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Prediction of clinically important acquired cardiac disease without an echocardiogram in large breed dogs using a combination of clinical, radiographic and electrocardiographic variables☆

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Abstract *Introduction:* Large breed (LB) dogs develop dilated cardiomyopathy (DCM) and myxomatous mitral valve disease (MMVD). Echocardiography is required for a definitive diagnosis but is not always available. Our objective was to assess the clinical utility of thoracic radiographs alone and in combination with physical examination and electrocardiography findings for the prediction of clinically important DCM or MMVD in LB dogs.

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VLAS;
Murmur

Animals: Four hundred fifty-five client-owned dogs ≥20 kg with concurrent thoracic radiographs and echocardiogram.
Materials and methods: Medical records were reviewed and stored thoracic radiographs and echocardiographic images were measured to classify dogs as normal heart size (NHS), preclinical DCM, clinical DCM, preclinical MMVD (with cardiomegaly), clinical MMVD, or equivocal. Dogs with preclinical MMVD, without cardiomegaly, were classified as NHS. Vertebral heart size (VHS) and vertebral left atrial size (VLAS) were measured. Receiver operating characteristic curves and prediction models were derived.
Results: Prevalence of MMVD (39.3%) was higher than the prevalence of DCM (24.8%), though most MMVD dogs (67.0%) lacked cardiomegaly and were classified as NHS for analysis. The area under the curve for VHS to discriminate between NHS and clinical DCM/MMVD or preclinical DCM/MMVD was 0.861 and 0.712, respectively, while for VLAS, it was 0.891 and 0.722, respectively. Predictive models incorporating physical examination and electrocardiography findings in addition to VHS/VLAS increased area under the curve to 0.978 (NHS vs. clinical DCM/MMVD) and 0.829 (NHS vs. preclinical DCM/MMVD).
Conclusions: Thoracic radiographs were useful for predicting clinically important DCM or MMVD in LB dogs, with improved discriminatory ability when physical examination abnormalities and arrhythmias were accounted for.

Abbreviations

AUC	area under the curve
C-DCM	clinical dilated cardiomyopathy
C-MMVD	clinical myxomatous mitral valve disease
CHF	congestive heart failure
DCM	dilated cardiomyopathy
DP	Doberman Pinscher
ECG	electrocardiogram
LA	Ao: left atrial to aortic root ratio
LB	large breed
LVIDdN	normalized left ventricular internal diameter at end diastole
LVIDsN	normalized left ventricular internal diameter at end systole
MMVD	myxomatous mitral valve disease
NHS	normal heart size
PC-DCM	preclinical dilated cardiomyopathy
PC-MMVD	preclinical myxomatous mitral valve disease
PE	physical examination
ROC:	receiver operating characteristic curve
SI	sphericity index
VHS	vertebral heart size
VLAS	vertebral left atrial size

Introduction

In dogs, the likelihood of developing various cardiac diseases relates primarily to breed. While large-breed (LB) dogs are predisposed to idiopathic dilated cardiomyopathy (DCM) [1], they can also develop myxomatous mitral valve disease (MMVD), similar to small breed dogs [2], with a potential for their MMVD to progress faster and carry a more guarded prognosis [3]. When any acquired heart disease is suspected in a dog, echocardiography is the test of choice to obtain a definitive diagnosis and stage severity. Echocardiography is not routinely available, may be cost-prohibitive, and requires additional training to obtain and interpret echocardiographic images. Thoracic radiographs and electrocardiograms, in comparison, are readily available and may be used as screening tools in dogs that are suspected of having underlying cardiac disease, particularly in primary care settings.

Two objective radiographic measurements of cardiac size have been described in dogs, vertebral heart size (VHS) [4] and vertebral left atrial size (VLAS) [5]. The VHS measurement has been widely studied in the setting of small breed dogs with MMVD [6—10], as well as across a number of individual dog breeds to develop breed-specific normal reference ranges [10—18]. It has not been investigated in LB dogs with MMVD, however. Similarly, the relatively new VLAS measurement was proposed as a tool to recognize left atrial enlargement in MMVD dogs; however, most of the studied

population comprised small breed dogs [5]. Large breed dogs with MMVD have been noted to have a different echocardiographic phenotype compared with small breed dogs [3]; thus, previously proposed VHS and VLAS cut-offs derived from small breed MMVD studies may not be applicable to LB dogs with MMVD.

In the setting of DCM, neither VHS nor VLAS has been thoroughly investigated. Because of the potential for ventricular systolic dysfunction to precede the development of substantial diastolic ventricular dilation in preclinical DCM, the clinical utility of thoracic radiographs as a screening tool for this disease is suspected to be low, but objective data are lacking. Recent recognition of increased cases of diet-associated DCM phenotypes in dogs of all ages and sizes highlights the need for veterinarians to screen for a DCM phenotype in more dogs than ever [19,20].

The objectives of this study, therefore, were (1) to evaluate the clinical utility of thoracic radiographs for prediction of clinically important preclinical and clinical DCM and MMVD and (2) to explore the potential utility of prediction models incorporating radiographic measurements, physical examination (PE) findings and electrocardiogram (ECG) findings to predict clinically important DCM and MMVD in the absence of an echocardiogram in a large population of LB dogs. For both objectives, clinically important disease was defined as disease meeting a threshold for initiation of medical treatment.

Animals, Materials and Methods

The Texas A&M University Veterinary Medical Teaching Hospital's veterinary medical information system was searched to identify dogs evaluated by the cardiology service that were $\geq$20 kg in body weight that had an echocardiogram and thoracic radiographs performed concurrently between February 2010 and February 2019. Concurrent was defined as thoracic radiographs taken within 30 days of the echocardiogram if the dog was not receiving furosemide, or, if the dog was receiving furosemide, the radiographs and echocardiogram had to have been performed on the same day. Exclusion criteria included a diagnosis of congenital cardiac disease, infectious endocarditis, cardiac neoplasia, on-going heartworm disease or chronic right-sided cardiomegaly persistent after heartworm treatment, right-sided cardiomegaly secondary to Chagas disease, moderate to severe pulmonary arterial hypertension, pleural or pericardial effusion if more than trace in volume, and on-going systemic illness at the time of evaluation that could substantially alter volume status. In addition, if the echocardiogram and thoracic radiographs were not performed on the same day and pimobendan was initiated in the interim between the two diagnostic tests, dogs were ineligible for inclusion. Only one echocardiogram and thoracic radiograph pairing was used for any individual dog.

Data collected from the medical record on all dogs included breed, body weight, sex, date of birth, indication for cardiac evaluation, date echocardiogram and thoracic radiographs were performed, cardiac medications received, presence or absence and (if applicable) grade of heart murmur, presence or absence of gallop sounds, presence or absence of an auscultable arrhythmia and arrhythmia diagnoses at the time of the visit based on six-lead ECG or contemporaneous ECG monitoring during the echocardiographic examination.

Stored thoracic radiographs were assessed and measured by board-certified veterinary cardiologists (SG, AS) or cardiology residents (KC, BJ, DM) who were blinded to the echocardiographic diagnosis at the time of measurement. Each rater was provided with a list of patient identification numbers and dates to allow retrieval of each dog's digitally stored thoracic radiographs on a DICOM viewer, with lists arbitrarily divided among participators. Radiographs were not anonymized. The VHS measurement was performed as previously described [4], with the ventral border of the caudal vena cava used as the starting point for the short-axis measurement for consistency. The VLAS measurement was performed as originally described [5]. The quality of the landmarks required for each of the radiographic measurements was rated as excellent, diagnostic, poor, or inadequate to obtain a measurement.

Stored echocardiographic cine loops were reviewed by a single board-certified veterinary cardiologist who was blinded to the thoracic radiographs in all dogs. Images were not anonymized, with studies viewed in the order in which they were entered into the master data spreadsheet. The following echocardiographic measurements were remeasured on all stored studies, as available: left ventricular internal diameter at end-diastole, left ventricular internal diameter at end-systole, fractional shortening, left atrial to aortic root ratio (LA:Ao) [21], left ventricular area shortening (measured on the right parasternal short-axis view at the level of the papillary muscles), ejection fraction by Simpson's method of discs (measured on either the left apical four-

chamber views or the right parasternal long-axis view, pending availability of stored images and optimal alignment), E-point to septal separation, and sphericity index (SI). A subjective assessment of whether right atrial enlargement was present was also recorded. Left ventricular measurements were preferentially obtained from M-mode short-axis images, however two-dimensional short-axis images or two-dimensional long-axis images were also used if short-axis M-mode images were not available and/or if short-axis images were interpreted as oblique and inadequate for representation of accurate left ventricular dimensions. If the stored echocardiographic cine loops were inadequate in number or quality to allow for a diagnosis to be reached the dog was excluded from the study.

Included dogs were assigned to one of six study groups based on their echocardiographic diagnosis:

Group 1: Normal heart size (NHS). Normal echocardiographic chamber size and systolic function. In non-Doberman Pinscher dogs, normalized left ventricular internal diameter at end-diastole (LVIDdN) was defined as normal if <1.85 [22], while normalized left ventricular internal diameter at end-systole (LVIDsN) was defined as normal if <1.2. The LVIDsN criteria was based on the inclusion criteria used in a large, multicenter retrospective study of non-Doberman Pinscher dogs with occult DCM[c]. In Doberman Pinschers (DP), normal left ventricular dimensions were defined based on breed-specific data: left ventricular internal diameter at end-systole $< 0.142 \times$ body weight $+$ 35.3; left ventricular internal diameter at end-diastole $< 0.1749 \times$ body weight $+$ 40.3. Normal 2D short-axis LA:Ao was defined as ≤ 1.6 [21], with Boxers considered normal if ≤ 1.73 [23]. Right-sided cardiac chambers were evaluated subjectively. Systolic function was evaluated globally after considering all available measurements in a given dog, including LVIDsN, FS (normal $\geq$20% [24]), left ventricular area shortening (normal $\geq$40.7% [24]) and ejection fraction (normal $\geq$46.7% [24]), with LVIDsN required to be <1.2 for inclusion in this group. If one of the other measurements was abnormal, FS, for example, but the others (LVIDsN, left ventricular area shortening and ejection fraction) were normal, then the systolic function was considered normal. Dogs included in group 1 could have preclinical MMVD, but only if both left atrial and left ventricular dimensions were normal as defined above. In addition, dogs with arrhythmias were not excluded from group 1 as long as chamber dimensions and systolic function were normal as defined.

Group 2: Preclinical DCM (PC-DCM). Echocardiographic DCM phenotype with no current or previous clinical signs of congestive heart failure (CHF). In non-Doberman Pinschers, LVIDsN ≥ 1.2 was used for diagnosis[c]. In DP, weight-adjusted breed-specific left ventricular cut-offs were used [25].

Group 3: Clinical DCM (C-DCM). As per Group 2 (PC-DCM) with active CHF or CHF requiring chronic medical management.

Group 4: Preclinical MMVD (PC-MMVD). Echocardiographic confirmation of MMVD with clinically relevant left atrial and/or left ventricular dilation. This group included dogs classified as stage B2 according to the 2019 ACVIM consensus statement [2], as well as dogs in which the left atrium or left ventricle (but not both chambers) were enlarged based on the criteria outlined for group 1. Dogs with evidence of significant systolic dysfunction could be categorized into group 4 if their echocardiogram suggested that MMVD was the primary underlying disorder (i.e. severe mitral regurgitation and mitral valve remodeling), with systolic dysfunction interpreted as a secondary change.

Group 5: Clinical MMVD (C-MMVD). As per Group 3 (PC-MMVD) with active or compensated CHF requiring chronic medical management.

Group 6: Equivocal (EQ). Dogs were considered equivocal if they did not meet inclusion criteria for Group 2 (PC-DCM) and did not meet the inclusion criteria for Group 1 (NHS). For DP, this included dogs with left ventricular dimensions at end-diastole that were higher than breed-specific data used to define normal DP (as defined for group 1) but with end-systolic left ventricular dimensions beneath the cut-off required for inclusion in group 2 [25].

Statistical analysis

Descriptive statistics are reported as median (interquartile range), minimum and maximum for continuous variables based on significant Shapiro–Wilk tests for all but one continuous variable. In addition, descriptive statistics relating to VHS and VLAS for more commonly occurring breeds were reported as median (interquartile range), minimum and maximum, as well as mean $\pm$ standard deviation. Categorical variables

[c] Gordon SG, Boswood A, Häggström J, Summerfield NJ, Wess G. SHIELD Study Lead Investigator Committee. Snapshot SHIELD retrospective study. Abstract presentation: International Cardiology Veterinary Symposium (ICVS). Dubrovnik, Croatia. October 21–23, 2016.

are reported as proportions. Categorical variables were collapsed into groups for further analysis where appropriate based on clinical relevance or to increase numbers in categories with few observations. Selected groupwise comparisons of continuous variables used the Mann-Whitney test. Selected correlations were explored, and significant Spearman correlation coefficients (r_s) are reported.

Receiver operator characteristic curves (ROCs) were constructed for VHS and VLAS to evaluate their ability to discriminate between the NHS group and other groups or combinations of groups. Three cut-offs were selected and used for all comparisons for both VHS and VLAS. All cut-offs were selected based on ROC inspection for the ROC of the NHS vs. all other groups comparison and review of relevant literature[d] [2]. The lower VHS and VLAS cut-offs (10.5, 2.0, respectively) were selected for relatively high sensitivity (>80%). The middle cut-off was selected based on optimization of overall accuracy. The highest cut-off was selected for a high specificity (>90%).

Exploratory binary forward stepwise logistic regression analysis was used to develop prediction models using nine predictors derived from PE (six predictors), a right lateral thoracic radiograph (two predictors entered as continuous variables: VHS, VLAS) and an ECG (1 predictor: 1 = atrial fibrillation, 2 = any ventricular arrhythmia, 3 = no arrhythmia or any arrhythmia other than atrial fibrillation or ventricular arrhythmias) [26,27]. The six PE predictors included breed (1 = other, 2 = Boxer, 3 = DP, 4 = Labrador Retriever), age (years), weight (kg), sex (male, female), murmur (1 = none or soft [Grade 1&2], 2 = moderate [Grade 3&4], 3 = thrilling [Grade 5&6]) and presence of arrhythmia on auscultation (yes, no). The binary response variable was NHS dogs vs. all other dogs as a single group, followed by two sub-analyses: NHS dogs vs. all preclinical dogs (PC-DCM + PC-MMVD) and NHS dogs vs. all clinical dogs (C-DCM + C-MMVD). The best models for each of the three comparisons were developed by entering all nine candidate variables into the forward stepwise analysis, which was then carried out by the statistical program. The criteria for model entry was P<0.05 and P>0.05 for removal. The stopping criteria were 100 iterations = 100 and convergence = 0.00001.

To test the stability of the best model from the comparison of NHS vs. all other groups, a random sample of approximately 20% of the full population (n = 100) was held back as a validation sample, with these 100 dogs not analyzed as part of the training sample. In addition, in an attempt to mimic the clinical approach to diagnosis and staging of heart disease and explore the relative additive value of additional diagnostic testing, the following model iterations were developed for each of the three comparisons: six PE predictors, six PE predictors and ECG predictor, six PE predictors and two radiographic predictors. Finally, to explore the relative stability of the model and relative value of the two radiographic predictors, VHS and VLAS, the analysis was repeated with 6 PE predictors, one ECG predictor and either VHS or VLAS. Overall model significance was assessed by the Likelihood, Score and Wald tests. Assessment of the significance of individual predictors was evaluated using the Wald Chi-squared statistic for the retained regression coefficients. Goodness of fit was assessed by the Hosmer–Lemes test. Predicted probabilities were assessed through the construction of classification tables to report specificity, sensitivity and percent of correct classifications using a cut-point threshold of 0.5. Models were compared using the area under the curve (AUC). Statistical tests were considered significant if the P-value was <0.05.

Decision tree analysis was then performed using a variety of combinations of predictors to develop two decision tree algorithms. For the purpose of simplification, the three-category murmur predictor used in the regression analysis was collapsed into two categories: 1 = none/soft (0 to Grade 2), 2 = moderate and thrilling (≥Grade 3).

Results

Data from 455 dogs were included in the study analysis. Descriptive statistics summarizing the age, weight and sex distribution of the entire population and the population of each of the six groups are presented in Table 1. Only one dog in the NHS group was less than one year of age, a 4.5-month-old Neapolitan Mastiff weighing 23 kg. Across the entire population, dogs from 50 breeds were represented in addition to mixed breed dogs. The most commonly represented breeds overall were DP (n = 83), Labrador Retriever (n = 82), Boxer (n = 69), Golden Retriever (n = 19), mixed breed (n = 19), Great Dane (n = 17), American Pit

[d] Vitt JP, Gordon SG, Fries RC, Rhinehart JD, Achen SE, Sosa I, Estrada AH, Carlson JA, Winter RL, Kadotani K, Lamb KE. Utility of VHS to predict echocardiographic EPIC Trial inclusion criteria in dogs with myxomatous mitral valve disease: a retrospective multicenter study. Abstract presentation: ECVIM Forum. St. Julian's, Malta. Sept. 14, 2017.

Table 1 Age, weight and sex distribution across groups in 455 dogs.

Group	Age (years)	Weight (kg)	Sex	
			Total Male (Male castrated)	Total Female (Female Spayed)
Total Population N=455	8.7 (6.1–10.8) [0.4–17.0]	32.8 (26.3–39.4) [20.0–90.0]	268 (196)	187 (172)
Normal heart size N=271	9.1 (6.2–11.1) [0.4–16.3]	31.6 (25.4–37.7) [20.0–86.7]	145 (106)	126 (114)
Preclinical DCM N=50	8.9 (6.2–10.6) [2.0–17.0]	34.4 (30.2–40.0) [22.5–53.9]	34 (23)	16 (14)
Clinical DCM N=63	7.1 (5.0–8.7) [1.8–13.1]	38.4 (29.3–44.0) [22.0–75.0]	46 (34)	17 (17)
Preclinical MMVD N=39	10.5 (8.3–11.3) [2.7–14.8]	29.1 (24.5–38.3) [20.2–75.0]	22 (16)	17 (17)
Clinical MMVD N=20	10.0 (8.5–11.2) [2.8–15.6]	28.5 (22.4–34.8) [20.8–90.0]	13 (10)	7 (7)
Equivocal N=12	7.1 (4.9–8.3) [1.3–10.1]	34.8 (31.0–36.7) [25.8–67.0]	8 (7)	4 (3)

Data are presented as median (interquartile range) [range]. DCM: dilated cardiomyopathy; MMVD: myxomatous mitral valve disease.

Bull Terrier (n = 17) and German Shepherd (n = 15). In the NHS group, the most commonly represented breeds were Labrador Retriever (n = 58; 21.4%), Boxer (n = 46; 17.0%) and DP (n = 26; 9.6%). The most commonly represented breeds in the combined PC-DCM and C-DCM groups were DP (n = 44; 38.9%), Boxer (n = 19; 16.8%) and Labrador Retriever (n = 16; 14.2%) with 20 total breeds represented. The most commonly represented breeds in the combined PC-MMVD and C-MMVD groups were Labrador Retriever (n = 8; 13.6%), Golden Retriever (n = 6; 10.2%) and Border Collie (n = 5; 8.5%) with 26 total breeds represented. The complete representation of included breeds and their frequencies in each of the six groups is presented in Supplemental Table A.

Cardiovascular diagnoses for all dogs in the NHS group are presented in Supplemental Table B. Dogs with normal cardiac size and function were most common at 113 (41.7%), followed by dogs with MMVD having normal cardiac size and function at 103 (38.0%), dogs with normal cardiac structure and function with arrhythmias or conduction disturbances at 19 (7.0%) and dogs with MMVD having normal cardiac size and function in addition to a second abnormality (arrhythmia, conduction disturbance, or positive Chagas titer) at 17 (6.3%). For analysis, dogs were classified as 271 NHS, 50 PC-DCM, 63 C-DCM, 39 PC-MMVD, 20 C-MMVD and 12 EQ.

The indications for cardiac evaluation are summarized in Supplemental Table C. The most common indication was the appreciation of an arrhythmia in 110 dogs (24.2%), the appreciation of a heart murmur in 98 dogs (21.5%) and the presence of respiratory clinical signs (cough, tachypnea/dyspnea, excessive panting) in 59 dogs (13.0%).

On PE, heart murmurs were present in 51.9% of the total population, while auscultable arrhythmias were appreciated in 22.4% of the total population and gallop sounds were appreciated in only 1.8% of the total population. Table 2 presents the frequency of cardiovascular PE abnormalities across groups. Dogs with C-MMVD and C-DCM had the highest proportion of heart murmurs at 90.0% and 68.2%, respectively. The C-MMVD and C-DCM groups also had the highest proportions of dogs with auscultable arrhythmias at 70.0% and 46.0%, respectively. Although rare overall, 6/8 dogs with gallop sounds were in the C-DCM group.

The various types of diagnosed arrhythmias and conduction disturbances are presented in Table 3. Again, the C-DCM and C-MMVD groups had the highest proportion of their populations with one or more of the listed ECG abnormalities in Table 3 at 82.5% and 80.0%, respectively. The most common arrhythmia diagnosed in the NHS, PC-DCM, C-DCM, and PC-MMVD groups was single ventricular premature complexes, while atrial fibrillation was the most common arrhythmia in the C-MMVD group.

Descriptive statistics for VHS and VLAS measurements are presented in Table 4. The quality of the VHS measurement landmarks was excellent in 219 dogs, diagnostic in 200 dogs, poor in 29 dogs and unmeasurable in seven dogs, while the quality of VLAS measurement landmarks was excellent in 175 dogs, diagnostic in 197 dogs, poor in 66 dogs and unmeasurable in 17 dogs. Median VHS in NHS

Table 2 Cardiovascular abnormalities detected on physical examination across study groups presented as N (%).

Cardiovascular abnormality	Entire Population (N = 455)	Normal heart size (N = 271)]	Preclinical DCM (N = 50)	Clinical DCM (N = 63)	Preclinical MMVD (N = 39)	Clinical MMVD (N = 20)	Equivocal (N = 12)
Heart murmur	236 (51.9%)	117 (43.1%)	23 (46.0%)	43 (68.2%)	30 (76.9%)	18 (90.0%)	5 (41.6%)
Grade 1	8 (1.8%)	6 (2.2%)	2 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Grade 2	71 (15.6%)	47 (17.3%)	9 (18.0%)	12 (19.0%)	1 (2.5%)	1 (5.0%)	1 (8.3%)
Grade 3	84 (18.5%)	42 (15.5%)	6 (12.0%)	22 (34.9%)	9 (23.0%)	4 (20.0%)	1 (8.3%)
Grade 4	50 (11.0%)	18 (6.6%)	4 (8.0%)	9 (14.3%)	9 (23.0%)	8 (40.0%)	2 (16.7%)
Grade 5	15 (3.3%)	0 (0.0%)	1 (2.0%)	0 (0.0%)	9 (23.0%)	5 (25.0%)	0 (0.0%)
Grade 6	2 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.1%)	0 (0.0%)	0 (0.0%)
Grade not recorded	6 (1.3%)	4 (1.5%)	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
Auscultable arrhythmia	102 (22.4%)	31 (11.4%)	16 (32.0%)	29 (46.0%)	10 (25.6%)	14 (70.0%)	2 (16.7%)
Gallop sounds	8 (1.8%)	1 (0.4%)	0 (0%)	6 (9.5%)	0 (0.0%)	1 (5.0%)	0 (0.0%)

DCM: dilated cardiomyopathy; MMVD: myxomatous mitral valve disease.

Table 3 Documented arrhythmias and their frequencies across groups presented as N(%).

Arrhythmia or conduction disturbance	Entire population (N = 455)	Normal heart size (N = 271)	Preclinical DCM (N = 50)	Clinical DCM (N = 63)	Preclinical MMVD (N = 39)	Clinical MMVD (N = 20)	Equivocal (N = 12)
N (%) of population with 1 or more of the listed abnormalities	205 (45.1%)	89 (32.8%)	29 (58.0%)	52 (82.5%)	17 (43.5%)	16 (80.0%)	2 (16.7%)
N (%) of population with 2 or more of the listed abnormalities	85 (18.7%)	31 (11.8%)	17 (34.0%)	28 (44.4%)	3 (7.7%)	6 (30.0%)	0 (0.0%)
Supraventricular premature contractions	27 (5.9%)	11 (4.1%)	7 (14.0%)	7 (11.1%)	1 (2.5%)	1 (5.0%)	0 (0.0%)
Supraventricular tachycardia	15 (3.3%)	6 (2.2%)	4 (8.0%)	2 (3.2%)	3 (7.7%)	0 (0.0%)	0 (0.0%)
Atrial fibrillation	39 (8.6%)	2 (0.7%)	1 (2.0%)	18 (28.6%)	5 (12.8%)	13 (65.0%)	0 (0.0%)
Ventricular premature complexes	137 (30.1%)	62 (22.9%)	24 (48.0%)	33 (52.4%)	11 (28.2%)	6 (30.0%)	1 (8.3%)
Malignant ventricular arrhythmias[a]	68 (14.9%)	27 (10.0%)	13 (26.0%)	23 (36.5%)	2 (5.1%)	2 (10.0%)	1 (8.3%)
Sinus tachycardia	13 (2.9%)	7 (2.6%)	0 (0.0%)	5 (7.9%)	0 (0.0%)	1 (5.0%)	0 (0.0%)
Sinus bradycardia	5 (1.1%)	4 (1.5%)	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
First degree AV block	12 (2.6%)	7 (2.6%)	3 (6.0%)	2 (3.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Second degree AV block	6 (1.3%)	4 (1.5%)	1 (2.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Bundle branch block	6 (1.3%)	5 (1.8%)	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

[a] Couplets, multiform ventricular arrhythmias, and/or ventricular tachycardia. DCM: dilated cardiomyopathy; MMVD: myxomatous mitral valve disease.

dogs was 10.9 (interquartile range:10.2—11.5), while median VLAS was 2.1 (interquartile range:1.9—2.4). Additional analysis of VHS and VLAS was undertaken in the three most represented dog breeds in the NHS group. Breed-specific VHS and VLAS data were normally distributed; thus, mean values are presented in Table 4 in addition to the median (interquartile range).

Table 4 Descriptive statistics in 455 dogs for radiographic measurements across groups.

Group	VHS	Not able to measure VHS	VLAS	Not able to measure VLAS
Entire population (N = 455)	10.9 (10.2–11.5) [8.6–14.5]	N = 7 (1.5%)	2.1 (1.9–2.4) [0.8–3.8]	N = 17 (3.7%)
NHS (N = 271)	10.6 (10.1–11.1) [8.6–12.7]	N = 4	2.0 (1.8–2.1) [0.8–3.0]	N = 12
NHS Labrador Retriever (N=58)	10.8 ± 0.6 [10.8 (10.3–11.1)]	N = 0	2.1 ± 0.3 [2.1 (1.9–2.3)]	N = 0
NHS Boxer (N=46)	10.9 ± 0.8 [11.1 (10.3–11.5)]	N = 0	1.9 ± 0.4 [2.0 (1.7–2.1)]	N = 0
NHS Doberman Pinscher (N=26)	10.1 ± 0.5 [10.2 (9.7–10.4)]	N = 0	2.0 ± 0.3 [1.9 (1.8–2.1)]	N = 0
Preclinical DCM (N=50)	11.1 (10.6–11.8) [9.7–13.0]	N = 0	2.2 (1.0–2.5) [1.4–3.1]	N = 1
Clinical DCM (N=63)	11.9 (11.2–12.9) [9.5–14.5]	N = 2	2.5 (2.2–2.9) [1.9–3.4]	N = 2
Preclinical MMVD (N=39)	11.1 (10.6–11.7) [9.4–13.5]	N = 0	2.4 (2.1–2.6) [1.3–3.0]	N = 1
Clinical MMVD (N=20)	12.5 (11.7–13.0) [10.6–13.8]	N = 1	2.9 (2.6–3.1) [2.1–3.8]	N = 1
Equivocal (N=12)	10.1 (9.9–10.2) [9.6–11.4]	N = 0	2.1 (1.8–2.2) [1.6–2.5]	N = 0

Data are presented as median (IQR) [range] for all groups, as well as mean ± standard deviation for breed-specific values.
DCM: dilated cardiomyopathy; MMVD: myxomatous mitral valve disease; NHS: normal heart size; SN: structurally normal; VLAS: vertebral left atrial size; VHS: vertebral heart size.

Table 5 Receiver operating characteristic curve analysis for VHS and VLAS to discriminate between different study groups.

Groups compared	VHS						VLAS					
	N	AUC	95%CI AUC	Cut-off (≥)	SE%	SP%	N	AUC	95%CI AUC	Cut-off (≥)	SE%	SP%
NHS vs ALL	448	0.747	0.700, 0.794	10.5	82.3	43.8	438	0.786	0.742, 0.830	2.0	87.2	42.1
				11.5	50.3	87.6				2.2	72.6	75.3
				12.3	24.3	98.5				2.8	25.7	98.5
NHS vs All Clinical (C-DCM + C-MMVD)	355	0.861	0.813, 0.910	10.5	95.0	45.1	347	0.891	0.854, 0.928	2.0	98.8	41.9
				11.5	67.5	88.0				2.2	86.3	74.9
				12.3	41.3	98.5				2.8	43.8	98.5
NHS vs All Preclinical (PC-DCM + PC-MMVD)	363	0.712	0.648, 0.776	10.5	80.9	45.3	353	0.722	0.658, 0.787	2.0	79.3	42.1
				11.5	41.6	88.0				2.2	64.4	75.2
				12.3	12.4	98.3				2.8	12.6	98.5

AUC: area under the curve; CI: confidence interval; C-DCM: clinical dilated cardiomyopathy group; C-MMVD: clinical myxomatous mitral valve disease group; DCM: dilated cardiomyopathy; MMVD: myxomatous mitral valve disease; NHS: normal heart size; PC-DCM: preclinical dilated cardiomyopathy; PC-MMVD: preclinical myxomatous mitral valve disease; SE: sensitivity; SP: specificity; VLAS: vertebral left atrial size; VHS: vertebral heart size.

Receiver operating characteristic curve analysis and subsequent AUC values for the utility of VHS and VLAS to discriminate between the NHS group and other individual groups or combinations of groups is presented in Table 5. In all three ROC comparisons, VLAS outperformed VHS as a sole measurement based on AUC though the magnitude of the difference was small. In this population, when comparing NHS dogs to all other dogs, 82.6% of dogs with a VLAS <2.0 had normal heart size while 92.0% of dogs with a VLAS ≥2.8 were abnormal. Similarly, 78.5% of dogs with a VHS <10.5 had normal heart size, while 91.7% of dogs with a VHS ≥12.3 were abnormal. When comparing NHS dogs to clinical dogs (C-DCM + C-MMVD), 99.1% of dogs with a VLAS <2.0 had normal heart size,

while 89.7% of dogs with a VLAS ≥2.8 were abnormal. For VHS, comparing NHS dogs to clinical dogs, 96.9% of dogs with a VHS <10.5 had normal heart size, while 89.2% of dogs with a VHS ≥12.3 were abnormal. Finally, when comparing NHS dogs to dogs with preclinical disease (PC-DCM + PC-MMVD), 86.2% of dogs with a VLAS <2.0 had normal heart size, while 73.3% with a VLAS ≥2.8 were abnormal. For VHS, comparing NHS dogs to preclinical dogs, 87.9% of dogs with a VHS <10.5 had normal heart size, while 73.3% of dogs with a VHS ≥12.3 were abnormal.

Echocardiographic data across all groups are summarized in Table 6. In this LB population of dogs, VHS was positively, but not strongly, correlated with LVIDdN (r_s = 0.514; CI: 0.438—0.538; P<0.0001) and LA:Ao (r_s = 0.572; CI: 0.501—0.636; P<0.0001). Similarly, VLAS was positively, but not strongly correlated with LVIDdN (r_s = 0.505; CI: 0.427—0.576; P<0.001) and LA:Ao (r_s = 0.543; CI: 0.468—0.610; P<0.0001). Compared to dogs with preclinical disease (PC-DCM + PC-MMVD), dogs with clinical disease (C-DCM + C-MMVD) had a significantly larger LVIDdN and LA:Ao measurement (P<0.0001).

All reported logistic regression models and retained predictors were significant, and goodness of fit for all reported models was confirmed with a non-significant (P>0.05) Hosmer—Lemeshow statistic. Regression models with the highest AUC for each of the three comparisons, termed the 'best' models, are summarized in Table 7, with regression equations for each of these models summarized in Supplemental Table D.

The best model for each comparison included predictors from all three diagnostic tests (PE, radiographs, ECG), however, the maximum number of retained predictors in any model was six out of nine possible predictors, and in the three best models, only four or five predictors were retained. Overall, the most common retained predictors in the three best models were murmur, breed, ECG, VHS and VLAS. Based on AUC, the best model for each comparison was good to excellent (0.829—0.978), with the highest AUC associated with comparisons of NHS dogs to dogs with clinical stages of disease. Overall, the classification table results demonstrate high specificity for all models that included predictors from at least two diagnostic tests (80—100%), while sensitivity was generally lower than specificity for all models. Sensitivity was highest for comparisons that included dogs with clinical stages of disease. The stability of the best model for the comparison between NHS dogs and dogs from all other groups was confirmed by rerunning the analysis with a randomized holdout set of approximately 20% (n = 100).

Models developed from two diagnostic tests (PE and radiographs with eight possible predictors or PE and ECG with seven possible predictors) demonstrated the magnitude of the clinical value of adding a third diagnostic test and are presented in Supplemental Table E. A model that included only six PE predictors also demonstrated a relatively good AUC for all three comparisons (0.747—0.866) with the highest AUC associated with the NHS vs. all dogs with clinical disease comparison. Models were also developed using all PE predictors and ECG plus one radiographic predictor (either VHS or VLAS) to evaluate the relative value of VHS vs. VLAS. Overall, when VLAS and VHS were entered as the only radiographic predictor, the AUC was reduced compared with the best model for each of the three comparisons, but the relative AUC was not subjectively different.

The three most commonly retained PE predictors from the regression analysis (breed, murmur, auscultable arrhythmia) were entered into a PE decision tree analysis (Fig. 1). A second decision tree was developed with VHS and VLAS entered in addition to these three PE predictors (Fig. 2).

Discussion

Practicing veterinarians must frequently make decisions about the likelihood of underlying cardiac disease without the benefit of an echocardiogram. Thoracic radiographs and ECGs are often used as initial screening tests to help gauge the need for specialist referral, more frequent monitoring or the initiation of cardiac medications, with considerable research aimed at the clinical utility of radiographic measurements of heart size. Although much is known about the clinical utility of these tools in small breed dogs with MMVD, LB dogs have received less study. Large breed dogs also have more potential than smaller breeds to develop not one but two different acquired cardiac diseases, DCM or MMVD, with radiographs previously suspected to be less useful for identification of preclinical DCM due to the predilection for systolic dysfunction to occur before diastolic dilation.

In the current population, the overall prevalence of DCM (both clinical and preclinical) was 24.8%, while the overall prevalence of MMVD was 39.3% if clinical and preclinical MMVD dogs with and without heart enlargement were combined. While DCM is an important differential diagnosis in LB dogs, within this population, MMVD was more

Table 6 Descriptive statistics for echocardiographic measurements across groups in 455 dogs.

Echocardiographic parameter	Entire population (N = 455)	Normal heart size (N = 271)	Preclinical DCM (N = 50)	Clinical DCM (N = 63)	Preclinical MMVD (N = 39)	Clinical MMVD (N = 20)	Equivocal (N = 12)
LVIDdN	1.57 (1.43–1.79) [1.04–2.83]	1.46 (1.36–1.56) [1.04–1.80]	1.82 (1.70–1.91) [1.48–2.53]	2.09 (1.88–2.29) [1.64–2.83]	1.76 (1.57–1.95) [1.26–2.78]	2.02 (1.88–2.17) [1.69–2.34]	1.68 (1.58–1.70) [1.47–1.76]
LVIDsN	1.02 (0.90–1.28) [0.50–2.42]	0.91 (0.83–1.01) [0.50–1.19]	1.39 (1.30–1.54) [1.23–1.97]	1.73 (1.53–1.89) [1.28–2.42]	1.07 (0.99–1.18) [0.85–1.65]	1.20 (1.07–1.29) [0.98–1.61]	1.14 (1.13–1.16) [0.99–1.32]
LA:Ao	1.34 (1.18–1.73) [0.90–3.30]	1.22 (1.13–1.33) [0.90–1.68]	1.54 (1.33–1.82) [0.99–2.41]	2.15 (1.81–2.55) [1.40–3.30]	1.76 (1.68–1.89) [1.31–3.19]	2.50 (2.18–2.69) [1.65–3.06]	1.28 (1.23–1.37) [1.13–1.58]
Fractional shortening (%)	29.2% (21.3–34.2) [3.4–60.1%]	31.4% (28.1–35.9 [17.2–60.1%]	16.0% (11.9–18.7) [6.8–31.7%]	10.8% (8.4–13.6) [3.4–21.4%]	33.2% (28.3–38.5) [21.3–51.3%]	35.5% (28.9–41.4) [14.3–50.0%]	25.4% (20.5–27.6) [14.7–35.2%]
Ejection fraction (%)	57.7% (44.5–64.4) [11.4–81.3%]	62.2% (57.2–66.3) [46.3–81.3%]	38.8% (33.4–43.2) [18.8–53.3%]	24.1% (20.0–30.3) [11.4–45.8%]	66.3% (55.0–69.8) [42.6–78.8%]	59.0% (52.5–62.5) [35.0–72.7%]	53.7% (46.9–55.8) [30.5–60.1%]
Ejection fraction N/A	165 (36.2%)	105 (38.7%)	15 (30.0%)	21 (33.3%)	12 (30.7%)	8 (40.0%)	4 (33.3%)
LV area shortening (%)	50.5% (38.0–56.0) [9.0–76.0%]	53.4% (49.2–58.8) [34.8–76.0%]	27.9% (22.0–33.8) [14.9–44.6%]	18.9% (14.7–23.6) [9.0–38.9%]	55.2% (49.6–61.1) [34.5–71.0%]	51.6% (45.4–59.6) [30.9–70.6%]	46.6% (38.9–49.1) [33.0–60.2%]
LV area shortening N/A	15 (3.2%)	8 (3.0%)	0 (0.0%)	4 (6.3%)	1 (2.5%)	2 (10.0%)	0 (0.0%)
Sphericity index	1.48 (1.31–1.64) [0.88–2.25]	1.60 (1.47–1.72) [1.15–2.25]	1.38 (1.25–1.50) [1.05–1.86]	1.23 (1.10–1.35) [0.88–1.60]	1.36 (1.19–1.53) [0.94–1.93]	1.18 (1.10–1.24) [0.96–1.48]	1.58 (1.48–1.64) [1.28–1.68]
Sphericity index N/A	117 (25.7%)	83 (30.6%)	8 (16.0%)	7 (11.1%)	13 (33.3%)	5 (25.0%)	1 (8.3%)
EPSS (cm)	0.57 (0.40–1.10) [0.18–4.22]	0.46 (0.32–0.55) [0.18–0.83]	1.15 (0.90–1.42) [0.63–2.35]	1.71 (1.29–2.34) [0.64–4.22]	0.41 (0.34–0.58) [0.25–1.27]	0.61 (0.42–0.71) [0.29–1.10]	0.65 (0.62–0.73) [0.56–0.78]
EPSS N/A	228 (50.1%)	155 (57.2%)	18 (36.0%)	20 (31.7%)	18 (46.1%)	10 (50.0%)	7 (58.3%)
Subjective RAE	21 (4.6%)	0 (0.0%)	2 (4.0%)	15 (23.8%)	0 (0.0%)	4 (20.0%)	0 (0.0%)

Data are presented as median (interquartile range) [range].

DCM: dilated cardiomyopathy; EPSS: E point to septal separation; LA:Ao: left atrial to aortic root ratio; LVIDdN: normalized left ventricular diameter at end diastole; LVIDsN: normalized left ventricular diameter at end systole; MMVD: myxomatous mitral valve disease; N/A: measurement not available, RAE: right atrial enlargement.

Table 7 Summary of prediction models with the best performance for each of the three studied comparisons, as well as the validation cohort (n = 100) used to test the normal heart size (NHS) vs. all other groups comparison.

Model [disease category proportion]		NHS vs. All other groups [40.8%]	NHS vs. All other groups Validation cohort T [37.7%] V [51.0%]	NHS vs. Preclinical DCM and MMVD [24.9%]	NHS vs. Clinical DCM and MMVD [22.8%]
PE (6), ECG (1) & Radiographs (2) (Total of 9 candidate predictors)	AUC	0.892	0.896	0.829	0.978
	Number of Retained Predictors	5	5	4	5
	Retained Predictors (Listed in order from most to least significant)	VLAS, Breed, VHS, ECG, Mur	VLAS, ECG, Breed, VHS, Mur	Mur, ECG, VHS, Breed	VHS, Breed, ECG, Mur, VLAS
	Classification Table: Specificity %	89.68	T: 91.13, V: 95.92	96.91	98.08
	Classification Table: Sensitivity %	71.25	T: 67.48, V: 70.59	43.02	84.42
	Classification Table: % Correct	82.16	T: 82.21, V: 83.00	83.48	94.96

Percentages in brackets beneath each group description reflect the percent of diseased individuals in the comparison relative to the number of structurally normal dogs for which the diagnostics were available.
C-DCM: clinical dilated cardiomyopathy group; C-MMVD: clinical myxomatous mitral valve disease group; DCM: dilated cardiomyopathy; ECG: electrocardiogram; Mur: murmur; MMVD: myxomatous mitral valve disease; PC-DCM: preclinical dilated cardiomyopathy group; PC-MMVD: preclinical myxomatous mitral valve disease group; PE: physical examination; T: training set; V: validation set; VHS: vertebral heart score; VLAS: vertebral left atrial score.

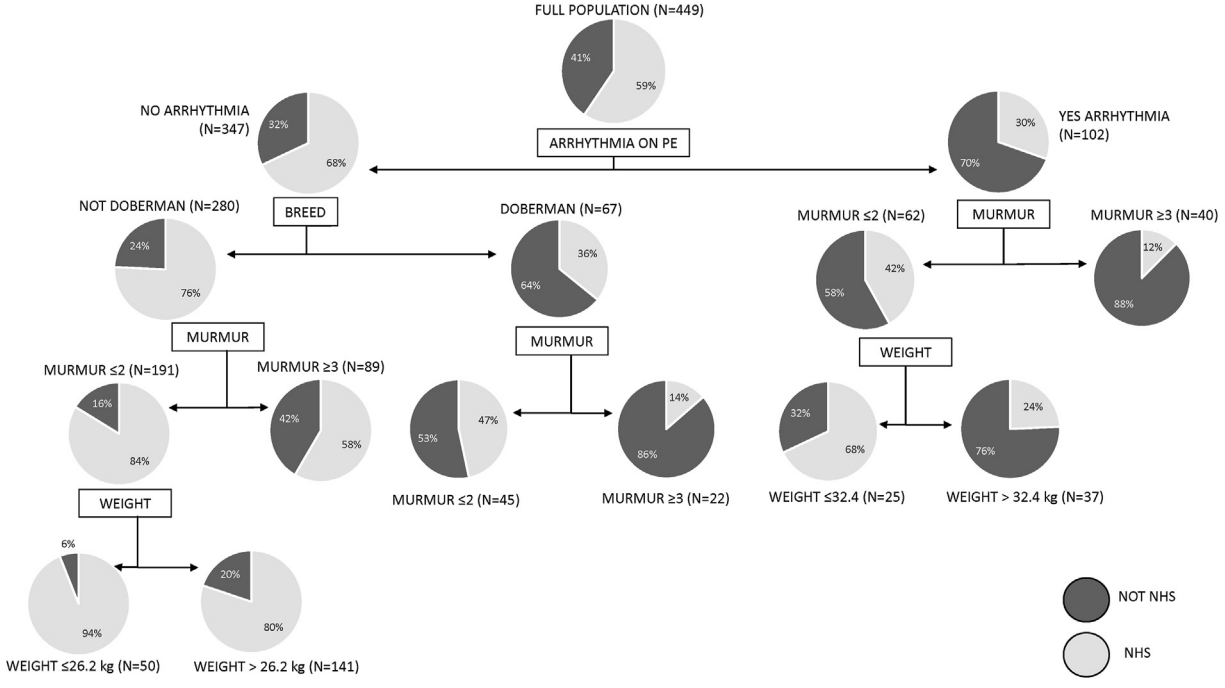

Fig. 1 Decision Tree Algorithm with three physical examination predictors. NHS: normal heart size; PE: physical examination.

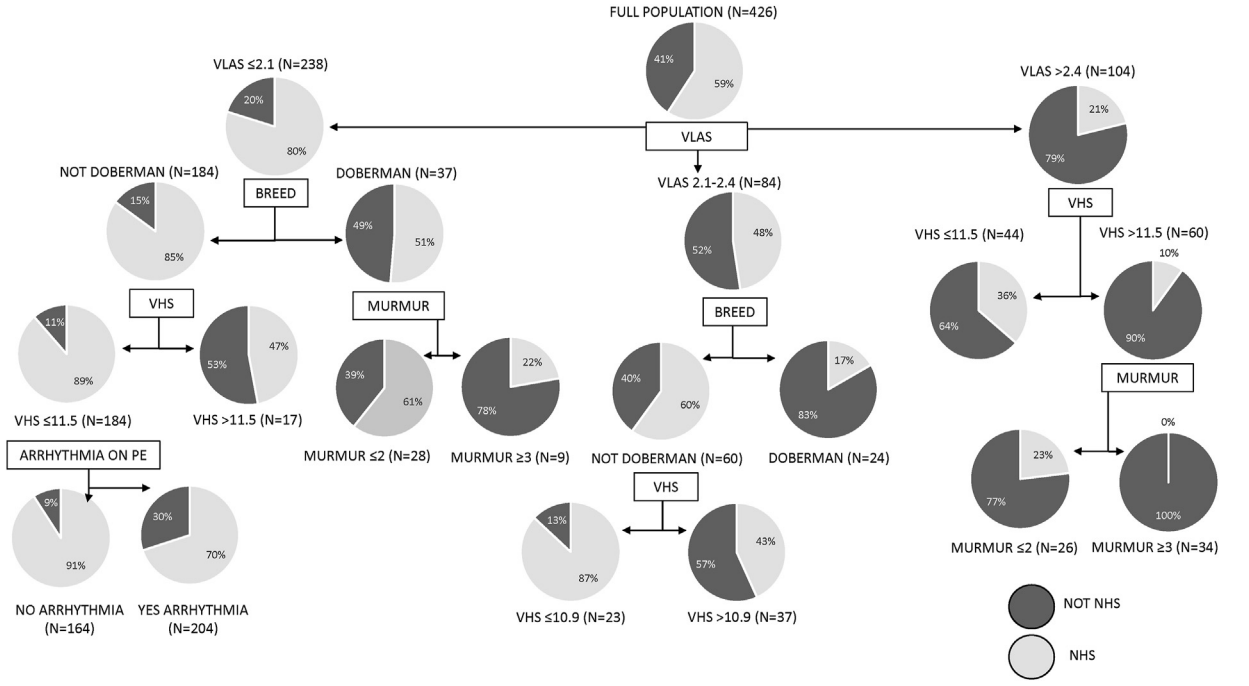

Fig. 2 Decision Tree Algorithm with murmur, breed, vertebral heart size and vertebral left atrial size. NHS: normal heart size; PE: physical examination; VHS: vertebral heart size; VLAS: vertebral left atrial size.

likely than DCM to actually be diagnosed. Of note, 67.0% of dogs with MMVD in this population had echocardiographic evidence of MMVD, but no secondary heart enlargement indicating that an early and clinically less important stage of this disease was most common. When considering dogs with clinically important disease, DCM was more likely to be diagnosed than MMVD, as only 13.0% of this population had MMVD that had either resulted in CHF or had advanced to the point that preclinical initiation of cardiac medication may have been considered.

Identification of asymptomatic LB dogs with clinically important DCM or MMVD arguably carries a similar level of importance from a treatment and follow-up perspective. Although there is strong evidence to initiate pimobendan in preclinical DP with DCM [25], as well as in preclinical Irish Wolfhounds with DCM [28], no studies have investigated the utility of pimobendan in other breeds with preclinical DCM. Similarly, while data from the EPIC trial strongly support the use of pimobendan in small breed dogs ($\leq$15 kg) with preclinical MMVD meeting specific criteria for left heart enlargement [29], evidence for the use of pimobendan in LB dogs with preclinical MMVD is lacking. Despite this, most practitioners extrapolate the knowledge gained from these prior trials [25,28,29] to non-DP/non-Irish Wolfhound DCM and LB MMVD populations and aim to institute treatment with pimobendan in the equivalent preclinical phase of both diseases. For a practicing veterinarian, the ability to identify LB dogs with clinically important phases of either disease is most clinically relevant. As such, we combined the PC-MMVD and PC-DCM groups and combined the C-MMVD and C-DCM groups for some analysis as a matter of practicality and clinical relevance.

With regard to radiographic measurements of heart size in this LB population, both VHS and VLAS were clinically useful screening tools. Within this population, for a dog with clinical signs that could be consistent with CHF, a VLAS $<$2.0 and a VHS $<$10.5 was associated with a 99.1% and 96.9% chance of having a normal-sized heart, respectively, suggesting CHF would be very unlikely. On the other hand, a similar dog with a VLAS $\geq$2.8 or a VHS $\geq$12.3 had an 89.7% and 89.2% chance of being abnormal, respectively, and was very likely to have underlying cardiac disease. Dogs with preclinical disease in this population were harder to identify with as much confidence, though a VLAS $<$2.0 and a VHS $<$10.5 still suggested an 86.2% and 87.9% chance of having a normal-sized heart, while a VLAS $\geq$2.8 or a VHS $\geq$12.3 both suggested a 73.3% chance of being abnormal and could reasonably prompt a recommendation for echocardiography to confirm whether disease is present.

In all three ROC analyses, VLAS marginally outperformed VHS in its ability to discriminate

between NHS dogs and dogs in other study groups despite slightly poorer correlations between VLAS measurements and echocardiographic measurements of left heart size compared to VHS. Currently, VHS is more widely used by veterinarians than VLAS. These findings suggest that training more veterinarians to use VLAS might be useful. In addition, median VLAS in this NHS population ($n = 271$) was 2.1 (IQR:1.9—2.4), which is closely aligned with the small control population ($n = 15$) of the study where VLAS was first proposed at 2.1 (IQR:1.8—2.3) [5], but larger than the median value of 1.9 (Range:1.3—2.2; proposed reference interval:1.4—2.2) from a larger population of 80 normal dogs [30]. The median body weight in both the previously published populations was substantially lower at 9.3 kg [5] and 11 kg [30] than the median body weight of 31.6 kg in this NHS population. Breed-specific differences in VLAS are likely important, similar to what has been appreciated with VHS across many breeds. A recent publication proposed a breed-specific VLAS reference interval in the Chihuahua breed (1.8 ± 0.2) [31] with our data providing additional insight into normal VLAS in the most commonly represented LB dogs in the NHS group (Labrador Retrievers, Boxers and DP), all of which appear to potentially range higher than the previously proposed general VLAS reference interval [30].

Going beyond thoracic radiographs alone, we sought to determine whether prediction models using information gained from the PE and ECG diagnoses in addition to VHS and VLAS could further improve a veterinarian's ability to predict clinically important DCM or MMVD in LB dogs. Our data revealed that these types of prediction models have good to excellent discriminatory potential, with several important points to highlight. First, both VHS and VLAS measurements remained significant in most of the models, suggesting an additive benefit to performing both measurements in all dogs despite the small advantage of VLAS over VHS when these measurements are used in isolation. In addition, for practitioners required to weigh which diagnostic test provides the most clinical utility in LB dogs suspected of having heart disease, prediction models were consistently better using PE findings in combination with VHS/VLAS compared to ECG diagnoses. This suggests that thoracic radiographs should always be the first diagnostic test of choice. If both radiographs and ECG can be performed, however, added predictive benefit is derived, with identification of ventricular arrhythmias and/or atrial fibrillation carrying important diagnostic weight. Furthermore, discriminatory potential using these models was quite good for both preclinical and clinical forms of DCM or MMVD but was particularly robust in clinical dogs that were in active or compensated CHF which may be especially helpful for improving practitioner confidence in a suspected diagnosis of CHF. Finally, while the regression equations derived from these prediction models can be used to calculate the probability of an individual LB dog having clinically important DCM or MMVD, the equations are cumbersome. They would be best used as a web-based tool for veterinarians in which all available data for a given dog can be input and a probability automatically derived, similar to what is available for a variety of diseases in humans. This is currently under development. In the interim, two decision tree algorithms developed based on data from this study can be useful for quickly gauging the probability that a given LB dog has clinically important DCM or MMVD. The PE algorithm emphasizes the relative clinical value of three PE predictors to identify dogs at the highest risk of clinically important DCM/MMVD and those most likely to have NHS. It clearly identifies DP to be different from all other breeds included in this study. The addition of VLAS and VHS to murmur, auscultable arrhythmia, and breed emphasizes the relative additive value of combining knowledge of key PE findings and objective radiographic measurement. Using this algorithm one can identify dogs with a very high probability of having NHS versus having clinically important disease in comparison to those that fall into more equivocal categories.

This study had several limitations. The retrospective nature of the study led to missing data points in some dogs, as well as an inability to consider the potential additive benefit that cardiac biomarkers may have provided to the prediction models, had they been measured. In addition, all of the study population had some indication that prompted the concurrent echocardiogram and thoracic radiographs; thus, selection bias is likely. Arrhythmia diagnoses were also made in some dogs based on arrhythmias appreciated during an echocardiogram, which reflects a longer period of ECG monitoring than would be typical for a standard six-lead ECG. It is possible, therefore, that some arrhythmias that were accounted for in this population may have been missed had only a shorter six-lead ECG been performed. With regard to radiographic measurements, operator training, radiograph quality and measurement repeatability all may impact the clinical utility of VHS and VLAS, with our data reflective of measurements made by board-certified cardiologists or cardiology residents. While our

measurements were made on radiographs with a range of diagnostic quality, they may not be interchangeable with measurements made by less experienced operators. It is also important to acknowledge that regression models have a tendency to overfit data. While we attempted to assess this by holding back a cohort of dogs to test our best model and model performance remained stable, ideally, all of our regression equations should be tested with a prospective validation cohort for confirmation of performance. In addition, multiple models that included multiple variables were included in this analysis, which may have increased the probability of type I error. Furthermore, a subanalysis of the models for the NHS vs. clinical disease and NHS vs. preclinical disease comparisons would ideally have had a higher ratio of events/variables to improve model stability, though the events/variable ratios were likely robust enough to support the nine variables included in these models. Finally, a few dogs in the MMVD groups were young enough that mitral valve dysplasia could be a plausible differential, despite a lack of obvious anatomic evidence on the echocardiographic cine loops available for review.

In addition to limitations inherent to the study as laid out above, there are also limitations related to the classification of disease in LB dogs. While DP have breed-specific criteria for DCM screening and diagnosis [25,32], the diagnosis of DCM in other breeds remains more challenging and open to debate. An updated DCM classification system for non-DP dogs is overdue. The last set of general guidelines for the diagnosis of DCM in dogs was published in 2003 and used a scoring system assigning point values for major and minor criteria [1]. Given the potential for missing data points in a retrospective study, we chose to use a simpler definition of DCM in non-DP dogs rather than the larger scoring system, and instead used LVIDsN $\geq$1.2 as our defining criteria, in accordance with an ongoing non-DP study of DCM in dogs[c]. When considering the previous DCM diagnosis guidelines, one measurement worth revisiting after a review of the present data is SI. An abnormal SI was defined in the 2003 guidelines as $<$1.65 and was classified as a major criterion supporting a DCM diagnosis [1]; however, this cut-off referenced data derived only from Newfoundland dogs [33]. Data from a subsequent study in DP supported the SI cut-off value of $<$1.65 to differentiate normal DP from those with DCM, but noted considerable overlap between control and DCM groups, calling into question the true utility of this measurement [34]. Data from our study further questions the utility of the previously proposed SI cut-off, as our NHS population with a more diverse breed population, had a median SI of 1.60. Future DCM guidelines should weigh whether SI remains a useful measurement for diagnosis of DCM, with a re-evaluation of the most clinically relevant cut-off.

Conclusions

Both VHS and VLAS were useful sole measurements for predicting clinically important DCM and MMVD in LB dogs in the absence of an echocardiogram. Prediction models that incorporate VHS and VLAS alongside PE abnormalities and arrhythmia diagnoses have an even greater discriminatory ability to identify clinically important DCM or MMVD.

Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvc.2021.07.003.

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