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Normal pulsed wave Doppler echocardiographic parameters of Turkmen horses of Iran

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Summary

Turkmen horse is one of the oldest and purest breeds in the world, but there is no information on Doppler echocardiographic parameters in this horse. In the present study, pulsed-wave (PW) Doppler echocardiography was performed on 42 clinically normal 3- to 15-year-old racing Turkmen horses. No cardiac disease was detected with two-dimensional (2-D) real-time, M-mode, and colour flow mapping. Doppler echocardiographic parameters and indices of tricuspid, mitral, pulmonary and aortic flows were measured in this study. Tricuspid inflow velocities during rapid filling (E) and atrial contractions (A) were significantly higher than mitral inflow ($P<0.05$). The Doppler waveforms, obtained from the aortic outflow, showed a significantly longer pre-ejection period (PEP) and shorter ejection time (ET) than the pulmonary artery waveforms ($P=0.001$ and $P=0.028$, respectively). The time taken from the onset of the QRS complex to the onset of the A wave for tricuspid flow, pulmonary PEP, and pulmonary PEP/ET, had a significant positive correlation with age. These values can be used as standard and reference values for evaluation of cardiovascular disorders in Turkmen horse.

Key words: Doppler echocardiography, Pulsed wave Doppler, Normal, Turkmen horses

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Introduction

Doppler echocardiography is a sensitive and non-invasive mean of demonstrating intracardiac blood flow and plays an increasingly important role in evaluating and managing patients or animals with cardiac diseases (Kriz and Rose, 2002; Boon, 2011). It is based on the Doppler principle, the frequency shift that occurs when sound of a known frequency is reflected by a moving structure, in this case the blood cells. When an ultrasonographic beam of any given frequency is directed into the heart, it will reflect at a different frequency determined by the velocity of the blood cells. Detection of the frequency shift allows the velocity of blood cells to be calculated. Blood cells which are moving towards the interrogating ultrasound beam produce an increase in frequency, a positive Doppler shift, whereas blood cells moving away from the interrogating sound beam produce a decrease in frequency, a negative Doppler shift (Marr and Patteson, 2010).

Diastolic (Plotnick and Vogel, 1989) and systolic ventricular function (Sabbah *et al*., 1986) can be assessed by measuring intracardiac flow velocities and other variables from Doppler waveforms. The area under the velocity waveform (velocity time integral [VTI]) is directly related to the stroke volume of the ventricle (Mehta and Bennett, 1986). In large animals, because of the size and the specific anatomy and physiology of their hearts, the non-invasive cardiac diagnostic tools have limited value (Reef, 1985). Doppler echocardiography allows an accurate quantitative assessment of cardiac blood flow velocities and systolic

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time intervals measurements (Kriz and Rose, 2002; Boon, 2011), which need normal echocardiographic reference values. Systolic time intervals (PEP and ET) are useful in detecting changes in ventricular performance. These can be measured from Doppler waveforms (Koito and Spodick, 1989). As different breeds have different biotypes and develop different attitudes, it is necessary to provide adequate information for each type of work or breed (Michima *et al*., 2004). Stadler *et al*. (1993) and Blissitt and Bonagura (1995) have reported a number of measurements from Doppler waveforms in warm blooded and Thoroughbred horses, respectively.

Turkmen horse is one of the oldest and purest breeds in the world. In spite of its small size the Turkmen horse always achieves high ranks in courses and jumping competitions (Golshan, 2005).

Regarding the lack of Doppler echocardiographic parameters data in healthy Turkmen horses, this study was aimed to determine quantitative reference values of Doppler flow profiles in this breed of horse.

Materials and Methods

Forty-two clinically normal Turkmen male and female horses (26 stallions and 16 mares), aged 3-15 years (Mean ± SD: 8.03 ± 3.62) and weighing 310-450 kg (Mean ± SD: 369 ± 37.8 kg), were included in this study. The Turkmen horse is bred in the northeast part of Iran near the borderline between Iran and Turkmenistan. This study was carried out in this region. All horses used in this study were racehorses, had at least one year of experience in competitions, and had been kept under similar hygienic, sanitary, and nutritional management. All horses were determined to be free from cardiac diseases on the basis of clinical, hematological, electrocardiographic, 2-D, and colour flow mapping Doppler echocardiographic examinations. Horses with an early systolic grade 2/6 ejection murmur with the point of maximal intensity over the aortic valve (7 of the 42 studied horses) were considered as normal. All horses were free from significant valvular regurgitation on Doppler echocardiography. Before imaging, the coat was bilaterally shaved from the 3rd to the 5th intercostal space between the midway of the point of the shoulder and the point of the elbow. The echocardiographic examination was performed with a Sonosite model MicroMaxx echocardiograph, using a 1-5 MHz phase array transducer, with a maximal depth of 25 cm and 2-D and brightness mode (B-mode), colour flow mapping, and spectral Doppler programs. The ultrasound machine had an integrated ECG function where the ECG traces were displayed simultaneously with the images. A base-apex lead system was used, with electrodes applied in the 6th intercostal space on the left side of the thorax along with a line parallel to the level of the point of the elbow and placed on the top of the right scapular spine. All of the imaging stages were recorded digitally for subsequent analysis. During examination, the animals were at rest, calm, and physically restrained only by halter, in a quiet and dark room.

PW Doppler measurements were made according to guidelines previously described by other researchers (Goldberg *et al*., 1988; Long *et al*., 1992; Blissitt and Bonagura, 1995). The sample volume was set at 5 mm for all measurements. Measurements were made from using computer software inbuilt in the Sonosite, model MicroMaxx. The heart rate was measured from the ECG tracings recorded together with the Doppler spectral images.

The tricuspid inflow was recorded from the right parasternal long axis view of right ventricular outflow tract (RVOT) (Fig. 1) and the mitral inflow from left parasternal long axis apical view of mitral valve (Fig. 2). Placing the sample volume on the ventricular side of tricuspid and mitral valves at the valve tips, velocity spectral recordings were obtained from the peak velocity of blood flow during the rapid ventricular filling (Peak E Wave) and during the atrial contraction (Peak A Wave). From those parameters, the ratio Peak E/Peak A (E/A) was calculated. The deceleration time (dt) of E waves was measured from the peak of the rapid filling signal (E) to the point where the downstroke intercepted the baseline. The time taken from the onset of the QRS complex to the onset of the E and

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A waves was recorded.

The pulmonary outflow velocity was recorded from the right 4th intercotal space with the transducer pointing towards the left 3rd intercostal space in right parasternal long axis view of RVOT (Fig. 3) and the aortic outflow from the left 4th intercostal space in left parasternal long axis view (5-chambered) of left ventricular outflow tract (LVOT) (Fig. 4). According to aortic and pulmonary velocity spectral recordings, the peak velocity of blood flow (Vmax) was measured by placing the sample volume on the arterial side (sino-tubular junction [JT]) of the aortic and pulmonary valves. The area under the velocity waveform (velocity time integral [VTI]) was measured by tracing the modal velocity (represented by the brightest line in the spectral Doppler waveform) envelope of the Doppler signal. The ejection time (ET) was measured from the onset to the end of the spectral waveform. The acceleration time was measured from the onset of the Doppler waveform to the start of the maximum velocity plateau. The pre-ejection period (PEP) was measured from the onset of the QRS complex to the onset of the spectral waveform. Flow velocities were recorded at resting heart rates. Measurements were made from 3 consecutive cardiac cycles.

The stroke volume and cardiac output derived from the Doppler measurements (SV-D and CO-D, respectively) were calculated as follows (Boon, 2011):

Fig. 1: A right parasternal long-axis PW Doppler echocardiogram of the right ventricular outflow tract (RVOT) and right atrium indicating the position or the sample volume to obtain a PW Doppler spectral trace of flow through the tricuspid valve. An ECG is displayed for timing of cardiac events

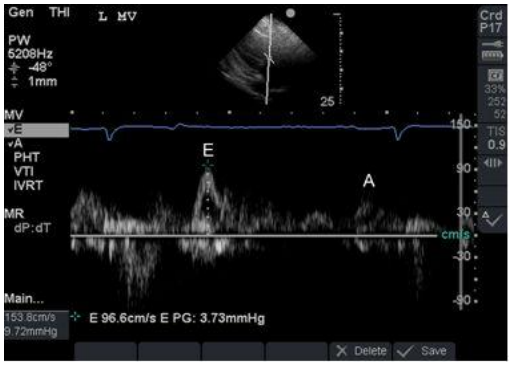

Fig. 2: A left parasternal long-axis PW Doppler echocardiogram of the left ventricle and left atrium indicating the position of the sample volume to obtain a PW Doppler spectral trace of flow through the mitral valve. An ECG is displayed for timing of cardiac events

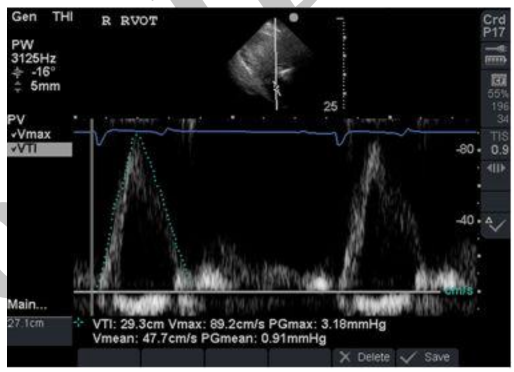

Fig. 3: A right parasternal long-axis PW Doppler echocardiogram of the right ventricular outflow tract indicating the position of the sample volume to obtain a PW Doppler spectral trace of flow through the pulmonic valve in systole (note: inverted PW Doppler spectral trace). An ECG is displayed for timing of cardiac events

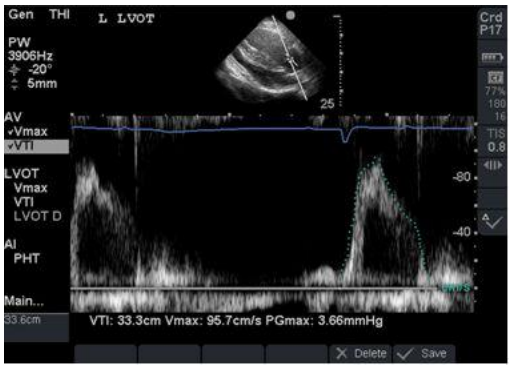

Fig. 4: A left parasternal long-axis PW Doppler echocardiogram of the left ventricular outflow tract (LVOT) and aorta indicating the position of the sample volume to obtain a PW Doppler spectral trace of flow through the aortic valve in systole (note: inverted PW Doppler spectral trace). An ECG is displayed for timing of cardiac events

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SV-D = VTI × [(AoJT/2)^2 × π]
CO-D = SV-D × HR

The stroke and cardiac index derived from the Doppler measurements (SI-D and CI-D, respectively) were calculated by dividing SV-D and CO-D, by the body weight of the horses (Boon, 2011).

Statistical analysis

Mean, standard deviation, maximum, and minimum for each Doppler parameter were reported. Comparisons between mitral and tricuspid inflow, and aortic and pulmonary outflow waveforms were evaluated by paired-sample t-test. PW Doppler parameters of Turkmen horses were compared with standard PW Doppler parameters value of Thoroughbred horses measured by Blissitt and Bonagura (1995) using one-sample t-test.

To identify the influence of sex, age, and body weight of the horses on the Doppler measurements, a linear regression analysis was performed. All explanatory variables were included in the regression model. A backward stepwise approach was used. Explanatory variables that were not statistically significant were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficient(s) for the retained variable(s) were significant at an alpha level of P<0.05. All the statistical analyses were performed using SPSS version 15.0.

Results

The horses used in this study had no history or evidence of cardiac dysfunction. In Table 1, the mean values of PW Doppler quantitative data of tricuspid and mitral flows are given, and in Table 2, the mean values of PW Doppler quantitative data of pulmonary and aortic flows obtained in adult normal Turkmen horses are described. In this study, the mean heart rate was 42 ± 2 beats/min, obtained during Doppler examination.

Comparisons between mean measurements obtained from the mitral and tricuspid inflow waveforms, showed the peak velocity of the tricuspid E and A signals to be significantly higher than E and A signals of the mitral, respectively (P=0.025, P=0.032) (Table 1). Measurements obtained from the mitral and tricuspid inflow waveforms were compared between Turkmen and Thoroughbred horses (Table 3).

Comparisons between measurements obtained from the aortic and pulmonary outflow waveforms are shown in Table 2.

Measurements obtained from the aortic and pulmonary outflow waveforms were compared between Turkmen and Thoroughbred horses (Table 4).

The results of linear regressions are summarized in Table 5. Amongst the Doppler-derived parameters, the following presented a significant positive correlation with age: The time taken from the onset of the QRS complex to the onset of the A wave for tricuspid flow, pulmonary PEP, and pulmonary PEP/ET. The heart rate had negative correlation with age. There were no PW Doppler parameters correlated to body weight and sex.

Discussion

In horses, Doppler echocardiography has been shown to be feasible, repeatable, and accurate, and has been demonstrated to be a powerful tool to detect cardiac abnormalities, drug or training-induced cardiac changes (Long *et al.*, 1992; Kriz and Rose, 2002).

The Doppler waveforms recorded from normal Turkmen horses are similar to those described in normal human subjects (Pye *et al.*, 1991), dogs (Brown *et al.*, 1991), Thoroughbred, and Thoroughbred cross horses (Blissitt and Bonagura, 1995).

Peak tricuspid flow velocities were less than peak mitral flow velocities in man, dog, Standardbred, and Thoroughbred (Reef *et al.*, 1989; Kirberger *et al.*, 1992). Human studies (Pye *et al.*, 1991) suggested that the early filling rate of the right ventricle is lower than that of the left ventricle. However, in this study, the peak velocity of the tricuspid E and A signals was significantly higher than E and A signals of the mitral, respectively. This may reflect differences in breeds and intercept angles between ultrasound beam and blood flow for tricuspid and mitral flows.

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In the present study, peak A velocity of tricuspid and mitral inflows were lower than Thoroughbred horses. This may reveal differences in breed and intercept angles between ultrasound beam and blood flow for tricuspid and mitral flows.

Other PW measurements consist of: E wave deceleration time, E wave onset time, and A wave onset time for both tricuspid and mitral inflows, and were shorter than Thoroughbred horses. This may reflect differences in breeds.

The aortic and pulmonary flow patterns are similar to those found in man, dog,

Table 1: Doppler echocardiographic parameters of tricuspid and mitral flows obtained in 42 normal adult Turkmen horses

Parameters	Tricuspid flow			
	Mean	Max	Min	SD
Peak E (cm/s)	95.34[a]	124	60.59	21.346
Peak A (cm/s)	45.59[a]	53.51	38.24	6.072
E/A ratio	2.085	2.598	1.584	0.363
E wave deceleration time (dt) (s)	0.151	0.22	0.08	0.042
E wave onset time (s)	0.553	0.63	0.47	0.045
A wave onset time (s)	1.35	1.64	1.18	0.16
	Mitral flow			
Peak E (cm/s)	69.85[b]	105.33	55.5	11.627
Peak A (cm/s)	37.876[b]	60.43	22.1	8.628
E/A ratio	1.926	3.19	1.226	0.489
E wave deceleration time (dt) (s)	0.152	0.2	0.07	0.033
E wave onset time (s)	0.571	0.630	0.51	0.034
A wave onset time (s)	1.329	1.66	1.03	0.18

Max: Maximum, Min: Minimum, SD: Standard deviation. [ab] Mean values of similar parameters of tricuspid and mitral flows followed by different letters are significantly different ($P<0.05$)

Table 2: Doppler echocardiographic parameters of pulmonary and aortic flows obtained in 42 normal adult Turkmen horses

Parameters	Pulmonary flow			
	Mean	Max	Min	SD
Peak velocity (Vmax) (cm/s)	106.355	161.9	86.9	17.005
Velocity time integral (VTI) (cm)	33.191	41.9	21.5	4.828
Acceleration time (dt) (s)	0.157	0.28	0.1	0.037
Pre-ejection perid (PEP) (s)	0.065[a]	0.11	0.04	0.016
Ejection time (ET) (s)	0.445[a]	0.51	0.34	0.046
PEP/ET	0.148[b]	0.244	0.094	0.034
	Aortic flow			
Peak velocity (Vmax) (cm/s)	101.948	143.2	79.1	15.341
Velocity time integral (VTI) (cm)	33.9	43.3	25.7	5.285
Acceleration time (dt) (s)	0.145	0.25	0.09	0.035
Pre-ejection perid (PEP) (s)	0.088[b]	0.13	0.06	0.018
Ejection time (ET) (s)	0.444[b]	0.53	0.4	0.031
PEP/ET	0.199[b]	0.321	0.125	0.046
SV-D (ml)	659.469	897.877	406.997	129.133
SI-D (ml/kg)	1.823	2.619	1.097	0.432
CO-D (l/min)	27.947	39.507	17.908	5.88
CI-D (l/min.kg)	0.077	0.112	0.048	0.019

Max: Maximum, Min: Minimum, SD: Standard deviation, SV-D: Stroke volume derived from the Doppler measurements, SI-D: Stroke index derived from the Doppler measurements, CO-D: Cardiac output derived from the Doppler measurements, CI-D: Cardiac index derived from the Doppler measurements. [ab] Mean values of similar parameters of pulmonary and aortic flows followed by different letters are significantly different ($P<0.05$)

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Table 3: Comparison among Doppler echocardiographic values of tricuspid and mitral flows obtained in current and Blissitt and Bonagura (1995) studies

Parameters	Tricuspid flow	
	Current study	Blissitt and Bonagura (1995)
	Mean	Mean
Peak E (cm/s)	95.34	89.9
Peak A (cm/s)	**45.59**	**68.7**
E/A ratio	**2.085**	**1.328**
E wave deceleration time (dt) (s)	**0.151**	**0.238**
E wave onset time (s)	**0.553**	**0.831**
A wave onset time (s)	**1.35**	**1.606**
	Mitral flow	
	Current study	Blissitt and Bonagura (1995)
Peak E (cm/s)	69.85	69.7
Peak A (cm/s)	**37.876**	**41.7**
E/A ratio	1.926	1.781
E wave deceleration time (dt) (s)	**0.152**	**0.216**
E wave onset time (s)	**0.571**	**0.608**
A wave onset time (s)	**1.329**	**1.442**

Statistically significant values are printed in **bold**

Table 4: Comparison among Doppler echocardiographic values of pulmonary and aortic flows obtained in current and Blissitt and Bonagura (1995) studies

Parameters	Pulmonary flow	
	Current study	Blissitt and Bonagura (1995)
	Mean	Mean
Peak velocity (Vmax) (cm/s)	**106.355**	**90.6**
Velocity time integral (VTI) (cm)	**33.191**	**25.740**
Acceleration time (dt) (s)	**0.157**	**0.208**
Pre-ejection perid (PEP) (s)	0.065	0.061
Ejection time (ET) (s)	**0.445**	**0.501**
PEP/ET	0.148	-
	Aortic flow	
	Current study	Blissitt and Bonagura (1995)
Peak velocity (Vmax) (cm/s)	**101.948**	**93.7**
Velocity time integral (VTI) (cm)	**33.9**	**25.369**
Acceleration time (dt) (s)	**0.145**	**0.122**
Pre-ejection perid (PEP) (s)	**0.088**	**0.075**
Ejection time (ET) (s)	**0.444**	**0.467**
PEP/ET	0.199	-
SV-D (ml)	659.469	-
SI-D (ml/kg)	1.823	-
CO-D (l/min)	27.947	-
CI-D (l/min.kg)	0.077	-

SV-D: Stroke volume derived from the Doppler measurements, SI-D: Stroke index derived from the Doppler measurements, CO-D: Cardiac output derived from the Doppler measurements, CI-D: Cardiac index derived from the Doppler measurements. Statistically significant values are printed in **bold**

Table 5: Linear regression analysis performed on PW Doppler measurements in 42 Turkmen horses

Dependent variable	Parameter	B	Std. error	R^2	P-value
A wave onset time for tricuspid flow (s)	Constant	1.105	0.107	0.512	
	Age	0.043	0.017		0.046
PEP for pulmonary flow (s)	Constant	0.054	0.005	0.178	
	Age	0.002	0.001		0.015
PEP/ET for pulmonary flow	Constant	0.115	0.011	0.275	
	Age	0.005	0.002		0.002
Heart rate (beats/min)	Constant	44.120	0.962	0.152	
	Age	-0.320	0.134		0.023

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Standardbred, and Thoroughbred. In the present study, no significant difference for Vmax, VTI, and acceleration time of flow was observed in the two vessels. This is probably due to differences in alignment with aortic and pulmonary artery flows in horses. However, this may represent a species variation in actual flow velocities. The Doppler waveforms obtained from the aortic outflow showed a significantly longer PEP than the pulmonary artery waveforms, indicating that right ventricular ejection begins earlier in systole, probably because of the lower diastolic pressure in the pulmonary artery compared to the aorta. This finding is similar in Thoroughbred horses. Blissitt and Bonagura (1995) have shown that ET was significantly shorter for the aortic outflow compared to the pulmonary one. In this study, the aortic waveforms had a significantly shorter ejection time than the pulmonary artery waveforms. As mentioned before, the aortic waveforms had a significantly longer PEP than the pulmonary artery waveforms. Therefore, the ratio of PEP/ET aortic outflow was significantly higher than the pulmonary artery waveforms ($P<0.001$).

Blissitt and Bonagura (1995) have published larger and smaller values to those acquired in the present study. In Turkmen horses, Vmax and VTI values of two vessels were more than Thoroughbred horses. In this study, ET of aortic and pulmonary outflows were shorter than Thoroughbred horses. Also, acceleration time of pulmonary outflow in Turkmen horses was shorter, and acceleration time of aortic outflow was longer than Thoroughbred horses. There was no significant difference in PEP of pulmonary outflow between Turkmen and Thoroughbred horses, but aortic PEP was longer in Turkmen horses. All of the differences in parameters values may reflect difference in breed and training.

Little or no correlation has been found between velocities spectra and age, sex, or breed in dogs (Yuill and O'Grady, 1991; Kirberger *et al*., 1992). The results concerning the relationship between body weight or heart rate and blood flow velocity in horses was more controversial. Some authors showed no correlation between blood flow measurements and body weight or heart rate, whereas other authors demonstrated a positive relationship between blood flow measurements and heart rate, and a negative relationship between blood flow measurements and body weight (Reef *et al*., 1989; Yuill and O'Grady, 1991; Kirberger *et al*., 1992; Yamamoto *et al*., 1993). In a study performed in 30 adult thoroughbred horses, tricuspid peak E negatively correlated with body weight, and pulmonary Vmax and VTI were positively correlated with body weight (Blissitt and Bonagura, 1995), the latter being thus the opposite of what was found in dogs. In the present study in the Turkmen horse, the time taken from the onset of the QRS complex to the onset of the A wave for tricuspid flow, pulmonary PEP, and pulmonary PEP/ET was also positively correlated to age. Also, the heart rate was negatively correlated to age. The other Doppler-derived parameters were not significantly related to age. There were no PW Doppler parameters correlated to body weight and sex.

These findings suggest that PW echocardiographic measurements in horses may be affected by breed, age, and possibly by athletic training. These values can be used as standard and reference values for evaluation of cardiovascular disorders in Turkmen horses.

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