

# Age-dependent electrocardiographic and echocardiographic changes in German Shepherd dogs

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## Summary

By causing numerous changes in the cardiovascular system, ageing leads to a decreased threshold for clinical manifestation of heart disease. The aim of this study was to define the existence of cardiac structural and functional changes in healthy dogs of different age. Radiographic, electrocardiographic (ECG) and echocardiographic examinations of 20 clinically healthy German Shepherd dogs were performed in order to define the values of relevant parameters. Afterwards, the values of cardio examinations were compared between young and old dogs and statistically analyzed. The ECG recordings did not show the appearance of clinically significant arrhythmias, nor was their appearance significantly different between dogs of different age. Statistically significant differences in QRS duration ( $P < 0.05$ ) and R wave amplitude ( $P < 0.05$ ) existed between groups, but all values were within the range of ECG reference values for healthy dogs. Concerning structural changes, the left ventricle wall thickness at end-diastole (LVWd) and end-systole (LVWs), and the relative wall thickness (RWT) between young and old dogs differed significantly ( $P < 0.001$ ,  $P < 0.05$ ,  $P < 0.05$ , respectively). These differences in heart structure were not accompanied by systolic dysfunction, estimated by a left ventricle shortening fraction. The diastolic wall stress index (WSID) was significantly decreased in old dogs ( $P < 0.05$ ). Young and old dogs showed similar electrical and systolic function. Old dogs had different cardiac structure compared to the young dogs, which could result in diastolic function change.

**Key words:** Ageing, Cardiology, German Shepherd dog

## Introduction

It was generally recognized that the frequency of cardiovascular diseases increases with ageing in dogs (Bonagura, 1981; Hamlin, 2005). Disorders of the cardiovascular system are one of the most commonly encountered disease entities in the ageing population (Saunders, 2012) and furthermore, are indicated as the second most common cause of death in dogs (Eichelberg and Seine, 1996). The mortality rate from heart disease, however, is dependent on breed, age and gender of dogs, and it was shown to be increased for Irish Wolfhound, Cavalier King Charles Spaniel and Great Dane (Egenvall *et al.*, 2005) or defined like leading cause of death in Newfoundland, Maltese, Chihuahua, Doberman Pincher, Fox Terrier and Bernese (Fleming *et al.*, 2011).

Ageing results in numerous changes of the cardiovascular system that could be observed from morphological, functional, endocrinological, genetic and biochemical points of view. The precise relationship between the negative modifications of cardiovascular function with ageing and development of specific cardiovascular diseases is difficult to ascertain. In healthy humans, age-associated cardiac changes seem to have relevance to the steep increases in left ventricular (LV) hypertrophy, heart failure and arterial fibrillation (Lakatta and Levy, 2003; Redfield *et al.*, 2005). Although the existence of cardiovascular functional

modification with ageing was shown in anesthetized and unanesthetized dogs under experimental conditions (Miller *et al.*, 1976; Templeton *et al.*, 1976, 1979; Haidet, 1993; Haidet *et al.*, 1996; Strasser *et al.*, 1997), extensive clinical studies were not performed in dogs. Besides, the studies in dogs as experimental animals (Gan *et al.*, 2013; Kim *et al.*, 2013; Xu *et al.*, 2013a, b) have been designed as researches in human cardiology. Knowing that the number and proportion of older dogs in the canine population is constantly increasing, the results of these studies could be very useful in canine cardiology.

Breed predisposition for certain acquired cardiac diseases imposes the need for studies in dogs of the same breed. German Shepherd dogs have low risk of death due to heart disease in comparison with other breeds (Egenvall *et al.*, 2005), which makes them suitable for assessment of cardiac morphology and function in relation to ageing. Although data concerning values of electrocardiographic (ECG) (Rezakhani *et al.*, 1990) and echocardiographic parameters (Kayar *et al.*, 2006; Muzzi *et al.*, 2006) in the healthy German Shepherd dog were published, these studies were not designed to analyze age-dependent cardiac changes. The study presented here was performed to define the existence of age-dependent changes of cardiac structural and functional characteristics in German Shepherd dogs, which could be registered with the conventional diagnostic procedures widely used

in canine cardiology.

## Materials and Methods

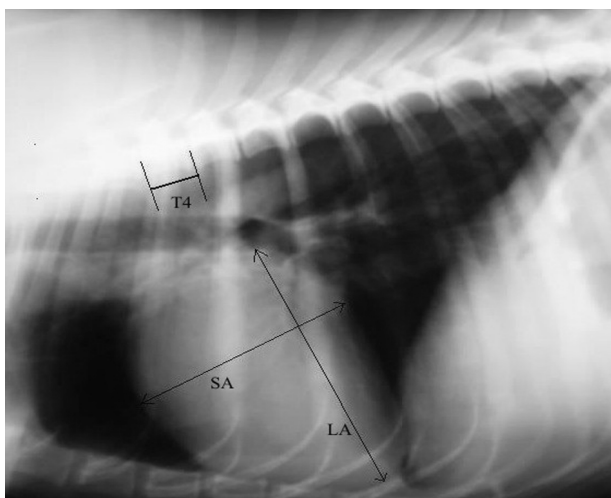
### Animals

In this study, 20 clinically healthy German Shepherd dogs (16 males and 4 females) were examined. The dogs were divided into two groups on the basis of their age: young dogs from 1 to 3 years of age (n=10 dogs, 8 males and 2 females; average age 2 years) and old dogs from 8 to 13 years of age (n=10 dogs, 8 males and 2 females; average age 10.5 years). Knowing that different breeds age at different speeds, the groups of young and old dogs were defined in accordance with available data on the subject of ageing in German Shepherd dogs (Egenvall *et al.*, 2005).

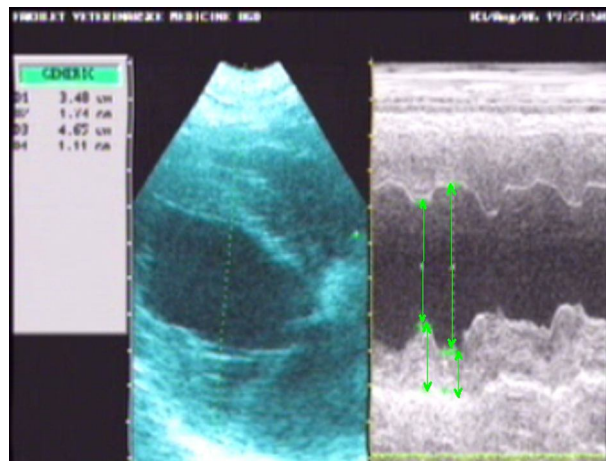
All dogs were assessed by clinical examination including history of each dog, physical examination, complete blood count and serum chemistry, thoracic radiography, electrocardiography and echocardiography. All examinations were performed with manual restraint of the animals, without using sedation or anesthesia.

### Methods

Radiographic examinations were performed from both laterolateral and dorsoventral radiographic views (Gierth HF 200, Japan). Radiographs of all dogs were analyzed subjectively by an experienced veterinary radiologist. Then, the vertebral heart size (VHS) was calculated from laterolateral thoracic radiographs of the dogs, according to modified Buchanan and Bücheler's measurement (Buchanan and Bücheler, 1995; Spasojević Kosić *et al.*, 2007). Briefly, the long axis (LA) and short axis (SA) of the heart were placed on the cardiac silhouette. By using calipers and ruler, the LA and SA were measured in mm, and then transformed into VHS unit (v) by dividing them with the length of the body and caudal disc of fourth thoracic vertebra (T4). Finally, the values of the LA and SA were summed up in order to define VHS (Fig. 1).



**Fig. 1:** Laterolateral radiograph with marked LA and SA of the heart and VHS unit (body and disc of T4) according to the modified VHS measurement



**Fig. 2:** Two-dimensional guided M-mode echocardiogram with LV parameters measurements

Electrocardiographic examinations were done separately as resting electrocardiography in dogs' right lateral recumbent position (Cardiopan, Philips and Schiller AT-1, Schiller AG, Switzerland) according to the Standards for Canine Electrocardiography (Hamlin and Patterson, 1977). The values of ECG parameters received from lead II were collected in a computer data base. The ECG recordings were analyzed according to the standard procedure (Edwards, 1987).

Echocardiographic examinations were performed in the standing position of dogs as two-dimensional (2D) (both longitudinal and transversal from right parasternal location, left caudal and left cranial parasternal location) and M-mode echocardiography, using a 5 MHz mechanical sector transducer (300 S Pandion Vet, Esaote Pie Medical, the Netherlands). Measurements were performed in accordance with recommendations of the American Society of Echocardiography (Sahn *et al.*, 1978). LV parameters: left ventricular internal diameter in diastole (LVIDd), left ventricular internal diameter in systole (LVIDs), left ventricular wall thickness in diastole (LVWd), and left ventricular wall thickness in systole (LVWs) were measured from right parasternal location by using two-dimensional guided M-mode echocardiograms. The M-mode cursor line was placed at the LV chamber level, just below the tip of the mitral valve, with the beam directed perpendicular to the septum and LVW in order to avoid papillary muscles within the left ventricle (Fig. 2). The measurements were made from the leading edge of an echo (Kienle, 1998). For each parameter, the mean of three consecutive cardiac cycles was taken as the result of measurement. As indicator of LV geometric remodeling, the relative wall thickness (RWT) was calculated (Foppa *et al.*, 2005). The relative wall thickness, the left ventricular fractional shortening (FS), the left ventricle free wall percent thickening (LVW%T), the systolic wall stress index (WSIS) and the diastolic wall stress index (WSID) were calculated using the following formulas (Minors and O'Grady, 1998; Foppa *et al.*, 2005):

$$RWT = 2 \times LVWd / LVIDd$$

$$FS(\%) = LVIDd - LVIDs / LVIDd \times 100$$

$$LVW\%T = 100 \times (LVWs / LVWd) / LVWd$$

$$WSIS = LVIDs / LVWs$$

$$WSID = LVIDd / LVWd$$

### Statistical analysis

Statistical analyses were performed with a computerized statistical software package Statistica 12. Data are reported as the mean  $\pm$  standard deviation (SD). Values of radiographic, ECG and echocardiographic parameters between dogs of different age were compared using Student's t-test for independent samples. Rate of arrhythmias' occurrence between groups was assessed by Fisher test of probability. Multiple regression analysis was used to assess the association between measured LV echocardiographic parameters (LVIDd, LVIDs, LVWd, LVWs) and calculated echocardiographic parameters (FS, LVW%T, WSIS, WSID, RWT). The correlation between measured and calculated LV echocardiographic parameters was evaluated by correlation coefficients. Statistical significance was defined as  $P < 0.05$ .

### Results

In this study, 20 German Shepherd dogs without clinical signs of cardiovascular or respiratory system diseases were clinically assessed. Dogs did not have pathological heart sounds or heart murmurs. Clinical and laboratory examinations revealed no evidence of relevant systemic disease existence. Average values of VHS in young and old German Shepherd dogs were  $10.26 \pm 0.34$  v and  $10.26 \pm 0.61$  v, respectively. There was no significant difference in VHS between dogs of different age.

Heart rhythm of all dogs was sinus in origin (Table 1). All registered arrhythmias in dogs were benign. Certain dogs had several arrhythmias (for example, sinus arrhythmia with sinus pause, wandering pacemaker with sinus arrest and AV block 1°). The appearance of AV block 1° in aged dogs was not statistically significant. The values of R wave amplitude and QRS complex duration were significantly different between dogs of different age (Table 2). However, all ECG parameter values were within the references range for healthy dogs (Edwards, 1987).

**Table 1:** Heart rhythm in German Shepherd dogs of different age

Heart rhythms	Young dogs n (% of all dogs)	Old dogs n (% of all dogs)
Normal sinus rhythm	1 (5%)	1 (5%)
Sinus arrhythmia	5 (25%)	5 (25%)
Wandering pacemaker	3 (15%)	3 (15%)
Sinus bradycardia	1 (5%)	1 (5%)
Sinus block	1 (5%)	-
Sinus pause	-	1 (5%)
Sinus arrest	1 (5%)	3 (15%)
AV block 1°	-	3 (15%)

n: Number of dogs

**Table 2:** ECG parameters in German Shepherd dogs of different age

ECG parameters	Young dogs (mean $\pm$ SD)	Old dogs (mean $\pm$ SD)
HR (beats/min)	92.184 $\pm$ 13.577	87.458 $\pm$ 17.538
P time (s)	0.040 $\pm$ 0.000	0.044 $\pm$ 0.008
P ampl. (mV)	0.183 $\pm$ 0.073	0.229 $\pm$ 0.079
PR time (s)	0.116 $\pm$ 0.014	0.126 $\pm$ 0.021
R ampl. (mV)	2.228 $\pm$ 0.674	1.650 $\pm$ 0.501*
QRS time (s)	0.060 $\pm$ 0.000	0.055 $\pm$ 0.007*
Tampl. (+) (mV)	+0.400 $\pm$ 0.000	+0.450 $\pm$ 0.260
Tampl. (-) (mV)	-0.427 $\pm$ 0.293	-0.373 $\pm$ 0.083
QT time (s)	0.210 $\pm$ 0.019	0.206 $\pm$ 0.021

\*  $P < 0.05$

The echocardiographic parameters (Table 3), the values of the LVWd ( $P = 0.000597$ ) and LVWs ( $P = 0.009259$ ) in older dogs were significantly higher compared with values in young dogs. Also, the RWT was significantly different ( $P = 0.007798$ ) between the two groups. There was no significant difference for FS between dogs of different age, but WSID was significantly lower ( $P = 0.007107$ ) in older dogs compared to the young ones.

**Table 3:** Echocardiographic data for German Shepherd dogs of different age

Echo parameters	Young dogs (mean $\pm$ SD)	Old dogs (mean $\pm$ SD)
LVIDd (mm)	43.88 $\pm$ 3.78	45.29 $\pm$ 3.34
LVIDs (mm)	31.23 $\pm$ 5.51	32.04 $\pm$ 3.55
LVWd (mm)	8.75 $\pm$ 0.71	10.99 $\pm$ 1.55**
LVWs (mm)	10.52 $\pm$ 1.38	12.97 $\pm$ 2.27*
RWT	0.40 $\pm$ 0.03	0.48 $\pm$ 0.09*
FS (%)	28.94 $\pm$ 8.96	29.16 $\pm$ 6.77
LVW%T (%)	20.05 $\pm$ 10.78	18.17 $\pm$ 14.97
WSIS	3.03 $\pm$ 0.78	2.55 $\pm$ 0.62
WSID	5.03 $\pm$ 0.48	4.20 $\pm$ 0.72*

\*  $P < 0.05$ , and \*\*  $P < 0.001$

Results of the multivariable regression model by using echocardiographic parameters are shown in Table 4. Both echocardiographic parameters, RWT and WSID, were significantly correlated with LVWd (Figs. 3A-B).

### Discussion

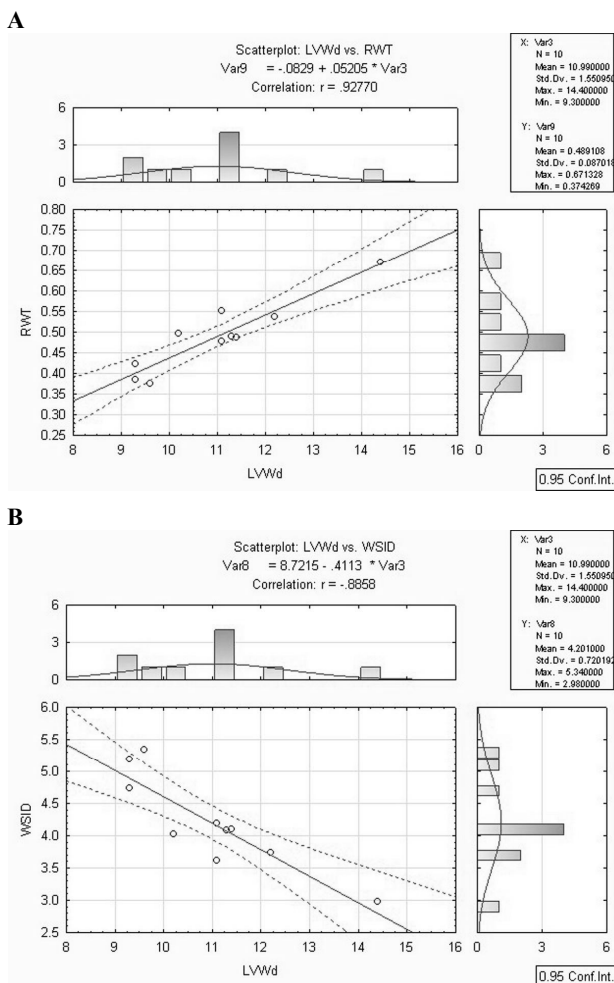
In this study, cardiac structural and functional differences were found between young and old German Shepherd dogs. An increase in LVW, and LV hypertrophy was registered with aging. A significant systolic dysfunction was not registered with aging and there was no correlation between LVW and FS in healthy German Shepherd dogs. Ageing leads to statistically significant decrease of electrical and diastolic function in German Shepherd dogs.

Earlier studies have shown changes of cardiovascular function with aging, such as decreased blood flow, blood velocity, arterial compliance and distensibility (Miller *et al.*, 1976; Haidet, 1993; Haidet *et al.*, 1996), as well as increased systemic vascular resistance and decreased

**Table 4:** The echocardiographic parameters examined for association: multiple regression results. The values are expressed as coefficient b (SE)

Dependent variables	Age	Independent variables			
		LVIDd	LVIDs	LVWd	LVWs
FS	Young	0.68 (0.04)**	-1.34 (0.03)**	NS	NS
	Old	0.71 (0.03)**	-1.07 (0.03)**	NS	NS
LVW%T	Young	NS	NS	-0.87 (0.04)**	1.47 (0.03)**
	Old	NS	NS	-1.02 (0.04)**	1.36 (0.03)**
WSIS	Young	NS	0.70 (0.07)**	NS	-0.50 (0.07)*
	Old	NS	0.58 (0.05)**	NS	-0.71 (0.06)**
WSID	Young	0.95 (0.02)**	NS	-0.88 (0.03)**	NS
	Old	0.46 (0.06)**	NS	-0.65 (0.08)**	NS
RWT	Young	-0.93 (0.05)**	NS	0.86 (0.05)**	NS
	Old	-0.38 (0.02)**	NS	0.81 (0.03)**	NS

\* P<0.05, and \*\* P<0.001. NS: Non-significant



**Fig. 3:** Correlations between LVWd and RWT (A), and LVWd and WSID (B) in old German Shepherd dogs. The graphics illustrate the linear regression line with a 95% confidence interval

cardiac output (Haidet *et al.*, 1996). Also, decreased contractile function, increase in LV systolic and diastolic stiffness and prolonged duration of contraction were demonstrated in senescent heart (Templeton *et al.*, 1976, 1979). According to the results of our study, aging leads to changes of cardiac electrical characteristics in German Shepherd dogs. Both parameters of ventricles depolari-

zation, QRS duration and R wave amplitude were significantly different between dogs of different age. However, values of QRS duration and R wave amplitude of both groups were within the reference range. Significant difference in R wave amplitude could be explained by the existence of low R wave amplitudes (R <1.5 mV) in 3 older dogs. Among the geriatric dogs, three dogs had first degree AV block (AV block 1°). Generally speaking, this type of impulse conduction disturbance increases with age in dogs. Although clinically important, the appearance of first degree AV block did not show statistically significant differences between the two groups. Our results are slightly different from the previously published ECG values of German Shepherd dogs (Rezakhani *et al.*, 1990), and higher values of P wave amplitude, PR duration and R wave amplitude were measured in our study.

The recognition of interbreed variations in cardiac dimensions has already led to the development of breed-specific ranges for echocardiography (Morrison *et al.*, 1992; Kayar *et al.*, 2006) and researches have suggested their importance in radiographic measurement as well (Lamb *et al.*, 2000, 2001). The values of VHS and LV echocardiographic parameters in our study were similar to previously published values for German Shepherd dogs (Lamb *et al.*, 2001; Kayar *et al.*, 2006; Muzzi *et al.*, 2006). There was no significant difference in VHS between young and old dogs, and average value of 10.26 v for VHS in German Shepherd dogs in our study is in the reference range for this breed (Lamb *et al.*, 2001). In the previous studies in German Shepherd dogs, measurements of LVWd and LVWs were 0.88 cm and 13.0 cm (Muzzi *et al.*, 2006), and significant correlation with body weight and gender was observed with no age correlation (Kayar *et al.*, 2006; Muzzi *et al.*, 2006). However, in our study, the echocardiographic results showed the existence of significant differences in LVWs (P=0.009259) and LVWd (P=0.000597) values between dogs of different age. A possible explanation could be the different age spans which were included in these studies. The study of Kayar *et al.* (2006) was performed in German Shepherd dogs from 12 months to 8 years of age, whilst in the study of Muzzi *et al.* (2006), German Shepherd dogs ranging in age from 1 to 5 years were

examined. In our study, comparison between young dogs (1-3 years of age) and aged dogs (8-13 years of age) was performed. The study group of dogs in our study, although smaller, included dogs on both ends of the scale of age span.

In addition, in our study the RWT was significantly higher in old dogs. Parietal thickness and its relation to LV chamber size have been recognized as measure of hypertrophy for more than 30 years (Sjogren, 1971). A RWT provides information regarding LV geometry independent of other calculations (Li *et al.*, 2001), precluding the requirement of most corrections. In the old dogs in our study, significant correlation was shown between LVIDd, LVWd and RWT. In humans, the reference cut point value for increased RWT derived from upper limits of normal samples is 0.44 (Ganau *et al.*, 1992) or 0.45 (Savage *et al.*, 1987). There are no data concerning reference values for the RWT in canine cardiology. In healthy Beagle dogs, the LV geometry estimated by the sphericity index and the RWT was not significantly different between young and old ones (Suzuki *et al.*, 2013). There was no significant difference in FS values between young and aged dogs in our study. These findings may suggest that there was no significant change in cardiac pump function in systole with ageing. According to our study no significant correlation was found between systolic cardiac function and LVWd and LVWs both in young and old dogs. Since increased LVW dimension leads to increased stiffness of the left ventricle and contributes to diastolic dysfunction, it could be assumed that diastolic function changes with aging. In humans, combined ventricular-vascular stiffening may contribute to the increased prevalence of heart failure with preserved ejection fraction in elderly person, particularly women (Redfield *et al.*, 2005). This is in accordance with findings of different myocardial diastolic functions between young and old Beagle dogs documented by a more sensitive echocardiographic technique (Suzuki *et al.*, 2013). In our study, the WSID, as an echocardiographic parameter of diastolic function, was significantly lower in old dogs. Since WSID represents a measure of LV preload (Aurigemma and Gaasch, 2007), it could be presumed that preload is reduced in old dogs.

Because of the simplicity of their calculation, both RWT and WSID should be assessed more frequently in dogs. They can be useful as screening methods for LV hypertrophy and diastolic dysfunction, as it was shown in our study. Further studies are needed in order to prove this assumption. In spite of the fact that systemic hypertension in dogs is usually secondary to another disease, further studies with blood pressure measurement should additionally explain the structural cardiac changes in old dogs. The small number of dogs and the use of a specific breed (German Shepherd) in our study may have an influence on the extrapolation of these findings to other breeds.

In conclusion, conventional methods of cardiac examination, such as electrocardiography and echocardiography register structural and functional changes

in aged German Shepherd dogs. Cardiac electrical activity changes were benign with no significant difference in the appearance of arrhythmias between dogs of different age. Systolic cardiac function was similar in young and old dogs. Significant differences in the values of LVW in systole and diastole, RWT and WSID were found between dogs of different age, highlighting that age should be taken into account in the assessment of LV hypertrophy and diastolic function in German Shepherd dogs.

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