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Echocardiographic reference intervals for end-diastolic and end-systolic left ventricle volumes obtained by Simpson's method of discs in healthy showjumping and polo Argentinian Saddlebred horses

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Abstract: The left ventricle volume quantification is a critical component of standard echocardiography. Therefore, this study aimed to establish breed-specific echocardiographic reference intervals for end-diastolic and end-systolic volumes using Simpson's method of discs (SMOD) in healthy showjumping and polo Argentinian Saddlebred horses. The central 90% reference limit was calculated, and its confidence and reference intervals were reported with 90% confidentiality. A comparison was performed between cardiac SMOD-derived and Teichholz-derived volumes and between males and females. A total of 117 horses (43 females and 77 males) weighing 445–690 kg (521 ± 56 kg) with similar training were selected to create the reference intervals. SMOD-derived end-diastolic volume indexed (EDVI) was 1.32 ml/kg, RI from 1.26 to 1.37 ml, and SMOD-derived end-systolic volume indexed (ESVI) was 0.47 ml/kg, RI from 0.43 to 0.50 ml. There was a significant difference between EDVI SMOD-derived and EDVI Teichholz-derived volumes ($p < 0.001$, $d = 1.14$). In addition, ESVI SMOD-derived volumes differed from Teichholz-derived volumes ($p < 0.05$, $r = 0.33$). EDVI was statistically similar between males and females ($p = 0.721$). ESVI was statistically similar between males and females ($p = 0.122$). In conclusion, it is feasible and reproducible to obtain SMOD-derived volumes in horses as in humans and dogs with standard views modifications and reference intervals are reported for clinical and research purposes, especially when accurate quantification of ventricular volumes and ejection fraction are needed.

Keywords: Horses, cardiac volumes, echocardiography, left ventricle, reference intervals

1. Introduction

Echocardiography is currently the most reliable tool for diagnosing congenital and acquired heart disease in horses (Schwarzwald, 2016; Stucchi et al., 2023). The number of clinical situations in which it is useful has progressively increased in recent years, particularly when evaluating the impact and origin of cardiac murmurs, arrhythmias, and muffled heart sounds during routine clinical examination (Ferraro et al., 2023; Schwarzwald, 2019). In terms of research, efforts to identify heart disease induced by strenuous exercise (Flethøj et al., 2016; Trachsel et al., 2016) and the study of the impact of cardiac diseases on athletic performance have focused on echocardiographic evidence in an attempt to understand the underlying pathophysiology of these conditions (Fraipont et al., 2011; Ramos et al., 2024). In addition, echocardiography plays an important role in the pre-purchase evaluation of athletic horses, and influences transactions and monetary decisions (Verdegaal et al., 2002).

From a clinical perspective, the use of echocardiography in horses is challenging when working with breeds other than Thoroughbreds or Standardbreds (Khalesi et al., 2022; Vitale et al., 2023). Likewise, interpretation of echocardiographic parameters that are highly influenced by weight, age, and sex requires special attention (Al-haidar et al., 2013; Marr, 2016; Trachsel et al., 2016). Horse sport levels and training create adaptive changes that veterinary cardiologists must consider in their analyses and interpretation (Dufourni et al., 2024; Gehlen & Schlaga, 2019). Hence, considering that understanding the average values of cardiac chambers is essential for detecting alterations in the heart, efforts have been made to establish normal reference values in other horse breeds to overcome these limitations (Al-haidar et al., 2013; Marzok et al., 2023; Schwarzwald, 2019).

Several studies have used M-mode echocardiography in horses to create reference intervals for cardiac wall thickness, ventricular diameter, and ventricular volumes using the Teichholz formula (Marzok et al., 2023; Ven et al., 2016). However, the latest updated guidelines for cardiac chamber quantification in humans and small animals have recommended that two-dimensional (2-D) measurement of cardiac volume is preferable under most circumstances (Fallahtafti et al., 2024; Lang et al., 2015; Wess et al., 2021). Volume measurements, such as those performed using the M-mode, are less suitable for quantitative analysis because they are limited to a single dimension (Smets et al., 2014). Therefore, the complex three-dimensional configuration of the ventricles is not considered, and the Teichholz formula mathematically assumes that the geometry of the left ventricle is fixed, which is not valid for the cardiac anatomy of all mammals (Seckerdieck et al., 2015; Wess et al., 2021).

The most recent guidelines of the American Society of Echocardiography (Fallahtafti et al., 2024; Lang et al., 2015), as well as clinicians in veterinary medicine, emphasize the benefit of embracing the area and volume measurements to evaluate cardiac chambers. Particularly when dilatation of the chambers or myocardial dysfunction is suspected (Wess et al., 2021). Irregular enlargement of the chambers or there are alterations in the geometry of the ventricle, due to wall contractile abnormalities (Pérez et

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al., 2020). In such cases, the measurement of cardiac volumes using the length-area method or Simpson's method of disc (SMOD) is theoretically more accurate because it considers the bidimensionality of the measurement and includes the chamber's area. Therefore, the aim of this cross-sectional observational study was two-fold. First, we established breed-specific echocardiographic reference intervals for the end-diastolic volume (EDV) and end-systolic volume (ESV) of the left ventricle using Simpson's method of discs in healthy Showjumping and Polo Argentinian Saddlebred horses with similar training programs. Second, we compared the cardiac volumes obtained using biplane Simpson's method of discs with those obtained using the Teichholz formula through traditional M-mode echocardiography.

2. Materials And Methods

2.1. Study population

This study was conducted during the routine annual general examination of the official sports horses of Colombia National Police Carabinieri School, and written informed consent was obtained from a legally authorized representative of the institution. Ethical approval for this study was obtained from the Institutional Review Board of the University of Caldas Ethics Committee in April 2018 (Act April 01 24, 2018).

2.2. Inclusion criteria

Showjumping and Polo Argentinian Saddlebred horses of any sex, age, or body weight and similar training programs consisting of 3-4 times a week flatwork, and 2-3 times a week conditioning and strength training were included if appropriate echocardiographic window and proper standard views and image quality were obtained. The health status of horses was guaranteed by reviewing their clinical history, preventive care programs, and recent events. A complete physical examination, serum biochemistry, and normal hematological results were obtained. Non-significant cardiac murmurs were accepted if they were < 3/6 according to the six-point grading system (Marr & Bowen, 2010), and if Doppler color characteristics during echocardiography suggested trivial regurgitation in the absence of other remodelations, such as ventricular dilation or atrial dilation (Mcconachie et al., 2013). During data acquisition, horses were included if there was no evidence of insidious arrhythmias on the electrocardiogram and no evidence of abnormal echocardiographic findings associated with either congenital malformations or acquired myocardial or valvular diseases. Horses that fulfilled these requirements were included in the statistical analysis.

2.3. Exclusion criteria

Elderly horses, individuals taking medications with cardiorespiratory effects during the three weeks before echocardiographic examination, pregnant or lactating females, and horses with clinical or laboratory test results that indicated systemic or metabolic disease were excluded. Horses with cardiac murmurs of > 3 grade accompanied by volume overload and remodeling, abnormal echocardiographic findings such as congenital or acquired heart disease, and malignant arrhythmias in the electrocardiogram, such as atrial fibrillation, were excluded. Horses without an adequate echocardiographic window or poor image quality were excluded from the statistical analysis.

2.4. Data collection

All horses were examined in a quiet barn without sedation and were restrained by an experienced handler. When necessary, the right forelimb was slightly protracted and abducted with animal weight-bearing. Images were acquired using a phased array 2.5/4.5 MHz transducer with a simultaneous base-apex electrocardiogram in a Mindray M5Vet laptop ultrasound system (Mindray Building, Keji 12th Road South, High-Tech Industrial Park, Nanshan, Shenzhen, China). The ultrasound machine settings were similar whenever possible for each individual, with a depth setting of 24–26 cm, a frame rate of 50 frames/s without harmonic imaging, and a focal point at 15–20 cm.

All echocardiographic measurements were performed by the same veterinary cardiologist (JMP), following the recommendations of the echocardiography committee of the American College of Veterinary Internal Medicine (Thomas et al., 1993), the standardized imaging technique for guided M-mode echocardiography in horses published by Long (Long et al., 1992), and the last updated guidelines proposed by the American Society of Echocardiography for cardiac chamber quantification (Lang et al., 2015). The same observer (JMP) repeated random measurements twice to evaluate intra-observer variability, with a minimum of two weeks apart, and a second observer measured the same measurements (C.A.) for inter-observer variability.

The limitations of the Equidae family, based on thoracic characteristics and size, were considered in order to overcome the limitations of image acquisition. To decrease intraobserver variability, focused standardized echocardiography was performed for each patient to simulate clinical reality. Cine-loop cardiac cycles were digitally stored, and measurements were calculated offline. Each measurement was performed in three consecutive cardiac cycles. Measurements were not taken from the cycle immediately following a second-degree atrioventricular block, or when the heart rate exceeded forty-five beats per minute. The average measurements for each horse were used for statistical analysis.

2.5. M-mode echocardiography

A right parasternal short-axis view was obtained at the chordae tendineae level, guided by simultaneous base-apex electrocardiography for the M-mode measurements. The reference for the end-diastole was the onset of the QRS complex. The reference for end-systole was defined as the point of maximal excursion of the interventricular septum and the left ventricular free wall. The leading-edge-to-leading-edge method was used, and the following structures were measured: the interventricular septum in systole (IVSs), diastole (IVSd), left ventricular internal diameter in systole (LVIDs) and diastole (LVIDd), left ventricular free

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wall in systole (LVFWs), and diastole (LVFWd). The end-diastolic volume (EDV) and end-systolic volume (ESV) were calculated using the Teichholz formula (7.0/ (2.4 + D) × D3).

2.6. Simpson's method of discs

To calculate the SMOD-derived EDV and ESV, apical four- and two-chamber views are routinely obtained in humans. However, because of the inability to obtain the same image planes for horses, we arbitrarily modified the views to obtain rational and comparative images. From the right hemithorax, we obtained a 2-D right parasternal long-axis 4-chamber view that was slightly modified to include the real apex of the left ventricle (figure 1-A, 1-B). From the left hemithorax, we obtained a 2-D left parasternal long-axis view of the left atrium and ventricle, which was optimized to produce the best image of the left ventricle (figure 1-C, 1-D). The reference for end-diastole was considered the first frame when the mitral valve was closed and at the onset of QRS on simultaneous ECG. The end-systole reference was the last frame before the mitral valve opening. To obtain the cardiac volumes in a specific frame, the left ventricle area was calculated by tracing the edge of the endocardium (blood-tissue interface), and the maximum length of the ventricle was calculated from the point of mitral valve coaptation to the edge of the endocardium at the ventricular apex. In the left parasternal long-axis view, the maximal LV length was measured from the mitral annulus in diastole and the coaptation point of the aortic valve in systole (figure 1-D), considering that it is difficult to obtain appropriate quality images including the mitral annulus in systole in some horses because of overlapping and poor visualization. The EDV and ESV were automatically calculated by the ultrasound software using the following formula (Simpson's rule): V=π/4 ∑ (i=I) ^20 (ai bi. L/20). The volumes obtained from the right and left hemi-thorax views were recorded individually for each horse. To allow statistical comparisons between the parameters in each horse, the results of the SMOD-derived and Teichholz-derived volumes were indexed to B.W. The left ventricular ejection fraction (LVEF) was calculated only with values from the right parasternal view in each horse.

2.7. Statistical analysis

Statistical criteria have been previously reported to create reference intervals for animal species (Friedrichs et al., 2012). First, all data were evaluated using histograms, and the Kolmogorov-Smirnov test was used to determine normality. The authors graphically reviewed the data to identify and eliminate outliers using Horn's algorithm and Tukey's interquartile range. Finally, the central 90% reference limits were calculated; and the reference intervals were reported with 90% confidentiality to determine their confidence intervals.

All 2-D measurements obtained from the right and left parasternal views were compared using a paired t-test for statistical purposes only. The final reference intervals only considered the results of the right parasternal view. The limits of agreement between these echocardiographic views were presented with a graphical analysis of the Bland-Altman. A paired t-test was used to compare SMOD- and Teichholz-derived volumes using an unpaired t-test and Wilcoxon Signed-rank test, and a comparison was made between females and males using a paired t-test to evaluate the influence of sex.

For the variability estimate, the Coefficient of Variation (CV) was calculated for the volumes obtained by both methods using the formula CV%= SD/μ × 100, where μ is the obtained mean of each method. The absolute intra- and inter-observer variability was calculated using the following formula:

$$\sqrt{\frac{(Measure1 - Measure2)^2}{2}}$$

The relative intra- and inter-observer variability was calculated using the following formula:

$$\frac{\sqrt{\frac{(Measure1 - Measure2)^2}{2}}}{\frac{(Measure1 + Measure2)}{2}}$$

Subsequently, verification was performed by examining the distribution with (n–1) degrees of freedom: (μ)/S.D., where μ is the mean of the absolute difference between measurements for variability assessment, and < 0.975 confirmed no difference (Popovic & Thomas, 2017). All the statistical analyses were carried out using the statistical software R V.2.15.3, except for the creation of the reference intervals that were carried out using MedCalc Statistical Software version 16.4.3 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2016). The effect size was calculated and reported using Cohen's d and Pearson's coefficients for normally and non-normally distributed data. The correlation metric for the effect size is summarized in (Table 1). For all variables, the significance level was set at $p < 0.05$.

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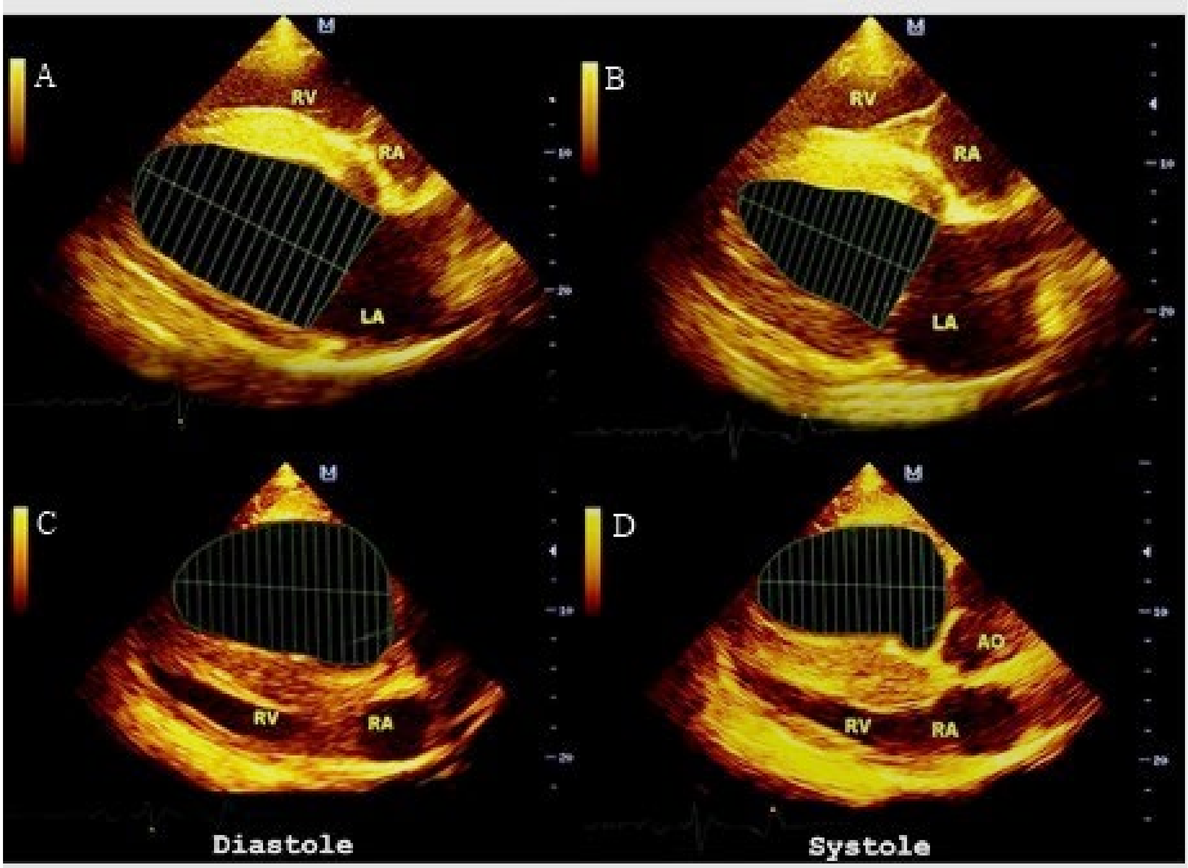

Figure 1 – Simpson's method of disc measurement performed on a standard 2-D right parasternal long-axis view of the left atrium and ventricle in diastole (A) and systole (B), and from the 2-D left parasternal long-axis view in diastole (C) and systole (D). A.O.: aorta; LA: left atrium; LV: left ventricle; R.A.: right atrium; R.V.: right ventricle.

3. Results

One hundred twenty-seven horses were evaluated. Seven horses were excluded from the analysis because of the following findings: two horses had loud murmurs (grade > 3) and severe aortic regurgitation; three horses had atrial fibrillation; and two horses had metabolic syndrome and abnormalities in laboratory test results. None of the horses were excluded because of an inadequate echocardiographic window. The remaining hundred twenty horses met the inclusion criteria and were included in the statistical analysis. 43 horses were females (35.7%) and 77 were males (64.28%), weighing between 445 and 690 kg (521 ± 56 kg). The median horse age was 12 years (range: 5 – 22 years). Seven horses presented with mild pulmonary regurgitation (< grade 3), three had mild mitral regurgitation (< grade 2), six had mild aortic regurgitation (< grade 3), and three had at least one atrioventricular blockage during the echocardiographic examination. Only three horses were eliminated after the outliers were inspected to decrease the dispersion of the reference ranges. The 117 remaining horses were used to create the reference intervals.

Cardiac volumes obtained in the right and left parasternal longitudinal-axis views were indexed to the B.W., and a Wilcoxon Signed-ranks test indicated that SMOD-derived end-diastolic volume indexed (EDVI) in the right-parasternal view was statistically equal (median 1.32 ml/kg, RI = 1.26 -1.37) to SMOD-derived EDVI in the left-parasternal view (median 1.3 ml/kg, range 1.22 – 1.45). The Wilcoxon signed ranks test indicated that SMOD-derived ESVI in the right-parasternal view was similar (median 0.47 ml/kg, RI 0.43 – 0.50) to SMOD-derived ESVI in the left-parasternal view, independent of the modification of the standard view in this left parasternal view (median 0.51 ml/kg, range 0.43 – 0.54).

Variables /comparison	Mean difference	Effect size	*r* equivalent to *d* †/Cohen's *d*	*p-value*
SMOD-EDVI right and left parasternal views	-0.005 ‡	0.12	0.10 (small effect)	0.44
SMOD-ESVI right and left parasternal views	-0.01 ‡	$r = 0.22$	0.16 (small effect)	0.22
SMOD and Teichh-EDVI	0.30	$d = 1.14$	1.14 (large effect)	0.01 *
SMOD and Teichh-ESVI	0.10 ‡	$r = 0.33$	0.50 (medium effect)	0.03 *
SMOD-EDVI in females and Males	0.06 ‡	$r = 0.39$	0.50 (Medium effect)	0.72
SMOD-ESVI in females and Males	-0.15 ‡	$r = 0.33$	0.50 (Medium effect)	0.12

Table 1 – A correlational metric of effect size for all comparisons with SMOD-derived volumes.

†: Where r = $\frac{d}{\sqrt{d^2+4}}$ (Rosenthal and Rosnow, 2008). ‡: Hodges-Lehmann median difference. *: level of significance t-test/Wilcoxon Signed-ranks test. Teich-EDVI: end-diastolic volume indexed to body weight (B.W.) derived from Teichholz formula; Teichh ESVI: end-systolic volume indexed to B.W. derived from Teichholz formula; SMOD-EDVI: end-diastolic volume indexed to B.W. derived of Simpson's method of disc; SMOD-ESVI: end-systolic volume indexed to B.W. derived of Simpson's method of disc.

The results for SMOD-derived and Teichholz-derived volumes are presented in Table 2. The Bland-Altman plots (Figure 2) for SMOD-derived volumes indexed to the B.W. showed good agreement in both right and left parasternal views, even with the arbitrary modification of the standard view from the left parasternal view. The differences were plotted against the average values of EDVI and ESVI for both views, and the limits of agreement are shown as dotted lines (+/- 1.96 SD of the mean difference).

Variables	Mean/median	RI	Range
EDV right parasternal (ml)	646.58/673.80	613.51-679.63	502.2-809
EDV left parasternal (ml)	645.88/666.67	610.2-681.51	490-840
ESV right parasternal (ml)	220.5/238.76	198.41-242.59	100-330
ESV left parasternal (ml)	229.4/239.5	205.2-253.46	100-350
EDVI right parasternal (ml/Kg)	1.32/1.32	1.26-1.37	1.02-1.6
EDVI left parasternal (ml/Kg)	1.32/1.32	1.26-1.38	0.98-1.6
ESVI right parasternal (ml/Kg)	0.47/0.48	0.43-0.50	0.22-0.67
ESVI left parasternal (ml/Kg)	0.48/0.51	0.44-0.53	0.21-0.73
SMOD-EDVI (ml/Kg)	1.32/1.33	1.26-1.37	1.07-1.59
SMOD-ESVI (ml/Kg)	0.48/0.48	0.44-0.51	0.25-0.70
Teichh-EDV (ml)	891.7/911.3	844.76-938.7	630.3-1119
Teichh-ESV (ml)	357.76/355.51	313.13-402.38	128.23-586
Teichh-EDVI (ml/Kg)	1.63/1.64	1.52-1.74	0.99-2.21
Teichh-ESVI (ml/Kg)	0.60/0.61	0.52-0.69	0.20-1.09
Teich-EF (%)	64/62	60-67	44-80
SMOD EF (%)	63/64	60-66	48-81

Table 2 – Left ventricle volumes obtained by Simpson's method of disc and left ventricle volumes obtained with the traditional Teichholz formula obtained from M-mode.

EDV: end-diastolic volume; EDVI: end-diastolic volume indexed to body weight (B.W.); ESV: end-systolic volume; ESVI: end-systolic volume indexed to B.W.; RI: reference intervals; SMOD-EDV: end-diastolic volume derived of Simpson's method of disc; SMOD-ESV: end-systolic volume derived of Simpson's method of disc SMOD-EDVI: end-diastolic volume indexed to B.W. derived of Simpson's method of disc; SMOD-ESVI: end-systolic volume indexed to B.W. derived of Simpson's method of disc; SMOD-EF: Ejection fraction derived of Simpson's method of disc; Teichh-EDV: end-diastolic volume derived from Teichholz formula; Teichh-ESV: end-systolic volume derived from Teichholz formula; Teichh-EDVI: end-diastolic volume indexed derived of Teichholz formula; Teichh-ESVI: end-systolic volume indexed derived of Teichholz formula; Teichh-EF: Ejection fraction derived of Teichholz formula.

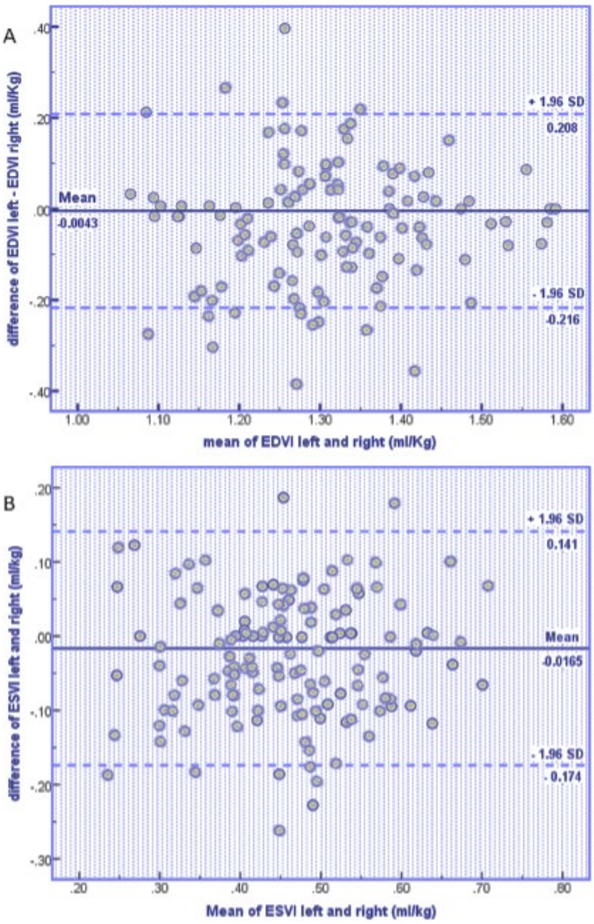

Figure 2 – The Bland-Altman plot for SMOD-derived volumes indexed to the B.W. between measurements of EDVI (A) and ESVI (B) obtained from the right parasternal. EDVI right: end-diastolic volume index to body weight (B.W.) in right and left parasternal views; EDVI left end-diastolic volume indexed to B.W. in left parasternal view; ESVI right: end-systolic volume indexed to B.W. in right parasternal view; ESVI left end-systolic volume indexed to B.W. in the left parasternal view.

The difference between the Teichholz and SMOD-derived volumes was independent of the indexation to B.W. There was a statistically significant difference between both methods, SMOD-derived EDVI (Mean = 1.32 ml/Kg, SD = 0.17) and Teichholz EDVI (Mean = 1.62 ml/Kg, SD = 0.32), $p < 0.01$. A Wilcoxon signed-ranks test indicates that SMOD-derived ESVI was statistically different (median 0.48 ml/Kg, range = 0.42 - 0.53) from Teichholz-derived ESVI (median 0.61 ml/Kg, range 0.39 – 0.83), $p < 0.03$.

The Wilcoxon Signed-ranks test indicates that SMOD-EDVI in males was statistically similar (median 1.48 ml/kg, range = 1.17 – 1.57) to SMOD-EDVI in females (median 1.34 ml/Kg, range 1.14 – 1.48), $p = 0.72$. The Wilcoxon Signed-rank test indicated that SMOD-ESVI in males was statistically similar (median 0.61 ml/Kg, range 0.50 – 0.74) to SMOD-ESVI in females (median 0.48, range 0.41 - 0.53), $p = 0.12$. Absolute end-diastolic volumes were more prominent in males than in mares but irrelevant for clinical purposes.

The CV for the SMOD-derived volumes was 12.81 % for the EDVI and 18.43 % for the ESVI, respectively. For volumes obtained using the Teichholz formula, the CV was 13.25 % for EDVI and 14.49% for ESVI. The repeatability and reproducibility of SMOD-derived and Teichholz-derived volumes are presented in Table 3.

	intraobserver variability		interobserver variability	
variable	Absolute	Relative %	Absolute	Relative %
SMOD-EDV	2.01±1.39†	2.00±1.19	7.17±3.55	4.03±1.33
SMOD-ESV	6.93±2.51	4.23±1.30	9.57±2.04	7.02±2.79
Teichh-EDV	1.81±0.25†	1.00±0.53	2.10±1.95†	1.00±0.93
Teichh-ESV	2.51±1.74	1.00±0.50	3.79±1.42	1.00±0.87

Table 3 – Repeatability and reproducibility of Simpson's method of disc and Teichholz formula to obtain cardiac volumes in horses.

†: <0.975 t-distribution interpreted as no difference between repeated measures. Teichh-EDV: end-diastolic volume derived from the Teichholz formula; Teichh-ESV: end-systolic volume derived from the Teichholz formula; SMOD-EDV: end-diastolic volume derived from Simpson's method of disc; SMOD-ESV: end-systolic volume derived from Simpson's method of disc.

4. Discussion

In the current study, we compared Teichholz- and SMOD-derived volumes finding a statistically significant difference between both methods in volume estimation. The Teichholz-derived volumes obtained using the M-mode were higher than the SMOD-derived volumes in this population of sports horses. Although both methods had a similar coefficient of variation, the M-mode has the benefit of high resolution and high pulse repetition, allowing the operators to obtain better quality images to estimate parameters, making the results less variable in repeated measurements. Hence, the similarity in the CV in both methods makes the SMOD-derived volumes feasible and reproducible in equine cardiology, at least at the same level as Teichholz-derived volumes, considering that volumetric measurements dependent on multiple linear dimensions are subject to more variability between observers (Kruckman et al., 2022). However, further comparison with gold-standard methods, such as real-time three-dimensional echocardiography, cardiac magnetic resonance imaging, and ECG-gated multidetector computed tomography angiography (ECG-MCTA) is necessary to demonstrate the accuracy and consistency of SMOD-derived volumes across different horse's breeds.

The study of Scollan (Scollan et al., 2016) found agreement between SMOD, real-time three-dimensional echocardiography, and MT-CTA in dogs' ventricular volumes suggesting that SMOD measurements were repeatable and correlated with reference standards; however, there was a significant bias between measurements, and the authors recommended that the results should not be used interchangeably and to the date similar data is absent in equine cardiology. Repeatability and reproducibility in Teichholz- and SMOD-derived volumes showed no differences between repeated measurements. SMOD-derived volumes can be reproducible at least at the same level as the M-mode to obtain volumes and can be replicated in other scenarios, for example in other breeds. Hence, the measurements of cardiac volumes using SMOD that fall within a specific value can be the same in any laboratory that applies the same measurement process among any horse with weight and conditions, as in this study (Popovic & Thomas, 2017).

An essential characteristic of our results was the similarity between the SMOD-derived volumes obtained in the left- and right-parasternal views, as no difference was evident between views in the bland-Altman plot. This agreement indicates that both views are valid for obtaining cardiac volumes and similar results were obtained in dogs in previous studies (Seckerdieck et al., 2015; Smets et al., 2014). However, the authors recommend obtaining only the volumes in the right parasternal view, as per the standard protocol for echocardiography in horses, does not commonly include left-parasternal views, and the comparisons in this study were performed only for statistical purposes. The measurements obtained in males and females were not statistically different, and the weight of the adult animals in these horse breeds was similar; nonetheless, these results should be interpreted with caution because of the small number of females included in the study.

Compared with a single-dimension measurement, the application of models consisting of the sum of different geometric figures, such as the model based on SMOD, apparently improves the estimation of the ventricular volumes in horses since this approach uses the area and length of the chamber in the mathematical approach (Lang et al., 2015; Seckerdieck et al., 2015). Indeed, obtaining the ventricular stroke volume and ejection fraction by SMOD has a good correlation with invasive methods in humans, but still, variable results in dogs, and no reported data to this date is available in horses (Behnia et al., 2013; Gehrcke et al., 2016). Considering that the entire area of the ventricle is obtained using the SMOD approach and that any changes in each chamber segment are included within the complete analysis, the ejection fraction results can be more accurate (Naser et al., 2022; Seckerdieck et al., 2015).

The evaluation of left ventricle volumes in horses by SMOD can overcome certain limitations of the M-mode and can help with a more appropriate way to detect ventricular dilation or volume overload, impacting the current approach in diseases, such as aortic regurgitation (Perez & Zucca, 2024; Ven et al., 2018). Nevertheless, its sensitivity, specificity, and likelihood is expected in further studies evaluating horses with cardiovascular conditions and this study aimed solely to prove the feasibility and reproducibility of SMOD promoting its use as the standard of care during routine echocardiography in equine cardiology and the reference range provided serve as a starting point.

One of the first studies to estimate cardiac volumes through 2-D echocardiography in horses was the study by (Voros et al., 1991), which evaluated the accuracy of 2-D echocardiography in the assessment of ventricular volumes in vitro with a consequent validation of 2-D echocardiography where volume calculation was based on a comparison with the results of ventricular volumes obtained from equine hearts disposed after necropsy showing good agreement (Vörös et al., 1990; Voros et al., 1991).

Few published studies have evaluated or obtained cardiac volumes in horses using SMOD, but they have not reported validated reference intervals at least within appropriate clinical settings (Fries et al., 2020; Giguère et al., 2005; Mcconachie et al., 2013). Considering that state-of-the-art echocardiography currently includes real-time three-dimensional echocardiography, 2-dimensional speckle tracking, and tissue Doppler imaging, it is interesting how they have evolved, and a lack of SMOD-derived volume reference intervals in equine cardiology persists. The absence of reference intervals for SMOD-derived volumes in horses limits its use, and the capacity to discriminate healthy horses from those with cardiovascular disease remains unclear, simply because there is not a reported cut-off. Therefore, studies analyzing ROC curves, sensitivity, specificity, and group control trials are warranted (Friedrichs et al., 2012).

The relationship between cardiac height and body size is recognized in all mammals, and the scale at which any morphometric measurement of the heart should be indexed to different body sizes or weight is essential for establishing reference intervals in any species (Brown et al., 2003; Gehlen et al., 2007; Sneddon et al., 2004). In our study, we indexed the cardiac volumes to the weight because, during routine echocardiography, the weight is the most practical value to index the parameters. Besides, different allometric studies confirm that cardiac volumes must be linearly related to B.W and the area, or wall thickness must be indexed to body surface area (BSA). In contrast, linear cardiac dimensions must be linearly related to body length (Al-haidar et al., 2013; Brown et al., 2003; Cornell et al., 2004; Rovira & Muñoz, 2009; Trachsel et al., 2016).

In horses, transthoracic echocardiography plays a pivotal role in investigating cardiovascular diseases linked to exercise training and conditioning, mainly because cardiac output and ejection fraction can be calculated noninvasively (Reef et al., 2014; Schwarzwald, 2016). Numerous studies have reported cardiac volumes (Stadler et al., 1994; Voros et al., 1991), cardiac output (Giguère et al., 2005; McConachie et al., 2013), and cardiac mass using the M-mode with the traditional Teichholz formula (Buhl et al., 2004; Young, 1999; Young et al., 2002). Similarly, the M-mode has been used to predict the weight of athlete horse's hearts during training (Buhl et al., 2005; Sleeper et al., 2014; Young & Wood, 2000). To date, no data have been published regarding the utility of SMOD assessing cardiovascular diseases associated to exercise in sports horses, and the authors hypothesized that SMOD can be a critical tool with a superior ability to study equine sports-associated cardiovascular diseases and athletic performance.

The cardiovascular effects of sports training are well known in humans and include enlargement of the ventricular chambers, reduction in heart rate, and increase in stroke volume (Sessa et al., 2018). Similarly, in horses, it has been found that diastolic dimensions of the left ventricle increase during sports training (Gehlen & Schlaga, 2019; Khalesi et al., 2022; Schwarzwald, 2016). These changes are associated with contractile and filling properties and confer protection against adverse cardiac effects (Sessa et al., 2018). However, there is growing evidence that human athletes and horses performing strenuous exercise for an extended period experience subsequent impairment of cardiac function, now known as exercise-induced cardiac fatigue (EICF) (Flethøj et al., 2016; Sessa et al., 2018).

The study of EICF with the investigations published to date is far from convincing and still controversial regarding systolic dysfunction. Specifically, the heterogeneous results cannot provide definite conclusions in horses (Flethøj et al., 2016). The standardization of SMOD-derived volumes should be of interest for the study of EICF because the bidimensionality of the measurements can recognize hypothetically slight changes in volumes if allometric scales are used. Additionally, interpreting these changes using SMOD can effectively discriminate volume overload from other load status conditions (Dufourni et al., 2024; Khalesi et al., 2022).

Some limitations must be considered, such as the breed of horses included in the study, which was initially considered exclusively to have a homogeneous size of subjects in the study to create the reference intervals, and it was the most common breed in the place where this study was performed; however, the authors recommend creating reference intervals with random populations of horses with different size using allometric scales (Friedrichs et al., 2012). Another limitation was the number of females, which could generate bias, and the interpretation of the difference between sex should be interpreted with caution.

Heart murmurs were allowed in the study, considering these as factors that could bias the variables despite being classified as cardiac murmurs without remodeling or mild intensity (< 3 grade) on a six-point grading system. It is essential to recognize that these heart murmurs are associated with physical training and that some patients included in the study were likely to undergo cardiac remodeling at the time of enrollment (Gehlen & Schlaga, 2019). Although healthy animals with heart murmurs were included, it is normal to find some degree of regurgitation in horses without clinical signs or cardiac remodeling during training, the prevalence of which has been estimated to be high in mixed populations of horses (Leroux et al., 2013), racehorses (Young et al., 2008), and thoroughbred horses (Young et al., 2008).

Another limitation is that the true cardiac apex in horses cannot be adequately obtained because the sternum overlaps in some animals (Vörös et al., 1990). Thus, it is not feasible to measure the total length of the ventricle in all horses. Finally, the 2-D linear and area measurements from the inner edges of the endocardial echoes could result in an error related to the amplification setting. However, this is probably less important in equine hearts because the structures are relatively large (Decloedt et al., 2017).

In conclusion, this study demonstrated that obtaining SMOD-derived volumes is both feasible and reproducible in horses, with breed-specific reference intervals provided. The end-diastolic and end-systolic volumes derived from SMOD measurements in Showjumping and Polo Argentinian Saddlebred horses represent the first data of this kind for these breeds and this information is valuable for both clinical and research applications. Notably, effect size was reported alongside the variables, which is important for designing and interpreting future research and meta-analysis in this field.

Consequently, studying the relationship between left ventricle volumes and athletic performance, as well as the effects of various training regimens and sports on cardiac volumes, would benefit from these data, improving our understanding of cardiovascular health in athletic horses. Finally, SMOD-derived volumes are a simple, non-invasive echocardiographic measure that can be used before more advanced imaging techniques, such as real-time three-dimensional echocardiography or ECG-gated multidetector computed tomography angiography are available in equine cardiology.

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