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Transthoracic echocardiographic reference intervals in 214 adult Golden retrievers

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ABSTRACT. This study describes the echocardiographic reference intervals in a large sample of Golden retrievers from the database of the Italian Veterinary Observatory for Cardiac Diseases. The echocardiographic variables and the cardiac functionality indexes obtained from 2012 to 2022 in 214 (122 females, 92 males) healthy adult Golden retrievers were compared with the previously published data for this breed and with data from the general canine population and other breeds. The diastolic left ventricular internal diameter and the left ventricle's systolic and diastolic allometric indices were higher than those reported for the general canine population ($P<0.05$). The same was true for the left ventricular end-diastolic and end-systolic volumes measured by Simpson's method of discs ($P<0.01$). Fractional shortening of the left ventricle was lower ($P=0.002$) and aortic flow velocity was higher ($P<0.001$) than the value previously reported for Golden retrievers. The reference intervals obtained in this study are useful for clinical echocardiography and for echocardiographic screening programs in Golden retrievers.

KEYWORDS: breed screening, cardiology, dog, echocardiography, Golden retriever

INTRODUCTION

Echocardiography is the main non-invasive diagnostic tool for the evaluation of canine heart, and it is essential for the cardiological screening of dogs [4, 6, 10, 12, 42, 53, 58]. There has been a growing interest in the identification of breed-specific echocardiographic reference ranges [5, 19, 24, 25, 31, 32, 37, 39, 43], especially in breeds predisposed to mitral valve disease or dilated cardiomyopathy (DCM) [1–3, 5, 18, 22, 30, 33, 36, 46, 49–51]. To our knowledge, few Golden retriever breed-specific echocardiographic reference intervals exist. The few published studies mostly focused on the normalized left ventricular volumes or internal diameters [2, 7, 21, 27, 56]. Therefore, echographers struggle to compare their clinical echocardiographic findings with published data. A recent small study described data from a small sample of echocardiographically healthy Golden retrievers [2]. Some of the echocardiographic parameters, including those considered indicative of DCM, were either at or above the upper limit reported in multi-breed studies [15, 21]. Furthermore, looking at the results of the published breed analysis in detail, almost all measurements of the Golden retrievers in that study were either within the generated body-weight-dependent prediction intervals or at least within the defined limits for "nondeviant breeds" (a breed was identified as a deviant breed if more than 10% of the measurements of dogs of this breed were above or below the corresponding prediction intervals) [21]. Consequently, investigators concluded that the generally applicable prediction intervals could be used for Golden retrievers, although breed-specific M-mode values, if available, might be preferred [15, 31]. Therefore, it is not possible to affirm whether some values are potential indicators of ventricular remodeling or are normal findings for Golden retrievers [2]. The higher values of the parameters suggestive of ventricular dilation and lower values of those predictive of reduced systolic function, associated with low levels of serum taurine, support the hypothesis of a possible greater predisposition of Golden retrievers to develop DCM or simply suggest a peculiar breed characteristic that is not necessarily related to a lower

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plasma concentration of taurine, and not even to an extreme body morphotype. Such differentiation would require examining a large sample of dogs fed a diet replete in taurine. For all these reasons, it is difficult to correctly interpret the echocardiographic findings in a Golden retriever, risking interpreting as abnormal something that could be normal for the breed. To the authors' knowledge, no veterinary study has published breed-specific echocardiographic data on Golden retrievers.

Based on these premises, we sought to 1) create reference intervals for transthoracic echocardiography in Golden retrievers, and 2) compare these values with previously published reference ranges for the general canine population and other medium-large breed dogs.

MATERIALS AND METHODS

This was a retrospective observational study. The database of the Italian Veterinary Observatory for Cardiac Diseases (Osservatorio Veterinario Italiano Cardiopatie−OVIC) [38] and the database of the cardiology section of the veterinary teaching hospital of the Department of Veterinary Medicine and Animal Science of the University of Milan (Lodi), were reviewed for echocardiographic examinations performed on Golden retrievers that had undergone cardiology screening from 2012 to 2022. Many of these dogs have been evaluated because, in Italy, there are breed screenings sponsored by kennel clubs and breeders and private owners willingly adhere to such evaluations. Data on signalment, clinical history, physical examination, and echocardiographic results, comprehensive cardiac functionality indexes, were collected. Dogs aged less than one year, and dogs affected by congenital or acquired cardiac disease or systemic diseases were excluded, such as dogs with echocardiographic parameters suggestive of DCM [14]. Pregnant and nursing females were also excluded from the study.

All echocardiographic examinations were performed by experienced operators, accredited by the OVIC board, using different ultrasound systems with phased-array transducers. Dogs were not sedated for the echocardiographic evaluation and were gently restrained both in right and left lateral recumbency on dedicated tables for echocardiographic evaluation. Standard M-mode, two-dimensional, Doppler echocardiographic images and videos were recorded with simultaneous ECG monitoring [48].

Echographers obtained the following 2D-guided M-mode measurements from the right parasternal short-axis view: the left ventricular internal diameter (LVID) in diastole (d) and systole (s) [15, 30, 35, 42], the left ventricular septal (IVSD) and free wall (LVFW) thickness. All the measurements were obtained with the leading edge-to-leading edge method. Diastolic and systolic LVID normalized for body weight (BW) (LVIDDn and LVIDSn −cm/kg) were calculated according to the formulas: $(LVIDd/10)/(BW)^{0.294}$ and $(LVIDs/10)/(BW)^{0.315}$ [7, 21]. The diastolic LVID (mm) normalized for body weight was also calculated using two other exponents (0.315 and 0.333), according to the recent literature [21, 57]. The left ventricular (LV) fractional shortening (FS) was calculated as (LVIDd-LVIDs/LVIDd) X 100 [7, 55, 56, 58]. The E-point to septal separation (EPSS) was obtained from the right parasternal short-axis view at the level of the mitral valve leaflets [7, 56]. Dilated cardiomyopathy was diagnosed if both LV dilatation and LV systolic dysfunction were present, defined as LVIDd >51 mm, LVIDs >35 mm [15, 30, 35], and FS <25% [56], EF <40% [7, 55, 58], EPSS >7 mm [7, 56].

The left atrial-to-aortic ratio (LA/Ao) was measured from the right parasternal short-axis view with B-mode, at the aortic valve level, in early diastole just after the aortic valve closure [41]. The B-dimensional aortic (Ao) and pulmonic (PA) annulus were measured from the right parasternal long-axis and short-axis views, respectively [11]. The index of aortic root dimension (wAo), useful as a conceptual aid to interpretations of results obtained in dogs with different weights, was calculated with the formula Ao_m/Ao_w, where Ao_m is the aortic root and Ao_w is calculated as $kW^{1/3}$, where W is body mass (kg) and k is a species-dependent constant (k=0.795 for dogs) [9]. The LVIDd was also indexed to Ao root annulus (LVIDd/Ao) [7]. The LV diastolic length was measured with B-mode from the apex to the mitral valve annulus in right parasternal long-axis view [20]. The LV sphericity index was calculated as the ratio of the LV long-axis diameter to short-axis diameter in end-diastole from the right parasternal 4-chamber long-axis view [20]. The LV volume in diastole (LVEDV) and systole (LVESV), and EF (as stroke volume/end-diastolic volume × 100) were obtained in B-mode from the left apical four chambers view [59], using Simpson's method of discs (SMOD) [7, 56, 57]. The LVEDV and the LVESV were then indexed to bodyweight (LVEDV-I and LVESV-I) and compared to reference intervals proposed in multi-breed studies [7, 21, 56–58]. These volumes were also indexed to body surface area (EDVI−end-diastolic volume index−and ESVI−end-systolic volume index) [59], calculated with the following formula: $0.101 \times BW\ (kg)^{2/3}$ [58].

Peak pulmonary flow velocity was measured with pulsed wave Doppler (PA_{Vmax}) with the sample volume placed distal to the pulmonic valve from the right parasternal short axis view optimized for the evaluation of the pulmonary trunk, without angle correction [8]. Peak aortic flow velocity (Ao_{Vmax}) was obtained from the retro-xiphoid view using continuous wave Doppler [10]. Transmitral flow was obtained from the left apical 4-chamber view with the pulsed wave sample volume placed between the tips of the opened mitral valve leaflets. Doppler E-wave and A-wave maximal velocities (E_{max}, A_{max}) were measured [44].

Statistical analysis was performed using SPSS™ 29.0 (IBM, Armonk, New York, NY, USA). Descriptive statistics were generated. All echocardiographic indices were collected and tabulated, tested for normality using a Shapiro-Wilk test, and tested for outliers using Tukey's method. The median, upper, and lower reference limits and 90% confidence intervals of the limits were calculated using an open-source spreadsheet add-in (Reference Value Advisor v. 2.1) [23, 53]. The outliers were identified, and the reference limits and confidence intervals were calculated using the non-parametric approach provided by that add-in. The echocardiographic variables were evaluated in a multivariable regression analysis (backward stepwise analysis), and regression coefficients were estimated (age, sex, and weight were considered as covariates). The goal of multiple regression analysis in this study was to describe and understand the relationship between echocardiographic measurements and age, sex, and weight of the dogs. A Student's *t*-test was used to compare the mean values of each echocardiographic variable with previously published studies on other breeds or the general canine population. If a study provided a median value, this was converted to the mean as previously described [29]. The results found in our sample were compared to previously published mean values obtained from a general population of dogs regarding LVIDDn, LVISDn, and LA/Ao [12, 41]. Simpson's derived EDVI, ESVI, EF, and SI were compared to the published reference values available for Dobermans and Boxers, and the general population of sighthound and not sighthound dogs [8, 15, 17, 21, 26–28, 34, 45, 47, 54, 57, 59]. The aortic size was compared using the wAo with previously reported values in the general canine population [9]. Lastly, Ao_{Vmax} and PA_{Vmax} were compared to the previously published mean values [8, 47]. Values of $P<0.05$ were considered significant.

Finally, to look for systematic bias in echocardiography by individual echographers, we plotted LVIDd, LVIDs, and LA:Ao against bodyweight for the six echographers who submitted 95% of the cases, and visually examined the plots to see if any echographer appeared to measure these variables differently from others. If they all measured similarly, all their data would be superimposed; if one or more echographers demonstrated systematic measurement bias, their data would separate from the other measurements. We did not formally test for bias, because visual inspection of the plots failed to reveal any obvious outlier observers.

RESULTS

We identified 546 Golden retrievers in the two databases (OVIC and University), but we excluded 332 dogs for the following reasons: incomplete clinical and echocardiographic data (51 dogs), impossibility of checking the echocardiography with a double check (crossing reported sheet data and echocardiography) (23 dogs), diagnosis of congenital heart diseases (40 dogs), myxomatous mitral valve disease (34), and DCM phenotype (184 dogs). Therefore, we included 214 apparently healthy Golden retrievers, consisting of 92 (43%) males and 122 (57%) females. The median BW was 31 kg (range 20.6–42 kg), and the median age was 2.51 years (range 1–11.5 years). The median heart rate was 98 ± 20 bpm (range 63–127 bpm). Cardiac auscultation revealed a low-grade systolic left apical murmur for 3/214 dogs. There were no signs of valvular anomalies in these three dogs, but the heart rate was above references (>140 bpm) with sinus tachycardia, so the murmurs were considered functional (for example, during exercise and stressful situations). Forty-eight dogs had trivial transvalvular tricuspid or mitral regurgitation but no signs of valve dysplasia or degeneration (thickening or prolapse). The reference intervals for all echocardiographic variables are reported in Tables 1 and 2. Multivariable regression analysis demonstrated that body weight accounted for all effects on left ventricular linear and volumetric measurements.

When we compared our results to previously published data for Golden retrievers we found that: 1) $LVIDDn^{0.294}$ did not differ from the value reported by Bagardi and colleagues in 2022 in a small study of 10 Golden retrievers (1.56 [CI 1.28–1.82] vs. 1.63 ± 0.17; P=0.373) [2]; 2) FS was lower than the value reported by Morrison and colleagues in 1992 in a study of 20 Golden retrievers

Table 1. M-mode and two-dimensional echocardiographic reference intervals obtained in a population of 214 healthy adult Golden Retrievers

	Variable	n	Median	Lower RI	(90% CI)	Upper RI	(90% CI)
M-mode	LVIDd (mm)	214	42.45	34.60	33.00–36.20	50.80	49.10–51.30
	LVIDs (mm)	214	28.75	20.20	17.90–21.40	36.30	35.40–37.00
	FS (%)	214	31.87	20.00	19.00–21.00	49.00	46.00–57.00
	EPSS (mm)	214	4.70	1.20	1.20–2.40	8.40	6.90–9.20
	$LVIDDn^{0.333}$ (cm/kg)	214	1.36	1.11	1.06–1.16	1.6	1.56–1.71
	$LVIDDn^{0.315}$ (cm/kg)	214	1.45	1.19	1.12–1.23	1.7	1.66–1.82
	$LVIDDn^{0.294}$ (cm/kg)	214	1.56	1.28	1.20–1.32	1.82	1.79–1.95
	LVIDSn (cm/kg)	214	0.92	0.65	0.58–0.67	1.17	1.13–1.20
	LVIDd/Ao	211	1.91	1.49	1.36–1.56	2.59	2.48–2.76
B-mode	Ao (mm)	211	17.40	14.70	13.10–14.90	24.8	23.10–26.00
	wAo	211	0.89	0.69	0.67–0.72	1.09	1.07–1-12
	PA (mm)	33	19.70	15.00	14.00–16.00	25	23.00–26.00
	Ao/PA	33	0.91	0.40	0.30–0.40	1.50	1.30–1.50
	LA/Ao	211	1.35	1.03	1.01–1.09	1.63	1.60–1.68
	SI	166	1.58	1.18	1.10–1.27	2.07	1.96–2.19
	EF (%)	156	59.55	38.64	35.22–41.29	78.11	76.08–79.69
	EDVI SMOD (mL/m^2)	103	66.81	45.14	40.47–47.09	97.04	90.34–101.18
	ESVI SMOD (mL/m^2)	105	26.10	12.09	10.79–14.10	45.05	44.07–47.13
	LVEDV SMOD (mL)	201	55.50	15.60	6.50–19.40	97.10	89.70–111
	LVESV SMOD (mL)	201	21.50	9.80	7.90–12.00	47.20	43.40–59.80
	LVEDV-I (mL/kg)	201	1.80	0.50	0.30–0.60	3.10	2.90–3.70
	LVESV-I (mL/kg)	201	0.70	0.30	0.29–0.30	1.60	1.40–2.00

RI, reference interval; CI, confidence interval; LVID, left ventricular internal diameter; d, diastole; s, systole; FS, fractional shortening; EPSS, E-point to septal separation; LVIDDn, left ventricular internal diameter in diastole normalized for body weight; LVIDSn, left ventricular internal diameter in systole normalized for body weight; LVIDd/Ao, left ventricular internal diameter in diastole to aortic annulus ratio; Ao, aortic annulus; wAo, index of aortic root dimension; PA, pulmonic annulus; Ao/PA, aortic annulus to pulmonic annulus ratio; LA/Ao, left atrial-to-aortic ratio; SI, sphericity index; EF, ejection fraction; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; SMOD, Simpson method of discs; LVEDV, end-diastolic left ventricular volume; LVESV, end-systolic left ventricular volume; LVEDV-I, left ventricular end-diastolic volume indexed to body weight; LVESV-I, left ventricular end-systolic volume indexed to body weight.

Table 2. Spectral Doppler echocardiographic reference intervals obtained in a population of 214 healthy adult Golden Retrievers

Variable	n	Median	Lower RI	(90% CI)	Upper RI	(90% CI)
Ao_{vmax} (m/sec)	208	1.51	0.66	0.58–0.81	2.2	2.13–2.24
PA_{vmax} (m/sec)	207	0.89	0.49	0.48–0.54	1.4	1.30–1.80
MV E (m/sec)	202	0.72	0.43	0.36–0.50	1.05	0.99–1.14
MV A (m/sec)	202	0.5	0.31	0.29–0.32	0.86	0.81–0.93
E/A	202	1.43	0.8	0.72–0.85	2.25	2.21–2.50

RI, reference interval; CI, confidence interval; Aovmax, peak aortic flow velocity; PAvmax, peak pulmonary flow velocity; MV E, mitral valve E wave velocity; MV A, mitral valve A wave velocity; E/A, mitral valve E and A velocities ratio.

(31.87% [CI 20.00–49.00%] vs. 39% [CI 27–55%]; P=0.002) [35]; 3) Ao_{Vmax} (m/sec) obtained with the PW Doppler was higher than the value reported by Hamabe in 2022 in 16 Golden retrievers (1.51 [CI 0.66–2.20] vs. 0.98 ± 0.15; P<0.001) [27]; 4) the E/A ratio (1.43 [CI 0.80–2.25] vs. 1.24 ± 0.33; P=0.013) was larger than the value reported by Hamabe in 2022 in 16 Golden retrievers [27].

Table 3 shows the comparison between study results and published data for the general canine population and some breeds predisposed to DCM phenotype development and systolic dysfunction, such as Dobermans and Boxers [15, 17, 21, 34, 47, 57, 59]. The LVIDd and LVIDDn were higher than non-sighthound breeds (P=0.009 and P<0.001, respectively) [21]. The LVIDSn was higher than the general canine population and non-sighthound breeds (P=0.002 and P<0.001, respectively) [15, 21]. The EDVI (SMOD) was higher than the general canine population (P<0.001), but lower than Dobermans (P=0.008) [45, 59]. The ESVI (SMOD) was higher than the general population (P<0.001) but lower than Boxers and Dobermans (P=0.016 and P=0.004, respectively) [45, 47, 59]. The LVEDV-I and LVESV-I were both lower than non-sighthound breeds (P<0.001 and P=0.0267, respectively) [57]. The aortic annulus was larger (P=0.009) [34] and the wAo was smaller (P<0.001) [17] than Boxers, whereas the pulmonary annulus was larger (P<0.001) [34]. The EF was lower than Boxers (P<0.001) [47]. The Ao_{Vmax} was higher than the general canine population and lower than Boxers (P=0.0165 and P<0.001, respectively) [8, 47]. The PA_{Vmax} was lower than Boxers (P<0.001) [47].

Finally, we could not identify any obvious systematic bias by echocardiographers for the tested variables (LVIDd, LVIDs, LA, and Ao) (Supplementary Fig. 1).

DISCUSSION

Our study provides echocardiographic reference intervals for Golden retrievers. Our data were acquired by multiple echocardiographers, satisfying the requirement for external validity (as compared to single-observer or single-institution-based studies). Finally, our study contains over 200 observations, substantially more than previous studies of Golden retrievers. Therefore, clinicians can consider our results robust.

Golden retrievers in our study had larger diastolic left ventricular internal diameters (absolute and normalized to body weight) than those reported by Esser for the population of all non-sighthound dogs [15, 21]. Similarly, dogs in our study had higher systolic diameters normalized to body weight than those reported by Esser and Cornell for both the general canine population and all non-sighthound dogs [15, 21]. These differences are likely due to breed-specific cardiac morphology. This confirms the recent literature reporting that the LVIDDn is breed-dependent [21]. The observed difference for LVIDd is in line with Esser: Golden retriever was found to be a "deviant breed" (more than 10% of the measurements of dogs of this breed were above or below the corresponding prediction interval) for this specific parameter [21]. The existence of specific reference limits for breeds that do not conform to allometric models of the general population should reduce the misclassification of healthy dogs as having left ventricular enlargement [40].

Our Golden retrievers had a larger aortic and pulmonary annulus than that reported in Boxers by Cunningham *et al*. 2008 [17] and Menegazzo *et al*. 2012 [34]. The dogs in our study had a lower EF than Boxers [47]. The values ESVI and EDVI obtained with B-mode with the SMOD method, were higher than those reported by literature for the general canine population [45], but lower than Dobermans [59]. The ESVI was lower than reported in Boxers [45, 47]. It is therefore possible that the differences observed in this study are due to the influence of the morphotype itself on cardiac volumes and geometry [40]. The ventricular morphology can also be a consequence of genetic selection. This is confirmed by the lower LVEDV-I and LVESV-I compared to the general canine population of non-sighthound breeds [40, 57].

The Ao_{Vmax} was higher than values reported by the literature for the general dog population, Boxers, Labrador, and Flat-Coated Retrievers [26, 27, 29, 47]. This value was also higher than the previous studies on Golden retrievers, most likely because of a difference in autonomic tone [27]. However, compared to the Japanese Golden retriever population, our dogs had a slightly smaller aortic annulus, which could also account for the slightly higher Ao_{Vmax} [27]. Conversely, Golden retrievers had lower pulmonary flow velocity than Boxers, consistent with their larger PA annular diameters [47]. The E and A waves were normal, but the upper limit of the ratio of the two waves was higher than the published limit (<2), maybe due to a better diastolic filling [8].

To assess left ventricular dilation, the LVEDV, LVIDd, and LVIDDn, the LVIDd/Ao and the SI need to be considered [7, 56]. To evaluate the systolic function, the LVESV, LVIDs, LVIDSn, EF, FS, and EPSS should be considered [7, 56]. The Golden retrievers included in our study showed mildly increased LV dimensions and decreased systolic function indices, compared to published reference limits. It is therefore important to stress the need to refer to specific echocardiographic breed parameters during routine

Table 3. Comparison between the obtained reference ranges and literature values for different parameters and general canine population and different breeds reported to be predisposed to dilated cardiomyopathy and systolic disfunction or that have body morphology similar to Golden Retrievers

Variable	Our study (n=214) Median and RI	Literature ranges (Median and RI or Mean ± SD)	*P* value	Reference	Comparison with:
M-mode					
LVIDd (mm)	42.45 (34.60–50.80)	42 (35–50)	0.121	Cornell *et al.* 2004 (n=494) [15]	General population (30 kg)
		41 (35–49)	**0.009**	Esser *et al.* 2020 (n=6,097) [21]	General population (All non-sighthound dogs)
LVIDs (mm)	28.75 (20.20–36.30)	28 (21–37)	0.178	Cornell *et al.* 2004 (n=494) [15]	General population (30 kg)
		28 (23–35)	0.607	Esser *et al.* 2020 (n=6,097) [21]	General population (All non-sighthound dogs)
LVIDDn (cm/kg)	1.56 (1.28–1.82)	1.5 (1.27–1.85)	0.159	Cornell *et al.* 2004 (n=494) [15]	General population (30 kg)
		1.38 (1.17–1.68)	**<0.001**	Esser *et al.* 2020 (n=6,097) [21]	General population (All non-sighthound dogs)
LVIDSn (cm/kg)	0.92 (0.65–1.17)	0.95 (0.71–1.26)	**0.002**	Cornell *et al.* 2004 (n=494) [15]	General population (30 kg)
		0.87 (0.70–1.09)	**<0.001**	Esser *et al.* 2020 (n=6,097) [21]	General population (All non-sighthound dogs)
2D					
Ao (mm)	17.40 (14.70–24.80)	17.4 ± 2.0	**0.009**	Menegazzo *et al.* 2012 (n=1,154, including dogs with subaortic stenosis) [34]	Boxers
wAo	0.89 (0.69–1.09)	0.91 (0.74–1.07)	**<0.001**	Cunningham *et al.* 2008 (n=81) [17]	Boxers
PA (mm)	19.70 (15.00–25.00)	17.4 ± 2.2	**<0.001**	Menegazzo *et al.* 2012 (n=1,126) [34]	Boxers
EF (%)	59.55 (38.64–78.11)	66.5 ± 6.4	**<0.001**	Smets *et al.* 2014 (n=85) [47]	Boxers
		49 ± 6	0.906	Wess *et al.* 2010 (n=123) [59]	Dobermans
EDVI SMOD (mL/m^2)	66.81 (45.14–97.04)	47.6 ± 8.4	**<0.001**	Serres *et al.* 2008 (n=24) [45]	General population
		70 ± 10	0.171	Smets *et al.* 2014 (n=85) [47]	Boxers
		72 ± 8	**0.002**	Wess *et al.* 2010 (n=123) [59]	Dobermans
ESVI SMOD (mL/m^2)	26.10 (12.09–45.05)	15.9 ± 3.9	**<0.001**	Serres *et al.* 2008 (n=24) [45]	General population
		36 ± 7	**0.016**	Smets *et al.* 2014 (n=85) [47]	Boxers
		38 ± 6	**0.004**	Wess *et al.* 2010 (n=123) [59]	Dobermans
LVEDV-I (mL/kg)	1.80 (0.50–3.10)	2.15 (1.25–3.27)	**<0.001**	Wess *et al.* 2021 (n=1,211) [57]	General population (Non-sighthound breeds)
LVESD-I (mL/kg)	0.70 (0.30–1.60)	0.88 (0.3–1.54)	**0.027**	Wess *et al.* 2021 (n=1,211) [57]	General population (Non-sighthound breeds)
Doppler					
Ao_{vmax} (m/sec)	1.51 (0.66–2.20)	1.15 ± 1.53	**0.017**	Bonagura *et al.* 1998 (n=15) [8]	General population
		1.80 ± 0.27	**<0.001**	Smets *et al.* 2014 (n=85) [47]	Boxers
PA_{vmax} (m/sec)	0.89 (0.49–1.40)	1.06 ± 1.38	0.169	Bonagura *et al.* 1998 (n=15) [8]	General population
		1.24 ± 0.25	**<0.001**	Smets *et al.* 2014 (n=85) [47]	Boxers
MV E (m/sec)	0.72 (0.43–1.05)	0.74 ± 0.89	0.747	Bonagura *et al.* 1998 (n=15) [8]	General population
MV A (m/sec)	0.50 (0.31–0.86)	0.46 ± 1.1	0.087	Bonagura *et al.* 1998 (n=15) [8]	General population

RI, reference interval; LVID, left ventricular internal diameter; d, diastole; s, systole; LVIDDn, left ventricular internal diameter in diastole normalized for body weight; LVIDSn, left ventricular internal diameter in systole normalized for body weight; EPSS, E-point to septal separation; Ao, aortic annulus; wAo, index of aortic root dimension; PA, pulmonic annulus; EF, ejection fraction; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; SMOD, Simpson method of discs; LVEDV-I, left ventricular end-diastolic volume indexed to body weight; LVESV-I, left ventricular end-systolic volume indexed to body weight; Aovmax, peak aortic flow velocity; PAvmax, peak pulmonary flow velocity; MV E, mitral valve E wave velocity; MV A, mitral valve A wave velocity.

echocardiographic evaluations. Only in this way, it is possible to discriminate healthy dogs and to properly diagnose DCM.

Unlike other studies of echocardiographic reference intervals in various dog breeds, we found no correlation between the echocardiographic measurements and sex [5, 16, 35, 52] that could not be accounted for by body weight alone. Consequently, we did not propose sex-specific reference intervals. This study has several limitations. First, the included dogs were enrolled only from Italy, although from different areas of the country. This could be an inclusion bias, with inadvertent over-representation of closely related dogs, which could affect echocardiographic measurements. Second, the dogs were fed with different diets, and data on food history were not recorded for all the included dogs. For this reason, we did not consider diet among inclusion criteria. Consequently, it is not possible to calculate the amount of taurine in the diet and to completely rule out mild myocardial dysfunction associated with taurine deficiency in a breed that is predisposed to the development of taurine deficiency [1–3, 20, 22, 36, 49]; however, dogs with overt echocardiographic evidence of DCM were excluded. Furthermore, due to the retrospective nature of the study, we could not follow dogs with borderline values longitudinally to exclude those with occult (echocardiographically undetectable) DCM. Third, the repeatability, intra-observer, and inter-observer measurement variability were not assessed for all the echocardiographic variables and all echocardiographers. For the repeatability test, we chose the six operators that performed the higher percentage of echocardiographic evaluation and the variables with the higher number of observations (LVIDd, LVIDs, LA, and Ao) (Table 1). However, all the echocardiographic examinations were performed by experienced operators belonging to the Italian Veterinary Observatory for Cardiac Diseases, which establishes a selective echocardiographic exam for the enrolment, including intra- and inter-observer measurement variability tests supervised by a recognized board including a board-certified cardiologist. This test is periodically carried out (every 1-to-2 years) to keep the quality standard of the echocardiographic skills of the group constant over time. To be approved and confirmed as an observer, the intra-observer must be lower than 10% and the inter-observer variability lower than 15% for all the echocardiographic variables included in the study [13]. We compared the results for LVIDd, LVIDs, LA, and Ao for six echocardiographers who submitted most of the echocardiographic data and could see no individual systematic bias by any observer. Fourth, we did not collect body condition score data, which could affect the reference intervals, if the sample population was particularly lean or underweight. The last limitation was that cases have not been followed up, so a subclinical cardiac disease such as DCM cannot be completely ruled out. However, because we created the reference intervals using a non-parametric approach, a small number of incorrectly categorized dogs (i.e., those with early signs of DCM) would be unlikely to alter the reference limits.

In conclusion, this is the first study providing echocardiographic reference intervals in a large number of healthy adult Golden retrievers. This breed tends to have bigger ventricular volumes and lower EF than the general canine population. In addition, the Golden retrievers show an increased aortic flow velocity compared to the general population. For this reason, the possible incidence of functional murmurs due to the increase in heart rate in healthy adult Golden retrievers should be considered during the physical examination for screening and/or clinical reasons and the aortic annulus must be always measured to rule out the presence of subaortic stenosis in a predisposed breed. These breed-specific echocardiographic features should be taken into consideration for an accurate echocardiographic interpretation and screening every time the cardiologist must evaluate a Golden retriever.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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