

ELECTROCARDIOGRAPHY, ECHOCARDIOGRAPHY, AND VERTEBRAL HEART SCORE IN HEALTHY LOCAL EGYPTIAN MALE DOGS: BIAS CORRECTED BOOTSTRAP REFERENCE INTERVALS

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Abstract: The Egyptian domestic (Baladi) dog has been increasingly adopted as a companion animal. Breed-specific cardiovascular reference intervals (RIs) are useful for clinicians in identifying abnormalities. The study aimed to estimate RIs for different heart indices in healthy male Baladi dogs. Twenty-six dogs were recruited to the study. Dogs were approved to be healthy. Afterwards, they were consciously examined by electrocardiography, echocardiography, and radiography. RIs were estimated using a bias-corrected bootstrapping approach. Electrocardiographic RIs were heart rate: 103 – 132 (beats/minute); P: 0.02 - 0.04, T: 0.03 - 0.04 waves durations (seconds); P: 0.13 - 0.23, R: 1.19 - 1.51, T: 0.20 - 0.27 waves amplitudes (mV); and PR: 0.11 - 0.17, QRS: 0.06 - 0.08, QT: 0.41 - 0.44 intervals (seconds). Echocardiographic parameters included end (diastolic: 29.95 - 46.49, systolic: 5.54 - 10.32) volumes (ml); ejection fraction: 76.23 - 83.41 (%); stroke volume 25.13 - 37.41 (ml); fractional shortening: 0.43 - 0.51 (%); inter-ventricular septal thickness (diastole: 7.61 - 8.55; systole: 12.19 - 14.12 mm); left atrial to aortic root ratio (0.98 - 1.06); left ventricular diameter (diastole: 27.86 - 33.34, systole: 14.42 - 18.14 mm) and left ventricular posterior wall thickness (diastole: 9.71 - 13.5; systole: 14.73 - 18.33 mm). Right lateral radiographs heart axes (long: 5.31 - 5.67, short 3.67 - 4.03 vertebrae) and VHS: 9.04 - 9.64 (vertebrae). No significant associations were identified between the measurements and body weight, age, and heart rate. In conclusions our results emphasized the importance of establishing Baladi-specific RIs.

Key words: Baladi dogs; cardiac indices; ECG; VHS; heart

Introduction

Recently, the Egyptian local (Baladi) dogs have been growingly used as pets in many Egyptian houses. Non-profit organizations (e.g., HOPE - Baladi Rescue & Rehabilitation and Egyptian Society for Mercy to Animals ESMA)

are exerting a potential effort in rescuing and adopting hundreds of stray dogs every day. Baladis are characterized by being smart, alert, easy to train, obedient, and friendly. They are well-adapted to Egypt's climate conditions and they are more resistant to most endemic diseases in Egypt as compared to other foreign dog breeds (e.g. Bulldog, German Shepherd and Husky) (1).

Heart suffers from variety of infectious, non-infectious, genetic, nutritional, environmental, and parasitic diseases leading to its compromised function and finally death. The canine cardiac diseases are common, complex, devastating and considered as silent killer (2). According to the American Veterinary Medical Association, one out of 10 dogs suffer from heart disease (3). Modern non-invasive and cost-effective diagnostic tools including electrocardiography (ECG), echocardiographic (M-mode) modality and radiologic imaging techniques have been routinely used to identify dogs with suspected cardiac diseases and heart rhythm disturbances. More specifically, ECG has been utilized to monitor the heart rate (HR), cardiac rhythm, and electrical conduction integrity (4, 5). Echocardiography has been implemented in the evaluation of the cardiac anatomy and functions (6-9). Thoracic radiography has been helping in establishing the severity of the cardiovascular disease and assessing changes (10-13). However, a substantial variability among dog breeds considering ECG (14); echocardiographic (14,15) and radiologic imaging parameters (16) has been reported in the previous literature. Such variability is mainly attributable to the genetic differences, variations in the shape of thoracic cavity and other anatomical differences among dog breeds. Moreover, it limits the usage of data of one breed for another and consequently necessitates the development of breed-specific RIs.

To the best of our knowledge, no standard intervals for ECG, echocardiographic, and radiologic imaging parameters have been previously published for Egyptian Baladi dogs. Therefore, the aim of this study was to establish RIs for ECG, echocardiographic, and radiographic measurements in healthy Baladis, which subsequently can be used by veterinarians and clinicians to identify cardiac abnormalities.

Material and methods

Study design

A cross-sectional study was conducted between January and December 2020 on Baladi dogs admitted to the veterinary teaching hospital, Faculty of veterinary medicine, Zagazig University. Twenty-six male Baladi dogs were admitted to the hospital for non-elective surgeries (e.g., tail

docking, nail cutting, dental scaling or castration), semen analysis, regular checkups (blood and fecal samples examination), anti-parasitic and vaccination programs. Written consent was obtained from the owners before dogs were included in the study. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Zagazig University, Zagazig, Egypt (approval number ZU- IACUC/2/F/132/2019).

Definition of “healthy” dog

Since the term “healthy dog” or “clinically healthy” can imply different criteria or presumptions, we used a four-stage approach including history, clinical symptoms, laboratory analysis and echocardiography to define the dog as “healthy” or normal (Figure 1). In the first stage, all dogs with no previous disease history (as reported by the owner) and vaccinated against infectious diseases known to affect cardiac function mainly, Parvovirus, leptospirosis and Canine distemper viruses (17, 18) were included.

A thorough physical examination was subsequently carried out and vital parameters [respiration rate (per minute), heart rate (beats per minute), rectal temperature (C)] were assessed. Dogs whose parameters were within the established reference ranges (19) and had no evidence of heart diseases as defined by Forfia et al. (20) proceeded to the laboratory analysis stage (third stage). In this stage, two venous blood samples were collected via cephalic venipuncture. One sample collected in labelled EDTA (ethylenediaminetetraacetic acid) tubes was used for evaluation of hematological indices including total red blood cell count (RBCs), Hemoglobin concentration (Hb), Total leukocytic count (TLCs) and packed cell volume (PCV). Plasma obtained from this sample after centrifugation was used for estimating cardiac troponin-I. The other blood sample was used for serum extraction to evaluate liver function enzymes (ALT, AST), kidney function enzymes (BUN and serum creatinine) and cardiac CK-MB.

Dogs whose hematological parameters were within the normal levels (21), were examined using a two-dimensional (2DE) and M-mode echocardiography to rule out any anatomical or congenital defects. Echocardiographic images were evaluated by an experienced cardiologist (B.M.).

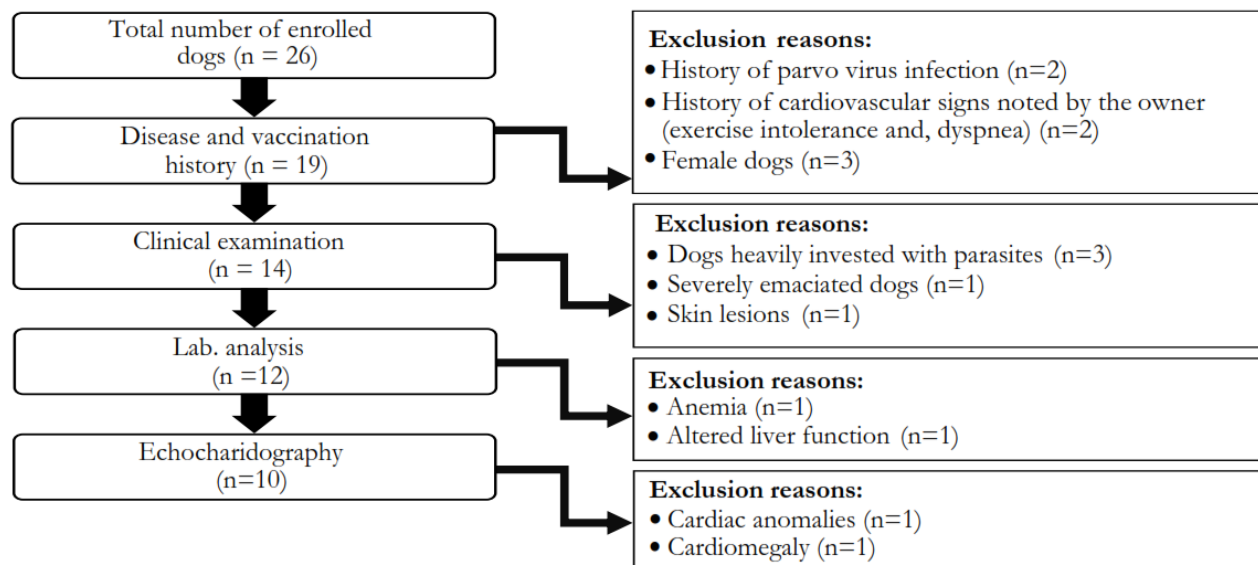


Figure 1: Flowchart shows the four-step approach for defining a healthy Baladi dog

Baseline demographic and laboratory characteristics of the enrolled dogs

Twenty-six Baladi dogs were initially examined at the beginning of the study, 16 animals were excluded from the final analysis due to the

Table 1: Baseline and laboratory characteristics of the healthy dogs (n=10)

	Mean \pm SD
Age (years)	2.33 \pm 0.22
Body weight (Kg)	23.27 \pm 3.0 kg
Cardiac function enzymes	
CTNI (ng/ml)	0.04 \pm 0.01
CK-MB (U/l)	33.41 \pm 3.55
Haematological indices	
RBCs ($\times 10^6$)	5.33 \pm 0.09
Hemoglobin (gm/dl)	12.04 \pm 0.31
Packed cell volume (PCV, %)	34.21 \pm 0.33
Total leucocytic count (TLC, 1000/ml)	10.94 \pm 0.83
Liver function enzymes	
ALT(U/l)	28.02 \pm 1.61
AST (U/l)	30.04 \pm 2.93
Kidney function enzymes	
Urea(mg/dL)	20.76 \pm 1.54
Creatinine(mg/dL)	0.64 \pm 0.07

following reasons: history of parvo virus infection (n=2), history of cardiovascular signs (exercise intolerance and dyspnea) as noted by the owner (n=2), female dogs (n=3), dogs heavily infested with parasites (n=3), severely emaciated dogs (n=1), skin lesions (n=1), anemia (n=1), altered liver function (n=1), cardiac anomalies (n=1), cardiomegaly (n=1) (Figure 1). Therefore, 10 dogs were approved to be healthy based on the results of physical examinations and laboratory tests. Their age ranged between 2 and 3 years (mean 2.33 \pm 0.22 years), and weight ranged between 15.1 to 25.7 kg (mean 23.27 \pm 3.0 kg) (Table.1).

Electrocardiography (ECG)

In the ECG unit, dogs were restrained in right lateral recumbency for ECGs evaluation while conscious. After applying ECG gel, the electrodes were placed above the stifle and elbow joints. A single-channel ECG recorder (Cardioline Delta-1 plus, Remco Italia Cardioline) with a speed of 50 mm/s, and calibration set to 10 mm/mV was used. The following ECG parameters were recorded: the duration of P wave (seconds), P wave amplitude (mV), duration of PR interval (seconds), duration of QRS complex (seconds), R wave amplitude (mV), T wave duration (seconds), T wave amplitude (mv) and heart rate (HR) were assessed. ECGs were recorded using standard bipolar limb Lead II.

Echocardiography

After taking the ECG readings, echocardiographic examinations were conducted following the procedures and precautions outlined by the American Society of Echocardiography (22). The right thoracic wall in the region between the 3rd and 7th ribs and adjoining 1-5 cm area lateral to the sternum was shaved and a coupling gel was applied. Dogs were manually restrained on right lateral recumbency on a table with a slit in the middle while conscious for imaging.

Echocardiographic imaging

A transducer was placed on the dependent side of the body through the slit at a point where the strongest palpable apex beat was felt. Transcutaneous 2D echocardiography examination of the heart was carried out according to the method describe by Singh et al. (23). 2D and M-mode echocardiography were performed using a Sonoscape A5V ultrasound unit with a 3.5MHZ transducer. The M-mode echocardiographic assessment was done simultaneously while displaying 2D images. The standardized image planes obtained by B mode imaging were used to guide measurements for M modes imaging.

Echocardiographic measurements and calculations

The following indices were obtained using the inbuilt electronic caliper of the ultrasound machine: left ventricular internal diameters in both diastole (LVDD) and systole (LVDs) were measured, and they were subsequently used in calculating the left ventricular end diastolic as well as systolic volumes (abbreviated as EDV and ESV, respectively) using the formula of Thomas et al. (22) as follows: $EDV(\text{ml})=7(LVDD)^3 / (2.4+LVDD)$ and $ESV(\text{ml})=7(LVDs)^3 / (2.4+LVDs)$. To evaluate the efficacy of the contractile strength of the heart and its hemodynamic characteristics, we used the following formulas (25, 26) to calculate the stroke volume (ml) $SV=EDV-ESV$; ejection fraction (%)= $((EDV-ESV)/EDV) \times 100$; and left ventricle fractional shortening (LVFS %)= $(LVDD -LVDs) \times (100/LVDD)$. In both diastole and systole, septal and posterior wall thicknesses were measured. LAD : AOD ratio was calculated using the aortic root diameter (AOD) and left atrial diameter (LAD) measurements.

Radiography

Without sedation at the time of full inspiration, thoracic radiographs were taken using standard exposure techniques. A Toshiba Rotande (Pox-300BT[®]) x-ray machine was used. We included left and right lateral views on dorsoventral and ventrodorsal projections. The heart's short and longitudinal axes were measured for quantitative evaluation of the radiographs. The vertebral heart scale (VHS) was then calculated by summing both measurements. These measurements were performed according to the methodology described by Buchanan and Bücheler (27). All radiographs were interpreted by an experienced radiographer (BM) at the animal hospital.

Statistical analysis

All statistical analyses and results reporting were done following the recommendations of Friedrichs et al.(28). Descriptive statistics included mean, median and standard deviation (SD) for all the cardiac measurements. Dot (jitter) and box plots were used to examine the data distribution and identify potential outliers 1.5 * inter quartile range (IQR). Data analysis was conducted in STATA (version 15) (29). Reference intervals were computed using nonparametric bootstrapping method. This involves different steps including: (1) recording the point estimate of the statistic of interest, which in our case was the mean $\hat{\theta}$, obtained from the original N observations; (2) drawing a random sample of size n with replacement from N and computing the mean, in our case we decided to draw samples of the same size as the original data i.e. (N = n); (3) repeat step 2 *itb* times, obtaining *itb* number of averages, that would be subsequently used in estimating the standard errors and 95% confidence intervals. Although, 50–200 replications might be adequate for estimating normally-approximated confidence intervals (30), we used 10000 replications to allow for the estimation of the percentile or bias-corrected confidence intervals; (4) finally, bias was estimated as the difference between the average of the bootstrap samples $\bar{\theta}$ and the average obtained from the observed data $\hat{\theta}$. The mean of the original sample and the bootstrap 95% confidence intervals for all the measured parameters were calculated. Sensitivity analysis was done to assess how suspected outliers affect the final estimates. A Pearson correlation test was used to assess whether there was correlation between the dogs' weight, age, and heart rate with cardiac parameters.

Results

Electrocardiographic findings

Descriptive statistics and reference intervals for the various electrocardiographic parameters are presented in Table 2. However, sensitivity analysis revealed no substantial change in the standard error and 95% confidence intervals. The Lead-II's ECG is also shown in (Figure 2). A couple of potential outliers were identified in the P wave amplitude and T wave duration (Figure 3).

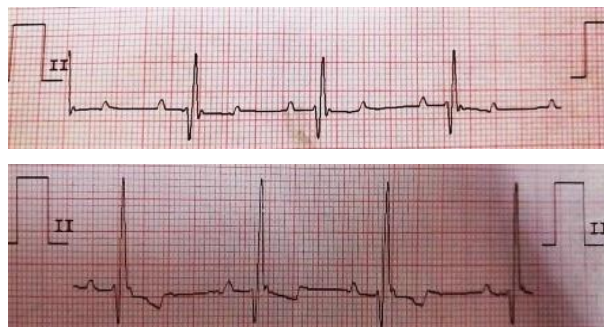


Figure 2: ECG of normal Baladi dog (lead II): A: showing normal ECG tracing with inverted (negative) T wave appeared in one examined dog; B: showing normal ECG with positive T w.

Parameters (units)	Mean* ± SE	Range (min-max)	95% reference interval
Heart rate (beats/min)	117.65±7.38	(60 - 152)	(103.19 - 132.12)
P wave duration (seconds)	0.03±0.004	(0.02 - 0.06)	(0.02 - 0.04)
QT interval (sec)	0.43±0.01	(0.4 - 0.46)	(0.41 - 0.44)
R wave amplitude (mV)	1.35±0.08	(0.9 - 1.7)	(1.19 - 1.51)
QRS interval (sec)	0.07±0.004	(0.05 - 0.08)	(0.06 - 0.08)
P wave amplitude (mV)	0.18±0.03	(0.1 - 0.4)	(0.13 - 0.23)
PR interval (sec)	0.14±0.02	(0.08 - 0.22)	(0.11 - 0.17)
T wave amplitude (mv)	0.24±0.02	(0.15 - 0.3)	(0.2 - 0.27)
T wave duration (sec)	0.04±0.004	(0.02 - 0.06)	(0.03 - 0.04)

Table 2: Descriptive statistics and proposed reference intervals for electrocardiographic measurements from 10 healthy Baladi Egyptian dogs

*: mean obtained from the original dog sample

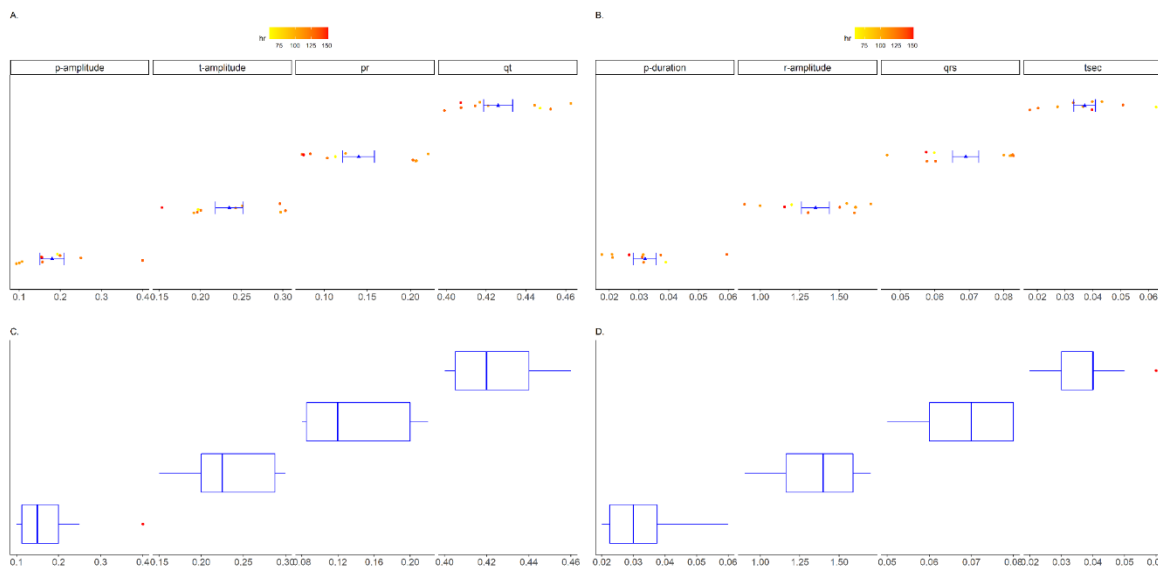


Figure 3: Jitter(A &B) and box plots (C&D) illustrating the Electrocardiographic easurement in normal Egyptian Baladi Dogs. The horizontal line depicts the mean, while the whiskers show the maximum and minimum values in this group

Table 3: Descriptive statistics and proposed reference intervals for echocardiographic measurements from 10 healthy Baladi Egyptian dogs

Parameters (units)	Mean* \pm SE	Range (min-max)	95% reference interval
End Diastolic Volume (mL)	38.22 \pm 4.22	(20.7 - 65.9)	(29.95 - 46.49)
Ejection Fraction (%)	79.81 \pm 1.83	(69.6 - 89.5)	(76.23 - 83.41)
End Systolic Volume (mL)	7.93 \pm 1.22	(2.86 - 15.6)	(5.54 - 10.32)
Stroke Volume (mL)	31.25 \pm 3.13	(17.9 - 50.2)	(25.13 - 37.41)
Fractional Shortening (%)	0.47 \pm 0.02	(0.38 - 0.59)	(0.43 - 0.51)
Interventricular Septal Thickness at Diastole (mm)	8.08 \pm 0.24	(7 - 9.3)	(7.61 - 8.55)
Interventricular Septal Thickness at Systole (mm)	13.15 \pm 0.49	(10.8 - 15.9)	(12.19 - 14.12)
left atrial to aortic root ratio	1.02 \pm 0.02	(0.92 - 1.1)	(0.98 - 1.06)
Left Ventricular Diameter at Diastole (mm)	30.6 \pm 1.4	(24.3 - 39)	(27.86 - 33.34)
Left Ventricular Diameter at Systole (mm)	16.28 \pm 0.95	(11.3 - 21.7)	(14.42 - 18.14)
Left ventricular posterior wall Thickness at Diastole (mm)	11.61 \pm 0.97	(8.2 - 17.5)	(9.71 - 13.5)
Left Ventricular Posterior Wall Thickness at Systole (mm)	16.53 \pm 0.92	(12.5 - 20.8)	(14.73 - 18.33)

*: mean obtained from the original dog sample.

Echocardiographic findings

Two-dimensional echocardiographic views of the right parasternal longitudinal and short axes are presented in (Figure 4). The distribution of echocardiographic parameters obtained by M-mode are presented in (Figure 5) and the proposed RIs are summarized in Table 3. No obvious measurement errors were identified on the measurements determined to be statistical outliers.

Radiographic findings:

The observed mean heart's longitudinal and short axes as well as VHS are presented in (Table 4). Lateral thoracic radiographs are shown in (Figure 7 A and B). There was no significant correlation between the VHS and the dogs' weight and age.

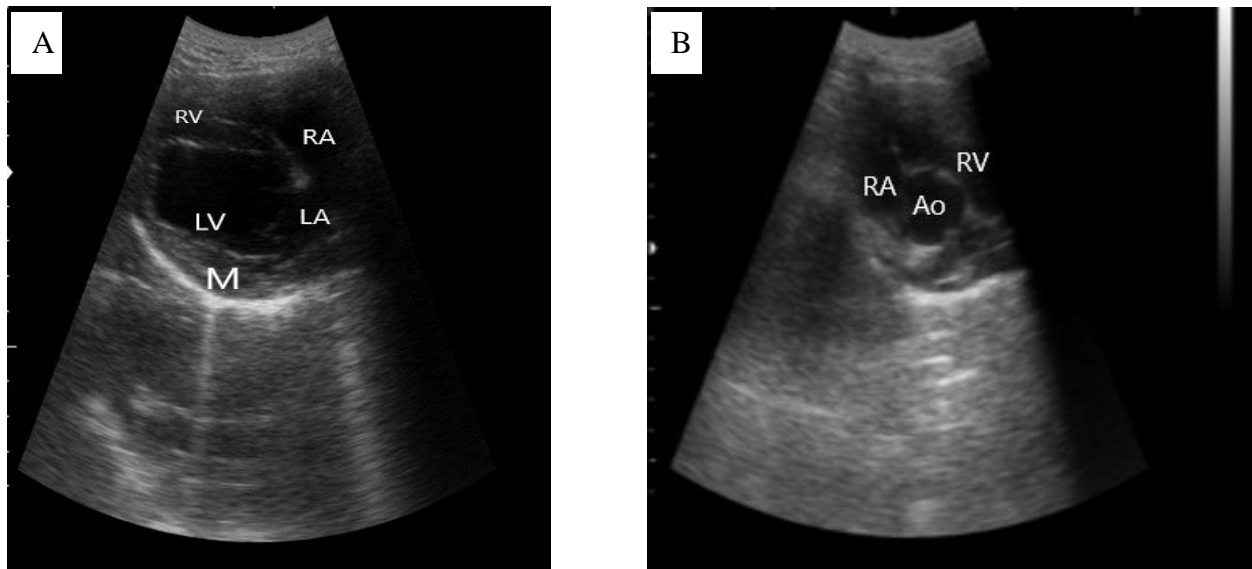


Figure 4: Echocardiographic examination: 2D-Echocardiographic image. A: Right arasternal Longitudinal Axis Four Chamber CG of normal Baladi dog. LV: left ventricle ; LA: left atrium ; RV: right ventricle; RA: right atrium; M: myocardium. B: Right Parasternal short Axis: RV: right ventricle; RA: right atrium; Ao:Aorta

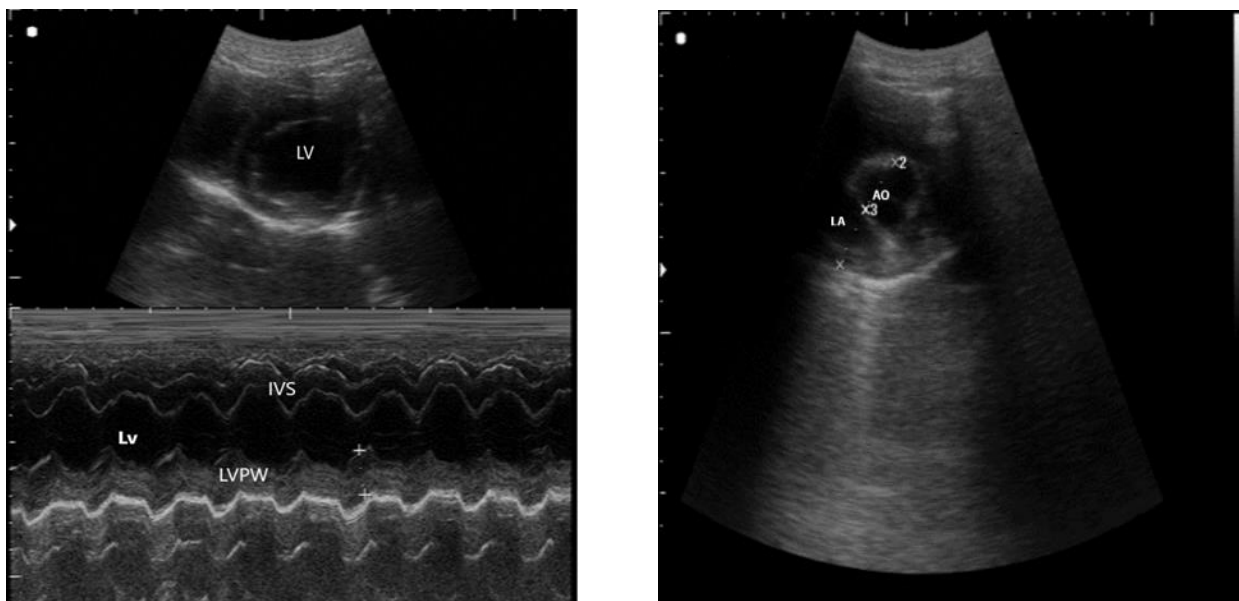


Figure 5: Echocardiographical examination. A: M-mode - left ventricular papillary muscle level. LV: left ventricle ; IVS: interventricular septum ; LVPW: left ventricular posterior wall. B: 2D-echocardiographic image - LAD: AOD: left atrial to aortic root ratio. Ao: Aorta; LA: left atrium

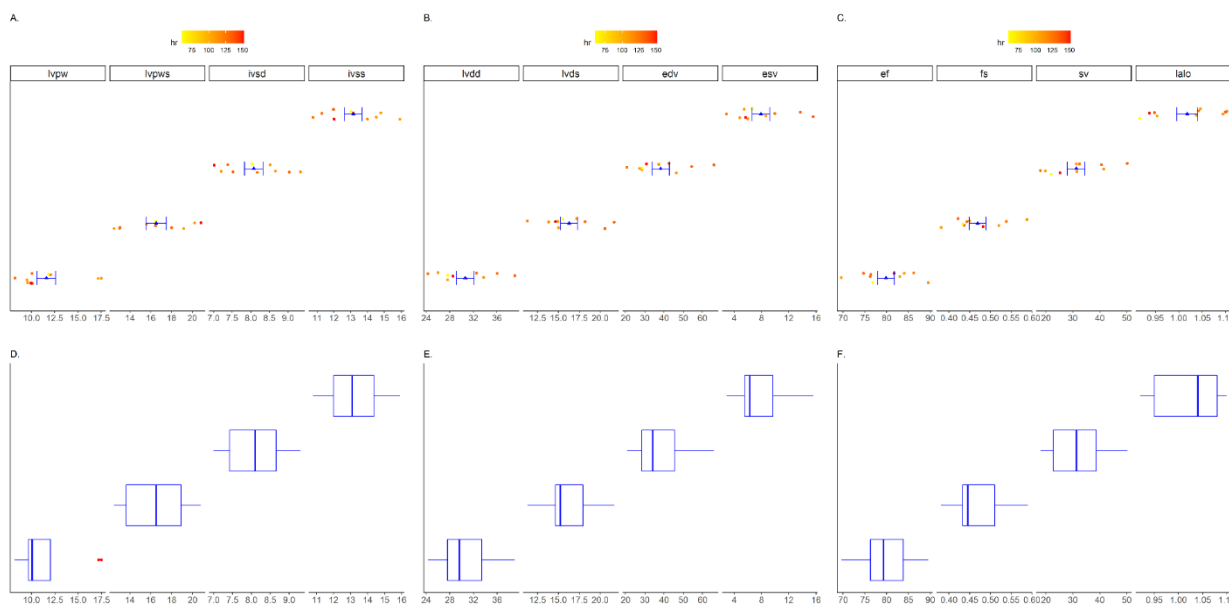


Figure 6: Jitter (A-C) and Box plots (D-F) illustrating the echocardiographic measurements and calculations in normal Egyptian Baladi Dogs. The horizontal line depicts the mean, while the whiskers show the maximum and minimum values in this group

Table 4: Descriptive statistics and proposed reference intervals for radiographic measurements from 10 healthy Baladi Egyptian dogs

Parameters (units)	Mean* ± SE	Range (min-max)	95% reference interval
Heart's long axis (vertebrae)	5.49±0.09	(5.1 - 6)	(5.31 - 5.67)
Heart's short axis (vertebrae)	3.85±0.09	(3.5 - 4.2)	(3.67 - 4.03)
Vertebral Heart Scale (vertebrae)	9.34±0.16	(8.6 - 10.1)	(9.04 - 9.64)

*: mean obtained from the original dog sample

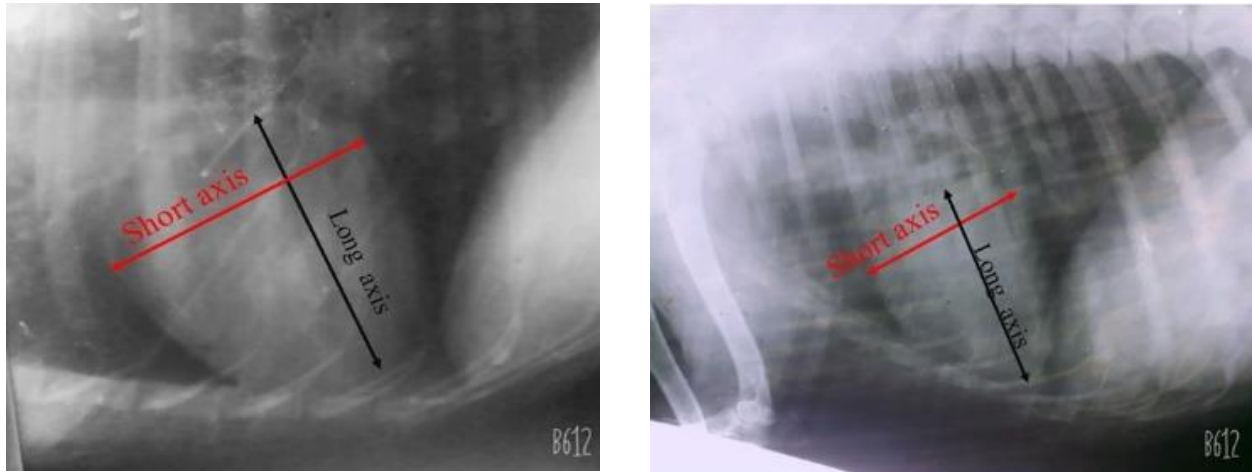


Figure 7: Long axis (LA) and short axis (SA) measurements of heart in lateral recumbency for calculation of VHS in two different animals. A and B are two different dogs

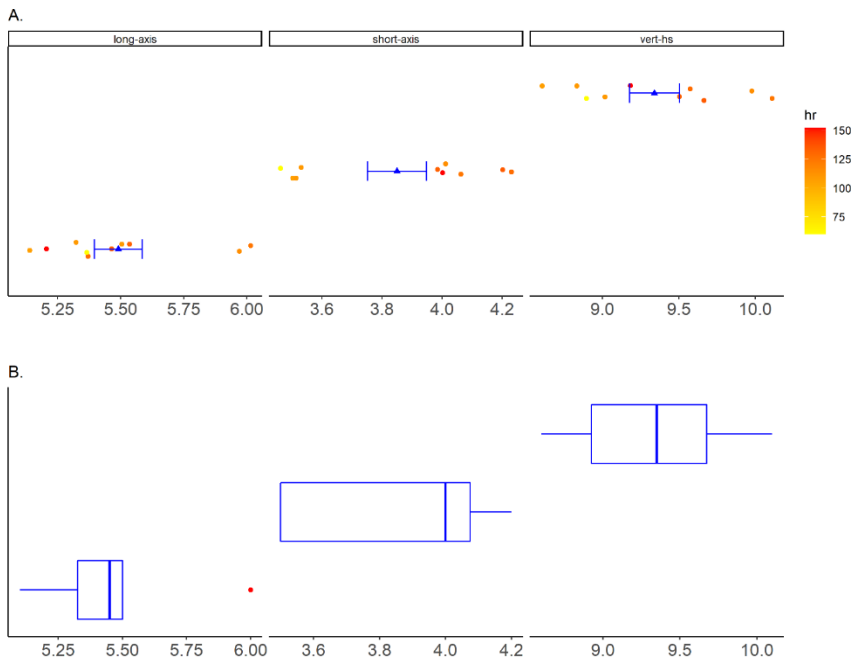


Figure 8: Jitter (A) and Box plots (B) illustrating VHS in normal Egyptian Baladi Dogs. The horizontal line depicts the mean, while the whiskers show the maximum and minimum values in this group

Discussion

Unlike other dog breeds, the Egyptian Baladi has not been officially recognized as a dog breed, and therefore, there is no reference ranges for their cardiovascular parameters. The electrocardiographic, echocardiographic, and radiographic indices recorded in this study can help to understand the structural and functional changes of the heart, and consequently identify cardiovascular abnormalities in Baladis. There was a substantial variability in some of the cardiac indices (e.g. QRS duration, ESV, EDV and VHS) among the

Baladis and other dog breeds; however, other indices fell within the limits of other dog breeds.

Electrocardiography:

The heart rates for our animals under investigation were within the normal range (up to 220 bpm for puppies and 70 - 160 bpm for adult dogs (31). P-wave duration, amplitude, and morphology's accurate analysis is important in identifying the atria's morphological and functional changes (32). Our P-wave values agreed with the results observed in golden retrievers (4), Indian Chippirai dogs (33) and other dog breeds (31, 34). On

the contrary, higher values were reported in mongrel, shepherd and karafuto (Sakhalin Husky) dogs (35,36).

R-wave amplitude is a precise indicator for ventricular contractility and compliance (14). In our study, the R-wave amplitude values agreed with standard reference values of most of the other dog breeds (31). However, lower and higher values have been recorded for the Labrador and the Golden Retriever, respectively (4).

The PR interval represents the time required for an impulse to travel from Sinoatrial node to the ventricle (33). In this study, PR interval values were close to the intervals reported by Tilley (31). Additionally, Mukherjee (4) recorded similar results in German Shepherds while Gugjoo et al. (14) reported relatively lower values in conscious Labrador retriever dogs.

The QRS interval represents the duration of ventricular depolarization. In the present study, our values did not exceed the normal range in dogs (31). However, these values were higher than what was reported in German Shepherd, Labrador, Golden Retriever (4), and Beagle dogs (37).

The amplitude and duration of T-wave have a direct relationship with the ventricular myocardial cells' repolarization (4). In our study, T-wave amplitude measurements were within normal ranges reported by Tilley (31). The inverted T-wave (negative) appeared in some dogs. The T-wave's genesis has a high complexity and its polarity's determinants have not yet been well understood. However, in Lead II, T-wave's altered polarity may happen owing to diaphragmatic elevation during respiration (319). In addition, Mukherjee (4) also recorded similar findings in German Shepherds and Golden Retrievers. However, Gugjoo et al. (14) recorded higher values in conscious Labrador retriever dogs.

Echocardiography

The dimensions of the left ventricle have a significant relationship with the clinical manifestation of cardiac diseases (8, 9). RI of LVIDd was similar to the Mongrel (38) and Spitz (39) dogs. Lower values were presented in the Dachshunds (40), Chippiparai (33) dogs. On the other hand, higher values were recorded in German Shepherds (41) and Labradors (8). Overall, the LVIDd

was found to be greater than LVIDs which indicates the normal ventricular morphology and contractility, as previously described (14,39, 41).

FS is the most used to assess the myocardium contractility and left ventricular capacity (42, 43) proposed a FS of less than 0.25 as indicator to heart hypovolemia. Our study findings agreed with values reported in the Italian Greyhounds (44). However, lower values were recorded in German Shepherds and Labradors (14, 41).

In this study, the mean EF value agreed with the findings of Crippa et al. (45). The ratio of normal left atrial to aortic root (LAD: AOD) in our study was within the normal range of 0.8 and 1.2 reported by Boon (46). However, lower values were recorded in German shepherds (41), and Italian Greyhounds, (44).

Other cardiac function indicators including the values of ESV and EDV observed in this study were lower than 100 mL/m² and 30 mL/m², respectively. However, based on the observations by Holt (47), these values can still be considered normal, similar values were also reported (44).

Radiography

Baladis had lower VHS intervals (**9.34±0.16**) than those reported by Buchanan (10). Similar values to our results were recorded by Ghadiri et al. (48) and Pinto(49). Conversely, higher values were recorded in German shepherd, Boxer, Doberman breeds (12), Greyhounds, Rottweilers (13), Cocker spaniel (50), Beagle (51), and Bulldogs (52). Baladi dogs have a smaller mean VHS compared to other dog breeds and thus on average, heart sizes and the thoracic vertebrae of Baladi dogs are slightly smaller in comparison to other large dog breeds.

The authors acknowledge that while this is the first study to report RIs specific to the Baladi dogs, there were some limitations due to the sample size and sampling technique. However, we mitigated the potential biases through the bootstrapping analytical approach that is more robust to be influenced by sample size and distributional assumptions. We recommend additional studies using a more diverse study population of Baladi dogs to evaluate possible variations associated with sex, body weight and age and further estimate sex-specific RIs. Additionally, validation studies are required to assess intra-observer repeatability.

Conclusion

In conclusion, our results emphasize the importance of using breed-specific RIs for Baladi dogs. Although most of the cardiac parameters are within published parameters for other breeds, some parameters are outside these ranges. We observe that for Baladi dogs, VHS values and heart sizes may be lower than those of other species. Overall, the authors present these as the baseline parameters for Baladi dogs, upon which further studies and validation can be done for the different parameters to be used in veterinary practice.

Authors disclose no conflict of interest.

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