The quantitative evaluation of cardiac structures and major thoracic vessels dimensions by means of lateral contrast radiography in Wistar albino rats (*Rattus norvegicus*)

Mahir KAYA[1,a], Mehmet Alper ÇETİNKAYA[2,4,b,✉], Doğa BESNE[3,c]

[1]Medical Imaging Techniques Program, Health Services Vocational College, Akdeniz University, 07070 Antalya, Türkiye; [2]Laboratory Animal Research and Application Center, Surgical Research Laboratory, Hacettepe University, 06100 Sıhhıye-Ankara, Türkiye; [3]Laboratory Animal Research and Application Center, Akdeniz University, 07070 Antalya, Türkiye; [4]Near East University Faculty of Veterinary Medicine, Department of Surgery Nicosia - Lefkosa, Turkish Republic of Northern Cyprus 99138

[a]ORCID: 0000-0002-2979-8832, [b]ORCID: 0000-0001-5097-6368, [c]ORCID: 0000-0002-3277-9907

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✉**Corresponding author**
macetinkaya@gmail.com

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ABSTRACT

The aim of this study was to define reference values for vertebral heart score (VHS) and modified left atrium (LA)-VHS, cardiac structures, and major thoracic vessels measurements and ratios obtained from thoracic contrast radiography Wistar albino rats. VHS, modified LA-VHS, left (LV) and right (RV) ventricles, interventricular septum (IVS), aorta (Ao), caudal vena cava (CaVC), and fourth thoracic vertebrae (v) length (T4) were measured from left lateral thoracic contrast radiographs of 50 young, healthy adult male Wistar albino rats. LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, and CaVC/ Ao ratios were calculated from these values. Measurements were performed by two observers unaware of the signalment data for interobserver repeatability analysis. Median values and references ranges were 8.2v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.1.37) for CaVC/Ao. Further studies are now needed to determine whether measurement values obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be used as a reference baseline for both pet and laboratory rats.

Introduction

Cardiac diseases are seen in both pet (12) and laboratory (36) rats. Spontaneous cardiomyopathy is reported to be relatively common in elderly rats, and the presence of left atrial and ventricular thrombosis is associated with this cardiac disease (30). Rat models are frequently used in cardiovascular research. These models include coronary ligation-induced cardiac hypertrophy, aortic banding (overload-induced cardiac hypertrophy), diabetic cardiomyopathy, the desoxycorticosterone acetate/salt model of hypertension, renal ischemia via renal artery stenosis, and aortocaval fistula (3, 10). Imaging methods are important for the diagnosis and follow-up of the progression of cardiac diseases in both pet rats and experimental cardiovascular rat models. Although echocardiography is the primary diagnostic method in cardiac diseases, it is not routinely used in rats. The size and shape of the heart and thoracic vessels can be evaluated using thoracic radiography (21), which is easier to perform and less expensive than echocardiography.

Since 1995, a quantitative assessment of the size of the cardiac silhouette from thoracic radiography has been performed via the vertebral heart score (VHS) system, which is simple, objective, and repeatable (8). Using this system, cardiac axes measured from thoracic radiographic views are compared with the length of specific thoracic vertebrae, thus minimizing the error associated with subjective interpretations and interpatient variability (37).

Normal VHS values have been established for Sprague-Dawley and Wistar albino rats (13, 15), as well as for various other animal species (5, 11, 14, 16, 17, 26, 32, 37, 38).

Radiographic indices that permit evaluation of the left atrium (LA) at lateral thoracic radiography are available. Vertebral left atrial size (29) and radiographic left atrial dimension (34) are widely recognized indices for dogs. LA-VHS, modified from VHS, was defined by Schober et al. (35) for cats. However, these indices have not been reported for rats.

Obtaining thoracic radiography at the peak of inspiration in rats is problematic due to their high respiration rate (13). The cranial border of the cardiac silhouette on lateral radiographs is also insufficiently clear due to soft tissue opacity of the cranial and ventral mediastinum. The apex of the heart may be indistinct on radiographs obtained during expiration as it may be located beyond the diaphragm. These factors can all adversely affect VHS measurements in rats. Our study hypothesis was that the sizes of the heart and LA can be directly evaluated with their distinct borders and that the widths of the left (LV) and right (RV) ventricles, the thickness of the interventricular septum (IVS), the widths of the aorta (Ao) and caudal vena cava (CaVC) can be measured using contrast radiography, independently of the cardiac silhouette.

The aim of this study was to define normal values for radiographic indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, fourth thoracic vertebrae length (T4)), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/ Ao) obtained from left lateral thoracic contrast radiography of 50 healthy, adult male Wistar albino rats.

Materials and Methods

Animals: The experimental protocol was approved by the Akdeniz University animal care ethics committee on the use of animals, Türkiye (No: B.30.2.AKD.0.05.07.00/27). Fifty healthy, young adult, intact male rats (*Rattus Norvegicus*, Albinus, Wistar) were housed in the Akdeniz University Experimental Research and Application Center (Türkiye), in groups of four to six animals (of the same sex) at 50-60% humidity and 20-21°C in a 12-h dark/light cycle. Standard rat chow and free access to water were provided.

Rats underwent daily physical examinations during the study. Neither cardiovascular nor pulmonary abnormalities (murmur, arrhythmia, abnormal respiratory sounds, etc.) were detected in any of the rats included in the study, and their hydration status was normal.

Anesthesia protocol: Radiographs were obtained from all rats under general anesthesia. The anesthesia protocol was applied as previously described by Dias et al. (13). Briefly, the rats were placed in an induction chamber, and anesthesia was induced using 5% isoflurane (Aerrane Volatil®, Eczacıbası-Baxter, Istanbul, Türkiye) plus oxygen (2 L/min). Anesthesia was maintained using 1–3% isoflurane delivered in oxygen at 1 L/min, by means of a small face mask and non-rebreathing circuit. All animals maintained spontaneous breathing throughout the entire procedure, except for the exposure time. Intravenous access was established in the tail vein of each animal under anesthesia for the administration of contrast medium.

Thoracic contrast radiography: Each animal was imaged in the left lateral projection (X-ray tube: ORIX-65, Ardet®, Istanbul, Türkiye. Parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance*).* The animal was placed lying on its left side on the X-ray cassette. The thoracic legs were then pulled cranially, and the beam was centered at the level of the thorax between the scapulohumeral joint and the last rib. Contrast radiography was performed after plain radiography (Figure 1A). However, radiographic measurements were performed only from contrast radiography. Contrast radiographs were obtained by bolus injection of 0.75 ml of non-ionic opaque contrast agent (300 mg I/ml, Iohexol, Omnipaque®, Opakim, Türkiye) from the tail vein. Exposure was performed as soon as the contrast agent injection was completed (Figure 2B). A positive pressure breath-hold was performed at the time of radiographic exposure in order to obtain radiographic images at the inspiration peak. While positive pressure breath-hold was being employed, care was taken not to cause pulmonary hyperinflation, to preserve the opacity of the CaVC, and not to cause excessive caudal displacement of the diaphragm. Otherwise, all animals continued breathing spontaneously throughout the entire procedure.

Radiographs with substantial symmetry/superposition of the ribs and scapulae and widely accessible cardiothoracic structures with adequate contrast and pulmonary inflation were considered to be of sufficient diagnostic quality.

Radiographic images obtained using a computed radiography reader (FCR Prima T2, FujiFilm®, Tokyo, Japan) were stored. These radiographic images were anonymized and randomized and then evaluated by two observers using commercially available computer software (Image Intelligence™, FujiFilm®, Tokyo, Japan). The observers were blinded to the age and body weight of each rat. They were able to manipulate the images as needed, including by changing the window width, window level, and magnification, and measurements were made of the radiographic cardiac indices and ratios. Corresponding mean radiographic cardiac indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, T4), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/Ao) were calculated for each observer, these being used for statistical purposes.

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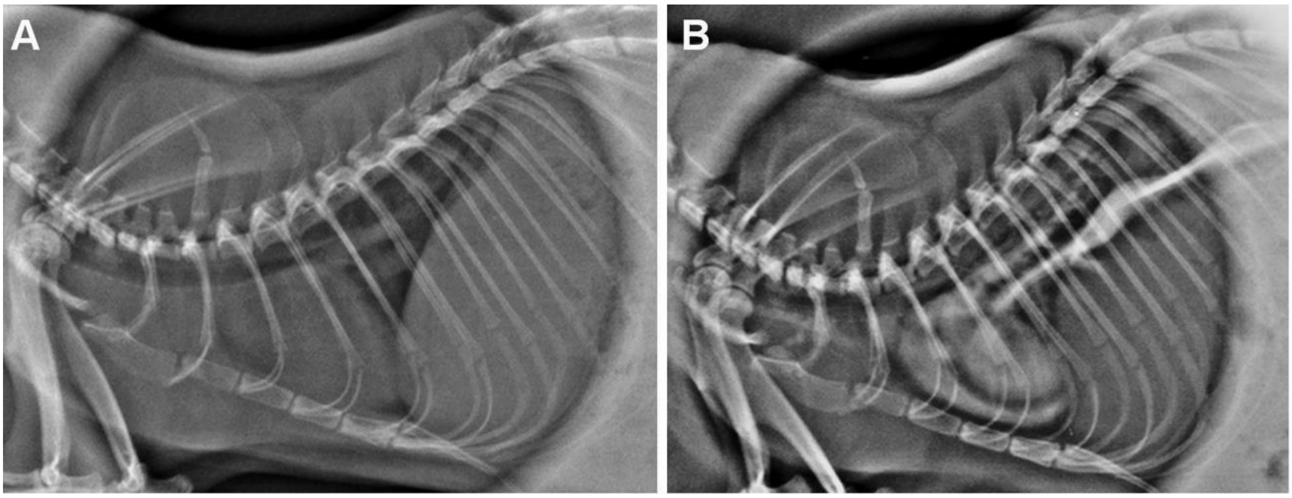

Figure 1. Plain (**A**) and contrast (**B**) radiographic studies of the thorax in the left lateral recumbency (parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance).

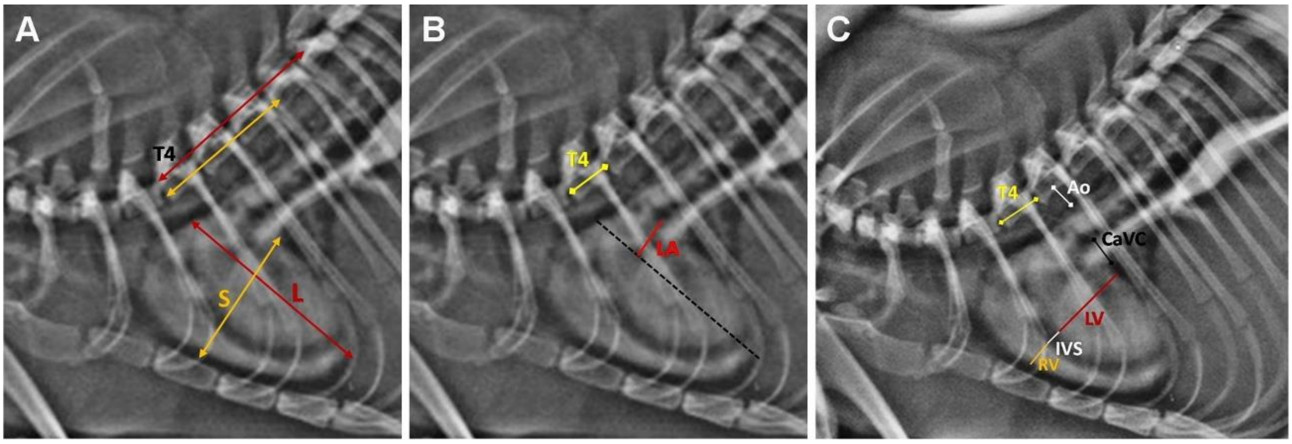

Figure 2. Examples of VHS (**A**), modified LA-VHS (**B**), aorta (Ao), caudal vena cava (CaCV), left ventricle (LV), interventricular septum (IVS), right ventricle (RV), fourth thoracic vertebrae (T4) (**C**) measurements on left lateral contrast views. L: long axis of VHS, S: short axis of VHS, LA: left atrium.

Measurements of radiographic cardiac indices and structures and major thoracic vessel: The heart was assessed by measuring the VHS, as described by Buchanan (9). On left lateral contrast rat radiographs, the cardiac long axis (L) was measured from the ventral border of the left mainstem bronchus (carina) to the cardiac apex. The cardiac short axis (S) was determined by measuring the widest distance between the cranial and caudal borders of the heart. Commercially available computer software was used to apply a 90-degree rotation between L and S. These two axes were then repositioned over the thoracic vertebrae from the cranial edge of T4, parallel to the vertebral column, and each length was then expressed in terms of the number of thoracic vertebrae (v) to the nearest 0.1v. The sum of the two values was used as VHS (Figure 2A).

The LA-VHS method previously described by Schober et al. (35) was subjected to some modification. A line was drawn between the carina and cardiac apex. The distance between this line and the intersection of the dorsal wall of the CaVC and the caudal border of the heart was measured perpendicular to this line. This measurement was divided by the T4 and used as modified LA-VHS (Figure 2B).

The widest parts of the LV and RV were measured perpendicular to IVS (Fig 2C). IVS thickness was measured from the same level. The widths of the Ao and CaVC were obtained by measuring the distance between the dorsal and ventral borders of each vessel. The Ao was measured caudally to the carina and dorsocaudally to the base of the heart. CaVC was measured immediately caudally to the intersection of the caudal border of the heart with this vessel. T4 was obtained by measuring the distance between the cranial and caudal edges of the vertebral body (21, 22) (Figure 2C). The measurements were performed digitally using the software. Except for VHS and modified LA-VHS, all the data obtained were expressed in mm. LV/T4, RV/T4, IVS/T4, Ao/T4,

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CaVC/T4, and CaVC/Ao ratios were calculated from these measurements.

***Statistical analysis**:* All statistical analyses were performed on Statistical Package for the Social Sciences software version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were generated. The normality of data distribution was assessed using the Shapiro-Wilk test. None of the variables were normally distributed, and results were reported as the median and interquartile range (IQR). Correlation between radiographic cardiac indices, measurements, and ratios was calculated using Spearman's rank-order correlation coefficient. Interobserver variabilities were assessed for radiographic cardiac indices, measurements, and ratios via intraclass correlation coefficient (ICC) estimates and their 95% confidence intervals (CI) based on a single rater, absolute agreement, and a two-way random (interobserver) effect. An ICC value >0.9 was considered excellent, 0.75 to 0.9 good, 0.5 to 0.75 moderate, and <0.5 poor (23). *P* values < 0.05 were considered significant for all analyses.

Results

The study population consisted of 50 healthy, male Wistar albino rats with median age and weight of 12 weeks (10-16) and 370 g (275-460), respectively.

No anesthesia or contrast agent-related complications or death occurred in any animals. Measurements of radiographic cardiac indices and structures and major thoracic vessels were performed uneventfully from all contrast radiographs.

The radiographic cardiac indices and measurements and ratios for cardiac structures, major thoracic vessels, and T4 reported by the observers are summarized in Table 1-3.

Median values and references ranges were 8.2v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.1.37) for CaVC/Ao.

Table 1. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for vertebral heart score (VHS) and modified left atrium-vertebral heart score (LA-VHS) recorded by two observers in healthy male Wistar albino rats.

Observer		**Radiographic Cardiac Indices**	
		VHS	**Modified LA-VHS**
Observer1	Mean±SD	8.21±0.36	1.15±0.12
	95% CI	8.12-8.32	1.11-1.18
Observer2	Mean±SD	8.25±0.4	1.17±0.13
	95% CI	8.14-8.36	1.13-1.21

Table 2. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for measurements of radiographic cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in healthy male Wistar albino rats.

Observer		**Measurements (mm)**					
		LV	RV	IVS	Ao	CaVC	T4
Observer1	Mean±SD	7.69±0.69	3.51±0.39	2.09±0.29	2.13±0.29	2.19±0.26	3.97±0.24
	95% CI	7.49-7.88	3.39-3.62	2.0-2.17	2.05-2.21	2.05-2.21	3.89-4.03
Observer2	Mean±SD	7.88±0.72	3.48±0.43	2.15±0.26	2.13±0.21	2.26±0.26	3.95±0.21
	95% CI	7.68-8.09	3.35-3.60	2.08-2.23	2.1-2.22	2.18-2.34	3.9-4.04

LV: Left ventricle, RV: Right ventricle, IVS: Interventricular septum, Ao: Aorta, CaVC: Caudal vena cava, T4: Fourth thoracic vertebra length.

Table 3. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for ratios of cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in male Wistar albino rats.

Observer		**Ratios**					
		LV/T4	RV/T4	IVS/T4	Ao/T4	CaVC/T4	CaVC/Ao
Observer1	Mean±SD	1.94±0.20	0.89±0.12	0.53±0.08	0.54±0.08	0.55±0.06	1.04±0.13
	95% CI	1.89-2.0	0.85-0.92	0.50-0.55	0.52-0.56	0.54-0.57	1.00-1.08
Observer2	Mean±SD	1.99±0.21	0.88±0.13	0.55±0.07	0.55±0.06	0.56±0.07	1.05±0.12
	95% CI	1.93-2.05	0.84-0.92	0.52-0.57	0.53-0.56	0.54-0.57	1.01-1.09

LV: Left ventricle, RV: Right ventricle, IVS: Interventricular septum, Ao: Aorta, CaVC: Caudal vena cava, T4: Fourth thoracic vertebra length.

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Positive correlation was observed between VHS and modified LA-VHS (r= 0.327, P< 0.021), between Ao and CaCV (r= 0.471, P< 0.001), between LV/T4 and RV/T4 (r= 0.505, P< 0.000), between RV/T4 and IVS/T4 (r= 0.321, P< 0.023) between Ao/T4 and CaCV/T4 (r= 0.472, P< 0.001), and between CaCV/Ao and CaCV/T4 (r= 0.307, P< 0.03). Negative correlation was observed between CaCV/Ao and Ao/T4 (r= -0.681, P< 0.000).

ICC evaluation revealed an agreement of 0.91 (95% CI: 0.85-0.95) for VHS, 0.76 (95% CI: 0.66-0.89) for modified LA-VHS, 0.93 (95% CI: 0.89–0.96) for LV, of 0.78 (95% CI: 0.64-0.87) for RV, 0.68 (95% CI: 0.49-0.80) for IVS, 0.78 (95% CI: 0.64-0.87) for Ao, 0.77 (95% CI: 0.63-0.89) for CaVC, 0.92 (95% CI: 0.88-0.97) for T4, 0.95 (95% CI: 0.92-0.97) for LV/T4, 0.86 (95% CI: 0.77-0.92) for RV/T4, 0.75 (95% CI: 0.60-0.85) for IVS/T4, 0.81 (95% CI: 0.69-0.89) for Ao/T4, 0.71 (95% CI: 0.54-0.83) for CaVC/T4, and 0.53 (95% CI: 0.29-0.70) for CaVC/Ao. Agreement between the observers may be therefore considered excellent for VHS, LV, T4, and LV/T4, good for modified LA-VHS, RV, Ao, CaCV, RV/T4, IVS/T4, and Ao/T4, and moderate for IVS, CaVC/T4, and CaVC/Ao.

Discussion and Conclusion

VHS, derived from measurements of the cardiac silhouette from thoracic radiographs, is based on a good correlation between silhouette size and the length of the thoracic vertebral body (34). This radiographic cardiac index is affected by respiratory phases and the cardiac cycle (7, 31). While the effect of the cardiac cycle on this index cannot be eliminated, the effect of respiration can be minimized by radiographs obtained at the inspiratory peak. Rats have a high respiration rate and cardiac cycle. Unfortunately, it is not possible to obtain thoracic radiographs in the inspiratory phase in non-intubated rats because intubation is both difficult and not routinely performed (13). In the present study, respiratory movement-related motion artifact was minimized with the radiographs obtained by positive pressure breath-hold at the time of radiographic exposure. The soft tissue opacity of the rat cranial ventral mediastinum due to soft tissues such as the thymus and intrathoracic fat often causes insufficient prominence of the cranial border of the heart (Figure 1A), especially if pulmonary inflation is poor. False VHS values may therefore be obtained from plain radiographs. Moreover, since only the cardiac silhouette is evaluated via this index, high VHS values can be obtained in the presence of pericardial effusion or pericardial fat. VHS is a reliable index for evaluating cardiac enlargement associated with eccentric hypertrophic cardiomyopathy (HCM) in patients with suspected heart disease, particularly due to volume overload (8, 9). However, the cardiac silhouette may be normal in size in concentric HCM, and VHS values can thus be obtained within the reference range. Contrast radiography made it possible to define all the cardiac borders, LV, RV, IVS, Ao, CaCV and the heart was also evaluated directly rather than using the cardiac silhouette in this study (Figure 2A).

VHS values obtained from healthy rats have been reported in previous studies (13, 15). The VHS values presented in the present study were higher than those obtained elsewhere from Sprague-Dawley rats (13). The difference between our values and those reported by Dias et al. (13) can be attributed to VHS being breed-specific in rats as well as dogs (4, 6, 33). Although Dogan et al. (15) also measured VHS in Wistar albino rats, the difference between the VHS values may be due to the fact that they used a different VHS measurement method (27) and obtained VHS values from elderly rats (10 months of age). Further studies are now needed to evaluate whether breed-associated variations and age would affect VHS in rats.

Quantitative evaluation of LA size is important in both pet and laboratory rats with suspected heart disease, similar to cats (19, 35) and dogs (29, 34). LA was subsequently further quantified by applying a VHS system for radiographic measurement of the LA, known as LA-VHS, in cats (35). Rats are comparable to cats due to their relatively standard thoracic conformation and size. The present study, therefore, evaluated the left atrium using the modified LA-VHS. Previous studies involving healthy cats have reported 1.0v (range: 0.72-1.30) (35) and 0.87±0.21v (19) values for LA-VHS. Schober et al. (35) explained that the portion of LA measured on thoracic radiographs is small, that it is difficult to divide a thoracic vertebra into 10 equal segments in cats, and that this is a potential cause of the error. We, therefore, modified the LA-VHS using the LA/T4 ratio. The LA can be evaluated more objectively in rats with this modification.

The cardiac cycle is a factor that directly affects ventricle and IVS measurement values. However, these measurement data may be useful as baseline values in the radiographic differential diagnosis of different forms of cardiomyopathy. Further studies are now needed to determine whether the differential diagnosis of cardiomyopathies (dilated *vs* hypertrophic, and eccentric *vs* concentric hypertrophic cardiomyopathy) can be established with these measurement values.

CaVC is visible on lateral thoracic radiographs as it runs from the diaphragm to the caudal border of the cardiac silhouette. Right heart function can be assessed by measuring the CaVC (2). In dogs and cats, dilatation of the CaVC is often used as an indicator of right-sided congestive heart failure (18, 24). In addition, dilatation of these vessels is also useful as a radiographic manifestation of heartworm disease (1, 25, 28), pericardial disease (18), pulmonic stenosis (20), tricuspid valve regurgitation, and dilated cardiomyopathy (24). The ratio of CaVC to other anatomic structures may permit quantitative analysis of the CaVC. Quantitative evaluation is therefore performed to compare the average diameter of the CaVC with the diameter of the Ao, and the length of the T4 (20, 24). The CaVC/Ao ratio is sensitive in cats with right heart failure compared to healthy animals (39). The CaVC/T4 ratio is less than 1 in healthy dogs and cats (24, 39). In the present study, the median CaVC/T4 ratio was 0.53. Similar to our results, the CaVC/Ao ratio is approximately equal to 1 in dogs (21).

This study has several limitations. The cardiac cycle, phase of respiration (despite our efforts to obtain radiographs at the inspiratory peak), hydration status, and intra-thoracic pressure may have interfered in the radiographic cardiac indices, measurements, and ratios obtained from this study.

The radiographs generated in this study were diagnostic, permitting accurate radiographic cardiac indices, measurements, and ratios in all animals.

In conclusion, this study established reference values for VHS, modified LA-VHS, and ventricle, IVS, Ao, and CaVC measurements on left lateral contrast thoracic radiographs from healthy, male Wistar albino rats. The results show that lateral thoracic contrast radiography is a simple, uncomplicated, and effective imaging technique for the quantitative evaluation of cardiac size and chambers and major thoracic vessels in healthy rats. Further studies are now needed to determine whether radiographic indices and ratios obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be useful as a reference baseline for both pet and laboratory rats.

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Conflict of Interest

We declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Author Contributions

MAÇ and MK conceived and planned the experiments. MK and MAÇ carried out the experiments. DB helped with experimental protocols. MK and MAÇ contributed to the interpretation of the results. MK and MAÇ wrote the original draft and contributed to reviewing and editing.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Animal Care Ethics Committee of Akdeniz University (no: B.30.2.AKD.0.05. 07.00/27).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Annette L, Clarke A, Rick A, et al** (2005): *Radiographic cardiac size in cats and dogs with heartworm disease compared with reference values using the vertebral heart scale method: 53 cases*. J Vet Cardiol, **7**, 33-40.
2. **Aycock KI, Campbell RL, Manning KB, et al** (2014): *A computational method for predicting inferior vena cava filter performance on a patient-specific basis.* J Biomech Eng, **136,** 081003.
3. **Bader M** (2010): *Rats models of cardiovascular diseases.* Methods Mol Biol, **567**, 403-410.
4. **Bagardi M, Locatelli C, Manfredi M, et al** (2022): *Breed-specific vertebral heart score, vertebral left atrial size, and radiographic left atrial dimension in Cavalier King Charles Spaniels: reference interval study.* Vet Radiol Ultrasound, **63**, 156-163.
5. **Black PA, Marshall C, Seyfried AW, et al** (2011): *Cardiac assessment of African hedgehogs (Atelerix albiventris).* J Zoo Wildlife Med, **42**, 49-53.
6. **Bodh D, Hoque M, Saxena AC, et al** (2016): *Vertebral scale system to measure heart size in thoracic radiographs of Indian Spitz, Labrador retriever and Mongrel dogs*. Vet World, **9**, 371-376.
7. **Brown CS, Johnson LR, Visser LC, et al** (2020): *Comparison of fluoroscopic cardiovascular measurements from healthy dogs obtained at end-diastole and end-systole.* J Vet Cardiol, **29**, 1-10.
8. **Buchanan JW, Bucheler J** (1995): *Vertebral scale system to measure canine heart size in radiographs.* J Am Vet Med Assoc, **206**, 194–199.
9. **Buchanan JW** (2000): *Vertebral scale system to measure heart size in radiographs.* Vet Clin North Am Small Anim Pract, **30**, 373-393.
10. **Camacho P, Fan H, Liu Z, et al** (2016): *Small mammalian animal models of heart disease*. Am J Cardiovasc Dis, **6**, 70-80.
11. **de Moura CR, das Neves Diniz A, da Silva Moura L, et al** (2015): *Cardiothoracic ratio and vertebral heart scale in clinically normal black-rumped agoutis (Dasyprocta prymnolopha, Wagler 1831).* J Zoo Wildlife Med, **46**, 314-319.
12. **Dias S, Anselmi C, Casanova M, et al** (2017): *Clinical and pathological findings in 2 rats (Rattus norvegicus) with dilated cardiomyopathy.* J Exot Pet Med, **26**, 205-212.
13. **Dias S, Anselmi C, Espada Y, et al** (2021): *Vertebral heart score to evaluate cardiac size in thoracic radiographs of 124 healthy rats (Rattus norvegicus).* Vet Radiol Ultrasound, **6**, 294-401.
14. **Dickson KV, Davies CW, Routh A, et al** (2016): *Radiographic cardiac silhouette measurement in captive*

livingstone's fruit bats (Pteropus livingstonii). J Zoo Wildl Med, **47**, 963-969.

15. **Dogan E, Okur S, Hayırlı A, et al** (2023): *Vertebral heart score and cardiothoracic ratio in Wistar rats.* Ankara Univ Vet Fak Derg, **70**, 43-47.
16. **Doss GA, Mans C, Hoey S, et al** (2017): *Verterbral heart size in chinchillas (Chinchilla lanigera) using radiography and CT*. J Small Anim Pract, **58**, 714-719.
17. **Garcia EB, Eshar D, Thomason JD, et al** (2016): *Cardiac assessment of zoo-kept, black-tailed prairie dogs (Cynomys ludovicianus) anesthetized with isoflurane*. J Zoo Wildl Med, **47,** 955-962.
18. **Gidlewski J, Petrie JP** (2005): *Therapeutic pericardiocentesis in the dog and cat.* Clin Tech Small Anim Pract, **20**, 151-155.
19. **Guglielmini C, Baron Toaldo M, Poser H, et al** (2014): *Diagnostic accuracy of the vertebral heart score and other radiographic indices in the detection of cardiac enlargement in cats with different cardiac disorders.* J Feline Med Surg, **16**, 812-825.
20. **Hashiyama JS, Gonda Y, Ishikawa H, et al** (2006): *Radiographic evaluation of caudal vena cava size as a useful parameter for the diagnosis of heart disease in dairy cattle.* J Vet Med Sci, **68**, 995-998.
21. **Johnson V, Hansson H, Mai W, et al** (2008): The heart and major vessels. 97-98. Schwar T and Johnson V (Eds). BSAVA Manual of Canine and Feline Thoracic Imaging. Wiley, ISBN: 978-0-905-21497-9.
22. **Jojima FS, Lucina SB, Santos AL, et al** (2019): *Use of measurements from thoracic radiographs to identify high mean left atrium pressure in dogs with myxomatous mitral valve disease.* Semina: Ciencias Agrarias, **40**, 191-202.
23. **Koo TK, Li MY** (2016): *A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research*. J Chiropr Med, **15**, 155-163.
24. **Lehmkuhl LB, Bonagura JD, Biller DS, et al** (1997): *Radiographic evaluation of caudal vena cava size in dogs*. Vet Radiol Ultrasound, **38**, 94-100.
25. **Lister A, Atkins C, Atwel R, et al** (2005): *Radiographic cardiac size in cats and dogs with heartworm disease compared with reference values using the vertebral heart scale method: 53 cases.* J Vet Cardiol, **7**, 33-40.
26. **Litster A, Buchanan JW** (2000): *Vertebral scale system to measure heart size in radiographs of cats.* J Am Vet Med Assoc, **216,** 210-214.
27. **Ljubica SK, Krstic N, Trailovic RD** (2007): *Comparison of three methods of measuring vertebral heart size in German shepherd dogs*. Acta Veterinaria (Beograd), **57**, 133-141.
28. **Losonsky JM, Thrall ED, Lewis RE** (1983): *Thoracic radiographic abnormalities in 200 dogs with spontaneous heartworm infestation*. Vet Radiol Ultrasound, **24**, 120-123.
29. **Malcom EL, Visser LC, Phillips KL, et al** (2018): *Diagnostic value of vertebral left atrial size as determined from thoracic radiographs for assessment of left atrial size in dogs with myxomatous mitral valve disease.* J Am Vet Med Assoc, **253**, 1038-1054.
30. **McInnes EF** (2012): Wistar and Sprague-Dawley rats. 15-17. In: McInnes EF (Ed), Background Lesions in Laboratory Animals A Color Atlas. 1st ed. Elsevier, St. Louis, Missouri.
31. **Olive J, Javar R, Specchi S, et al** (2015): *Effect of cardiac and respiratory cycles on vertebral heart score measured on fluoroscopic images of healthy dogs*. J Am Vet Med Assoc, **246**, 1091-1097.
32. **Onuma M, Ono S, Ishida T, et al** (2010): *Radiographic measurement of cardiac size in 27 rabbits.* J Vet Med Sci, **72**, 529-531.
33. **Puccinelli C, Citi S, Vezzosi T, et al** (2021): *A radiographic study of breed-specific vertebral heart score and vertebral left atrial size in Chihuahuas.* Vet Radiol Ultrasnoud, **62**, 20-26.
34. **Sanchez Salguero X, Prandi D, Labres-Diaz F, et al** (2018): *A radiographic measurement of left atrial size in dogs.* Ir Vet J, **71**, 25.
35. **Schober KE, Maerz I, Ludewig E, et al** (2007): *Diagnostic accuracy of electrocardiography and thoracic radiography in the assessment of left atrial size in cats: comparison with transthoracic 2-dimensional echocardiography*. J Vet Inter Med, **21**, 709-718.
36. **Sharp P, Villano J** (2012): Important biological features. 1. In: Sharp P, Villano J (Eds), The Laboratory Rat. 2nd ed. Boca Raton: CRC Press.
37. **Sleeper MM, Roland R, Drobatz KJ** (2013): *Use of the vertebral heart scale for differentiation of cardiac and noncardiac causes of respiratory distress in cats: 67 cases (2002–2003).* J Am Vet Med Assoc, **242**, 366-371.
38. **Stepien RL, Benson KG, Forrest LJ** (1999): *Radiographic measurement of cardiac size in normal ferrets*. Vet Radiol Ultrasound, **40**, 606-610.
39. **Vosugh D, Nazem N** (2019): *Radiological evaluation of caudal vena cava in domestic shorthair cats with regard to right heart failure diagnosis.* Bulg J Vet Med, **2**, 220-226.