



RESEARCH ARTICLE

Transthoracic M-mode echocardiographic assessment of pulmonary vein-to-pulmonary artery ratio in healthy horses

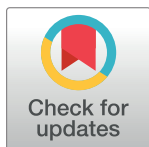
Domenico Caivano¹ , Andrea Corda² *, Mark Rishniw^{3,4}, Maria Elena Giorgi¹, Maria Luisa Pinna Parpaglia², Maria Beatrice Conti¹, Francesco Porciello¹ ‡, Francesco Biretoni¹ ‡

1 Department of Veterinary Medicine, University of Perugia, Perugia, Italy, **2** Department of Veterinary Medicine, University of Sassari, Sassari, Italy, **3** Veterinary Information Network, Davis, California, United States of America, **4** Department of Clinical Sciences, Cornell University, Ithaca, New York, United States of America

 These authors contributed equally to this work.

‡ These authors also contributed equally to this work.

* andracorda@uniss.it



OPEN ACCESS

Citation: Caivano D, Corda A, Rishniw M, Giorgi ME, Parpaglia MLP, Conti MB, et al. (2019) Transthoracic M-mode echocardiographic assessment of pulmonary vein-to-pulmonary artery ratio in healthy horses. *PLoS ONE* 14(8): e0221154. <https://doi.org/10.1371/journal.pone.0221154>

Editor: Jacopo Guccione, Università degli Studi di Napoli Federico II, ITALY

Received: June 28, 2019

Accepted: July 31, 2019

Published: August 14, 2019

Copyright: © 2019 Caivano et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: The authors declare that the funder [VIN] provided support in the form of salaries for author [MR] but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: One of Authors (MR) has a commercial affiliation with VIN but this does not

Abstract

Equine cardiovascular structures and function are routinely assessed by transthoracic echocardiography. Recently, investigators have described the echocardiographic visualization of equine pulmonary vein ostia. In companion animals, the right pulmonary vein (RPV) to right pulmonary artery (RPA) ratio has been used as an index to estimate the severity of cardiac diseases resulting in left ventricular volume overload. We sought to assess the feasibility of measuring RPV and RPA dimensions, and sought to provide various previously examined RPV and RPA variables in clinically healthy horses that could be used to assess cardiopulmonary disease status. Echocardiographic examination was prospectively performed in 70 healthy horses. The RPV and RPA were visualized using a modified right parasternal long-axis view and maximum and minimum diameters of both vessels were measured from 2D guided M-mode traces. The aortic diameter (Ao) was measured from the right parasternal short-axis view in early diastole. These measurements were then used to produce various ratio indices. RPV and RPA were imaged in all 70 horses. Median of the minimum and maximum RPV/RPA was 0.51 and 0.60, respectively. Median fractional dimensional change of vessels was 33% for RPV and 22% for RPA. The medians of the minimum and maximum RPV/Ao and RPA/Ao were 0.18, 0.28, 0.35 and 0.46, respectively. No relationships between either bodyweight or heart rate and any of the vein or artery variables were identified (maximum $r^2 = 0.04$). Inter- and intra-observer measurement variability was very good for all RPV and RPA measurements. Measuring of RPV and RPA diameters using M-mode transthoracic echocardiography is feasible in healthy horses. Further studies of these variables in horses with cardiac diseases are needed to determine the clinical applicability and utility.

alter our adherence to PLOS ONE policies on sharing data and materials.

Introduction

Equine cardiovascular structures and function are routinely assessed by transthoracic echocardiography [1,2]; left atrial (LA) and left ventricular diameters can provide useful information in horses with atrial fibrillation, aortic insufficiency and mitral regurgitation [1–3].

Recently, investigators have described the echocardiographic visualization of equine pulmonary vein ostia [4,5]. These authors suggested that the size and flow patterns of the pulmonary veins might help the assessment of cardiovascular disease in horses [4,5].

Investigators have evaluated the right pulmonary vein ostium (RPV) and right pulmonary artery ostium (RPA) in dogs and cats as an echocardiographic index for estimating severity of canine myxomatous mitral valve disease [6,7] and feline cardiomyopathies [8], and for identifying pulmonary hypertension in dogs [9,10]. These studies demonstrated that the RPV/RPA ratio, obtained from a modified right parasternal long axis view, imperfectly discriminated dogs with congestive heart failure from dogs with subclinical mitral valve disease [7,11]. However, selected RPV and RPA variables performed better than LA to aorta ratio in discriminating cardiomyopathic cats with and without congestive heart failure [8]. Whether similar vascular ratios would provide additional information in equine cardiac disease remains unknown.

However, to the authors' knowledge, no studies exist describing echocardiographic assessment of the third pulmonary venous ostial and RPA diameters in healthy horses. Therefore, we sought to assess the feasibility of measuring the third pulmonary venous ostial and RPA dimensions, and to characterize previously reported pulmonary venous ostial and RPA variables in clinically healthy horses that could allow clinicians to assess cardiopulmonary disease status.

Materials and methods

Study population

We prospectively recruited 70 client-owned healthy horses from various farms in Italy (Sardinia, Umbria and Tuscany regions). Horses were considered healthy based on history, physical examination, routine electrocardiography and echocardiography. The study protocol was approved by the Ethics Committee of the University of Sassari (approval number 5731). Owners gave informed consent for their animals' inclusion in the study.

Echocardiography

Echocardiograms were performed by 2 experienced sonographers (AC and MEG) using 2 different portable ultrasound units (MyLab 30 Gold and MyLab Alpha, Esaote, Italy) both equipped with multi-frequency 1–4 MHz phased array transducers. Echocardiographic examination was performed in all horses without sedation and in according to previously described imaging standards [12–14] with some modifications. Briefly, the sonographers modified the right parasternal long axis view as follows: to visualize the third pulmonary venous ostium and RPA, the sonographers moved the ultrasound probe slightly apically and angled it dorso-cranially, to obtain a somewhat oblique view of the heart base (Fig 1). To conserve nomenclature that has been adopted in companion animals, we refer to the third pulmonary venous ostium as the right pulmonary vein (RPV) throughout the remainder of this manuscript, as this ostium receives blood from the largest part of the right lung [4,5], and is analogous to the right pulmonary vein in dogs and cats [6–8].

In this view, the RPA appeared as a circle just below the linear RPV. The sonographers then positioned the M-mode cursor for M-mode acquisition so that it perpendicularly bisected the

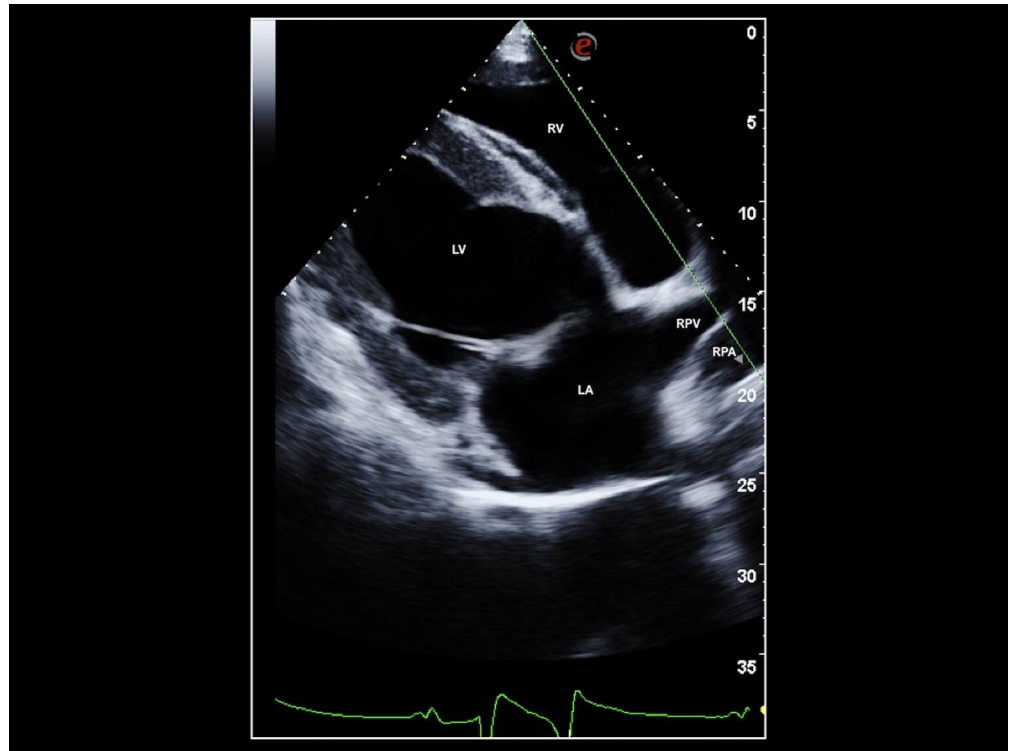


Fig 1. Echocardiographic image. Two-dimensional echocardiographic right parasternal long-axis view optimized for the third pulmonary vein ostium (RPV) and right pulmonary artery ostium (RPA) in a healthy horse, showing placement of the M-mode cursor (green line) for M-mode acquisition. RPV: right pulmonary vein ostium; RPA: right pulmonary artery ostium; RV: right ventricle; LA: left atrium; LV: left ventricle.

<https://doi.org/10.1371/journal.pone.0221154.g001>

RPV, where the venous walls were reasonably parallel, and to pass through the center of the adjacent RPA (Fig 1). The sonographers then acquired 2D guided M-mode traces that displayed at least two consecutive cardiac cycles and stored all data for subsequent off-line analysis (Fig 2).

Aortic diameter (Ao) was measured from the right parasternal short-axis view at aortic valve level, where the valve cusps were visible at the onset of the diastole (Fig 3), as previously described for dogs and cats [6,8].

Because not all horses tolerated electrocardiographic clips or lead wires, no simultaneous ECG tracing was recorded in all horses. At least 3 nonconsecutive cardiac cycles were stored in cine-loop format for each measured variable (RPV, RPA, Ao).

Because clinicians often perform echocardiography on horses “in the field” without simultaneous ECG monitoring, we opted to examine the RPV and RPA variables using mechanically timed events. One investigator (DC) measured all the echocardiographic variables off-line from archived M-mode images, at the subjective maximum (RPV_{max} and RPA_{max}) and minimum diameters (RPV_{min} and RPA_{min}) of both vessels, using the inner edge-to-inner edge method, as previously described [6]. Three representative, nonconsecutive cardiac cycles were measured, and individual measurements were subsequently averaged. Cycles during marked transient tachycardia, following a sinus pause or a 2nd-degree atrioventricular block were excluded from analysis.

From the measured vessels diameters, we calculated the following variables:

- RPV_{min}/RPA_{min} : the ratio between RPV and RPA at their minimum diameter

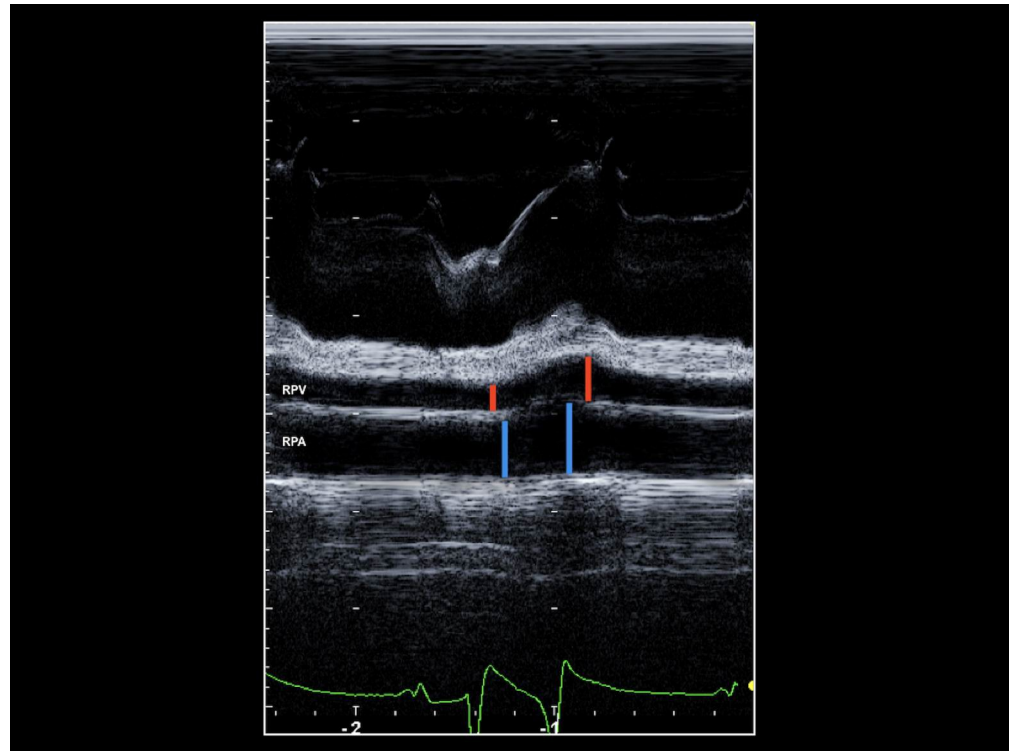


Fig 2. Echocardiographic image. M-mode echocardiographic image of the third pulmonary vein ostium (RPV) and right pulmonary artery ostium (RPA) obtained from the right parasternal long axis view. Red and blue lines show visually identified maximal and minimal diameters of the RPV and RPA, respectively, using the blood tissue interface method. RPV: right pulmonary vein ostium; RPA: right pulmonary artery ostium.

<https://doi.org/10.1371/journal.pone.0221154.g002>

- RPV_{max}/RPA_{max} : the ratio between RPV and RPA at their maximum diameter
- RPV_{min}/Ao : the ratio between RPV at its minimum diameter and Ao
- RPV_{max}/Ao : the ratio between RPV at its maximum diameter and Ao
- RPA_{min}/Ao : the ratio between RPA at its minimum diameter and Ao
- RPA_{max}/Ao : the ratio between RPA at its maximum diameter and Ao
- ΔRPV (fractional dimensional change - $\Delta RPV\%$): $(RPV_{max}-RPV_{min})/RPV_{max} \times 100$
- ΔRPA (fractional dimensional change - $\Delta RPA\%$): $(RPA_{max}-RPA_{min})/RPA_{max} \times 100$

Data analysis

We assessed the distributions of the data on variables with a Shapiro-Wilk test. Because several of the variables did not conform to requirements for parametric reference intervals, we adopted a non-parametric approach for all variables to maintain consistency and avoid confusion [15] and presented the measures of central tendency as medians and interquartile ranges. To examine the effects of bodyweight (BW) and heart rate (HR) on the dependent variables, we drew scatter plots to visually examine the data. After examining for major violations of assumptions (and confirming that none existed), we performed linear regression analysis to determine if any substantive relationship existed.

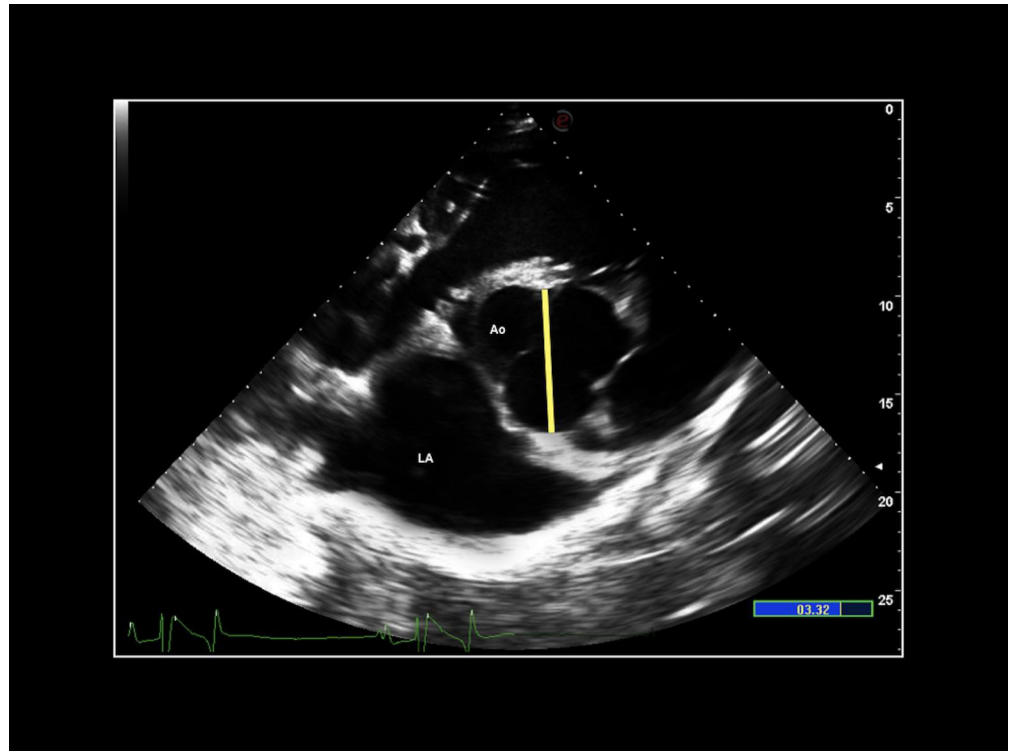


Fig 3. Echocardiographic image. Two-dimensional echocardiographic right parasternal short-axis view at the heart base. Aortic diameter (yellow line) was measured at aortic valve level, where the valve cusps were visible at the onset of the diastole. LA: left atrium; Ao: aorta.

<https://doi.org/10.1371/journal.pone.0221154.g003>

To examine inter- and intra-observer variability in measuring the variables, ten studies were selected randomly (using a random number generator). Each investigator (AC and DC) then measured the minimal and maximal RPV and RPA dimension in each of these selected studies. One investigator (DC) measured the same studies on 2 separate occasions, separated by at least one month, and without knowledge of the previous measurements. Coefficients of variation are problematic when calculated for pairs of measurements, because the standard deviation of a pair of observations cannot be accurately determined. Therefore, we calculated the percent difference and the absolute difference between the 2 sonographers for each variable in each study and for the 2 measurements by the same sonographer as has been previously described [8,11].

Results

The study population consisted in 27/70, female horses, 34/70 geldings and 9/70 stallions of the following breeds: Anglo-Arabian (n = 22), warmblood (n = 23), Arabian (n = 10), Quarter Horse (n = 6), Thoroughbred (n = 3), Italian Trotter (n = 2), Haflinger (n = 1), Holstein (n = 1), Appaloosa (n = 1) and Quarab (n = 1), with a median BW of 480 kg (range 300–670 kg) and a median age of 8 years (range 2–27 years). Body condition score ranged from 5 to 6 on 9-point scale (Henneke scale) in all horses. During the echocardiography, horses had a mean HR of 40 ± 8 beats per minute. We were able to image and measure the RPV and RPA in all 70 horses. The M-mode tracing was characterized by multiple phasic deflections both of the RPV and RPA wall (Fig 2). Twenty out of 70 (28.6%) horses did not tolerate electrocardiographic clips or lead wires, so we could not acquire simultaneous ECG tracings. In the 50/

70 (71.4%) horses with available ECG tracings, a positive RPV deflection began immediately after the QRS complex, reaching its azimuth near to the end of the T wave and then declined to a nadir just after the QRS complex (before the onset of the positive deflection). We additionally identified a positive deflection, followed by a gradual decrement in the RPA tracings. The positive deflection reached its azimuth during the T wave and declined to a nadir at the end of the QRS complex (Fig 2). In horses without ECG tracings, RPV_{min} and RPV_{max} were measured at the onset and at the azimuth of positive deflection, respectively. Similarly, RPA_{min} and RPA_{max} were measured at the onset and at the azimuth of positive deflection, respectively.

The medians and reference limits of the various RPV and RPA variables are displayed in Table 1.

We identified no relationships between either BW or HR and any of the RPV or RPA variables (maximum $r^2 = 0.04$) (S1 File).

Inter-observer measurement variability was very good for all RPV and RPA measurements. The largest percentage difference between the 2 sonographers was 8% (for RPV_{min}). In all other measurements, the percentage difference was <7%. Similarly, intra-observer variability was very good. As with inter-observer variability, the RPV_{min} measurement had the greatest intra-observer variability, with the percentage difference approaching 8%, whereas all other measurements had a percentage difference <6% for intra-observer variability. When assessed on an absolute difference, the average measurements for RPV and RPA differed between sonographers and within sonographer by <1.5 mm.

Discussion

We have, for the first time, demonstrated the feasibility of measuring RPV and RPA diameter in healthy horses and have also generated reference intervals for various RPV and RPA variables, as has previously been reported in dogs and cats. We could reliably image the RPV and RPA diameters by transthoracic echocardiography in healthy horses using a modified right parasternal long axis view to obtain the relevant M-mode images. We could easily detect the anatomical landmarks that corresponded to vessels identified by previous investigators [6,16]. Whether these variables will prove useful in assessing equine cardiac pathology, identifying

Table 1. Reference intervals for right pulmonary vein and pulmonary artery variables in 70 clinically healthy horses.

Variable	Median	Lower reference limit (90% CI ^a)	Upper reference limit (90%CI)
RPV_{min}^b/RPA_{min}^c	0.51	0.32 (0.29–0.36)	0.81 (0.75–0.83)
RPV_{max}/RPA_{max}	0.60	0.41 (0.38–0.45)	0.87 (0.83–0.88)
RPV_{min}/Ao^d	0.18	0.11 (0.10–0.13)	0.27 (0.25–0.29)
RPV_{max}/Ao	0.28	0.18 (0.17–0.19)	0.39 (0.37–0.40)
RPA_{min}/Ao	0.35	0.26 (0.25–0.27)	0.46 (0.42–0.50)
RPA_{max}/Ao	0.46	0.36 (0.34–0.37)	0.57 (0.53–0.59)
ΔRPV^e (%)	33	17 (17–19)	51 (48–53)
ΔRPA^f (%)	22	11 (10–12)	37 (34–39)

^aCI—confidence interval

^bRPV—third pulmonary vein ostium

^cRPA—right pulmonary artery

^dAo—aorta

^e ΔRPV —pulmonary vein fractional dimensional change

^f ΔRPA —pulmonary artery fractional dimensional change

<https://doi.org/10.1371/journal.pone.0221154.t001>

congestive heart failure, or predicting success of cardioversion in atrial fibrillation, remains to be determined.

Because clinicians often perform echocardiographic evaluations of horses without simultaneous ECG monitoring, we opted to use mechanically timed events to measure RPV and RPA variables (minimal and maximal vessel distensions), rather than ECG-timed events. In horses with ECG available, minimum and maximum diameter of the RPV and RPA occurred before the onset and during the positive deflection of both vessels, respectively. Although mechanically timed events are subject to a “best guess” by the sonographer, our inter- and intra-observer variability analyses suggest that, at least in this study, both observers consistently identified maximal and minimal diameters of both vessels. Furthermore, previous investigators have demonstrated that mechanically or electrically timed RPV and RPA measurements work equally well in dogs and cats, and that the mechanically based method more accurately determined vessel fractional dimensional change [6,8].

We visualized phasic deflections of the RPV and RPA on the M-mode tracings of horses that resembled the M-mode deflections observed in dogs and cats [6,8]. Right pulmonary vein ostium diameter in the horse changes during the cardiac cycle, reflecting the different phases of LA function (reservoir, conduit and booster pump phase), as previously described in humans and small animals [6,8,17,18]. The RPV reaches its maximum diameter at ventricular end systole. Indeed, during ventricular systole, the mitral annulus is pulled apically and the LA acts as an aspiration pump. Thus, the LA, including the RPV, relaxes and is stretched apically with increasing of both volumes just prior to mitral valve opening [18]. The RPV diameter then decreases when the mitral valve opens, during the rapid ventricular filling phase. The RPV reaches its minimum diameter just before the ventricular systole: in humans and horses, atrial myocardial fibers, oriented circumferentially, extend into the pulmonary vein ostia [17,19], therefore, when the atrium contracts, pulmonary vein ostia also constrict, thereby reducing their dimensions. The positive systolic RPA deflection begins just after the QRS (at the same instant as achieving the minimal diameter), consistent with blood entering the pulmonary artery from the right ventricle and distending it. The RPA then reaches its maximal diameter during the T wave, and then the deflection declines throughout diastole, reaching its nadir at the end of the QRS complex.

The echocardiographically derived RPV/RPA in healthy horses is approximately half that observed in healthy dogs, for both RPV_{\min}/RPA_{\min} (0.51 vs 1.15) and RPV_{\max}/RPA_{\max} (0.60 vs 1.07), but similar to that observed in healthy cats (0.51 vs 0.51 and 0.60 vs 0.67, respectively). Moreover, computed tomographic studies in humans reported an upper limit of the RPA diameter around 19.8 mm [20] while the diameters of pulmonary vein ostia ranged from 9 mm to 12.4 mm [21], which would result in RPV/RPA of approximately 0.45–0.62. These findings suggest that humans, cats and horses have similar RPV/RPA.

By comparing the RPV and RPA dimensions to the aortic dimension, one can determine if the small RPV/RPA is due to a larger RPA or a smaller RPV (or both). Horses and cats appear to have a relatively smaller RPV/Ao , and a relatively larger RPA_{\min}/Ao compared to dogs. However, horses had a smaller RPA_{\max}/Ao than dogs or cats. The fractional dimensional change of the RPA and RPV, calculated in our horses, had a median ΔRPA of 22%, similar to the value reported in cats (23%) [8], but lower than what has been reported in dogs (37% and 43%) [6,9]. Moreover, equine ΔRPA values were closer to the human value (28%) than the value reported for dogs. Pulmonary artery fractional dimensional change is inversely related to pulmonary arterial pressure in dogs [9,10] and humans [22,23]. Healthy horses have higher pulmonary arterial pressures [24] than healthy dogs [25] and humans, therefore we speculated that they could have decreased fractional dimensional change of the right pulmonary artery compared to other species. On the other hand, we found a median ΔRPV of 33%, lower than

that obtained in dogs (37%) [6], cats (40%) [8] and humans (39% for right superior pulmonary vein and 31% for right inferior pulmonary vein in a MRI-derived study) [26].

We did not find any relationships of any of the indexed RPV or RPA variables with either BW or HR. This is not surprising, as indexing removes the effect of size, and allows for a single threshold value to be established for healthy horses.

We have measured the aortic diameter from the right parasternal short-axis view at aortic valve level, where the valve cusps were visible at the onset of the diastole, as previously described for dogs and cats [6,8]. Commonly, aortic diameter is measured from the right parasternal long axis (left ventricular outflow tract) view at the end of diastole in horses [12–14]. We preferred to use the view described in dogs and cats to compare the results obtained in the three different species. Indeed, aortic measurements differ significantly throughout diastole and are not clinically interchangeably in dogs [27]. Whether this occurs also in horses remains unknown. However, clinicians measuring RPV and RPA variables and indexing them to the aorta should note that aortic measurements in different views and different phases of the cardiac cycle might yield different results.

We did not include any small ponies or miniature horses in our study—therefore, whether these values apply to equids different from our study population remains to be determined. Furthermore, we did not evaluate any horses with cardiac pathology to determine whether any of the variables will prove useful in assessing cardiac status.

Conclusions

Our study supports the feasibility of measuring RPV and RPA diameters using transthoracic M-mode echocardiography and provides reference intervals for various pulmonary vascular variables in healthy horses. Further studies of these variables in horses with cardiac diseases, are needed to determine the clinical applicability and utility.

Supporting information

S1 File. Raw data and linear regression analysis from the study. Clinical and echocardiographic variables in 70 healthy horses. Linear regression analysis to examine the effects of bodyweight and heart rate on the RPV and RPA variables. (XLSX)

Author Contributions

Conceptualization: Domenico Caivano, Andrea Corda, Mark Rishniw, Maria Elena Giorgi, Maria Luisa Pinna Parpaglia, Maria Beatrice Conti, Francesco Porciello, Francesco Birettoni.

Data curation: Domenico Caivano, Mark Rishniw, Maria Elena Giorgi.

Formal analysis: Mark Rishniw.

Investigation: Domenico Caivano, Andrea Corda, Maria Elena Giorgi.

Methodology: Domenico Caivano, Andrea Corda, Mark Rishniw, Maria Luisa Pinna Parpaglia, Francesco Birettoni.

Project administration: Francesco Porciello.

Supervision: Francesco Porciello, Francesco Birettoni.

Writing – original draft: Domenico Caivano, Andrea Corda.

Writing – review & editing: Domenico Caivano, Andrea Corda, Mark Rishniw, Maria Elena Giorgi, Maria Luisa Pinna Parpaglia, Maria Beatrice Conti, Francesco Porciello, Francesco Birettoni.

References

1. Schwarzwald CC. Disorders of the Cardiovascular System. In: Reed SM, Bayly WM and Sellon DC, editors. *Equine Internal Medicine*, 4th edition. St. Louis: Saunders Elsevier; 2018. p. 387–541.
2. Boon JA. Evaluation of size, function, and hemodynamics. In: Boon JA, editor. *Veterinary Echocardiography*, 2nd edition. Oxford: Wiley-Blackwell; 2011. p. 153–266.
3. Marr CM. Cardiac murmurs: valvular regurgitation and insufficiency. In: Marr CM and Bowen M, editors. *Cardiology of the Horse*, 2nd edition. Amsterdam: Saunders Elsevier; 2010. p. 207–16.
4. Vandecasteele T, van Loon G, Vandevelde K, De Pauw B, Simoens P, Cornillie P. Topography and ultrasonographic identification of the equine pulmonary vein draining pattern. *Vet. J.* 2016; 210: 17–23. <https://doi.org/10.1016/j.tvjl.2016.01.009> PMID: 26876622
5. Vandecasteele T, Cornillie P, van Steenkiste G, Vandevelde K, Gielen I, Vanderperren K et al. Echocardiographic identification of atrial-related structures and vessels in horses validated by computed tomography of casted hearts. *Equine Vet. J.* 2019; 51: 90–6. <https://doi.org/10.1111/evj.12969> PMID: 29808486
6. Birettoni F, Caivano D, Patata V, Moise NS, Guglielmini C, Rishniw M. Canine pulmonary vein-to-pulmonary artery ratio: echocardiographic technique and reference intervals. *J. Vet. Cardiol.* 2016; 18: 326–35. <https://doi.org/10.1016/j.jvc.2016.07.004> PMID: 27670068
7. Merveille AC, Bolen G, Krafft E, Roels E, Gomart S, Etienne AL et al. Pulmonary vein-to-pulmonary artery ratio is an echocardiographic index of congestive heart failure in dogs with degenerative mitral valve disease. *J Vet Intern Med.* 2015; 29: 1502–509. <https://doi.org/10.1111/jvim.13634> PMID: 26415640
8. Patata V, Caivano D, Porciello F, Rishniw M, Domenech O, Marchesotti F, Giorgi ME, Poser H, Guglielmini C, Spina F, Birettoni F. Pulmonary vein to pulmonary artery ratio in healthy and cardiomyopathic cats. *J Vet Intern Med.* 2018; 32: 583–84.
9. Venco L, Mihaylova L, Boon JA. Right Pulmonary Artery Distensibility Index (RPAD Index). A field study of an echocardiographic method to detect early development of pulmonary hypertension and its severity even in the absence of regurgitant jets for Doppler evaluation in heartworm infection. *Vet Parasitol.* 2014; 206: 60–6. <https://doi.org/10.1016/j.vetpar.2014.08.016> PMID: 25218885
10. Visser LC, Im MK, Johnson LR, Stern JA. Diagnostic Value of Right Pulmonary Artery Distensibility Index in Dogs with Pulmonary Hypertension: Comparison with Doppler Echocardiographic Estimates of Pulmonary Arterial Pressure. *J Vet Intern Med.* 2016; 30: 543–52. <https://doi.org/10.1111/jvim.13911> PMID: 26893108
11. Birettoni F, Patata V, Rishniw M, Caivano D, Giorgi ME, Porciello F. Pulmonary Vein-to-Pulmonary Artery Ratio Helps Identify Dogs with Congestive Heart Failure Secondary to Mitral Valve Disease. *J Vet Intern Med* 2017; 31: 247–48.
12. Long KJ, Bonagura JD, Darke PG. Standardised imaging technique for guided M-mode and Doppler echocardiography in the horse. *Equine Vet J* 1992; 24: 226–35. PMID: 1606937
13. Patteson MW, Gibbs C, Wotton PR, Cripps PJ. Echocardiographic measurements of cardiac dimensions and indices of cardiac function in normal adult thoroughbred horses. *Equine Vet J Suppl* 1995; 19: 18–27.
14. Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of echocardiographic assessment of left atrial size and mechanical function in horses. *Am. J. Vet. Res.* 2007; 68: 735–47. <https://doi.org/10.2460/ajvr.68.7.735> PMID: 17605609
15. Geffre A, Concordet D, Braun JP, Trumel C. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Vet Clin Pathol.* 2011; 40: 107–12. <https://doi.org/10.1111/j.1939-165X.2011.00287.x> PMID: 21366659
16. Brewer FC, Moise NS, Kornreich BG, Bezuidenhout AJ. Use of computed tomography and silicon endocasts to identify pulmonary veins with echocardiography. *J Vet Cardiol.* 2012; 14: 293–300. <https://doi.org/10.1016/j.jvc.2012.02.004> PMID: 22386718
17. Hassink RJ, Aretz HT, Ruskin J, Keane D. Morphology of atrial myocardium in human pulmonary veins: a postmortem analysis in patients with and without atrial fibrillation. *J Am Coll Cardiol* 2003; 42: 1108–14. [https://doi.org/10.1016/s0735-1097\(03\)00918-5](https://doi.org/10.1016/s0735-1097(03)00918-5) PMID: 13678939
18. Bowman AW, Kovács SJ. Prediction and assessment of the time-varying effective pulmonary vein area via cardiac MRI and Doppler echocardiography. *Am J Physiol Heart Circ Physiol.* 2005; 288: 280–6.

19. Vandecasteele T, Van Den Broeck W, Tay H, Couck L, van Loon G, Cornillie P. 3D reconstruction of the porcine and equine pulmonary veins, supplemented with the identification of telocytes in the horse. *Anat Histol Embryol*. 2018; 47: 145–152. <https://doi.org/10.1111/ahc.12334> PMID: 29314148
20. Bozlar U, Ors F, Deniz O, Uzun M, Gumus S, Ugurel MS et al. Pulmonary artery diameters measured by multidetector-row computed tomography in healthy adults. *Acta Radiol*. 2007; 48: 1086–91. <https://doi.org/10.1080/02841850701545755> PMID: 17963079
21. Kim YH, Marom EM, Herndon JE, McAdams HP. Pulmonary vein diameter, cross-sectional area, and shape: CT analysis. *Radiology*. 2005; 235: 43–9. <https://doi.org/10.1148/radiol.2351032106> PMID: 15731371
22. Bogren HG, Klipstein RH, Mohiaddin RH, Firmin DN, Underwood SR, Rees RS et al. Pulmonary artery distensibility and blood flow patterns: a magnetic resonance study of normal subjects and of patients with pulmonary arterial hypertension. *Am Heart J*. 1989; 118: 990–9. [https://doi.org/10.1016/0002-8703\(89\)90235-4](https://doi.org/10.1016/0002-8703(89)90235-4) PMID: 2816711
23. Jardim C, Rochitte CE, Humbert M, Rubinfeld G, Jasinowdolinski D, Carvalho CR et al. Pulmonary artery distensibility in pulmonary arterial hypertension: an MRI pilot study. *Eur Respir J*. 2007; 29: 476–81. <https://doi.org/10.1183/09031936.00016806> PMID: 17135232
24. Erickson BK, Erickson HH, Coffman JR. Pulmonary artery and aortic pressure changes during high intensity treadmill exercise in the horse: effect of frusemide and phentolamine. *Equine Vet J*. 1992; 24: 215–9. PMID: 1606935
25. Johnson L. Diagnosis of pulmonary hypertension. *Clin Tech Small Anim Pract*. 1999; 14: 231–6. [https://doi.org/10.1016/S1096-2867\(99\)80016-4](https://doi.org/10.1016/S1096-2867(99)80016-4) PMID: 10652841
26. Lickfett L, Dickfeld T, Kato R, Tandri H, Vasamreddy CR, Berger R et al. Changes of pulmonary vein orifice size and location throughout the cardiac cycle: dynamic analysis using magnetic resonance cine imaging. *J Cardiovasc Electrophysiol*. 2005; 16: 582–8. <https://doi.org/10.1046/j.1540-8167.2005.40724.x> PMID: 15946353
27. Dickson D, Caivano D, Patteson M, Rishniw M. The times they are a-changin': Two-dimensional aortic valve measurements differ throughout diastole. *J Vet Cardiol*. 2016; 18: 15–25. <https://doi.org/10.1016/j.jvc.2015.11.001> PMID: 26810488