EQUINE VETERINARY JOURNAL
Equine vet. J., Suppl. **19** (1995) 38-46

Pulsed wave Doppler echocardiography in normal horses

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Keywords: horse; Doppler; echocardiography; cardiac

Summary

Reference values were established for selected Doppler derived variables from a group of 40 normal Thoroughbred and Thoroughbred cross horses. Standard two-dimensional (2-D) images used for guiding the Doppler sampling site allowed accurate alignment with flow. Tricuspid inflow velocities during rapid filling (E) and atrial contraction (A) were significantly higher when recorded from a right parasternal angled view than from a right parasternal long-axis view. In 8 horses the tricuspid inflow peak A velocity was higher than the peak E velocity. The peak acceleration of blood flow was higher (P=0.000) in the aorta (mean 8.01 m/s/s) than in the pulmonary artery (4.45 m/s/s). Significant differences were also noted in the pre-ejection period, ejection time and acceleration time between the 2 vessels. Horses with functional ejection murmurs had lower peak aortic acceleration and a longer acceleration time than horses without flow murmurs. Horses with filling murmurs over the left hemithorax had a significantly higher peak mitral E velocity than horses without such murmurs. Measurements from Doppler waveforms were repeatable and may prove useful in assessing ventricular function in this species.

Introduction

Doppler echocardiography is an established technique for the noninvasive investigation of cardiac disease in man, and has substantially improved the understanding of blood flow in the normal and diseased heart (Simpson and Camm 1990). Diastolic (Devereux 1989; 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 maximum acceleration of flow is considered to be a sensitive index of myocardial contractile function (Noble *et al.* 1966). The area under the velocity waveform (velocity time integral) is directly related to the stroke volume of the ventricle (Mehta and Bennett 1986). Indices of ventricular function measured from Doppler waveforms can distinguish human patients with normal and abnormal left ventricular function (Gardin *et al.* 1983; Sabbah *et al.* 1986), can be used to monitor clinical responses to therapy (Elkayam *et al.* 1983; Bennett *et al.* 1984; Sabbah *et al.* 1988) and provide information about mechanisms and haemodynamic effects of therapeutic agents (Harrison *et al.* 1988, 1991). Blood flow patterns and velocities are altered in human subjects with cardiac malformations and valvular disease (Oh *et al.* 1989; Marcus *et al.* 1990) and Doppler echocardiography can be used to monitor the response of the heart to valvular dysfunction (Goldberg *et al.* 1988). Despite the use of Doppler echocardiography to identify valvular regurgitation in horses with cardiac murmurs (Reef 1988a,b; Marr *et al.* 1990; Reimmer *et al.* 1991), there have been few studies to evaluate Doppler echocardiography in normal horses. Systolic time intervals (pre-ejection period and ejection time) are useful in detecting changes in ventricular performance (Borow 1989). These can be measured from Doppler waveforms (Sequeira *et al.* 1976; Koito and Spodick 1989), but in horses only the ejection time has been measured from Doppler waveforms (Weinberger 1991). Reef *et al.* (1989) reported blood flow velocities from normal Standardbred horses; however, the angles between the ultrasound beam and blood flow were large and required angle correction for velocity determination. This potentially causes considerable error in the estimation of flow velocities (Goldberg *et al.* 1988). The standard 2-D images reported by Long *et al.* (1992), may allow more accurate alignment of the ultrasound beam with blood flow. Weinberger (1991) has reported a number of measurements from Doppler waveforms in Warmblood horses. The present study recorded Doppler waveforms from standard 2-D images in normal Thoroughbred and Thoroughbred cross horses. Indices of systolic and diastolic ventricular function were measured from the Doppler waveforms.

Methods

Doppler spectra were recorded from 40 normal Thoroughbred or Thoroughbred cross horses (3 stallions, 21 geldings and 16 mares). The horses were age 2–17 years, weighed 428–648 kg (mean 513 kg) and had no history or evidence of cardiac dysfunction on clinical, electrocardiographic (ECG) and 2-D echocardiographic examinations. Horses with the following functional murmurs were included as normal: early systolic ejection murmurs with the point of maximum intensity (PMI) over the aortic valve; presystolic murmurs (murmur audible between the fourth and first heart sounds); early diastolic murmurs (murmur audible between the second and third heart sounds). Functional murmurs were present in 62.5% of the horses.

A Vingmed CFM 700 ultrasound system (Sonotron Ltd, Bedford, UK) with a 2.25 MHz annular phased array transducer and 2-D, M-mode, colour flow mapping and spectral Doppler functions, was used for all studies. During spectral Doppler recording the transducer was used in the high pulsed repetition frequency mode (HPRF) at a frequency of 2 MHz. The velocity scale was set at 1.5 m/s, so that only one sample volume was available for velocity recording. Flow velocities were displayed

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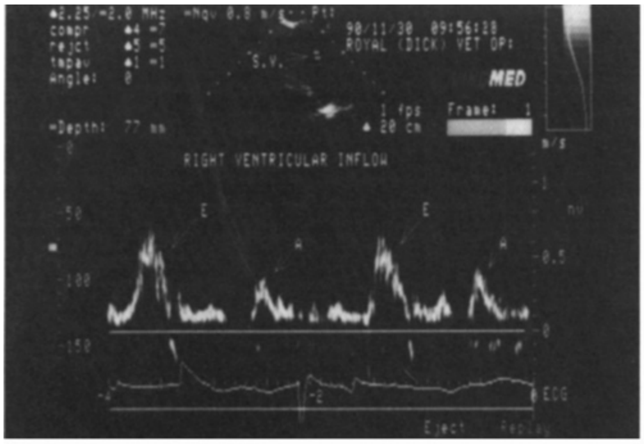

Fig 1: Pulsed wave Doppler study of the tricuspid inflow from a right parasternal long-axis apical view. Flow velocities are recorded from a sample volume positioned on the ventricular side of the tricuspid valve. The sample volume is displayed on the small image sector at the top of the figure. The velocity scale, m/s, is shown on the right of the Doppler display. Blood is flowing towards the transducer and is therefore displayed above the baseline of the Doppler display. S.V. = sample volume, A = flow due to atrial contraction, E = rapid filling wave.

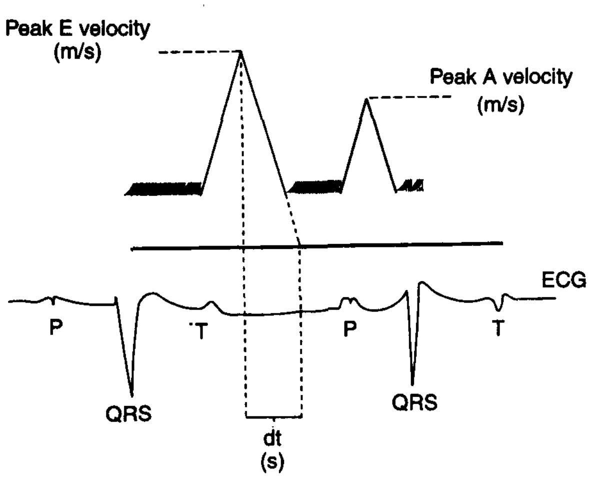

Fig 2: Diagram representing the ventricular filling velocities recorded by Doppler echocardiography. The peak E and A velocity are measured in m/s. The deceleration time (dt) is measured by extrapolating the downstroke of the E signal to the baseline. The deceleration time is given in seconds.

graphically with velocity in m/s on the y axis and time in seconds on the x axis. Flow towards the transducer was displayed above the baseline whereas flow away from the transducer was displayed below the baseline.

Accurate velocity recordings are obtained when the Doppler ultrasound beam is aligned parallel to the direction of flow (Hatle and Angelsen 1985). Alignment with blood flow was initially assessed from a 2-D ultrasound image. A colour flow study from that image was then used to guide placement of the sampling site into an area of maximal blood velocity. Minor alterations in beam angulation were made to obtain the clearest audio and visual signals. Accurate alignment with blood flow was assumed when the audible signal was clear and the spectral envelope of the Doppler waveform was complete. Measurements were made from videotape recordings of the Doppler waveforms, using computer

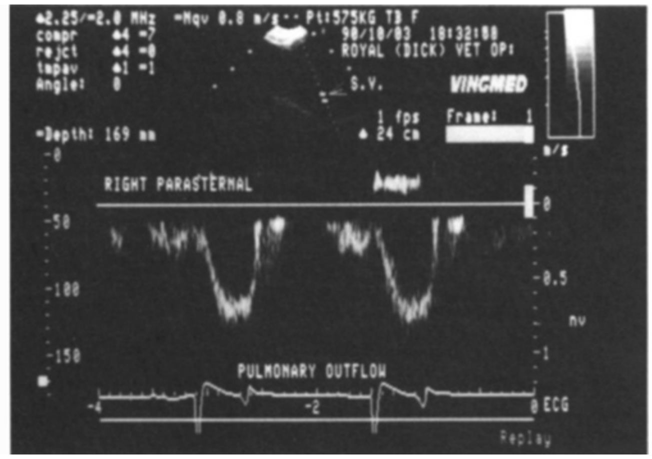

Fig 3: Pulsed wave Doppler study from the pulmonary artery. The pulsed wave Doppler sampling site (S.V.) is represented by the two dots which can be seen positioned in the pulmonary outflow on the 2-D image. The velocity scale, m/s, is shown on the right of the Doppler display. Blood is flowing away from the transducer and therefore is displayed below the baseline of the Doppler display.

software inbuilt in the Vingmed CFM 700.

The tricuspid inflow was recorded from the right parasternal long-axis apical view and from the angled view dorsal location right ventricular outflow tract (Long *et al.* 1992). The sample volume was placed on the ventricular side of the tricuspid valve at the valve tips so that it remained between the valve leaflets in diastole (Fig 1). Minor adjustments in transducer angulation were made to obtain the maximum flow velocity. The peak velocity was measured during the rapid filling phase of the ventricle (E wave) and during the atrial contraction (A wave). The deceleration time (dt) of the E wave was measured from the peak of the rapid filling signal (E), to the point where the downstroke intercepted the baseline (Fig 2). The time taken from the onset of the QRS complex to the onset of the E and A waves was recorded. Simultaneous R-R intervals were also measured. Where peak E velocities were obtained from a different location than peak A velocities, the R-R intervals relate to the peak E recording.

Spectral Doppler traces from the right parasternal angled view showed E and A waveforms similar to those recorded from the long-axis view. In this view initial alignment with flow was determined from the colour flow signal not from the 2-D image. Signals were measured as for the long-axis view.

The pulmonary outflow velocity was recorded from the right parasternal short-axis view at the pulmonary artery level (Long *et al.* 1992) with the sample volume on the arterial side of the pulmonary valve (Fig 3). Care was taken to ensure that the sample volume remained in the centre of the vessel during systole. The peak velocity (VMax), peak acceleration (dv/dt), velocity time integral (VTI), pre-ejection period (PEP) and ejection time (ET) were measured from the Doppler waveforms (Fig 4). The acceleration time (dt) and R-R interval were also measured. The acceleration time was recorded from the onset of the Doppler waveform to the start of the maximum velocity plateau. The velocity time integral was measured by tracing the modal velocity of the Doppler signal.The modal velocity was represented by the brightest line in the spectral Doppler waveform (Goldberg *et al.* 1988). When baseline crossing was obscured by low velocity signals or the use of low velocity filters, the point of baseline crossing was determined by extrapolation of the downstroke. To minimise error during this procedure low velocity filters were set as low as possible (0.05 m/s). The pre-ejection period was measured from the onset of the QRS complex to the onset of the spectral waveform. The ejection time was measured from the

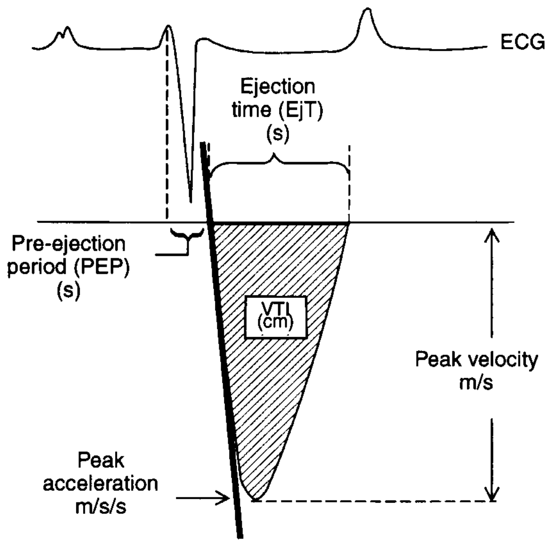

Fig 4: Diagram representing the ventricular outflow velocities recorded by Doppler echocardiography. The peak velocity (V_{max}) is measured in m/s. The peak acceleration, m/s/s, is measured from the middle of the upstroke of the Doppler waveform to the peak velocity. The area under the curve (VTI) is measured in cm. The pre-ejection period (PEP) and ejection time (ET) are given in seconds. The acceleration time, in seconds (dt), is measured from the onset of the Doppler waveform to the start of the maximum velocity plateau.

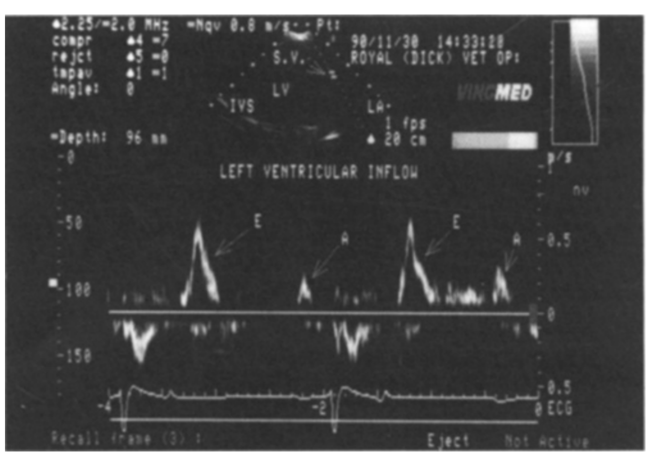

Fig 5: Pulsed wave Doppler study of the mitral inflow from a left parasternal long-axis view. Flow velocities are recorded from a sample volume positioned on the ventricular side of the mitral valve. The sampling site is displayed on the small image sector at the top of the figure. The velocity scale, m/s, is shown on the right of the Doppler display. Blood is flowing towards the transducer and therefore is displayed above the baseline of the Doppler display. S.V. = sample volume, A = flow due to atrial contraction, E = rapid filling wave, LA = left atrium, LV = left ventricle, IVS = interventricular septum.

onset to the end of the spectral waveform. For both measurements the calliper was placed in the centre of the ascending or descending limbs of the spectral waveform as it crossed the baseline.

The mitral inflow velocity (Fig 5) was recorded from the left parasternal apical view of the left ventricular inlet (Long *et al.* 1992). The sample volume location in relation to the valve, and the measurements derived from the spectral waveforms were as described for the tricuspid inflow. The aortic outflow (Fig 6) was

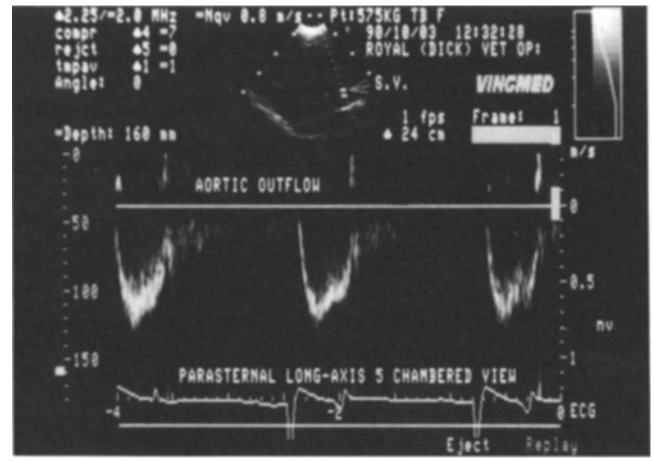

Fig 6: Pulsed wave Doppler study from the aorta. The pulsed wave Doppler sampling site is displayed on the small image sector at the top of the figure. The velocity scale, m/s, is shown on the right of the Doppler display. Blood is flowing away from the transducer and therefore is displayed below the baseline of the Doppler display.

recorded from the left parasternal long-axis 5-chambered view (Long *et al.* 1992). The sample volume location on the arterial side of the valve, and the measurements derived from the Doppler waveforms were as described for the pulmonary outflow.

Flow velocities were recorded at resting heart rates. Where horses showed second degree atrioventricular block, the immediate post block beat was not measured. Further Doppler studies were recorded from 6 horses on 3 consecutive days to assess the repeatability of the measurements.

Data analysis.

Measurements were made from 5 consecutive cardiac cycles, the maximum and median values from each individual horse being used for analysis. Data were tested for normality using box and whisker plots and normal probability plots. Parametric methods of analysis were used for normally distributed data, whereas data that were not normally distributed were analysed by nonparametric methods. Descriptive statistics were calculated for the median values of 5 consecutive Doppler spectra from 40 normal Thoroughbred and Thoroughbred cross horses (range, mean, standard deviation and coefficient of variation). Maximum values from 5 consecutive Doppler spectra were also analysed to allow comparisons to be made with a previous study (Reef *et al.* 1989). Correlation (Pearson's coefficient) was used to determine any relationship between measurements and bodyweight and between measurements and age. Student's *t* test was used to compare measurements from: the pulmonary and aortic outflow, the tricuspid and mitral inflow and the tricuspid inflow waveforms recorded from the right parasternal long-axis and angled views. The peak velocity, peak acceleration and time to peak velocity of aortic and pulmonary waveforms in 14 normal horses with ejection murmurs were compared with measurements in 26 normal horses without ejection murmurs (Student's *t* test). The mitral E velocity and the deceleration time in 14 horses with left sided early diastolic flow murmurs were compared to the same measurements in 26 horses without such murmurs (Student's *t* test). The tricuspid E velocity and deceleration time in 7 horses with right sided early diastolic flow murmurs was compared to the same measurements in 33 horses without such murmurs (Mann-Whitney test). Wilcoxon's test was used to compare measurements obtained from the same group of horses on different days.

Results

The summary statistics of median measurements from 40 normal Thoroughbred and Thoroughbred cross horses are given in Tables 1 and 2. Recording of tricuspid inflow velocities from the angled view was attempted only in 23 horses. An A wave was recorded in 17 of these horses. Many measurements had a large coefficient of variation, especially the peak velocities of the mitral and tricuspid A wave and the aortic and pulmonary pre-ejection periods. There was less variability in tricuspid inflow measurements from the angled view than from the long-axis view.

Comparisons between median measurements from the mitral and tricuspid inflow waveforms show the peak velocity of the tricuspid A signal recorded from the long-axis image to be significantly higher than that of the mitral signal (Table 1). There was no significant difference between the peak velocity of the mitral and tricuspid E signals but the deceleration time of the mitral E signal was shorter. The time to onset of the tricuspid E and A waveforms was shorter than the time to onset of the mitral E and A waveforms.

Significant differences were detected between tricuspid inflow velocities recorded from the right parasternal long-axis and angled views (Table 1). The velocity of the tricuspid E and A signals was significantly greater when recorded from the angled view than the long-axis view. The time to onset of the tricuspid E and A signals was significantly shorter when measured from the long-axis compared to the angled view.

Comparisons between measurements from the aortic and pulmonary outflow waveforms are shown in Table 2. The Doppler waveforms from the aortic outflow showed a significantly greater acceleration (P=0.000) and a shorter time to peak velocity than the pulmonary artery waveforms. The aortic waveforms also had a significantly longer pre-ejection period and a significantly shorter ejection time than the pulmonary artery waveforms.

There was no significant differences between measurements from geldings and mares except for the deceleration time of the mitral E wave which was significantly shorter (P=0.043) in mares than in geldings.

Correlation with age and bodyweight

A weak though significant negative correlation was detected between the peak E velocity of tricuspid inflow and age when waveforms were measured from the angled view (r=-0.55, P=0.006). Very weak, but statistically significant, positive correlations were demonstrated between age and the onset time of the tricuspid E signal from the angled view (r=0.44, P=0.042), the peak E velocity of mitral inflow (r=-0.32, P=0.045) and the R-R interval during left ventricular filling (r=0.38, P=0.016). No significant correlation was demonstrated between any measured

TABLE 1: Summary statistics of Doppler measurements from a group of Thoroughbred and Thoroughbred cross horses

Variable	n	Mean	Max	Min	s.d.	CV	P value
RR Interval (s)							
RR (TV angled)	23	1.896	4.21	1.33	0.229	13.23	0.089#
RR (TV long-axis)	40	1.695	2.49	1.24	0.248	**15.03**	0.730*
RR (mitral inflow)	39	1.685	4.16	1.06	0.273	**16.70**	
Peak E velocity (m/s)							
E (TV angled)	23	0.899	1.05	0.77	0.097	10.74	**0.000#**
E (TV long-axis)	40	0.650	0.87	0.44	0.104	**16.02**	0.100*
E (mitral inflow)	40	0.697	1.12	0.41	0.139	**19.96**	
Peak A velocity (m/s)							
A (TV angled)	17	0.687	1.07	0.48	0.136	**19.87**	**0.000#**
A (TV long-axis)	40	0.531	0.79	0.26	0.117	**22.01**	**0.000***
A (mitral inflow)	40	0.417	0.63	0.24	0.099	**23.74**	
E/A ratio							
E/A (TV angled)	17	1.328	1.974	0.870	0.292	**20.86**	0.810#
E/A (TV long-axis)	40	1.305	3.192	0.759	0.486	**37.26**	**0.000***
E/A (mitral inflow)	40	1.781	3.560	0.953	0.631	**35.43**	
E wave deceleration time (dt) (s)							
dt (TV long-axis)	40	0.238	0.34	0.16	0.041	**17.03**	**0.013***
dt (mitral)	37	0.216	0.27	0.14	0.032	14.97	
E wave onset time (QE) (s)							
QE (TV angled)	22	0.831	0.96	0.73	0.058	6.98	**0.000#**
QE (TV long-axis)	40	0.566	0.67	0.46	0.046	8.06	**0.000***
QE (mitral inflow)	37	0.608	0.89	0.52	0.061	10.75	
A wave onset time (QA) (s)							
QA (TV angled)	16	1.606	2.00	1.14	0.215	13.40	**0.000#**
QA (TV long-axis)	40	1.358	2.29	0.96	0.249	**18.32**	**0.004***
QA (mitral inflow)	37	1.442	2.23	0.92	0.271	**18.82**	

n = number of horses, Min = minimum value, Max = maximum value, s.d. = standard deviation, CV = coefficient of variation (s.d./mean), CV values >15% are printed **in bold**. P values marked# compare tricuspid inflow measurements from long-axis (TV long-axis) and angled views (TV angled) using Student's *t* test. P values marked *compare measurements from the tricuspid (TV long-axis) and mitral inflow (Student *t* test). Statistically significant values are printed in **bold.**

Doppler inflow variable and bodyweight except for the peak E velocity when waveforms were measured from the angled view (r=-0.46, P=0.026).

The peak velocity of the pulmonary artery waveforms, showed a statistically significant correlation with bodyweight (r=0.51, P=0.001). Velocity time integral measurements, from pulmonary artery waveforms showed a very weak, but statistically significant, relationship with age (r=0.38, P=0.015) and bodyweight (r=0.36, P=0.022). R-R intervals during recording of aortic waveforms outflow showed a very weak, but statistically significant, relationship with age (r=0.39 P=0.014).

Comparisons in horses with functional murmurs

Table 3 shows the results of comparisons between measurements from normal Thoroughbred and Thoroughbred cross horses with and without functional murmurs. Horses with ejection murmurs showed a significantly lower peak aortic acceleration (P=0.019) and a significantly longer time to peak velocity (P=0.004) than horses with no ejection murmur. However, there were no significant differences in the aortic peak velocity or velocity time integral between horses with and without ejection murmurs, nor in any of the measured variables from the pulmonary waveforms. Horses with left sided early diastolic filling murmurs had a significantly higher peak mitral E velocity than horses without murmurs (Table 4). There was no significant difference in the mitral E signal deceleration time between these horses. Horses with right sided early diastolic filling murmurs showed no significant differences in the peak tricuspid E velocity nor in the tricuspid E deceleration time when compared to horses without murmurs (Table 4).

Repeatability study

There were no significant differences between measurements from 6 horses on 3 separate days, with the following exceptions. The time to onset of the tricuspid A wave was significantly different between Days 2 and 3. The R-R intervals during mitral and tricuspid inflow recordings were significantly different between Days 1 and 3 and between Days 2 and 3. The R-R intervals from pulmonary artery recordings varied between Days 1 and 3. In all cases P=0.036.

Discussion

The Doppler waveforms and peak flow velocities recorded from normal Thoroughbred and Thoroughbred cross horses are similar to those described in normal human subjects (Gardin *et al.* 1984; Hatle and Angelsen 1985; Pye *et al.* 1991) and dogs (Brown *et al.* 1991). The peak flow velocities from the aortic outflow and mitral inflow in the present study are similar to those recorded in conscious horses using invasive techniques (Nerem *et al.* 1974).

Ventricular inflow velocities

The mean tricuspid E and A inflow velocities and the mitral A velocity were higher in the present study than in the study by Reef *et al.* (1989) despite analysis of the largest flow signals in the previous study. The mean velocity from the largest mitral (E)

TABLE 2: Summary statistics of Doppler measurements from a group of normal horses

Pulmonary outflow							
Variable	n	Mean	Max	Min	s.d.	CV	P value
RR interval (s)							
Pulmonary outflow	40	1.744	4.09	1.31	0.229	13.23	**0.002**
Aortic outflow	40	1.672	4.17	1.29	0.229	13.23	
Peak velocity (V_{max}) (m/s)							
Pulmonary outflow	40	0.906	1.04	0.78	0.082	9.09	0.061
Aortic outflow	40	0.937	1.15	0.78	0.094	10.07	
Peak acceleration (dv/dt) (m/s/s)							
Pulmonary outflow	40	4.453	6.71	2.99	0.742	**16.66**	**0.000**
Aortic outflow	40	8.015	10.83	5.19	1.448	**18.07**	
Acceleration time (dt) (s)							
Pulmonary outflow	40	0.208	0.27	0.16	0.027	13.19	**0.000**
Aortic outflow	40	0.122	0.17	0.09	0.021	**16.93**	
Velocity time Integral (VTI) (cm)							
Pulmonary outflow	40	25.740	36.710	20.370	3.072	11.93	0.580
Aortic outflow	40	25.369	32.900	20.610	3.209	12.65	
Pre-ejection period (PEP) (s)							
Pulmonary outflow	40	0.061	0.10	0.02	0.017	**28.23**	**0.000**
Aortic outflow	37	0.075	0.11	0.04	0.018	**24.80**	
Ejection time (ET) (s)							
Pulmonary outflow	40	0.501	0.58	0.45	0.030	6.03	**0.000**
Aortic outflow	40	0.467	0.55	0.41	0.031	6.72	

n = number of horses, Min = minimum value, Max = maximum value, s.d. = standard deviation, CV = coefficient of variation (s.d./mean). CV values >15% are printed **in bold**. P values compare measurements from the aorta and pulmonary artery (Student's *t* test). Statistically significant values are printed **in bold**.

TABLE 3: Comparison of the mean values of median measurements from the pulmonary artery and aorta in horses with and without systolic ejection murmurs, using Student's *t* test

Variable	n	No murmur	n	Ejection murmur	P value
Aortic outflow					
Peak velocity (s)	26	0.934	14	0.939	0.860
Peak acceleration (m/s/s)	26	8.430	14	7.240	**0.019**
Acceleration time (s)	26	0.115	14	0.135	**0.004**
VTI (cm)	26	24.630	14	26.750	0.064
Pulmonary artery					
Peak velocity	26	0.895	14	0.927	0.260
Peak acceleration	26	4.486	14	4.390	0.750
Acceleration time	26	0.202	14	0.218	0.150
VTI	26	25.830	14	25.570	0.790

Differences were considered significant when P<0.05. Significant values are printed **in bold**. n = number of horses studied.

TABLE 4: Comparison of mean values of median measurements from the mitral and tricuspid inflow in horses with and without early diastolic filling murmurs using Student's *t* test

Variable	n	No murmur	n	Filling murmur	P value
Tricuspid inflow E wave (long-axis)					
Peak velocity (m/s)	33	0.655	7	0.627	0.500
Deceleration time (s)	33	0.240	7	0.231	0.590
Mitral inflow E wave					
Peak velocity (m/s)	26	0.649	14	0.786	**0.400**
Deceleration time (s)	26	0.213	14	0.223	

Mitral flow velocities are compared in horses with and without left side murmurs, tricuspid flow velocities are compared in horses with and without right side murmurs. Differences are considered significant when P<0.05. Significant values are printed **in bold.** n = number of horses studied.

waveforms reported by Reef *et al.* (1989), was similar to the mean velocity of median measurements in the present study, but smaller than that of the maximum measurements. This may reflect differences in breed, sampling site between the two studies, instrumentation or closer alignment with flow in the present study. Reef *et al.* (1989) recorded tricuspid inflow velocities from the atrial side of the tricuspid valve rather than from the valve tips, where flow velocities have been shown to be higher (Weinberger 1991). However, the velocities recorded at the mitral and tricuspid valve by Weinberger (1991) were also lower than those recorded in the present study. The apical view used in the present study to guide placement of the sample volume, may have allowed more accurate alignment with flow, however the angle between the ultrasound beam and the blood flow was not measured. Although this angle can be estimated from the 2-D image, this only indicates alignment in 2 planes. In addition the 2-D image is fixed during the final adjustment of the transducer, when the most accurate alignment is achieved.

In some horses the peak A velocities of the mitral and tricuspid inflows, were not recorded from the same site as the peak E velocities. In these horses the sites of the maximum E and A flow velocities were indicated by the colour flow image. The peak A flow was often recorded nearer to the interventricular septum whereas the peak E flow was recorded from the centre of the ventricular inlet. The peak E and A velocities were recorded separately in these horses. Underestimation of the peak A velocity may have occurred in previous studies, if the transducer was adjusted to ensure optimum E waveforms.

Ventricular inflow velocities (E/A ratios)

In most horses the peak velocity of ventricular filling (E) was higher than the peak A velocity for both the mitral and tricuspid inflow, although in some horses this was reversed, producing a large coefficient of variation for the E/A ratio. Reversal of the E to A ratio was detected most commonly during right ventricular filling (E/A<1 in 8 horses), although it also occurred in one horse during filling of the left ventricle. In man the E velocity is normally higher than the A velocity (Nishimura *et al.* 1989; Pye *et al.* 1991), reversal of the E to A ratio is associated with abnormal ventricular diastolic function (Gottdiener 1991). The reversal of the E/A ratio in the present study, may reflect more accurate alignment with the A waveform (atrial contraction) than the E wave of rapid filling. An apical four chambered plane would allow more accurate alignment with both the E and A waveforms; however, this cannot be achieved in adult horses. The determination of E/A ratios from peak E and A velocities recorded from separate locations, is a limitation of the present study. To establish reference E/A ratios in normal horses the E and A waveforms should probably be recorded from a standard location, with E and A velocities measured from the same cardiac cycle.

Comparison of tricuspid inflow velocities from long-axis and angled views

The higher E and A velocities measured from the angled view implies more accurate alignment with the right ventricular inflow from this view or that the intraventricular blood flow velocity is higher at this site. In the angled view, blood at the sampling site is flowing towards the transducer. However the expected direction of flow, from the 2-D image, would be from right to left, perpendicular to the transducer. Flow in this direction would have resulted in a low Doppler frequency shift and poor signal quality. In contrast, the signal was strong and of high velocity. Comparison of the onset times of the E and A waveforms obtained from the angled and long-axis views shows that signals are recorded significantly later from the angled view than from the long-axis view (Table 1). This finding suggests that in the angled view the right ventricular inflow signal is not recorded as soon as it passes through the tricuspid valve, as in the long-axis view, but is recorded later in diastole. The colour flow studies from this view suggest that blood flows into the right ventricle towards the apex and up towards the right ventricular outflow tract. It then appears to curl back and flows towards but perpendicular to the tricuspid valve at which point the angled recordings were made (Blissitt and Bonagura 1995). Due to the higher velocities and lower coefficient of variation for E wave measurements from this site, it may be a more appropriate location from which to measure the equine E wave.

Comparison of mitral and tricuspid inflow velocities

The peak A velocity of the mitral inflow was significantly higher than the tricuspid inflow. This has also been reported in normal humans (Nishimura *et al.* 1989; Pye *et al.* 1991). Studies in humans (Nishimura *et al.* 1989; Pye *et al.* 1991) and horses (Reef *et al.* 1989) have shown the mitral E wave to be significantly higher than the tricuspid E wave. A similar trend was observed in this study (Table 1). Human studies (Zoghbi *et al.* 1990; Pye *et al.* 1991) suggest that the lower inflow velocities at the tricuspid valve reflect the greater area of the tricuspid valve annulus compared to the mitral valve annulus. Zoghbi *et al.* (1990) suggest that the early filling rate of the right ventricle is lower than that of the left ventricle. Tricuspid valve and mitral valve areas were not evaluated in the present study, nor was the rate of ventricular filling.

The onset of rapid filling and atrial contraction was earlier for the tricuspid inflow (long-axis) than the mitral inflow, despite similar R-R intervals. Earlier onset of right atrial contraction would be expected, as the sino-atrial node, located in the right atrial wall, will cause earlier stimulation of the right atrium (Holmes 1987). The E signal of rapid filling begins when the ventricular pressure falls below the atrial pressure. This study suggests right ventricular filling precedes left ventricular filling in normal horses. The deceleration time of the E signal was significantly shorter for the mitral inflow compared to the tricuspid inflow. This has also been reported in man (Pye *et al.* 1991). The deceleration time of the left ventricle reflects the decrease in the left atrial, left ventricular pressure gradient and is related to the peak E velocity (Nishimura *et al.* 1989). A decrease in the peak E velocity results in a prolonged deceleration time whereas an increase in the peak E velocity decreases the deceleration time. If similar factors affect the right ventricular deceleration time, the lower peak E velocity of right ventricular inflow would cause a prolonged deceleration time compared to the left ventricular inflow. Although the difference in peak E velocities between the 2 ventricles was not statistically significant, the peak E velocity of the mitral inflow tended to be larger, which may have resulted in the significantly reduced deceleration time.

Ventricular outflow velocities

In the present study aortic outflow velocities were recorded from the left hemithorax from a 5-chambered view. Pulmonary artery velocities were recorded from the right hemithorax from a short-axis view at the pulmonary artery level. Considerable angulation of the transducer was used in the present investigation to obtain good alignment with aortic flow. This was facilitated by the small round head of the Vingmed transducer. The small transducer also facilitated recording of pulmonary outflow velocities, for which it was necessary to push the transducer between the triceps mass and the thoracic wall. The peak velocity of the pulmonary and aortic outflow, recorded from the opposite hemithoraces, reported by Reef *et al.* (1989) were slightly higher than in the present study. However, these values (Reef *et al.* 1989) were corrected to allow for the underestimation of velocity which occurs when there is a large angle between the ultrasound beam and the blood flow. Estimation of this angle from a 2-D image, can lead to overestimation of the true flow velocity (Hatle and Angelsen 1985). Reanalysis of the results reported by Reef *et al.* (1989), without correction for the angle between the ultrasound beam and blood flow, revealed that the pulmonary and aortic blood flow velocities were considerably lower than those reported in the present study. This supports the work of Weinberger (1991), who showed that the aortic flow velocity recorded from the right hemithorax was lower than that recorded from the left.

Comparison of aortic and pulmonary outflow velocities

In this study the peak acceleration was significantly higher and the time to peak velocity and ejection time were significantly shorter, for the aortic outflow compared to the pulmonary outflow. There was no difference in the peak velocity of flow in the two vessels. The R-R intervals were significantly longer for the pulmonary artery studies, however the measured increase in R-R interval was slight and is unlikely to have significantly influenced the results. Similarly in man, the average acceleration is higher and the ejection time is shorter in the aorta compared to the pulmonary artery, but the aortic peak velocity is also higher (Gardin *et al.* 1984). Reef *et al.* (1989) also failed to show a difference in peak flow velocity between the equine pulmonary artery and aorta. This is probably due to differences in alignment with aortic and pulmonary artery flow in horses. However this may represent a species variation in actual flow velocities. In the present study a shorter pre-ejection period was recorded in the pulmonary artery, indicating that right ventricular ejection begins earlier in systole, probably because of the lower diastolic pressure in the pulmonary artery compared to the aorta.

Variability

The large coefficients of variation of some values indicates a large variation in these measurements between individual horses, possibly due to poor alignment with flow in certain animals. A large variation in the alignment with flow was reported by previous authors (Reef 1989; Weinberger 1991). The coefficient of variation of the tricuspid E wave was lower for measurements obtained from the angled view than for those obtained from the long-axis view. This suggests more consistent alignment with flow between individual horses from this view. The pulmonary artery and aortic pre-ejection period also showed marked variability between horses. This may be associated with inaccuracies, caused by extrapolation of outflow signals to the baseline.

Repeatability study

The values obtained on a day to day basis were not significantly different with the exception of the time to onset of the mitral and tricuspid A wave. However, changes in R-R intervals probably caused the observed changes in the onset time of the mitral and tricuspid A waves. Horses should be as relaxed as possible during Doppler echocardiography, as subtle changes in autonomic tone can markedly influence heart rate which may affect Doppler values.

Correlation between flow velocities and age

Studies in man have shown that the peak E velocity of the tricuspid and mitral inflows decreases with age, whereas the peak A velocity increases (Zoghbi *et al.* 1990; Kitzman *et al.* 1991). This was not demonstrated in the present study, except for a modest negative correlation between age and the peak tricuspid E velocity recorded from the angled view. The decrease in the peak E velocity with age in man, is similar to that which is recorded in patients with prolonged ventricular relaxation (Zoghbi *et al.* 1990). Although horses of a wide age range were investigated in the present study (2–17 years), it is possible that older horses would need to be examined to determine the effects of ageing on intracardiac flow velocities in this species.

A significant weak correlation with age was shown by R-R intervals measured during the mitral inflow and the aortic outflow studies. However, R-R intervals recorded during the pulmonary outflow studies and tricuspid long-axis and angled studies were not significantly related to age. It is unlikely that these findings represent a true relationship between R-R intervals and age. A similar weak correlation was detected between the pulmonary artery velocity time integral and age.

Flow velocities in horses with functional murmurs

Comparison of Doppler waveforms in horses with functional flow murmurs showed that horses with left sided early diastolic murmurs had a significantly higher peak mitral E velocity than horses which did not have left sided early diastolic murmurs. The E waveform is dependent on the active relaxation of the ventricle

(Devereux 1989) and atrioventricular pressure gradient. An increase in the velocity of blood flow into the ventricle during rapid filling may cause the critical Reynolds number to be exceeded such that turbulence develops. However, the audible Doppler signal in these horses was clear and the spectral Doppler tracing did not show spectral broadening. Horses with right sided inflow murmurs did not show an elevated inflow velocity. In many horses with early diastolic murmurs, the murmur only occurs at the end of rapid filling, at the time of the third heart sound. These murmurs may be related to the abrupt end of rapid active ventricular relaxation in some horses, rather than turbulence of the inflow itself.

Comparison of Doppler outflow measurements in horses with systolic ejection murmurs, to those with no ejection murmurs revealed no differences in the maximum recorded velocity in the aorta or pulmonary artery. This is in contrast to human studies where aortic velocity was significantly related to the production of flow murmurs following the administration of the inotrope dobutamine (Klewer *et al.* 1991). The peak acceleration of the aortic waveforms was significantly lower and the time to peak aortic acceleration was significantly longer, in horses with ejection murmurs. Acceleration has been shown to have a stabilising effect on fluid flowing above the critical Reynolds number for turbulent flow (Yoganathan *et al.* 1988). It is possible that the peak blood flow velocity recorded in both groups of horses was sufficiently high to produce turbulent aortic flow. However, the higher flow acceleration in the group with no murmurs may have prevented the formation of turbulent flow. There was a trend towards a higher velocity time integral in horses with functional murmurs, although this did not reach statistical significance. Examination of a larger group of horses may be required to determine an influence of velocity time integral on functional murmurs. No differences were detected between the pulmonary waveforms in the two groups of horses. The arbitrary grouping of horses based on the presence of a functional murmur prior to the Doppler examination was a limitation of this study, as it was not known whether the murmur was still present during data collection. Simultaneous echocardiographic and phonocardiographic studies are needed to detect any differences in Doppler waveforms in horses with functional murmurs.

This study shows that the standard views described by Long *et al.* (1992) are useful for guiding Doppler studies and allow closer alignment of the Doppler ultrasound transducer with blood flow than previously reported. The reference values recorded for selected Doppler measurements in a group of Thoroughbred and Thoroughbred cross horses have been shown to be repeatable and may prove useful for the diagnosis of cardiac dysfunction in horses.

Acknowledgements

Karen J. Blissitt (neé Long) was funded by a Veterinary Research Training Scholarship from the Horserace Betting Levy Board. The Vingmed CFM 700 was purchased with funds generously provided by the Home of Rest for Horses, the Royal College of Veterinary Surgeons Spencer Hill Charitable Trust, the University of Edinburgh Faculty of Veterinary Medicine and Diasonics Sonotron, BMS (Scotland) Ltd. We wish to thank Dr G. R. Scott who advised on the statistical analysis and the staff of the Large Animal Hospital, Royal (Dick) School of Veterinary Studies who patiently held the horses.

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