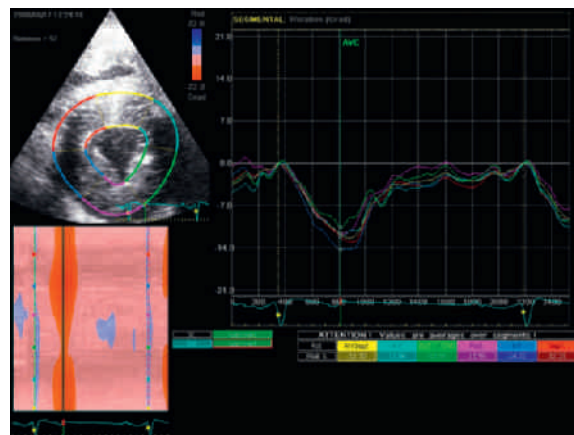


Fig. 3 The result screen in three parts: a grayscale image with the region of interest (A), a colour coded M - Mode (B) and the six curve profiles (C), corresponding to the average of each of the six myocardial segments. The vertical axis shows rotation values in degree, the horizontal axis shows the time (ms) and the electrocardiogram. The vertical green line shows the Aortic valve closure. | Der Ergebnisbildschirm in drei Abschnitten: Ein Graustufenimage mit der entscheidenden Region, eine farbkodierte M-Mode sowie Kurvenprofile (korrespondierend mit dem Durchschnitt aus jedem der sechs Myokardsegmente). Die Vertikalachse zeigt Rotationswerte in Graden, die Horizontalachse zeigt die Zeit (ms) und das Elektrokardiogramm. Die vertikale grüne Linie zeigt den Aortenklappenschluss.

Our results reveal that LVrot can be assessed with a high reproducibility and reliability at the scan plane PM using standard 2D ECG recordings of standing, unsedated, adult horses.

We determined that the LV rotation decreases from the AP to the basal level.

In contrast to humans (Kim et al. 2007) and dogs (Chetboul 2008, Suzuki et al. 2013), we were not able to assess a left ventricular torsion (LVtor). LVtor is the difference between AP and MV LVrot. The rotational deformation has to be measured on different planes simultaneously to assess the LVtor (Burns et al. 2008, Burns et al. 2010). We did not have the technical capabilities to do this.

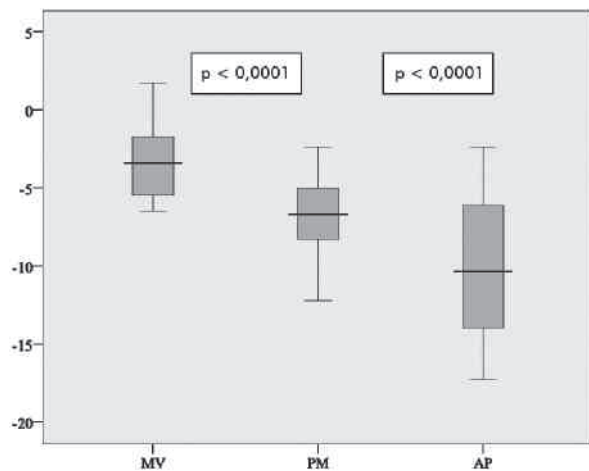


Fig. 4 Comparison of the rotational data in degree at the three levels. The vertical axis shows the rotation values degree. The horizontal axis shows the scan levels. There was a significant difference between the levels. | Vergleich der Rotationsdaten in Graden in allen drei Schallebenen. Die Vertikalachse zeigt die Rotationswerte in Graden. Die Horizontalachse dagegen zeigt die Schallebenen. Es existiert ein signifikanter Unterschied zwischen den einzelnen Schallebenen.

Table 1a, b Variability of left ventricular rotation in percent at the chordal (MV), equatorial (PM) and apical (AP) level expressed as coefficient of variation (CV), measured by two observers on three different days. Variabilität auf Höhe der Mitralklappen(MV)-, Äquatorial(PM)- und Apikal(AP)-Ebene, ausgedrückt als Variationskoeffizient (CV) in Prozent gemessen von zwei Tierärzten an drei unterschiedlichen Tagen.

Position	Intra-day		Between-day	
	Intrareader	Inter-observer	Intra-observer	Inter-observer
MV	26.58	37.57	28.43	35.05
PM	5.99	13.71	18.22	19.28
AP	4.66	31.64	19.28	24.61

	MV		PM		AP	
	Obs. One	Obs. Two	Obs. One	Obs. Two	Obs. One	Obs. Two
Day 1	-5.08° ± 3.29	-4.27° ± 2.24	-8.07° ± 1.99	-8.16° ± 1.87	-13.14° ± 3.08	-9.97° ± 4.13
Day 2	-5.31° ± 2.21	-5.33° ± 2.24	-8.39° ± 2.07	-7.64° ± 2.42	-13.62° ± 3.30	-10.35° ± 2.17
Day 3	-4.92° ± 2.37	-4.73° ± 2.37	-8.02° ± 2.99	-7.84° ± 1.96	-12.59° ± 4.54	-9.39° ± 4.42

MV = scans derived from the short axis images recorded at the chordal level of the mitral valve; PM = scans derived from the short axis images recorded at the papillary muscle; AP = scans derived from the short axis images recorded at the apical level

Several studies in dogs and humans revealed a difference between AP and basal (MV) rotation of the LV (Chetboul 2008, Notomi et al. 2005, Suzuki et al. 2013). In our study, the LVrot showed a similar progression at the AP, PM and MV level. As seen from the base, there is an initial clockwise rotation (positive rotation) followed by a dominant counter-clockwise rotation (negative rotation) at each level.

The LVrot at the MV level offers different characteristics. The segments "AntSept", "Ant" and "Lat", or rather the septum and the caudal free wall, rotate clockwise at the end of systole, whereas the segments "Post", "Inf" and "Sept", or rather the lateral and the cranial free wall, rotate counter-clockwise. A marginal part of the studies showed this phenomenon at the PM level. The AP level showed a smooth rotation, as described above (Table 2).

We measured a high observer and reader variability at the MV level. Other studies confirmed this (Declodt 2012, Schwarzwald et al. 2009). One of the reasons might be the "out of plain motion" due to the longitudinal motion of the heart. The high interobserver variability could be due to the fact that the examinations were performed consecutively and we had the influence of time on the horses. At the PM level both the intra- and inter-observer variability was moderate. At this level, we have a well-defined, standardized view. The thickness of the myocardial muscle allowed an exact artefact-free tracking by the software. At the AP level the intra- and inter-observer variability increased to again. This had already been observed in dogs (Chetboul et al. 2008). It might be explained by the more variable positioning of the sample volume at the apex. The AP short axis view is standardized more precisely in human medicine (Kim et al. 2007). In addition, the luminal obliteration was used as a landmark. That is, however, not applicable in horses because of the prominent olecranon.

A potential limitation of 2DST is that the longitudinal motion of the heart base leads to considerable through-plane motion when imaging the LV in the short axis, which may impair the tracking accuracy for radial and circumferential events by current 2D algorithms. This may explain the rotational data, where segments showed clockwise and counter-clockwise rotation simultaneously.

In conclusion, we could show that LV mechanical function can be evaluated non-invasively using 2DST-based wall motion analysis applied to standard 2D ECG recordings of the LV in a right-parasternal short axis view at the level of the MV, PM and AP. We showed that measurements at the PM level obtained data are acceptable. We also raised some questions: We were not able to measure LV Torsion (LV Tor), but we can as-

Table 2 Rotational data of the six tracking areas at the three scan planes. | Rotationsdaten der sechs Myokardregionen an den drei Schallebenen.

		MV	PM	AP
Ant Sept	Mean ± SD	-2.79° ± 1.20	-7.81 ± 2.27	-10.79 ± 3.05
	Min	-4.93	-10.99	-18.47
	Max	-0.5	-3.44	-2.94
	Range	4.43	7.55	15.53
Ant	Mean ± SD	-3.01 ± 1.75	-4.82 ± 2.37	-11.36 ± 4.41
	Min	-6.97	-9.63	-21.1
	Max	-0.5	-0.2	-1.93
	Range	6.47	9.43	19.16
Lat	Mean ± SD	-4.42 ± 3.28	-4.72 ± 2.40	-12.96 ± 5.22
	Min	-13.9	-9.01	-24.55
	Max	-0.34	-0.17	-1.39
	Range	13.56	8.84	23.16
Post	Mean ± SD	-6.84 ± 3.46	-8.65 ± 2.38	-14.78 ± 4.20
	Min	-12.55	-13.35	-24.9
	Max	0.57	-3.82	-4.22
	Range	11.98	9.53	20.68
Inf	Mean ± SD	-11.00 ± 3.13	-12.37 ± 2.93	-15.12 ± 3.28
	Min	-16.79	-17.24	-20.59
	Max	-2.36	-5.36	-5.25
	Range	14.43	11.87	15.34
Sept	Mean ± SD	-8.72 ± 2.13	-11.40 ± 2.80	-13.31 ± 3.03
	Min	-12.72	-15.59	-18.48
	Max	-4.6	-4.99	-3.34
	Range	8.16	10.96	15.14

Range = Total angular rotation in degrees

Table 3 Number of tracking areas, which had to be excluded, because of poor tracking. | Anzahl der Myokardregionen, die wegen eines zu schwachen Ergebnisses ausgeschlossen wurden.

	AntSept	Ant	Lat	Post	Inf	Sept
MV	7	12	11	5	0	1
PM	0	2	1	0	0	0
AP	1	0	0	0	0	1

sume that there is torsional deformation. Perhaps our AP level was not deep enough. As we defined above, torsion is the net difference between MV and AP rotation. Even if the segments do not rotate in the opposite direction, there is a difference which leads to torsional deformation. Thus, another important question arises: If torsional deformation is so important for myocardial deformation, why have horses less of it?

Further studies are required to answer this question and assess cardiac disease-related alterations in left ventricular rotation (LVrot), as described in humans, and its relation to disease severity, exercise capacity and prognosis.

Manufacturer's addresses

- ^a GE Vivid i, GE Medical Systems, Ultrasound Tirat Carmel, Israel; Application Software 6.1.1.110; Systemsoftware: 1.36.18
- ^b 3S-RS phased array transducer, GE Medical Systems
- ^c EchoPAC Software Only version 7.0.0, GE Vingmed Ultrasound A/S, Horten, Norway
- ^d PASW Statistics 17.0

Conflict of interest

The authors have no conflicts of interest to disclose.

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