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Doppler Echocardiographic Reference Parameters in Healthy Labrador Retriever Dogs

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ABSTRACT

Thirty-one clinically healthy Labrador retriever dogs of both sexes (18 males and 13 females) were selected for determining Doppler echocardiographic reference values. 2 D and Pulse wave Doppler echocardiography was carried out by using GE Logiq P5 Color Doppler machine. The effect of body weight, age and sex on various doppler echocardiographic parameters were recorded. Twenty four dogs were in body weight range of 20-40 kg and 7 dogs in 40-60 kg range. To study the effect of age on various Doppler echocardiographic measurements, dogs were divided into 4 age groups (1-2, 2-3, 3-5 and >5 years of age). The mitral A wave peak velocity (MA) and ME:MA ratio were significantly ($p<0.05$) affected by body weight. The pulmonic valve velocity and pulmonic valve pressure were significantly ($p<0.05$) affected and there was significant negative correlation of pulmonic valve peak velocity (Pulmonary V max) and pulmonary pressure with body weight with r^2 values of 0.160 and 0.120 respectively. Mitral valve (MV) deceleration time was significantly ($p<0.05$) affected by age. The tricuspid valve deceleration time (TVDecT) was significantly ($p\leq0.01$) higher in dogs > 5 years of age. Tricuspid A velocity was significantly ($p\leq0.01$) higher in 2-3 year age group dogs as compared to dogs belonging to age group 3-5 years and > 5 years of age group. The pulmonic valve velocity and pressure were significantly affected by age. The tricuspid valve TE: TA ratio was significantly ($p\leq0.05$) affected by gender and the ratio was significantly ($p\leq0.05$) higher in males as compared to females.

HIGHLIGHTS

- The mitral A wave peak velocity (MA) and ME:MA ratio were significantly ($p<0.05$) affected by bodyweight.
- The tricuspid valve deceleration time (TVDecT) was significantly ($p\leq0.01$) higher in dogs >5 years of age.

Keywords: Doppler echocardiography, Labrador retrievers, body weight, age

Echocardiography is considered as a standard diagnostic tool that helps in the non-invasive estimation of cardiac chamber size, ventricular mass and systolic function in various clinical conditions (Ryu, 2016). Now-a-days, echocardiography has become the principal imaging technique used for the investigation of cardiac morphology, function and hemodynamics (Maleki and Esmaeilzadeh, 2012). During the last thirty years, pulsed wave (PW) Doppler echocardiography was developed first in humans and then in different domestic animals including dogs, cats, horses, cattle and sheep (Leroux *et al.,* 2012). This technique has become a routine for the diagnosis and evaluation of heart diseases in veterinary medicine as well. Doppler echocardiography represents continuous

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display of blood flow velocities during the cardiac cycle (Anavekar and Oh, 2009). Pulse wave (PW), continuous wave (CW) and color flow doppler echocardiography are the three doppler formats commonly used in Veterinary medicine (Boon, 2011). Doppler echocardiography provides information on instant direction of blood flow relative to transducer, direct velocity of blood flow within the sample volume and presence of any regurgitation with in heart or blood vessels (Bonagura and Fuentes, 2000). Doppler studies are based upon the principle described by John Christian Doppler, who first described the frequency shift that occurs when ultrasound waves are reflected back by the moving object. Pulse wave doppler studies the doppler shift within defined anatomic area of interest or sample volume (Boon, 2011).

The normal echocardiogram values are required for comparison and evaluation of dogs suspected of having cardiac problems. Although some normal echocardiographic values for dogs have been published, but there is variation depending on breeds and body size (Stack *et al.,* 2020). In canine cardiology, reference echocardiographic values are obtained from a large population of healthy dogs of different breeds and ranged according to body weight or body surface area (BSA) (Henik, 2001). Variation between breeds is considered to be the most important factor affecting Doppler echocardiographic reference ranges. So, echocardiographic reference ranges derived from some breeds may be misleading for others (O'Leary *et al.,* 2003). There is a need for reliable, normal echocardiographic values for comparison and evaluation of dogs suspected for having cardiac problems. So, this study was carried out to determine normal Doppler echocardiographic parameters in healthy Labrador retriever dogs and relate the effect of body weight, age and sex on various Doppler echocardiographic parameters.

MATERIALS AND METHODS

Selection of animals

In this study, thirty-one clinically healthy Labrador retriever dogs of both sexes (18 males and 13 females) presented for routine deworming, vaccination and general health check at Teaching Veterinary Hospital of GADVASU were selected for determining Doppler echocardiographic reference values. The dogs with normal cardiovascular physical examination and no electrocardiographic and radiographic abnormality were undertaken for the study. Echocardiographic examination showed no valvular regurgitations and no myocardial lesions. For echocardiographic study, healthy Labrador retriever dogs were grouped into two groups based on their body weight. Out of which, 24 dogs with body weight range 20-40 kg and 7 dogs in 40-60 kg range were taken for study. To study the effect of age on various echocardiographic measurements, dogs were divided into 4 age groups (1-2, 2-3, 3-5 and >5 years of age).

Echocardiography

Standard 2D, Pulse wave Doppler (PW) and Color flow Doppler echocardiographic examinations of dogs were carried out by using GE Logiq P5 Color Doppler machine equipped with sector probe 3S, 5S and 7S depending on the size of the animal.

Patient preparation and positioning

All the dogs were clipped on the right and left thoracic wall from 4^{th} to 6^{th} intercostal space and placed in the lateral recumbency on a specially designed table with a semicircular cut on one side of the table (Boon, 2011). The transducer was placed through a hole underneath the table. Ultrasound gel was used for coupling of transducer with skin. None of the animals were given sedation or tranquilization.

Measurements

Doppler echocardiography

For pulse wave doppler measurements, the transducer positioning was performed according to the recommendations of Darke *et al.* (1993). Left parasternal apical four chamber plane was used to record transmitral and tricuspid flow. By placing the sample gate at the tips of the valvular leaflets on ventricular side when they were wide open, velocity 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 these parameters, the ratio Peak E/ Peak A (E/A) was calculated. The deceleration time (MV

Dec T) of E waves was measured from the peak of the rapid filling signal (E) along its deceleration slope to base line.

For recording the aortic flow, left apical five-chamber view was used. By placing PW Doppler gate just distal to the aortic valve, peak aortic valve velocity (Ao, m/sec) was measured in meters per second.Ao valve Pressure gradient was determined in mm of Hg by computer software of machine. Pulmonary flow was recorded from the right parasternal short-axis plane with aorta and pulmonary artery. The PW gate was placed distal to the valve within the pulmonary artery and peak Pulmonary valve velocity was measured in meters per second (PV velocity m/sec). PV Pressure gradient was determined in mm of Hg by computer software of machine.

STATISTICAL ANALYSIS

The statistical analysis was done with the help of multiple ANOVA with Tukey's Post hoc test. Linear regression and Pearson's correlation analysis was done by using software 'SAS' version 9.3.

RESULTS AND DISCUSSION

Mean ± SEM of Doppler echocardiographic reference parameters of healthy Labrador retriever dogs and the effect of body weight, age and gender on all Doppler echocardiographic parameters are presented in Table 1.

Mitral valve flow

During the diastole, the mitral valve flow was positive (over the baseline), laminar and was divided in two main triangular shaped phases of left ventricular filling called E and A waves (Fig. 1). The early phase of ventricular filling which extends from mitral valve opening to peak ventricular filling is called mitral E wave peak velocity (ME). The filling of ventricle due to atrial contraction is called mitral A wave peak velocity (MA). In present study, ME and MA velocity was 0.89 ± 0.02 m/s and 0.61± 0.02 m/s respectively in healthy Labradors (Table 1). The values of ME and MA in healthy Labrador fell in the normal range as described by Boon (2011). Normally, the A wave is lower than E wave as seen in present study and the relationship between the E and A waves (E/A) was greater than 1.0 as reported by Jeyaraja *et al*. (2016). There is importance of mitral E:A ratio in cases of cardiac diseases, which affect the diastolic function as in restrictive cardiomyopathy, making the ME wave much greater than the MA wave (Muzzi *et al.,* 2006). Similarly, a high velocity mitral E wave has been shown to be associated with a higher risk of death or decompensation in dogs with degenerative mitral valve diseases (DMVD) (Chetboul and Tissier, 2012). Mitral valve Deceleration time (M DecT) is the time from the point of maximal E velocity along its deceleration slope to the baseline. It is one of the important parameter for echocardiographic assessment of the diastolic function (Muzzi *et al.,* 2006). In present study, M DecT was 151.65±7.00 ms in healthy Labrador dogs. Kayar *et al.* (2006) observed the mitral valve deceleration time of 132.6 ± 16.44 ms in healthy German shepherd dogs.

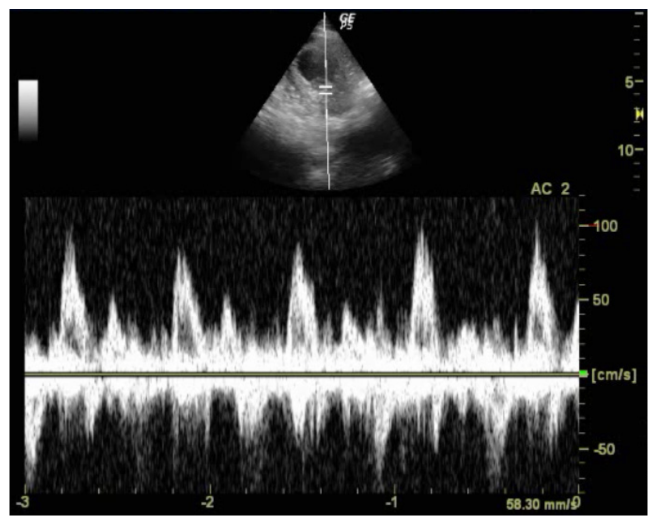

Fig. 1: Pulse wave Doppler measurement of mitral valve flow

Effect of body weight, age and sex on Mitral valve flow

The mitral valve peak A wave velocity (MA) and mitral valve ME:MA ratio were significantly ($p \leq 0.05$) affected by bodyweight in healthy Labradors. However, Muzzi *et al.* (2006) did not find any significant correlation between bodyweight and valve flows. However, Lobo *et al.* (2008) found a positive statistically significant correlation between M DecT and bodyweight in healthy Estrela mountain dogs. Schober and Fuentes (2001) also found increase in MV deceleration time with increasing body weight. However, Gaber (1987) reported significant

Table 1: Effect of Body weight, age and sex on Doppler echocardiographic reference parameters in healthy Labrador retriever dogs

	Mitral Valve				Tricuspid Valve				Aorta		Pulmonic Valve	
	M E (m/s)	**M A (m/s)**	**M E/M A**	**M Dec T (ms)**	**T E (m/s)**	**T A (m/s)**	**T TE/ T A**	**T Dec T (m/s)**	**AoV_max (m/s)**	**Ao Pressure mm Hg**	**PA V_max (m/s)**	**PA Pressure mm Hg**
Body weight (Kg)												
20-40 Kg (n= 24)	0.89 ± 0.02	0.64^{a} ± 0.02	1.45^{b} ± 0.04	157.00 ± 8.57	0.59 ± 0.02	0.43 ± 0.01	1.40 ± 0.04	204.74 ± 13.15	1.07 ± 0.03	4.73 ± 0.23	1.04^{a} ± 0.02	4.38^{a} ± 0.20
40-60 Kg (n= 7)	0.87 ± 0.02	0.53^{b} ± 0.03	1.69^{a} ± 0.09	132.55 ± 7.83	0.56 ± 0.02	0.43 ± 0.02	1.33 ± 0.07	195.03 ± 15.37	1.02 ± 0.02	4.15 ± 0.16	0.86^{b} ± 0.06	3.12^{b} ± 0.42
p value	0.6107	0.0269	0.0122	0.208	0.8036	0.4357	0.2662	0.8001	0.2731	0.2328	0.0023	0.0117
Age (years)												
1-2 years (n= 6)	0.94 ± 0.04	0.63 ± 0.05	1.58 ± 0.08	149.39^{ab} ± 9.40	0.56 ± 0.02	0.44^{ab} ± 0.02	1.39 ± 0.11	173.65^{b} ± 13.63	1.13 ± 0.06	5.28 ± 0.61	1.17^{a} ± 0.06	5.66^{a} ± 0.54
2-3 Years (n= 10)	0.91 ± 0.04	0.68 ± 0.03	1.36 ± 0.05	135.83^{b} ± 6.68	0.63 ± 0.03	0.50^{a} ± 0.02	1.28 ± 0.04	182.97^{b} ± 16.58	1.08 ± 0.04	4.75 ± 0.32	0.99^{b} ± 0.04	4.00^{b} ± 0.26
3-5 Years (n= 8)	0.90 ± 0.03	0.59 ± 0.03	1.59 ± 0.08	139.46^{ab} ± 7.99	0.55 ± 0.03	0.41^{b} ± 0.02	1.40 ± 0.06	187.73^{ab} ± 13.77	1.06 ± 0.03	4.54 ± 0.29	0.96^{ab} ± 0.05	3.82^{ab} ± 0.37
>5 Years (n= 7)	0.80 ± 0.03	0.54 ± 0.04	1.54 ± 0.09	191.87^{a} ± 25.59	0.56 ± 0.04	0.38^{b} ± 0.02	1.50 ± 0.10	271.53^{a} ± 31.46	0.98 ± 0.04	3.88 ± 0.29	0.89^{b} ± 0.02	3.22^{b} ± 0.16
p value	0.1091	0.1006	0.1012	0.0419	0.2567	0.0013	0.2471	0.0078	0.3349	0.3139	0.0014	0.0007
Gender												
Female (n = 13)	0.92 ± 0.02	0.66 ± 0.02	1.43 ± 0.05	138.57 ± 6.93	0.59 ± 0.02	0.46 ± 0.01	1.27^{b} ± 0.03	190.25 ± 15.26	1.12 ± 0.03	5.11 ± 0.27	1.03 ± 0.05	4.4 ± 0.38
Male (n= 18)	0.86 ± 0.03	0.58 ± 0.03	1.55 ± 0.05	159.5 ± 10.27	0.57 ± 0.02	0.41 ± 0.02	1.46^{a} ± 0.05	210.32 ± 14.60	1.02 ± 0.03	4.29 ± 0.24	0.98 ± 0.02	3.91 ± 0.20
p value	0.345	0.0776	0.0614	0.4631	0.7683	0.0915	0.0311	0.9506	0.0637	0.092	0.7397	0.5806
Overall Mean	0.89 ± 0.02	0.61 ± 0.02	1.51 ± 0.04	151.65 ± 7	0.58 ± 0.02	0.43 ± 0.01	1.38 ± 0.04	202.55 ± 10.72	1.06 ± 0.02	4.6 ± 0.19	1 ± 0.02	4.1 ± 0.19
Range	0.55-1.2 (0.65)	0.34-0.96 (0.62)	1.02-2.21 (1.19)	78.66-333.35 (254.69)	0.32-0.94 (0.62)	0.2-0.65 (0.45)	1.01-2.47 (1.46)	95.14-506.23 (411.09)	0.61-1.52 (0.91)	1.47-9.3 (7.83)	0.62-1.43 (0.81)	1.56-8.19 (6.63)

Values with different superscript within a parameter differ significantly.

correlation between body weight and velocities at atrio-ventricular valves. The difference in views of above 2 studies could be due to different sample size of population examined.

In healthy Labradors, MV deceleration time was significantly ($p \leq 0.05$) affected by age (Table 1). MV DecT of dogs > 5 year age was significantly ($p \leq 0.05$) higher than 2-3 year age group. Schober and Fuentes (2001) also found that transmitral valve E deceleration time was higher in dogs more than 10 years of age as compared to dogs less than 6 years of age. They further suggested that prolonged mitral valve deceleration time associated with increasing age as in our study indicated a gradual decrease in the rate of left ventricular relaxation, increased myocardial stiffness and enhanced late diastolic filling (Disatian *et al.,* 2008). In present study, the ME, MA velocity and ME:MA ratio were not affected with age. The ME velocity in dogs >5 years of age was lower as compared to younger dogs below 5 years of age although statistically non-significant ($P<0.05$). Schober and Fuentes (2001) also reported that there was a progressive decrease in mitral valve E wave peak velocity with age and found significant difference between ME velocity for dogs > 6 years age and for dogs < 2 years old. The decrease in ME velocity with increase in age is due to delayed relaxation of ventricles with advancing age (Disatian *et al.,* 2008). However, Schober and Fuentes (2001) observed significant effect of age on E:A ratio and mitral valve deceleration time. Disatian *et al.* (2008) also reported a significant weak negative relationship between age and MV peak E in healthy cats. They also observed significant change in peak E and peak A velocities in different age groups.

In healthy Labradors, no significant effect of gender was observed on mitral valve flow. Similarly, various previous workers reported no effect of sex on mitral valve flow velocity or time intervals (Schober and Fuentes, 2001 and Muzzi *et al.,* 2006). However, the higher values for peak velocity of the ME was found in women as compared to men by some previous workers (Caballero *et al.,* 2015).

Tricuspid valve flow

The diastolic flow of tricuspid valve was similar to the mitral valve flow with Peak E and A points above the baseline (Fig. 2). In present study, TE and TA velocity were 0.58 ± 0.02 m/s and 0.43 ± 0.01 m/s respectively in healthy Labradors (Table 1). The TE and TA velocities of tricuspid flow in the present study was lower as compared mitral flow velocities. This is attributed to the reduced pressure drop from right atrium to right ventricle as compared to the pressure change from the left atrium to the left ventricle (Kirberger *et. al*., 1992).

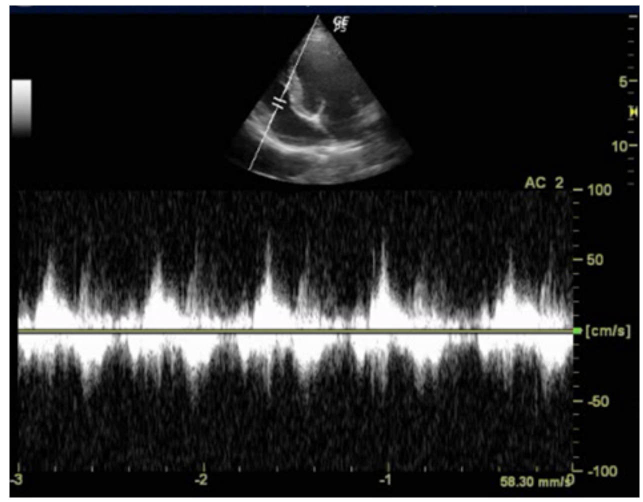

Fig. 2: Pulse wave Doppler measurement of Tricuspid valve flow

Effect of body weight, age and sex on tricuspid valve flow

The body weight had no effect on any parameter of tricuspid flow in healthy Labradors (Table 1). Similarly, Muzzi *et al.* (2006) did not find any correlation between body weight with tricuspid E wave, A wave, E:A ratio and Tricuspid Dec.time in healthy German shepherd dogs.

The tricuspid valve deceleration time (T DecT) was significantly ($p \leq 0.01$) higher in dogs > 5 years of age as compared to 1-2 and 2-3 year age group which could be due to increased wall stiffness with advancing age leading to decrease in rate of right ventricular relaxation (Yu and Sanderson, 1997). However no significant difference was found between dogs belonging to age groups 1-2 years, 2-3 years and 3-5 years. Tricuspid A velocity was significantly ($p \leq 0.01$) higher in 2-3 year age group dogs as compared to dogs belonging to age group 3-5 years and > 5 years of age group.

The tricuspid valve TE: TA ratio was significantly ($p \leq 0.05$) affected by gender and the ratio was significantly ($p \leq 0.05$)

higher in males as compared to females. However, Muzzi *et al.* (2006) did not observe any correlation between gender and tricuspid flow parameters in German shepherd dogs.

Aortic valve flow

The aortic valve flow was negative during systole with a rapid laminar acceleration phase (Fig. 3). The mean aortic valve velocity was 1.06 ± 0.02 m/sec in healthy Labrador dogs (Table 1) which was comparable to that recorded by Muzzi *et al*.(2006) in healthy German shepherd dogs (1.02 ± 0.143 m/s). The normal healthy dogs should have aortic flow velocities less than 200 cm/sec and aortic velocity above 250 cm/sec are considered abnormal (Boon, 2011).

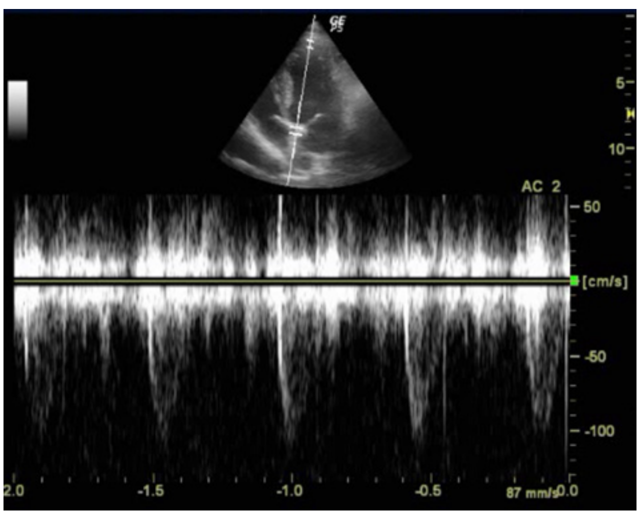

Fig. 3: Pulse wave Doppler measurement of aortic valve flow

Effect of body weight, age and sex on aortic valve flow

In the present study, the aortic valve flow was not significantly affected by body weight. This was in agreement with the results of previous studies (Muzzi *et al*., 2006). In healthy Labrador, the aortic valve flow and pressure were not significantly affected by age. However, no effect of age on aortic flow was observed by previous workers also (Kirberger *et al*., 1992). No significant effect of gender was observed on the mean values of Ao V max velocity and Ao pressure (Table 1). Similarly, Muzzi *et al.* (2006) observed no correlation between the peak aortic velocities with gender in healthy German shepherd dogs.

Pulmonic valve flow

The pulmonic valve waveform was negative (Fig. 4) which was similar in morphology to the findings reported by Jeyaraja *et al*. (2016). The peak pulmonic velocity was in the range of 0.62 to 1.43 m/sec in Labrador dogs (Table 1) which was comparable to 0.7-1.4 m/sec as reported by Jeyaraja *et al*. (2016).

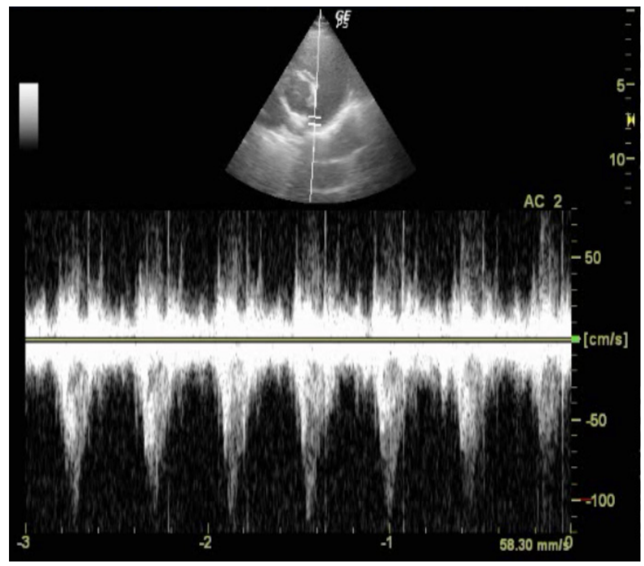

Fig. 4: Pulse wave Doppler measurement of pulmonic valve flow

Effect of body weight, age and sex on pulmonic valve flow

In healthy Labrador dogs, the values of PV max and pulmonary pressure was significantly ($P<0.05$) higher in dogs with lesser body weight as compared to heavy weight dogs. There was significant negative correlation of pulmonic valve peak velocity (Pulmonary V max) and pulmonary pressure with body weight with r^2 values of 0.160 and 0.120 respectively in healthy Labradors (Table 2). Similarly, Lobo *et al.* (2008) also reported negative linear correlation between PA Vmax and body weight. Increased body weight decreases the mean velocity and is found to be secondary to decreased heart rate in larger dogs (Kirberger *et al.,* 1992). In contrast to our findings, Muzzi *et al.* (2006) reported no significant correlation between the pulmonary valve flow and body weight.

In healthy Labradors, the PA V max was significantly

Table 2: Linear regression model showing relationship between body weight (x) (Kg) and Doppler echocardiographic parameters (y) in healthy Labrador retriever dogs

Doppler's Parameters	Regression (y =)	Correlation	(r^2)	p-Value
Mitral E (m/s)	(-0.021)x + 0.933	-0.060	0.000	0.64
Mitral A (m/s)	(-0.102)x + 0.838	-0.282	0.080	0.024*
Mitral E/A	0.241x + 0.969	0.324	0.110	0.009*
Mitral Dec Time (ms)	(-24.455)x + 205.914	-0.189	0.030	0.15
AoV Max (m/s)	(-0.056)x + 1.183	-0.137	0.020	0.28
AoPressure (m/s)	(-0.577)x + 5.881	-0.160	0.030	0.206
Tricuspid E (m/s)	(-0.027)x + 0.640	-0.092	0.010	0.475
Tricuspid A (m/s)	(-0.004)x + 0.443	-0.020	0.000	0.876
Tricuspid E/A	(-0.072)x + 1.544	-0.103	0.010	0.425
Tricuspid Dec Time (ms)	(-9.710)x + 224.160	-0.049	0.000	0.708
Pulmonary V max (m/s)	(-0.173)x + 1.381	-0.394	0.160	0.002*
Pulmonary Pressure (m/s)	(-1.262)x + 6.908	-0.348	0.120	0.006*

*Correlation and regression value is significantly ($P \leq 0.05$) related with that echocardiographic parameter.

($P<0.01$) higher in 1-2 year age group as compared to 2-3 year and > 5 year age groups (Table 1). The PA pressure was significantly ($P<0.001$) higher in 1-2 years age group as compared to 2-3 years and > 5 years of age group. Disatian *et al.* (2008) also reported that pulmonary venous velocity decreases when kittens become adults. Gender had no effect on pulmonary artery velocity and pressure. Many previous studies also observed no effect of gender on pulmonic valve velocity and pressure in healthy dogs (Muzzi *et al*., 2006).

In conclusion, body weight and age has significant effect on various doppler echocardiographic parameters. So within a given breed, doppler echocardiographic reference data need to be established in different body weight categories and age groups, so that reference data can be used for diagnosis of various cardiac problems in dogs of different body weights and belonging to different age groups.

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