

Radiographic cardiac indices for healthy New Zealand white rabbits: A reference interval study based on echocardiography

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ARTICLE INFO

Article History

Received : 29.11.2023

Accepted : 01.06.2024

DOI: 10.33988/auvfd.1396879

Keywords

Rabbit

Radiographic left atrial dimension

Reference range

Vertebral heart scale

Vertebral left atrial size

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How to cite this article: Kaya M, Çetinkaya MA (2025): Radiographic cardiac indices for healthy New Zealand white rabbits: A reference interval study based on echocardiography. Ankara Univ Vet Fak Derg, 72 (1), 105-112. DOI: 10.33988/auvfd.1396879.

ABSTRACT

This research was intended to identify reference intervals for the radiographic cardiac indices [vertebral heart scale (VHS), radiographic left atrial dimension (RLAD), and vertebral left atrial size (VLAS)] of 58 healthy, adult New Zealand white rabbits based on echocardiography. The VHS, VLAS, and RLAD measurements were taken from contrast right lateral (R) and ventrodorsal (VD) thoracic radiographs. The correlations between these radiographic cardiac indices and echocardiographic parameters were then evaluated. The mean values with a reference interval were 7.94 ± 0.31 vertebrae (v) (7.2-8.6 v) for R-VHS and 8.67 ± 0.33 v (7.8-9.2 v) for VD-VHS. The median values with a reference interval were 1.5 v (1-2 v) for VLAS and 1 v (0.7-1.4 v) for RLAD. Body weight and gender had no effect on radiographic cardiac indices. There were positive correlations between all radiographic indices obtained from the R contrast radiographs and the echocardiographic parameters ($r_s \geq 0.421$, $P < 0.0001$). Excellent intraobserver agreement was determined for the radiographic measurement methods (intraclass correlation coefficients ≥ 0.818). The contrast thoracic radiography appears to represent a useful technique for the accurate determination of radiographic cardiac indices. The findings can be used as reference values for radiographic cardiac evaluation in both pet and laboratory rabbits.

Introduction

Cardiac disease in rabbits, which are growing in popularity as pets, has become increasingly recognized (25). The prevalence of those diseases in pet rabbits is 2.6% (26). Cardiac disease can generally be classified as valvular valve diseases, cardiomyopathies, or arrhythmias (27). Rabbits have also been extensively used as animal models in experimental cardiovascular studies (9, 28, 35). Sophisticated diagnostic methods used in cardiac disease are becoming increasingly important for both pet and laboratory rabbits. Thoracic radiographs, electrocardiography, and echocardiography are useful in the definitive diagnosis, treatment planning, and monitoring of rabbit cardiac diseases (25). Although

echocardiography is one of the more important diagnostic processes in cardiac disease, it may not always be available to practicing veterinary clinicians (10). Thoracic radiographs provide critical information about cardiac disease by allowing the cardiac shape and size to be assessed. Vertebral heart scale (VHS) measurements, first described in 1995 for dogs (4), are an effective indicator of progressive heart enlargement. The VHS measurements have been reported in healthy rabbits (19, 24, 34), dogs (5), cats (16), and other animal species (3, 6, 8, 20). However, echocardiography-based VHS reference ranges in rabbits have not been reported. The left atrial (LA) dimension can be objectively assessed in dogs via vertebral left atrial size (VLAS) (17) and radiographic left

atrial size (RLAD) values (30). Radiographic cardiac indices have also been defined for healthy rats (6) and rats with hypertrophic cardiomyopathy (32) using the contrast thoracic radiography. However, they have not previously been reported for rabbits.

Similar to rats (13), the caudal vena cava (CaVC) and the cardiac cranial border in rabbits are usually unsatisfactory on lateral thoracic plain radiographs (25, 34). This can result in inaccurate measurements of radiographic cardiac indices. Our hypothesis was that the heart size and LA dimension can be evaluated more accurately, and independently of the heart silhouette since the measurement sites would be more prominent on thoracic contrast radiographs. This research was intended to identify reference intervals for the radiographic cardiac indices from the thoracic contrast radiographs of healthy, adult New Zealand white (NZW) rabbits based on echocardiography.

Materials and Methods

Animals: The experimental protocol was approved by the Akdeniz University animal care ethics committee (decision no. 2023.11.009/111). Fifty-eight NZW rabbits, 31 (53.44%) male and 27 (46.56%) female, were included in the analysis. The age and body weight range of the rabbits used in the study was between 9-17 months and 2.6-4.1 kg., respectively. All animals were normal based on physical examinations, thoracic radiographs, and echocardiography. Rabbits were considered normal if they

were free of abnormal heart and respiratory sounds, arrhythmia on auscultation, and in the absence of evidence of any pulmonary changes or radiographic findings of congestive heart failure, or thoracic vertebral abnormalities on the plain thoracic radiographs. They were also regarded as normal if no evidence of any cardiac morphological or hemodynamic changes was observed at echocardiography. The thoracic radiography and echocardiography were performed without using sedation or anesthesia.

Echocardiographic Measurements: Complete echocardiographic examinations were performed by a specialist (M.K.) with more than 20 years' veterinary echocardiography experience using an ultrasonographic unit (Mindray DC-80, Shenzhen Mindray Bio-medical Electronics, Guangdong, China) equipped with a sector probe (8-12 MHz). Echocardiographic measurements were obtained as previously described for rabbits (10, 34) based on the protocols established for standard views in dogs and cats (33). 2D-guided M-mode values were obtained between the papillary muscles and the mitral valve from the right parasternal short-axis view (Figure 1). The end-diastolic left ventricular internal diameter (LVIDd) measured from the short-axis M-mode was indexed to body weight (BW) according to the formula $LVIDdN=LVIDd\text{ (cm)}\div BW\text{ (kg)}^{0.294}$ (14). The method described by Hansson et al. (11) was used to calculate the LA to Ao ratio (LA/Ao) (Figure 2).

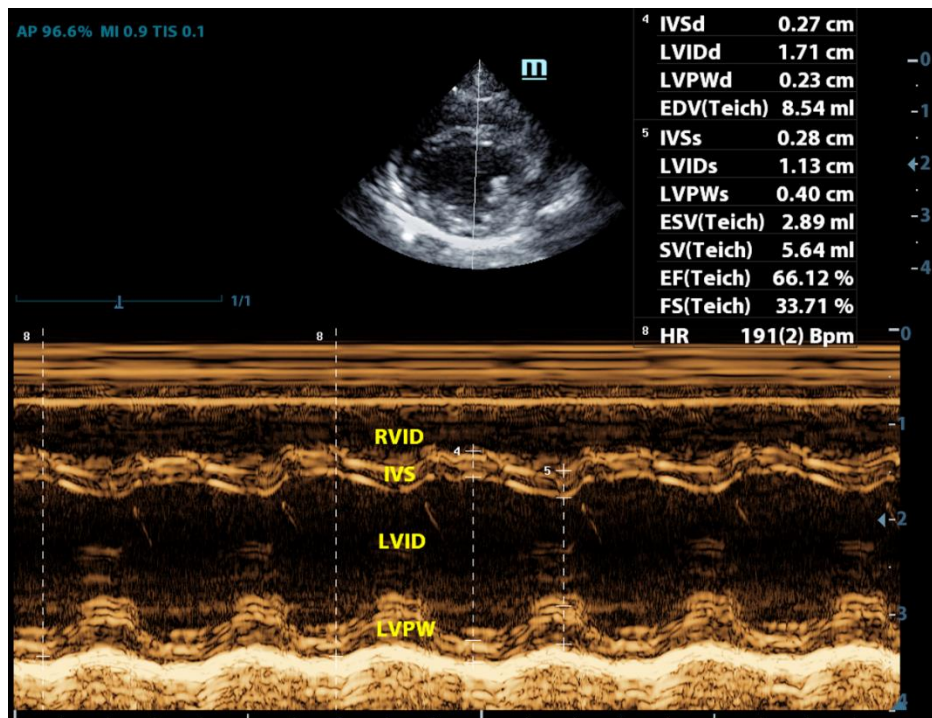


Figure 1. From 2D guided M-mode tracings between the papillary muscles and mitral valve in the right parasternal short axis view, interventricular septum (IVS) and left ventricular posterior wall (LVPW) thicknesses and left ventricular internal diameter (LVID) are measured in diastole and systole. RVID: right ventricular internal diameter, HR: heart rate.

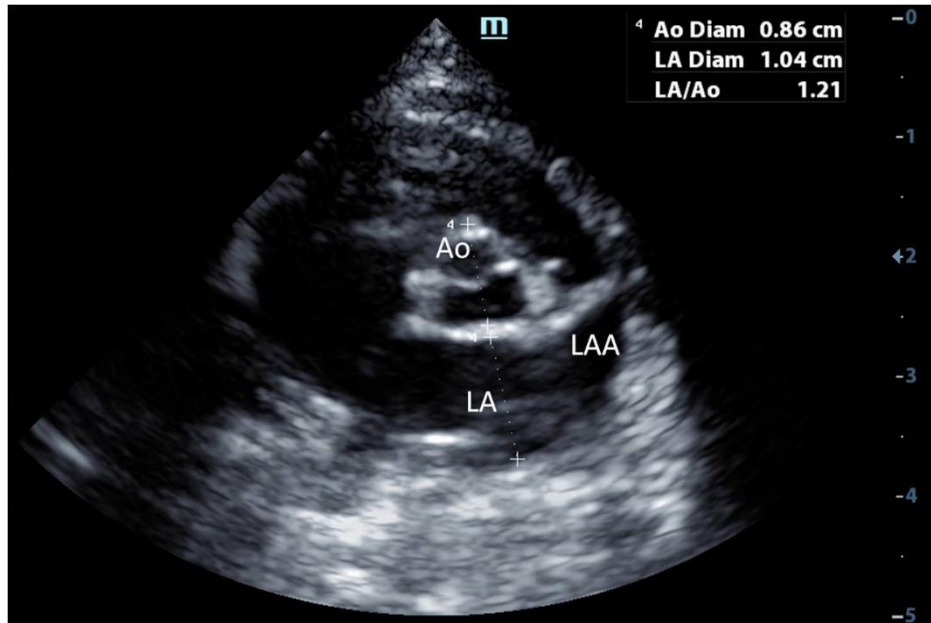


Figure 2. Measurement of the left atrium (LA) and aorta (Ao) from the 2D right parasternal short axis view at the level of the aortic root. LAA: left atrial appendage.

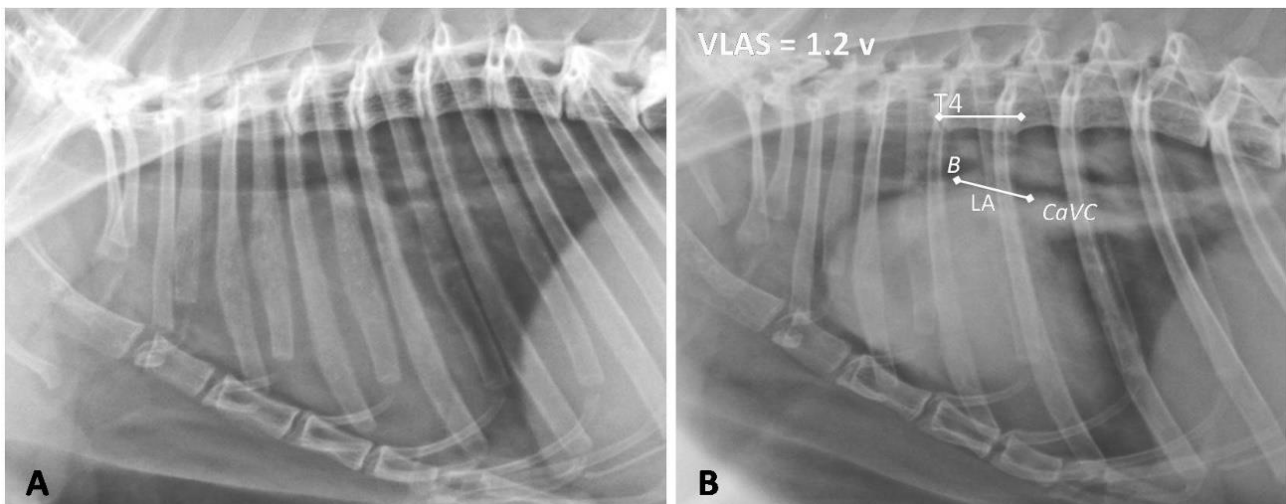


Figure 3. Plain (A) and contrast (B) right lateral rabbit radiographs (65 kVp, 8 mA, 0.6 s, 100 cm film-focus distance). VLAS measurement (LA) is made from the tracheal bifurcation to the intersection of most caudal aspect of the LA and the dorsal border of the caudal vena cava (CaVC) on a right lateral contrast radiograph. In contrast to the plain radiograph, the CaVC and the cranial border of the heart are visible in the contrast radiograph. T4: fourth thoracic vertebrae, B: tracheal bifurcation.

Radiographic Measurements: Each animal was imaged in the right lateral (R) and ventrodorsal (VD) positions. Both plain and contrast R radiographs were obtained (Figure 3). The VHS was measured on both contrast R and VD radiographs, while the VHS, VLAS, and RLAD were only measured from contrast R radiographs. The R contrast radiographs were obtained using a non-ionic opaque contrast agent (1 ml/kg Iohexol (300 mg I/ml), Opaxol®, Opakim, Istanbul, Türkiye) administered from the saphenous vein. Radiographic images were obtained using a computed radiography reader (FCR Prima T2, FujiFilm®, Tokyo, Japan). Two observers blinded to the

echocardiographic results performed the radiographic measurements using commercially available computerized software (Image Intelligence™, FujiFilm®, Tokyo, Japan).

The cardiac size was measured in two projections (R and VD), using the vertebral heart scale (VHS), as described by Buchanan (5). The cardiac long axis (L) (from the tracheal bifurcation to the cardiac apex) and short axis (S) (from the intersection of the caudal border of the heart with the dorsal border of the CaVC to the cardiac cranial border) were measured on the R view (Figure 3B). The L (from the intersection of the right mediastinal border with the cardiac silhouette to the apex)

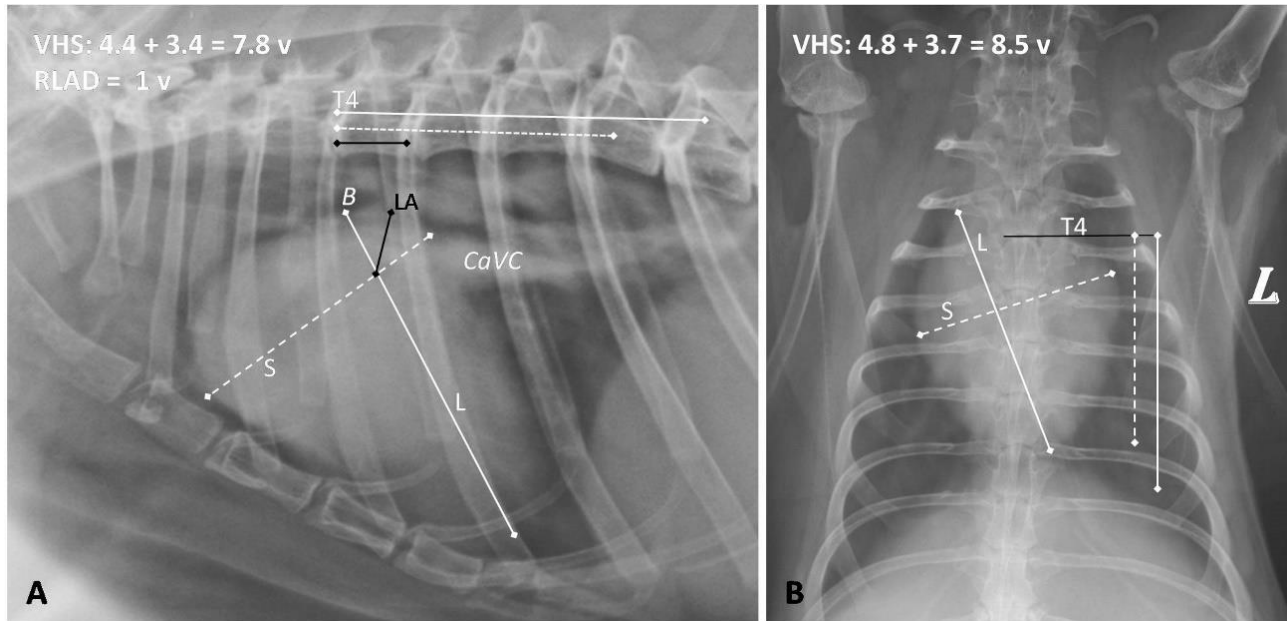


Figure 4. An example of VHS and RLAD measurements on contrast right lateral (A) and ventrodorsal (B) thoracic radiographs (65 kVp, 8 mA, 0.6 s, 100 cm film-focus distance). Long axes of VHS (L) are measured from the tracheal bifurcation to the cardiac apex for the right lateral view and from the intersection of the right mediastinal border with the cardiac silhouette to the apex for the ventrodorsal view. Short axes of VHS (S) are measured from the intersection of the caudal border of the heart with the dorsal border of the CaVC to the cardiac cranial border for the right lateral view and the widest part of the cardiac silhouette for the ventrodorsal view. RLAD measurement (LA) is performed from the intersection of L and S of the R-VHS to the dorsal edge of the LA at a 45° angle. T4: fourth thoracic vertebrae (v), B: tracheal bifurcation, CaVC: caudal vena cava, L: left side.

and S (widest part of cardiac silhouette) was measured on VD view (Figure 4B). In both projections the S was measured perpendicular to the L. The measurements of these two axes were converted to vertebral numbers (v) starting from T4. The sums of v on the L and S axes obtained from the R and VD views were used as the R-VHS and VD-VHS, respectively.

The VLAS (from the tracheal bifurcation to the intersection of most caudal aspect of the LA and the dorsal border of the CaVC) (Figure 3B) (30) and the RLAD (from the intersection of the L and S of the R-VHS to the dorsal edge of the LA) (Figure 4A) (17) were measured in the R projections. Similar to VHS, both measurements were converted to v, and VLAS and RLAD values were thus obtained.

Two different observers (M.A.Ç. and M.K.) reviewed the R and VD views of 10 randomly selected rabbits in order to assess interobserver agreement for the VHS, VLAS, and RLAD methods.

Statistical Analysis: To achieve a good concordance agreement with a literature and statistical significance α error (Type I) at β error (Type II, power) of 0.99, considering standard deviation (SD) for the VLAS as 0.45, sample size was calculated to be 21 (PS Power and Sample Calculations, Version 3.0 January 2009; Vanderbilt University, Nashville, TN). A commercial software (IBM SPSS Statistics 22.0, SPSS Inc., Chicago, IL) was used to

perform statistical analysis. The Shapiro-Wilk test was used to test data normality. Normally distributed data were expressed as mean \pm SD and skewed data as median and interquartile range (IQR). Gender differences were evaluated using the Mann-Whitney U test for the VLAS and RLAD and a two-sample t-test for the VHS values. The Pearson and Spearman's rank-order correlation coefficients (r_s) were applied to evaluate correlations between BW, all radiographic measurements (R-VHS, VD-VHS, RLAD, and VLAS), and echocardiographic data (LVIDd, LVIDdN, LA, and LA/Ao). Correlation coefficient values ranging from 0.1 to 0.3, 0.4 to 0.6, 0.7 to 0.9, or 1 signified weak, moderate, high, or perfect correlation, respectively (7). The differences between R-VHS in the current rabbit population and the mean reference values proposed in the previous literature were evaluated using a one-sample test. Values of 7.99 ± 0.58 v (24), 7.6 ± 0.32 v (19), 7.6 ± 0.39 v (34), and 7.55 ± 0.49 v (22) had previously been reported for R-VHS. Interobserver variabilities were evaluated for radiographic cardiac indices using intraclass correlation coefficient (ICC) estimations and 95% confidence intervals (CI) based on a single rater, absolute agreement, and a two-way random (interobserver) effect. ICC values greater than 0.81 were considered "good," 0.61 to 0.8 "significant," 0.41 to 0.6 "moderate," 0.21 to 0.4 "fair," and 0 to 0.2 "poor" (29). P values lower than 0.05 were considered statistically significant.

Results

The mean age, body weight (BW), and HR values of the 58 NZW rabbits were 11.5 ± 0.363 months, 3.17 ± 0.38 kg, and 231.4 ± 26.85 bpm, respectively. No animals exhibited any problems related to the contrast material, and no death mortality occurred. The cranial heart border, CaVC, and aorta were not visible on the R plain radiographs, whereas the borders of the heart, left atrium, and CaVC were well-defined on the R contrast radiographs (Figure 3A). The radiographic cardiac indices were thus measured with no difficulties using contrast R radiographs. The clarity of the heart borders on VD radiographs was sufficient to measure VHS.

Echocardiographic parameters are summarized in Table 1.

Table 1. The echocardiographic parameters in 58 New Zealand white rabbits.

Parameter	Mean \pm SD	95% CI	Range
LVIDd (cm)	1.34 ± 0.12	1.31-1.37	1.06-1.71
LVIDdN	0.97 ± 0.08	0.93-1	0.78-1.6
LA (cm)	0.89 ± 0.12	0.86-0.92	0.66-1.22
Ao (cm)	0.75 ± 0.09	0.73-0.78	0.59-0.95
LA/Ao	1.18 ± 0.14	1.15-1.22	1-1.46

Abbreviations: SD, standard deviation; CI, confidence interval; LVID, left ventricular internal diameter; LVIDdN, left ventricular end-diastolic internal diameter normalized for body weight; EF, ejection fraction; FS, fractional shortening; LA, left atrium; Ao, aort; LA/Ao, left atrial-to-aortic ratio; d, diastole.

The mean values (95% CI and minimum-maximum values) were 7.94 ± 0.31 v (7.83 - 8.04 v; 7.2 - 8.6 v) for R-VHS and 8.67 ± 0.33 v (8.58 - 8.77 v; 7.8 - 9.2 v) for the VD-VHS. The median values (IQR) were 1.5 v (1 - 2 v) for VLAS and 1 v (0.7 - 1.4 v) for RLAD. No differences in the radiographic cardiac indices were observed between females and males (Table 2).

Table 2. The mean \pm SD (minimum-maximum) and median (IQR) values of radiographic cardiac indices according to gender.

Gender	R-VHS	VD-VHS	VLAS	RLAD
Female	7.89 ± 0.36 (7.71-7.99)	8.62 ± 0.34 (8.41-8.72)	1.4 (1-1.9)	1.1 (0.7-1.4)
Male	8.04 ± 0.23 (7.95-8.12)	8.78 ± 0.26 (8.57-8.83)	1.5 (1.1-2)	1.1 (0.8-1.4)

Abbreviations: SD, standard deviation; ce interval; R, right lateral; VD, ventrodorsal; VHS, vertebral heart scale; VLAS, vertebral left atrial size; RLAD, radiographic left atrial dimension.

The NZW rabbits in this study exhibited a significantly higher R-VHS (7.94 ± 0.31 v) than the reference values of 7.6 ± 0.39 v, 7.6 ± 0.32 v, and 7.55 ± 0.49 v established by Moarabi et al. (19), Turner Garcia et al.

(34), and Ngosurachet et al. (22), respectively ($P < 0.002$), but were not significantly different from the reference value of 7.99 ± 0.58 v proposed by Onuma et al. (24) ($P = 0.637$).

No significantly significant correlation was found between BW and radiographic cardiac indices ($r_s = 0.031$, $P = 0.416$ for R-VHS; $r_s = 0.110$, $P = 0.223$ for VD-VHS; $r_s = 0.167$, $P = 0.124$ for RLAD; $r_s = 0.106$, $P = 0.231$ for VLAS). However, a positive weak correlation was determined between VD-VHS and LVIDd ($r_s = 0.289$, $P = 0.022$), and VD-VHS and LVIDdN ($r_s = 0.322$, $P = 0.011$), whereas there was a moderate positive correlation between R-VHS and LVIDd ($r_s = 0.538$, $P < 0.0001$), and R-VHS and LVIDdN ($r_s = 0.544$, $P < 0.0001$). A moderate positive correlation was observed between R-VHS and VD-VHS ($r_s = 0.687$, $P < 0.0001$). RLAD and VLAS exhibited moderate positive correlations with LA ($r_s = 0.421$, $P < 0.0001$ and $r_s = 0.506$, $P < 0.0001$, respectively) and LA/Ao ($r_s = 0.575$, $P < 0.0001$ and $r_s = 0.629$, $P < 0.0001$, respectively). A strong positive correlation was also determined between RLAD and VLAS ($r_s = 0.752$, $P < 0.0001$).

Interobserver variability assessed using ICC values demonstrated excellent agreement for all radiographic cardiac indices ($ICC \geq 0.818$, $P < 0.0001$, Table 3).

Table 3. Interobserver agreements for radiographic cardiac indices in New Zealand white rabbits.

Variable	ICC	95% CI	P value
R-VHS	0.894	0.827-0.935	0.0001
VD-VHS	0.818	0.711-0.888	
VLAS	0.961	0.935-0.977	
RLAD	0.953	0.932-0.975	

Abbreviations: ICC, intraclass correlation coefficients; CI, confidence interval; R, right lateral; VD, ventrodorsal; VHS, vertebral heart scale; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size.

Discussion and Conclusion

To the best of our knowledge, this is the first study to propose reference intervals for VHS, RLAD, and VLAS based on echocardiographic parameters in NZW rabbits. Based on cardiac measurements from thoracic plain radiographs, VHS is a valid method for assessing cardiomegaly associated with eccentric hypertrophy or dilated cardiomyopathy in patients with suspected heart disease (4, 5). The cardiac cranial border on lateral radiographs in normal rabbits is usually indistinguishable from the cranial mediastinum due to soft tissue opacity (thymus and intrathoracic fat) (34). The fat in the pericardium also causes the heart silhouette to appear larger, especially in obese rabbits (25, 27, 34). VHS measurements in rabbits may be adversely affected by all

these factors. Therefore, although the VHS reference intervals have been defined in healthy rabbits (19, 22, 24, 34), this is not widely used in clinical practice (25), in contrast to dogs and cats. Our mean R-VHS value was significantly higher than those in three previous studies (19, 22, 34), but not than that in Onuma et al. (24). The short axis of the VHS was measured from the dorsal border of the CaCV in order to include atrial enlargement (5). VHS measurements were carried out based on this slight difference in the present research, in contrast to previous studies (19, 22, 24, 34). Although the CaVC cannot be identified on lateral thoracic plain radiographs from rabbits (34), the visualization of the borders of the heart and the CaVC was quite adequate on R thoracic contrast radiographs, and we were able to measure VHS, RLAD, and VLAS independently of the heart silhouette in this study. R-VHS in our study was significantly higher than in three previous studies (19, 22, 34), but not compared to Onuma et al. (24). This difference is very likely due to both the use of contrast thoracic radiograms and to measurement of the cardiac short axis, in contrast to other studies.

VHS is a breed-specific index in dogs (29). Mean VHS values in some dog breeds such as the Bulldog, Boston terrier, Boxer, Pug, and Cavalier King Charles spaniel (1, 12, 15) are above the reference range reported by Buchanan and Bucheler (5). Although the feline thoracic structure is uniform, the VHS in Moon Cain cats is higher than in other cat breeds (23). Breed is also reported to affect VHS values in rats (6, 13). However, no difference has been determined between VHS values obtained from different rabbit breeds (22, 36). Although gender does not affect VHS in rabbits (19, 24, 34), BW does exhibit such an effect (24). Radiographic cardiac indices in the present study were not correlated with BW. This may be attributable to the values being obtained from rabbits weighing between 2.6 and 4.1 kg.

Ozawa et al. (26) reported that left-sided cardiac enlargement (54.1%) is relatively more frequently diagnosed than right-sided cardiac enlargement (51.4%), while the prevalence of both left- and right-sided cardiac enlargement was 27% in pet rabbits with cardiovascular disease. In that study, the most common diagnosis was degenerative valve disease (40.5%), followed by dilated cardiomyopathy (18.9%), unclassified cardiomyopathy (10.8%), restrictive cardiomyopathy (8.1%), and hypertrophic cardiomyopathy (5.4%). These cardiac pathologies, especially degenerative valve diseases, will eventually cause atrial enlargement. LA enlargement is a strong prognostic factor in dogs with mitral valve disease and a known prelude to congestive failure (17). LA dimensions can be objectively assessed in dogs. Myxomatous mitral valve disease can be diagnosed radiographically using VLAS and RLAD (15, 17, 18, 31).

Reported values in dogs without mitral valve disease are between 1.79 v and 2.1 v for VLAS (1, 2) and between 1.2 ± 0.34 v and 1 ± 0.23 v for RLAD (1, 17). The equivalent values in healthy rats are 1.95 v for VLAS and 1.3 v for RLAD (6). The VLAS and RLAD values in the present study were lower than those in both dogs and rats. Similar to VHS, VLAS and RLAD are breed-related indices in dogs (1, 2). Further research is now needed to determine whether these indices are breed-specific in rabbits. Echocardiographic LVIDdN (>1.7) is used as a criterion for determining the VHS threshold value for cardiomegaly in dogs (18, 30). The echocardiographic LA/Ao ratio (>1.6) is generally adopted as a criterion for determining VLAS and RLAD threshold values for predicting LA enlargement (17, 18, 30). A positive correlation has been shown between echocardiographic parameters (LVIDdN and LA/Ao) and radiographic cardiac indices (VHS, VLAS, or RLAD) (17, 21). The threshold values of echocardiographic LVIDdN and LA/Ao in rabbits have not been reported. According to the results of the present study, radiographic cardiac indices exhibit a positive correlation with echocardiographic parameters. The indices can thus permit quantitative assessment of cardiac size and LA dimension for veterinarians and researchers who experience difficulty in terms of echocardiographic evaluation. Further research is needed to determine the diagnostic value of these indices in rabbits with cardiac diseases characterized by cardiomegaly and LA enlargement.

In conclusion, the contrast thoracic radiography is an easy, effective, and uncomplicated imaging technique for an accurate measurement of the radiographic cardiac indices. The present study provides a reference interval for values of the VHS, VLAS, and RLAD and demonstrated that measurements of these indices are repeatable and reliable in rabbits.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study was approved by the Animal Care Ethics Committee of Akdeniz University (No: 2023.11.009/111).

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

MK and MAÇ conceived and planned the experiments. MK and MAÇ carried out the experiments. 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.

Animal Welfare

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

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