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Prevalence of subclinical heart disease in apparently healthy American Staffordshire Terriers

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Summary

The American Staffordshire Terrier (AST) is a breed characterized by a specific body build and high activity. The predisposition of the AST to cardiovascular diseases has not been investigated thoroughly yet. The study population consisted of 88 adult dogs showing no signs of cardiovascular diseases. The tested dogs were of different gender, age and body weight. Systolic blood pressure, clinical examination, echocardiography, electrocardiography, thoracic radiograph were performed in each dog, additionally, the blood was taken for laboratory tests. Subclinical heart disease was found in 67% of the tested dogs. Diseases of atrioventricular valves were most prevalent, significantly more common than aortic and pulmonary valve diseases. The most common subclinical heart disease was mitral valve regurgitation. A large part of the examined dogs had accelerated LVOT and RVOT in relation to the reference values adopted for the general canine population. The subclinical heart diseases are common in the Polish population of adult AST. Detection of the heart murmur in auscultation, respiratory sinus arrythmia in ECG, qR pattern of the QRS complex in ECG, PQ ≥ 0.11s, and LA/Ao ≥ 1.4 in an asymptomatic adult AST is suggestive of the subclinical heart disease and is an indication for a complete echocardiographic examination. Performing cardiac screening tests in adult AST dogs enables the elimination of sick individuals from breeding. Full echocardiographic examination including Doppler technique is a mainstay of the cardiologic diagnostics in modern veterinary medicine. It is the only method that allows the diagnosis of subclinical heart diseases.

Keywords: cardiology, dogs, cardiovascular diseases

Abbreviations:

A4C view – apical 4-chamber view
A wave – late diastolic transmitral flow
ALT – alanine aminotransferase
ANP – atrial natriuretic peptide
Ao – aortic root diameter
AS – aortic stenosis
AspAT – aspartate aminotransferase
AST – American Staffordshire Terrier
AUROC – area under receiver operating characteristic curve
BW – body weight
BSA – body surface area
CI 95% – 95% confidence interval
Cl – chloride
DCM – dilated cardiomyopathy
E wave – early diastolic transmitral flow
EDV – end-diastolic volume
EDVI – end-diastolic volume index
EF – ejection fraction
EPSS – end point to septal separation
ESV – end-systolic volume
ESVI – end-systolic volume index
FS – fractional shortening
fT4 – free thyroxine
HR – heart rate
IQR – interquartile range
IVSd – interventricular septum in diastole
IVSs – interventricular septum in systole
K – potassium
LA – left atrial dimension
LR_+ – likelihood ratio of positive result (positive likelihood ratio)
LR_- – likelihood ratio of negative result (negative likelihood ratio)
LVDd – left ventricular dimension in diastole
LVDs – left ventricular dimension in systole
LVDdN – left ventricular internal diameter in diastole normalized for body weight
LVDsN – left ventricular internal diameter in systole normalized for body weight
LVOT – left ventricular outflow tract
LVWd – left ventricular peripheral (free) wall in diastole
LVWs – left ventricular peripheral (free) wall in systole
MCH – mean corpuscular hemoglobin
MCHC – mean corpuscular hemoglobin concentration
MCV – mean corpuscular volume
MEA – mean electrical axis
MMVD – myxomatous mitral valve disease
MPV – mean platelet volume
MVD – mitral valve disease
MVR – mitral valve regurgitation
Na – sodium
OFA – Orthopaedic Foundation for Animals
PCT – platelet mass
PLAX – parasternal long axis
PLT – platelet count
PS – pulmonary stenosis
PV – parasternal view
RBC – red blood cell
RV – right ventricular
RVOT – right ventricular outflow tract
SBP – systolic arterial blood pressure
Se – diagnostic sensitivity of a test
Sp – diagnostic specificity of a test
SMOD – Simpson's method of discs
T4 – total thyroxine
TVR – tricuspid valve regurgitation
VHS – vertebral heart score (size)
WBC – white blood cell

The American Staffordshire Terrier (AST) is a dog breed characterized by an exuberant temperament and high activity. In terms of body structure AST dogs are similar to English Bull Terriers, which are predisposed to various heart diseases (8, 14). Historically, the AST dogs used to be considered as a generally healthy breed (American Kennel Club, available online: https://www.akc.org). There is no obligation to test representatives of the breed before allowing them to breed. The predisposition of the breed to cardiovascular diseases has not been investigated thoroughly yet. In 2020, an a large scale epidemiological study investing breed predisposition to congenital heart was published, in which AST accounted for only a small fraction of the study population. The study showed the following congenital heart defects: pulmonic and aortic stenosis, mitral valve dysplasia and double chamber right ventricle (4). It is possible that AST, like other terrier breeds, is predisposed to pulmonary artery stenosis (15, 20), and due to its size (large dog breed) it may have a tendency to develop dilated cardiomyopathy (16).

Full echocardiographic examination including Doppler technique is a mainstay of the cardiological diagnostics in modern veterinary medicine. Despite considerable improvement in the availability of specialist diagnostic procedures, they remain very expensive and still require scheduled consultations due to limited number of certified cardiologists. This fact discourages owners of apparently healthy dogs from regular prophylactic cardiologic consultations. They usually expect a general practitioner to examine their dog during routine visits (e.g. vaccinations) and let them know when a cardiologic consultation is necessary. To meet this expectation a general practitioner must have a simple and relatively inexpensive diagnostic method which will be good at ruling heart diseases out. Such a method, usually referred to as a screening diagnostic test, must yield highly trustworthy negative results so that a veterinarian knows that a dog in which the test is negative is almost certainly healthy. To do this a diagnostic test must have high capability of detecting diseased animals (i.e. high diagnostic sensitivity Se) and, as a result, a low likelihood ratio of negative result (LR_-) (18). The threshold LR_- usually considered as sufficient is ≤ 0.1 which means that a negative result is obtained in a diseased animal at least 10 times less often than in the healthy one (13). Whether any of broadly available cardiological diagnostic methods satisfies this requirement in AST remains unknown.

In this study, we aimed to describe the prevalence of subclinical heart diseases in the Polish population of adult AST without symptoms of cardiovascular disease. In addition, we tried to identify the alterations detectable in the basic clinical and auxiliary examination that could indicate the presence of subclinical heart disease in an apparently healthy AST with sufficient confidence and hence be used as a screening diagnostic test.

Material and methods

Study population and eligibility criteria. The analytical cross-sectional study was carried out at the Small Animal Clinic of the Institute of Veterinary Medicine, Warsaw University of Life Sciences between 2018 and 2022. The study was announced as a voluntary free-of-charge screening program for adult dogs (> 1 year-old) of AST breed documented by pedigree certificate issued by the Polish Kennel Club. The minimum number of dogs to be enrolled was 88. It was calculated so that the prevalence of subclinical heart diseases could be estimated assuming no prior knowledge on the prevalence (i.e. expected prevalence rate 50%), precision of estimation (accepted error) ± 10%, level of confidence 95%, and total Polish population of AST equal to 918 adult AST according to the Annual Breeding Report of The Polish Kennel Club for 2017 (Związek Kynologiczny w Polsce, available online: https://www.zkwp.pl/sprawozdania/spr_hod_2017.pdf.). To be enrolled in the study dogs had to be considered healthy by their owners (i.e. without any alarming symptoms) and satisfy the following criteria in the standard clinical examination: normal rectal body temperature (37.8 to 39.2°C), pink and moist mucous membranes of the gums and conjunctivae, normal submandibular, prescapular and popliteal lymph nodes in palpation, soft and non-tender abdominal cavity in palpation, no gait abnormalities, and no pathological sounds in the lung auscultation. Moreover, the dogs had to undergo a blood check-up including at least basic haematology, biochemistry and obtain results within the laboratory reference intervals (RI). The manual peripheral blood smear was performed, which was then analyzed under a microscope to assess the white blood cell system, the red blood cell system, and the platelet population. Complete blood count was performed using the Abacus Junior Vet (Diatron, Poland) hematology analyzer and included the following elements: total white blood cell count (WBC), percentage of: band neutrophils, segmented neutrophils, eosinophils, lymphocytes, monocytes, basophils, red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total platelet count (PLT), and mean platelet volume (MPV). Biochemistry was performed using the Miura One (TDI, Poland) biochemistry analyzer. The following parameters were determined: activity of aspartate aminotransferase (AspAT), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and concentration of glucose, creatinine, urea, total protein, and total cholesterol. The concentrations of potassium (K), sodium (Na) and chloride (Cl) ions were determined using the Diamond Diagnostics ProLYTE (ProLYTE, Poland) electrolyte analyzer. Concentrations of thyroid hormones – total thyroxine (T4) and free thyroxine (fT4) – were determined using the Adaltisanalyzer (Adaltis, Poland).

The dog owners were informed about the purpose and protocol of the study and they all granted informed written consent for participation in the study.

Basic cardiovascular examination. Basic examination of the cardiovascular system was carried out by one of two examiners. First, the heart was thoroughly auscultated using a human pediatric stethoscope (Littmann, USA). The

chestpiece was placed diaphragm to the skin at the points of maximal intensities (Latin: *puncta maxima*). The heart rate was counted for a minute, and the respiratory action was observed at the same time to detect respiratory sinus arrhythmia (defined as a relative acceleration of the heart rhythm during the inspiratory phase of the breathing cycle). Next, the auscultation was continued to detect heart murmurs which were classified in terms of their strength on the 6-point ordinal scale (I – the mildest through VI – the strongest).

Then, after approximately 10 minutes of acclimatization, the systolic arterial blood pressure (SBP) was measured using with the VET BP Doppler (VetBP Poland). The measurements were made in the standing position on the thoracic limb or tail. The cuffs were selected individually to the size of the dog, in most cases cuff no. 8 (limb circumference 13-20 cm) or no. 10 (limb circumference 18-26 cm) was used on the thoracic limb, the cuff no. 3 (circumference 4.3-8 cm) or no. 4 (circumference 7-13 cm) on the tail. SBP was measured 5 times, after which the two extreme values were discarded, and the remaining 3 measurements were averaged.

The electrocardiographic (ECG) examination was performed using the BTL-08-MD machine (BTL, Poland) in the standing position. Standard 6-lead ECGs (leads I, II, III, aVR, aVL, aVF) were recorded and the following ECG measurements were calculated from the lead II: P-wave amplitude and duration, PQ interval, Q-wave amplitude, R-wave amplitude, S-wave amplitude, QRS complex duration, QT interval, S-T segment elevation or depression, T-wave amplitude, mean electrical axis (MEA), and heart rate (HR). Heart rhythm and the presence of respiratory sinus arrhythmia were also recorded. All measurements were performed according to standard recommendations (19) MEA was calculated using the Mean Electrical Axis Calculator (Veterinary Information Network, available online: https://www.vin.com).

Thoracic radiography was performed in the right lateral projection and vertebral heart score/size (VHS) was calculated according to the method of Buchanan and Bucheler (5).

The transthoracic echocardiography was performed using Aloka 4000 ultrasound machine (Miro, Poland) with 3.5 MHz sector transducer and EsaoteMyLab X8 machine with 3.5 MHz sector transducer (Esaote, Poland) with simultaneous cardiac rhythm monitoring using electrodes connected to the skin folds. During the project, the ultrasound machine was replaced with a new one, therefore the tests were carried out on two different machines. Dogs were examined in the standing position. The hair was not clipped, only an appropriate amount of alcohol and coupling gel was used to ensure tight contact between the transducer and skin. The basic echocardiographic examination was performed in the two-dimensional (2D) presentation and the right parasternal short-axis view. The diameter of the left atrium (LA) and aortic root (Ao) were measured, and their ratio (LA/Ao) was calculated.

Full echocardiographic examination. The echocardiographic examination was continued in the right parasternal short-axis view and two-dimensional (2D-mode) presentation to measure the systolic blood flow velocity through the pulmonary artery (m/s) (right ventricular outflow tract, RVOT) (m/s) using Doppler ultrasonography. The right ventricular outflow tract obstruction (mmHg) were automatically calculated.

In the right parasternal short-axis view at the height of the papillary muscles, below the mitral valve annulus in a one-dimensional (M-mode) presentation the following measurements were taken (cm): the interventricular septum thicknesses in end-diastole (IVSd) and end-systole (IVSs), left ventricular peripheral (free) wall in end-diastole (LVWd) and end-systole (LVWs), end-diastolic right ventricular dimension (RV) and left ventricular dimension in end-diastole (LVDd) and end-systole (LVDs) (22). The LVDs and LVDd were indexed to BW according to Cornell's method (9) to obtain their normalized measurements (LVDdN and LVDsN, respectively). At mitral height, the distance of the septal mitral valve (point E) from the interventricular septum (EPSS) was measured (cm).

The left ventricular fractional shortening (FS) was calculated as follows:

$$FS = (LVDd - LVDs)/LVDd \times 100\%$$

The end-diastolic and end-systolic left ventricular volume (EDV and ESV, respectively) were measured in the 2D presentation the right parasternal long-axis view (PLAX) and left apical four-chamber view (A4C) using Simpson's method of discs (SMOD) (ml). In the A4C view mitral valve E wave velocity and mitral valve A wave velocity were also measured (m/s) and their ratio (E/A) was calculated. EDV and ESV were divided by body surface area (BSA) to obtain EDV and ESV indices in ml/m^2 (EDVI and ESVI, respectively) (23). BSA was calculated as follows (22):

$$BSA\ (m^2) = 0.101 \times BW\ (kg)^{2/3}$$

The ejection fraction (EF) was calculated as follows:

$$EF = (EDV - ESV)/EDV \times 100\%$$

In the left apical 5-chamber view and two-dimensional presentation the velocity of blood flow through the aorta (LVOT) was measured (m/s) and the left ventricular outflow tract obstruction (mmHg) was automatically calculated.

Subclinical heart diseases were defined as follows: mitral valve regurgitation (MVR) was diagnosed when the color doppler examination showed regurgitation through the mitral valve in the systolic phase. Myxomatous mitral valve disease (MMVD) was diagnosed when regurgitation through the mitral valve and thickening of its leaflets were observed, and mitral valve dysplasia when abnormalities in the construction of the valvular apparatus and mitral regurgitation were observed. Otherwise, the diagnosis of MVR of unknown character was held. Tricuspid valve regurgitation (TVR) was detected when the color doppler examination showed regurgitation through the tricuspid valve in the systolic phase. Pulmonary valve stenosis (PS) was diagnosed based on RVOT increased above 3 m/s. Values between 1.7 and 3 m/s were considered as borderline, potentially indicating an ongoing development of PS. Aortic valve stenosis (AS) was diagnosed based on LVOT increased above 3 m/s. Values between 2.45 and 3 m/s were considered as borderline, potentially indicating an ongoing development of AS. The RI for flows through the aorta and pulmonary artery that can be found in the literature is below 2 m/s (2).

In AST dogs, it was decided to recognize AS and PS above 3 m/s because below the mentioned flow no morphological changes were found in the outflow tracts of the left and right ventricle; moreover the tested dogs did not show any clinical changes and did not have heart murmurs. An example of a dog breed predisposed to aortic stenosis is the Boxer (American Kennel Club, available online: https://www.akc.org, 10). These dogs also have an acceptable aortic flow limit higher than the standard for the general population of dogs and it is 2.4 m/s (10). Cardiomegaly was defined as both LVDdN and LVDsN increased above 1.73 and 1.14, respectively (3). Dilated cardiomyopathy (DCM) was diagnosed when EDVI, ESVI, LVDd, and LVDs were elevated relative to the RI for AST dogs (17, 21), the FS and EF were decreased. On the basis of the obtained results, the dogs were divided into 2 groups: 29 dogs without heart disease (henceforth referred to as healthy) and 59 dogs with subclinical heart disease (henceforth referred to as diseased), and a statistical analysis was performed.

Statistical analysis. Numerical variables were presented as the median, interquartile range (IQR) and range, and compared between groups using the Mann-Whitney U test. Categorical variables were expressed as count and percentage, and compared between groups using the maximum likelihood G test or Fisher's exact test (if the expected count in any cell of the contingency table was < 5). Multivariable analyses were carried out using multivariable logistic regression with backward stepwise elimination procedure and the goodness-of-fit of the model was evaluated using Hosmer and Lemeshow (H&M) χ^2 test and Nagelkerke's pseudo-R^2 coefficient. The general diagnostic accuracy of numerical variables was determined by estimating the area under ROC curve (AUROC). For binary variables AUROC was calculated as the arithmetic mean of diagnostic sensitivity (Se) and specificity (Sp) (6). AUROCs were interpreted as follows: > 90% – a highly accurate test, > 80% to 90% – a moderately accurate test, > 70% to 80% – a fairly accurate test, ≤ 70% – a poorly accurate test (7, 11). Optimal cut-off value for numerical variables was determined by maximizing the Youden's index (J). For the optimal cut-off value Se and Sp as well as positive and negative likelihood ratios (LR_+ and LR_-) were provided to illustrate the magnitude of influence a given variable may have on clinical decision. LR_+ and LR_- were considered clinically important when their value was > 10 or < 0.1, respectively (13). The chance-corrected agreement between results of diagnostic tests was determined using Gwet's AC_1 coefficient (12) and categorized as follows: AC_1 = > 0.81 – very good, 0.61-0.80 – good (substantial), 0.41-0.60 – moderate, 0.21-0.40 – fair, and ≤ 0.20 – poor agreement (1). A significance level (α) was set at 0.05. All statistical tests were two-tailed. The statistical analysis was performed in TIBCO Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA). For the needs of this study, the test had to show $LR_- \leq 0.2$ to be considered as sufficiently accurate screening diagnostic method.

Results and discussion

The study population counted 88 adult AST, 39 males and 49 females, aged from 1.3 to 13.9 years with the median (IQR) of 4.2 (2.3-6.2) years. Age did not differ significantly between sexes (p = 0.108). Body weight ranged from 18 to 44 kg and males were significantly heavier (median 30 kg, IQR 28-33 kg) than females (median 28 kg, IQR 25-30 kg) (p < 0.001). Virtually all results of the blood check-up were within the RI (Tab. S1). Some abnormalities observed were mild and clinically unimportant.

Tab. S1. Blood check-up results and reference intervals (RI) used for their interpretation

Measurement	No. of dogs tested	Median, interquartile range (range)	Laboratory reference interval (RI)
Hematology			
WBC (G/L)	88	9.4, 7.2-11.7 (4.3-22.1)	6.0-16.5
RBC (T/L)	88	6.8, 6.4-7.3 (5.2-8.5)	5.5-8.5
Hemoglobin (g/dL)	88	15.9, 14.8-17.0 (12.7-19.8)	12-18
Hematocrit (%)	88	48, 43-51 (36-60)	37-55
MCV (fl)	87	70, 67-72 (62-77)	60-77
MCH (pg)	86	23.1, 22.4-23.7 (21.2-26.9)	19-24
MCHC (g/dL)	86	33.3, 32.5-33.8 (29.7-36.8)	32-36
Platelets (G/L)	88	254, 213-314 (94-507)	200-580
MPV (fl)	84	8.9, 8.0-9.5 (7.0-12.7)	6.1-10.1
Band neutrophils (G/L)	88	0.1, 0-0.2 (0-1.3)	< 0.5
Segmented neutrophils (G/L)	88	6.9, 5.1-9.0 (2.4-18.8)	3.6-12.7
Eosinophils (G/L)	88	0.1, 0-0.3 (0-1.8)	0.1-1.7
Lymphocytes (G/L)	88	1.5, 1.2-2.3 (0.5-4.1)	0.7-5.0
Monocytes (G/L)	88	0.1, 0-0.3 (0-0.9)	0.2-1.7
Biochemistry			
AspAT (U/L)	88	32, 27-40 (15-70)	< 45
ALT (U/L)	88	39, 29-57 (18-244)	< 60
ALP (U/L)	88	31, 24-42 (15-296)	< 155
Glucose (mg/dL)	88	99, 91-105 (34-127)	70-120
Total protein (g/dL)	88	61, 58-64 (52-72)	55-75
Urea (mg/dL)	88	33, 26-39 (16-59)	20-50
Creatinine (mg/dL)	88	1.2, 1.0-1.3 (0.7-1.9)	< 1.7
Total cholesterol (mg/dL)	86	203, 171-262 (114-428)	128-364
Na (mmol/L)	85	148, 146-150 (141-156)	139-157
K (mmol/L)	85	4.4, 4.2-4.6 (3.7-5.4)	4.1-5.4
Cl (mmol/L)	84	111, 109-115 (101-124)	99-116
T4 (ng/dL)	87	23, 17-31 (10-43)	10-40
fT4 (ng/dL)	86	1.7, 1.3-2.5 (0.5-3.5)	0.5-4.0

Tab. 1. Prevalence of subclinical heart diseases. The numbers and percentages do not add up to the total number of dogs (100%) as one dog could have more than 1 disease

Subclinical heart diseases	Number of diseased dogs and percentage of 59 dogs with subclinical heart disease	Prevalence (CI 95%) (%) in the study population (percentage of 88 study dogs)
Mitral valve regurgitation (MVR)	52 (88.1)	59.1 (48.6-68.8)
Myxomatous mitral valve disease (endocardiosis, MMVD):	20 (33.0)	22.7 (15.2-32.5)
of only mitral valve	16 (27.1)	18.2 (11.5-27.5)
of mitral and tricuspid valve	4 (6.8)	4.5 (1.8-11.1)
Mitral valve dysplasia	2 (3.4)	2.3 (0.6-7.9)
Tricuspid valve regurgitation (TVR)	12 (20.3)	13.6 (8.0-22.3)
Pulmonary valve disease:	12 (20.3)	13.6 (8.0-22.3)
Pulmonary stenosis (PS) (defined as RVOT > 3 m/s)	2 (3.4)	2.3 (0.6-7.9)
Borderline RVOT increase (1.7-3.0 m/s)	10 (16.9)	11.4 (6.3-19.7)
Aortic valve disease:	7 (11.9)	8.0 (3.9-15.5)
Aortic stenosis (AS) (defined as LVOT > 3 m/s)	0	0 (0-4.2)
Borderline LVOT increase (2.45-3.0 m/s)	7 (11.9)	8.0 (3.9-15.5)
Any atrioventricular valve disease	55 (93.2)	62.5 (52.1-71.9)
Any arterial valve disease	16 (27.1)	18.2 (11.5-27.5)
Dilated cardiomyopathy (DCM)	0	0 (0-4.2)
Cardiomegaly (defined as LVDdN > 1.73 and LVDsN > 1.14)	15 (25.4)	17.0 (10.6-26.2)

Explanations: LVDdN – normalized left ventricular dimension in end-diastole, LVDsN – normalized left ventricular dimension in end-systole; LVOT – left ventricular outflow tract; RVOT – right ventricular outflow tract

Any subclinical heart disease was diagnosed in 59/88 AST which yielded the prevalence of 67.0% (CI 95%: 56.7-76.0%) (Tab. 1). Diseases of atrioventricular valves were most prevalent, significantly more common than aortic and pulmonary valve diseases. The most common subclinical heart disease was MVR, found in 52/59 AST with subclinical heart disease. This was also the only subclinical heart disease detected more often as a solitary condition than concurrently with other diseases (Fig. 1). In only approximately one third of AST with MVR a definitive diagnosis of MMVD or mitral valve dysplasia was made based on the echocardiographic view. In the remaining cases the character of pathological process could not be clarified, on the heart valves in these dogs, changes typical of endocardiosis and dysplasia were not visible. TVR and pulmonary valve disease were observed equally often, in 12/59 AST with subclinical heart disease. They were usually detected concurrently with MVR, very rarely as an isolated condition (Fig. 1). Fully developed PS was diagnosed in only 2/59 dogs, the remaining 10 dogs had increased RVOT. Seven dogs had increased LVOT but none had fully developed AS. Fifteen dogs had cardiomegaly, of which 14 had MVR (along with TVR in 1 dog, with accelerated RVOT and accelerated LVOT in 1 dog, with TVR, accelerated RVOT and accelerated LVOT in 1 dog). The remaining dog with cardiomegaly had only accelerated RVOT. None of dogs had DCM.

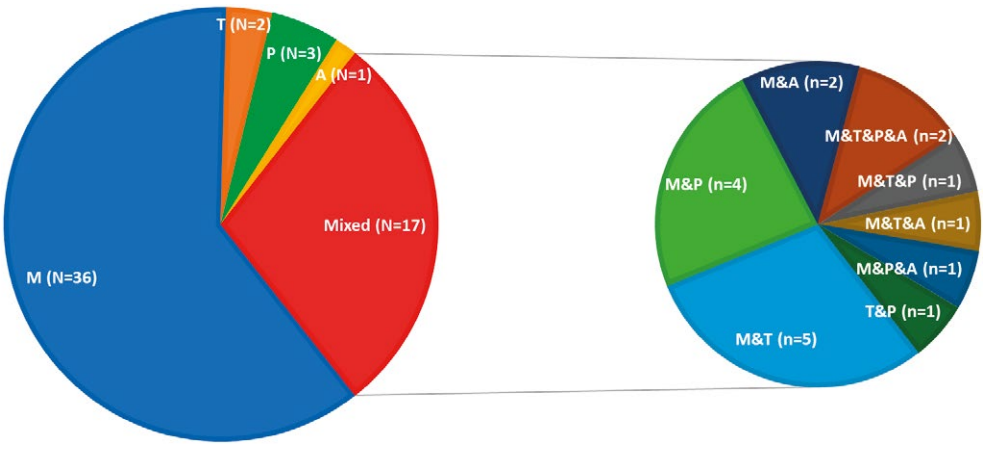

Fig. 1. Combinations of subclinical heart diseases in the study population of American Staffordshire Terriers
Explanations: M – mitral valve disease; T – tricuspid valve disease; P – pulmonary valve disease; A – aortic valve disease

None of demographic characteristics proved to be significantly related to the occurrence of subclinical heart disease (Tab. 2). Only mild heart murmur (from I/VI through III/VI) was detected and only in diseased dogs so it was 100% specific for the subclinical heart disease (Tab. 3). On the other hand, its Se was very low (Tab. 3) which indicated that this diagnostic test was unsuitable for ruling out subclinical heart disease. SBP, VHS, and detection of respiratory sinus arrhythmia in auscultation did

Tab. 2. Basic clinical and auxiliary diagnostic test results in American Staffordshire Terriers with and without subclinical heart disease

Potential indicators of the subclinical heart disease [a]	Group		p-value	Area under ROC curve – AUROC (CI 95%) (%)
	Diseased dogs (n = 59)	Healthy dogs (n = 29)		
Demographic characteristics				
Male sex	24 (40.7)	15 (51.7)	0.328	45 (32-57)
Age (years)	4.1, 2.2-6.4 (1.3-13.9)	4.5, 2.6-5.7 (1.5-10.1)	0.759	52 (40-64)
Body weight (kg)	29, 25-31 (18-40)	30, 27-32 (23.5-44)	0.115	60 (48-73)
Auscultation				
Heart rate (per min)	100, 88-112 (68-142)	100, 88-108 (72-124)	0.527	54 (42-67)
Heart murmur level I/VI-III/VI	24 (40.7)	0	< 0.001	70 (60-81)
Respiratory sinus arrhythmia	23 (39.0)	11 (37.9)	0.924	51 (38-63)
Systolic blood pressure				
Blood pressure measured on the tail (mmHg)	130, 115-150 (90-190) (56 dogs)	130, 115-145 (80-190) (23 dogs)	0.677	53 (39-67)
Blood pressure measured on the forelimb (mmHg)	130, 115-145 (87-210) (57 dogs)	138, 118-149 (80-180) (24 dogs)	0.784	52 (38-66)
Thoracic radiography				
Vertebral heart score (VHS)	11.1, 10.7-11.4 (10.1-12.4) (55 dogs)	11.0, 10.5-11.5 (9.9-12.2) (28 dogs)	0.105	61 (47-74)
Electrocardiography				
Heart rate (per min)	110, 90-120 (60-150)	110, 100-120 (70-150)	0.693	53 (40-65)
Respiratory sinus arrhythmia	34 (57.6)	8 (27.6)	0.007	65 (53-77)
qR pattern of QRS complex	7 (11.9)	0	0.015	56 (44-68)
P-wave duration (s)	0.05, 0.04-0.05 (0.03-0.08)	0.05, 0.04-0.05 (0.03-0.06)	0.572	53 (40-66)
P-wave amplitude (mV)	0.25, 0.20-0.25 (0.15-0.45)	0.25, 0.25-0.30 (0.15-0.45)	0.018	65 (53-78)
PQ interval duration (s)	0.11, 0.10-0.12 (0.09-0.18)	0.10, 0.10-0.12 (0.07-0.14)	0.021	65 (53-77)
QRS complex duration (s)	0.07, 0.06-0.07 (0.05-0.09)	0.07, 0.06-0.08 (0.04-0.08)	0.898	51 (37-64)
Q-wave amplitude (mV)	0.40, 0.20-0.60 (0.10-0.95)	0.45, 0.35-0.65 (0.20-0.90)	0.172	59 (47-71)
R-wave amplitude (V)	1.50, 1.10-1.85 (0.16-3.00)	1.80, 1.40-2.05 (0.90-3.20)	0.032	64 (52-76)
S-wave amplitude (mV)	0.15, 0.08-0.20 (0.00-0.30)	0.15, 0.10-0.20 (0.03-0.60)	0.240	58 (45-70)
QT interval duration (s)	0.22, 0.21-0.23 (0.14-0.29)	0.21, 0.20-0.22 (0.15-0.25)	0.034	64 (52-76)
ST segment amplitude (mV)	–0.15, –0.20 - –0.10 (–1.15 - –0.05)	–0.15, –0.2 - –0.1 (–0.35-0.25)	0.185	58 (45-71)
T-wave amplitude (mV)	0.15, 0.15-0.20 (0.05-0.40)	0.15, 0.15-0.25 (0.01-0.35)	0.501	54 (41-68)
Mean electrical axis (MEA)	30, 20-52 (4-150)	30, 15-46 (–10-62)	0.632	53 (40-66)
Basic echocardiography				
Left atrium to aortic root ratio (LA/Ao)	1.37, 1.31-1.43 (1.04-1.66)	1.26, 1.16-1.35 (1.01-1.49)	< 0.001	73 (62-84)

Explanations: a – numerical variables presented as the median, interquartile range and range; categorical variables presented as the count and percentage in parentheses

Tab. 3. Diagnostic performance (diagnostic accuracy) of selected clinical and auxiliary tests in detecting subclinical heart disease in American Staffordshire Terriers

Diagnostic test	Area under ROC curve – AUROC (CI 95%) (%)	p-value for AUROC	Optimal cut-off value [a]	Diagnostic sensitivity – Se (CI 95%) (%)	Diagnostic specificity – Sp (CI 95%) (%)	Likelihood ratio	
						positive – LR_+ (CI 95%)	negative – LR_- (CI 95%)
Presence of the heart murmur level I/VI-III/VI in chest auscultation	70 (60-81)	< 0.001	–	41 (29-53)	100 (88-100)	$+\infty$	0.59 (0.48-0.73)
Presence of the respiratory sinus arrhythmia in electrocardiography	65 (53-77)	0.015	–	58 (45-69)	72 (54-85)	2.1 (1.1-3.9)	0.59 (0.40-0.85)
PQ-interval duration (s)	65 (53-77)	0.017	≥ 0.11	71 (59-81)	52 (34-69)	1.5 (1.0-2.2)	0.56 (0.33-0.95)
Left atrium to aortic root ratio (LA/Ao)	73 (62-84)	< 0.001	≥ 1.4	63 (50-74)	72 (54-85)	2.3 (1.2-4.2)	0.52 (0.35-0.77)

Explanation: a – presented only for quantitative diagnostic tests

not show any diagnostic value (Tab. 2). Interestingly, the agreement between auscultation and ECG with respect to detection of respiratory sinus arrhythmia was low (AC_1 33%, CI 95%: 14-53%), and detection of respiratory sinus arrhythmia in ECG had some, still rather poor, diagnostic value (Tab. 3). Similarly to the detection of heart murmur, its Se was low which again indicated that this diagnostic test was unsuitable for ruling out subclinical heart disease.

Besides respiratory sinus arrhythmia in ECG as many as 5 ECG measurements were significantly different between diseased and healthy dogs. The qR morphology of QRS complex occurred only in dogs with subclinical heart disease so it was 100% specific for subclinical heart disease (CI 95%: 88.3-100%) but only 12% sensitive (CI 95%: 6-23%). This indicated that a dog with qR complex certainly had subclinical heart disease but detection of qRs morphology of the QRS complex did not provide any useful information. The P-wave and R-wave amplitudes were significantly lower and the PQ-interval and QT-interval duration were significantly longer in dogs with subclinical heart disease. Multivariable logistic regression analysis including respiratory sinus arrhythmia in ECG, P-wave and R-wave amplitudes, and the PQ-interval and QT-interval duration narrowed down the number of significant ECG measurements to two: detection of respiratory sinus arrhythmia in ECG (OR 3.1, CI 95%: 1.2-8.3, $p = 0.026$) and PQ-interval duration expressed (OR (per 1 ms) 1.03, CI 95%: 1.00-1.07, $p = 0.046$). The model fit data well (H&L $\chi^2 = 6.47$, $p = 0.486$; Nagelkerke's pseudo-R^2 coefficient = 0.18). The magnitude of PQ-interval duration difference between diseased and healthy dogs was very small and unlikely to be applicable in routine veterinary practice.

Dogs with subclinical heart disease had significantly higher LA/Ao ($p < 0.001$) although still its diagnostic accuracy was only fair (Tab. 3). The optimal cut-off value was LA/Ao $\geq$ 1.4 indicating subclinical heart disease. However, LA/Ao had low Se (only 63%) which, like in the case of detection of the heart murmur, indicated that this diagnostic test was completely unsuitable for ruling out subclinical heart disease.

Two thirds of the examined dogs were diagnosed with subclinical heart disease. Valvular changes were the most common, occurring in both young and older dogs. Changes on the atrioventricular valves occurring in young dogs may indicate valve dysplasia, while in older dogs – endocardiosis (MMVD). MMVD in large dog breeds may be poorly expressed in the echocardiographic scans, therefore the final diagnosis can be made only on the basis of histopathological examination. A large number of dogs had MVR without visible leaflet changes. Therefore, it is worth testing a larger population of dogs and monitoring it for a longer period of time to assess whether this change is clinically significant and progressing in breeding, pedigree AST dogs.

Although data from the literature indicated a predisposition of the breed to PS, in our study population this change occurred in only two dogs. Our study also did not confirm the predisposition to AS and DCM, none of the dogs showed the above changes. A large part of the examined dogs had accelerated LVOT and RVOT in relation to the RI adopted for the general canine population; however, they were within the RI established for the AST breed (17, 21).

Studies have shown that AST dogs have an enlarged heart silhouette (17). Comparing the obtained echocardiographic values to the RI for the general population of dogs, cardiomegaly may be incorrectly stated. Dogs with subclinical heart disease had higher LA/Ao, RVOT, LVOT and LVDdN, however all echocardiographic measurements obtained in our studies were within the RI adopted for the AST breed (17, 21). In addition, the absence of cardiomegaly is evidenced by an enlarged left ventricle with the correct dimension of the left atrium.

Most of the diagnosed changes in the AST's heart affected the left side. Our finding is consistent with the literature that reports that most heart disease in dogs affects the left side of the heart (20).

Only 41% of dogs with atrioventricular regurgitation had a heart murmur. AST dogs, due to their temperament and barrel chest, were very difficult to auscultation. Accordingly, echocardiography is the only method to rule out heart disease.

Most of the examined AST dogs had subclinical changes in the heart and echocardiography was the only method to detect them. There are differences in some echocardiographic parameters between healthy AST and the general dog population, AST dogs have an unusual heart shape; it is larger, what's more, they have higher LVOT. Therefore, only specific RI established for the breed should be used when testing this dogs (17, 21).

The main limitation of our study was the small number of dogs tested. Additionally, these dogs were assessed by their owners as healthy, showing no signs of cardiovascular disease, therefore a large part of them was healthy, the rest had only slight changes in the heart. Dogs were examined once, the progress of changes in the cardiovascular system was not monitored by us.

Our study shows that subclinical heart diseases are common in the Polish population of adult AST with MVR being the most common disease. What proportion of subclinical heart diseases progress into clinical heart diseases remains unknown. Detection of the heart murmur in auscultation, respiratory sinus arrythmia in ECG, qR pattern of the QRS complex in ECG, PQ $\geq$ 0.11 s, and LA/Ao $\geq$1.4 in an asymptomatic adult AST is suggestive of the subclinical heart disease and should prompt owners to seek cardiological consultation. None of these tests fulfils criteria for a screening diagnostic method. Only a full echocardiographic

examination performed by a cardiologist makes it possible to rule out the subclinical heart disease in adult AST.

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