Reference intervals for transthoracic echocardiography in the American Staffordshire Terrier

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Running head: Echocardiography in Amstaff dogs

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ABSTRACT

This study reports the echocardiographic reference intervals in the American Staffordshire Terrier (AST). The echocardiographic variables obtained in 57 healthy adult AST were compared with published data from the general canine population and other breeds. In the AST, the left ventricular volumes were lower than values reported in Boxers and Dobermans (P<0.0001), but higher than in small breeds (P<0.0001). The left ventricular ejection fraction was higher than Boxers and Dobermans (P<0.0001), but lower than small breed dogs (P=0.027). The aortic peak velocity values were similar to Boxers (P=0.55) but higher than the general canine population (P<0.0001). The reference intervals presented in this study are clinically useful for an accurate echocardiographic interpretation and screening in the AST.

KEY WORDS

Amstaff, canine, cardiology, echocardiography, screening.
Transthoracic echocardiography is the main non-invasive diagnostic tool for evaluating structural heart diseases, and it is crucial for cardiology screening of canine breeds predisposed to congenital or acquired diseases [4, 5, 9, 11, 43]. Specific breeding programs based on echocardiographic screening have been found to reduce the prevalence of certain inherited cardiac diseases in canine breeds [5, 11]. The most used echocardiographic reference intervals in dogs derive from the canine populations of many different breeds and mainly depend on body weight (BW) [8, 12, 23, 30]. However, breed is an important factor influencing echocardiographic measurement in addition to BW in dogs, thus limiting the use of between-breed data [14, 17, 29]. Specific echocardiographic reference intervals have thus been described for many canine breeds and significant differences regarding cardiac dimensions, shape and function have been found [3, 13, 14, 16, 20-22, 28, 31, 42]. The English Bulldog, the Bull Terrier and the American Pitbull terrier are the ancestors of the American Staffordshire Terrier (AST) [1, 18]. Like these breeds, ASTs are susceptible to congenital heart diseases, and especially valvular stenosis [32, 33]. The aim of the study was to describe the reference intervals for transthoracic echocardiography in the AST, and to compare these values with previously published reference values for the general canine population.

This was a retrospective observational study. The clinical database of the Italian Veterinary Observatory for Cardiac Diseases (OVIC) [34] was reviewed in terms of the echocardiographic examinations performed on AST that had undergone cardiology screening from 2013 to 2019. Data on signalment, physical examination and echocardiographic results were recorded. Exclusion criteria included dogs aged less than one year, and the presence of cardiac disease or systemic diseases. The exclusion of dogs with aortic and pulmonic stenosis was based on the presence of an elevated and turbulent aortic or pulmonic blood flow velocity (>2.25 m/sec) in conjunction with patho-anatomical abnormalities of the left or right ventricular outflow tract and/or the aortic or pulmonic leaflets [9]. All echocardiographic examinations were performed by experienced operators, accredited by the OVIC board, using ultrasound systems with phased-array transducers. Dogs were positioned unsedated and gently restrained, in right and left lateral recumbency on tables for echocardiographic evaluation. Standard M-mode, two-dimensional, Doppler echocardiographic images and video-loops were recorded with continuous ECG monitoring as previously reported [40]. From the right parasternal short-axis view,
the interventricular septal thickness (IVS), left ventricular internal diameter (LVID), and left ventricular free wall thickness (LVFW) were obtained in diastole (d) and systole (s) using the M-mode. Diastolic and systolic LVID normalized for BW (LVIDDn and LVIDSn) were calculated as previously described [12]. The left ventricular (LV) fractional shortening (FS) was then calculated. The left atrial-to-aortic ratio (LA/Ao) was measured from the right parasternal short-axis view, at the aortic valve level, in early diastole (first measurable frame after aortic valve closure) [35]. The aortic (Ao) and pulmonic (PA) annulus were measured from the right parasternal long axis and short axis views, respectively [10]. The index of aortic root dimension (wAo) was calculated as the Ao annulus divided by the weight-based aorta [8]. The LV diastolic length was measured in 2D from the apex to the mitral valve annulus using the right parasternal long axis view [17]. The LV sphericity index was calculated by dividing the LV diastolic length by the LVIDD obtained from M-mode images [17]. Peak pulmonary flow velocity was recorded with pulsed wave Doppler (PA\textsubscript{vmax}) with the sample volume placed just distal to the pulmonic valve from the right parasternal short axis view [8]. Peak aortic flow velocity (Ao\textsubscript{vmax}) was obtained from the subcostal view using continuous wave Doppler [9]. Transmitral flow was recorded 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 (MV E, MV A) were measured. Simpson’s method of discs derived LV end-diastolic volume (EDV), LV end-systolic volume (ESV) and ejection fraction (EF) were calculated using the right parasternal long-axis view [44]. Left ventricular volumes were indexed to body surface area (EDVI and ESVI) [44]. The body surface area was calculated with the following formula: 0.101 × BW \textsuperscript{(kg)}\textsuperscript{2/3} [43].

Statistical analysis was performed with a commercially available softwares (Graphpad Prism 5.0; SAS 7.0). Descriptive statistics were generated. All echocardiographic indices were tabulated, visually inspected using a dot plot, 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 application to calculate reference intervals (Reference Value Advisor v. 2.1) [19]. The nonparametric method to determine reference intervals was used with the entire eligible data set. Regarding echocardiographic variables, females were compared with males using analysis of covariance in a multivariate model with age, BW, and heart rate as covariates. 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 [25]. The results found in our sample population were compared to previously published mean values obtained from a general population of dogs regarding LVIDDn, LVISDn [11] and LA/Ao [36]. Simpson’s derived EDVI, ESVI, EF and SI were compared to the published reference values available in large-breed dogs (i.e. Dobermans [24, 44] and Boxers [39]) and small-breed dogs [38]. The aortic size was compared using the wAo with previously reported values in the general canine population [8]. Lastly, \( A_{v\text{max}} \) and \( P_{A\text{max}} \) were compared to the previously published mean values derived from a general population of dogs [6] and Boxers [39], a breed predisposed to aortic and pulmonic stenosis like the AST. A value of \( P < 0.05 \) was considered significant.

A total of 57 healthy AST was included in the study, consisting of 32 males and 25 females. The median BW was 27.7 kg (range, 19-36 kg), and median age was 2.6 years (range 1-10 years). The median heart rate was 114 bpm (range, 60-160 bpm), and cardiac auscultation revealed a left basilar systolic murmur in 4/57 (7%) dogs, with 1/6 grade in three dogs and 2/6 grade in one dog. The reference intervals for the echocardiographic variable included in the study are reported in Tables 1 and 2. According to the multivariable analyses, no differences in echocardiographic variables were found between females and males, thus no different reference intervals according to gender were calculated. In addition, there was no significant effect of age, BW and heart rate on echocardiographic variables. The mean value of LVIDDn in the present study (1.62) was significantly higher than values previously reported in a range of different breeds (1.53; \( P<0.001 \)). On the other hand, the mean LVISDn (0.96) did not differ from the general canine population (0.97; \( P=0.93 \)). The mean SI value in the present study (1.46) was lower than the mean value reported in Dobermans (1.86; \( P<0.0001 \)), but similar to the mean value in small-breed dogs (1.43; \( P=0.49 \)). The mean EDVI and ESVI in our population of ASTs (62 ml/m\(^2\) and 23 ml/m\(^2\), respectively) were significantly lower than the mean values reported in Boxers (70 ml/m\(^2\) and 36 ml/m\(^2\); \( P<0.0001 \)) and Dobermans (72 ml/m\(^2\) and 38 ml/m\(^2\); \( P<0.0001 \)), but higher than previously reported values in small breeds (48 ml/m\(^2\) and 16 ml/m\(^2\); \( P<0.0001 \)). The mean EF in our study (62%) was significantly higher than values reported in Boxers (49% \( P<0.0001 \)) and Dobermans (49%; \( P<0.0001 \)), but lower than previously reported in small breeds
The mean LA/Ao value in our population (1.37) was similar to the general canine population (1.35; P=0.54). The mean wAo was significantly lower (0.79) than previously reported in the general canine population (1.00; P<0.0001). Regarding Doppler findings, the mean Ao\textsubscript{vmax} in our study (1.77 m/sec) did not differ from values reported in Boxers (1.80 m/sec; P=0.55), but was higher than the general canine population (1.15 m/sec; P<0.0001). Lastly, the mean PA\textsubscript{vmax} (1.16 m/sec) did not differ from Boxers (1.24 m/sec; P=0.052) and the general canine population (1.06 m/sec; P=0.09).

To the best of our knowledge, this is the first study providing echocardiographic reference intervals in American Staffordshire Terriers for clinical use.

In the present study, 7% of healthy adult ASTs presented a soft left basilar murmur. The presence of non-pathological (functional) murmurs in healthy adult dogs has already been reported in other breeds such as Boxers and Whippets [2, 27]. The possible incidence of functional murmurs in healthy ASTs should be taken into account during the physical examination for screening and/or clinical reasons. Regarding echocardiographic variables, the mean LVIDDn in our AST population was significantly higher than values reported in a range of canine breeds. However only one dog presented an LVIDDn above the upper reference limit (1.85) reported for the general canine population established by Cornell et al. 2004 [12]. The relevance of this finding in clinical practice is thus questionable. A possible explanation may be the ventricular geometry in this breed. In our population of ASTs, the mean SI (1.41) was significantly lower than the mean values reported for Dobermans (1.86; P<0.0001) [24]. Previous studies reported a cutoff of <1.65 as an index of increased LV sphericity and a possible predictor of dilated cardiomyopathy [17, 28]. One reason for this difference in the SI between ASTs and other breeds could be the breed-specific cardiac morphology of ASTs, which based on our results have a more rounded heart than other breeds. Further studies on the influence of breed and morphotype on cardiac size and geometry are needed.

Regarding LV volumes, the EDVI and ESVI in our AST population were significantly lower than the mean values reported in Boxers and Dobermans [24, 39, 44], but higher than for small breed dogs [38]. Ventricular volumes are correlated with body size, however EDVI and ESVI are derived from indexing cardiac volume to body surface area, thus differences among canine breeds cannot only be explained
by the different body size. A possible influence of breed morphotype on cardiac volumes and geometry needs to be explored.

The EF in our AST population was significantly higher than previously reported in Boxers and Dobermans [24, 39, 44], but lower than in small breeds [38]. This finding is in line with previous evidence of a negative correlation between EF and body weight [41].

In terms of Ao annulus, our results suggest that the AST has smaller aortic size than the general canine population, similar to Boxers [14] and Bull Terriers [31]. In addition, we provided a normal range for the Ao/PA ratio in this breed, with a reference upper limit of 1.26. This is in line with the previously proposed cutoff of 1.2 for distinguishing between type A and B pulmonic stenosis [10]. These findings may be helpful in a more accurate screening of ASTs for aortic and pulmonic stenosis, especially in light that a recent study reported a progressive increase in the prevalence of pulmonic stenosis in AST from 1997 and 2017 [7].

Regarding the LA/Ao, the upper reference limit in our AST population was 1.75, with 6/57 (10%) dogs with a LA/Ao >1.6. The LA/Ao is the most widely used echocardiographic index to evaluate left atrial dimension in dogs, and several studies have used the upper reference limit of 1.6 to define left atrial enlargement [26, 35]. However, our results are in line with a study demonstrating that in around 10% of dogs, in particular in Boxers and Beagles, a small aortic root is present, which leads to an increase in the LA/Ao up to an upper reference limit of 1.73 [36].

With regard to the spectral Doppler variables, the Ao\textsubscript{vmax} in our AST population did not differ from values reported in Boxers [39], but was higher than in the general canine population [6], with an upper reference limit of 2.45 m/sec in our study. This is in line with previous studies reporting similar results in Boxers [14] and Bull Terriers [31], in which a smaller aortic annulus was associated with a higher aortic flow velocity. This evidence be may particularly important in the echocardiographic screening of ASTs for aortic valvular and/or subvalvular stenosis. However, the pulmonic flow velocity did not differ in this study from values previously reported in Boxers [39] and the general canine population [6].

Lastly, regarding the transmitral flow, the peak velocity of the E wave showed an upper reference limit of 1.29 m/sec, with 6/57 (10%) showing a peak velocity higher than 1.1 m/sec but with a normal E/A ratio. Usually, a peak velocity of ≥ 1.1 m/sec is predictive of an increased LV filling pressures and the risk of congestive heart failure [37]. This finding should thus be taken into consideration when screening
this breed, and further studies on transmirtal inflow in different breeds and canine morphotype may be useful.

This study presents some limitations. First, the sample size was relatively small compared to the usually recommended sample size of 120 dogs [41]. However, our sample population is similar or even larger than previous studies evaluating echocardiographic reference intervals in different canine breeds [13, 16, 21, 22, 28, 31]. Second, the study was retrospective, the echocardiographic examinations were performed by different operators and the repeatability, the intraobserver and interobserver measurement variability were not assessed. However, all the echocardiographic examinations were performed by experienced operators in echocardiography who belonged to the Italian Veterinary Observatory for Cardiac Diseases, which establishes a selective exam for the enrolment, including intra- and inter-observer measurement variability tests supervised by a recognized board. This test is periodically carried out in order to keep the quality standard of the echocardiographic skills of the group of observers constant by the time. Third, the echocardiographic values obtained in this study were compared with previous findings in other breeds and/or the general canine population, however the interoperator measurement variability among different studies should also be considered.

In conclusion, this is the first study providing echocardiographic reference intervals in healthy adult ASTs. This breed tends to have smaller ventricular volumes, lower SI and higher EF than Boxers and Dobermans and than the general canine population. In addition, the AST shows a smaller aortic annulus and an increased aortic flow velocity. These breed-specific echocardiographic features should be taken into consideration for an accurate echocardiographic interpretation and screening in the AST.

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POTENTIAL CONFLICTS OF INTEREST
The authors have nothing to disclose.
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Table 1. M-mode and two-dimensional echocardiographic reference intervals obtained in a population of 57 healthy adult American Staffordshire Terriers.

| Variable           | Median | Lower RI (90% CI) | Upper RI (90% CI) |
|--------------------|--------|-------------------|-------------------|
| **M-mode**         |        |                   |                   |
| IVSd (mm)          | 10.7   | 5.9               | 5.4-7.0           | 14.3               | 13.6-14.3         |
| LVIDd (mm)         | 43.0   | 34.4              | 34.0-37.0         | 51.2               | 48.8-52.4         |
| LVFWd (mm)         | 9.1    | 6.2               | 5.9-6.9           | 12.1               | 11.3-12.2         |
| IVSs (mm)          | 14.0   | 8.1               | 6.6-10.8          | 21.6               | 19.8-22.0         |
| LVIDs (mm)         | 27.6   | 17.6              | 16.0-28.8         | 36.9               | 33.7-38.4         |
| LVFWs (mm)         | 14.0   | 9.3               | 8.9-11.0          | 19.1               | 16.9-19.6         |
| FS (%)             | 34.3   | 20.1              | 18.4-27.8         | 56.4               | 47.9-56.4         |
| LVIDDn             | 1.64   | 1.27              | 1.27-1.42         | 1.87               | 1.79-1.90         |
| LVIDSn             | 0.99   | 0.64              | 0.60-0.73         | 1.31               | 1.21-1.38         |
| **2D**             |        |                   |                   |
| Ao (mm)            | 18.6   | 13.9              | 12.5-15.2         | 23.7               | 22.3-25.1         |
| wAo                | 0.78   | 0.61              | 0.55-0.65         | 0.97               | 0.92-1.02         |
| PA (mm)            | 18.2   | 14.0              | 12.7-15.4         | 23.3               | 21.9-24.6         |
| Ao/PA              | 1.02   | 0.80              | 0.73-0.85         | 1.26               | 1.19-1.32         |
| LA/Ao              | 1.39   | 1.00              | 1.00-1.04         | 1.75               | 1.66-1.75         |
| SI                 | 1.41   | 1.15              | 1.15-1.23         | 2.10               | 1.75-2.10         |
| EF (%)             | 60.7   | 40.5              | 35.3-46.0         | 82.5               | 76.5-88.0         |
| EDVI (ml/m²)       | 59.4   | 37.1              | 33.8-41.4         | 86.7               | 79.3-92.8         |
| ESVI (ml/m²)       | 21.9   | 9.5               | 6.3-13.0          | 35.3               | 31.4-38.4         |

RI, reference interval; CI, confidence interval; IVS, interventricular septal thickness; LVID, left ventricular internal diameter; LVFW, left ventricular free wall thickness; d, diastole; s, systole; FS, fractional shortening; LVIDDn, left ventricular internal diameter in diastole normalized for body weight; LVIDSn, left ventricular internal diameter in systole normalized for body weight; Ao, aortic annulus; wAo, index of aortic root dimension; PA, pulmonic annulus; LA/Ao, left atrial-to-aortic ratio; SI, sphericity index; EF, ejection fraction; EDVI, end-diastolic volume index; ESVI, end-systolic volume index.
Table 2. Spectral Doppler echocardiographic reference intervals obtained in a population of 57 healthy adult American Staffordshire Terriers.

| Variable     | Median | Lower RI | (90% CI)     | Upper RI | (90% CI)  |
|--------------|--------|----------|---------------|----------|-----------|
| $A_{ov_{\text{max}}}$ (m/sec) | 1.77   | 1.17     | 1.17-1.23     | 2.45     | 2.30-2.46 |
| $P_{av_{\text{max}}}$ (m/sec) | 1.13   | 0.76     | 0.74-0.88     | 1.70     | 1.60-1.72 |
| MV E (m/sec) | 0.92   | 0.50     | 0.45-0.67     | 1.29     | 1.13-1.31 |
| MV A (m/sec) | 0.67   | 0.36     | 0.35-0.41     | 1.16     | 0.99-1.18 |
| E/A          | 0.85   | 0.90     | 0.84-1.07     | 1.88     | 1.79-1.88 |

RI, reference interval; CI, confidence interval; $A_{ov_{\text{max}}}$, peak aortic flow velocity; $P_{av_{\text{max}}}$, 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.